OP 1
Diabetic Foot

1

High prevalence of foot ulceration in the Balkan region - a multicenter study from the BALKANDIAB network.

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Background and Aims: The epidemiology of foot ulceration (FU) is still not well understood because of the absence of reliable estimates of the true prevalence from the non-industrialized regions worldwide. Since much of our understanding of the pathophysiology of FU is derived from cross-sectional surveys this multicenter survey was undertaken to identify the prevalence of FU and potential risk factors related to peripheral neuropathy (DPN) in patients attending diabetic clinics in all the countries of the Balkan Region as a initiative of the former established Balkandiab Network
Materials and Methods: Data were obtained from 1491 diabetic subjects (mean age: 56.4±13.7 years and mean duration of diabetes 10±8.1 years, males 774 (52%), type 2 1160 (78%). The diagnosis of DPN was based on a standardized clinical examination applicable in every center according to the Neurodiab criteria. Motor and sensory deficits were assessed in both legs and scored (tendon reflexes - maximum 8 and reduced sensation of pain, touch, cold and vibration- maximum 8- respectively) using a modified scoring system of this proposed of P.Dyck. NDS≥3 established the diagnosis of DPN
Results:1) The prevalence of FU was 7.5% (SE= 1.4) and of motor deficits (MS≥1) 35.7% (SE=1.24) and of sensory deficits (NS≥2) 43.2% (SE=1.2). 2) Mean age and mean duration of diabetes were significantly higher in ulcerated patients (60.5 ±11.8 vs. 55.7±13.8 and 11.9±1.3 vs. 9.1±2.4 yrs respectively, ($p<0.05$ in both cases). FU was more prevalent in male population than in female (7.8% vs. 6.8%, $p<0.05$). 3) Furthermore FU was more common in patients with even small motor deficits (MS≥1 – reduced reflexes) (15% vs. 5% $p<0.05$) and sensory deficits (NS≥2) (22.96% vs. 8.9% $p<0.05$). The odds ratio for the patients with reduced reflexes or reduced sensations to get an ulcer are 3.5 and 2.36 respectively. The prevalence of FU did not differ between smokers and non smokers.
Conclusion: The present study showed that intrinsic factors related to neuropathy such as small abnormalities in motor or sensory function detectable in daily practice are important risk factors for FU. Further hypothesis is that extrinsic risk factors could also contribute to this high prevalence of FU. Preventive strategies should be addressed to both of the above groups of predisposing factors to FU.

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Reduction in diabetes related lower extremity amputations in the Netherlands: 1991 - 2000.

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Background and Aim: Lower extremity amputations are a prevalent complication among patients with diabetes worldwide. However, there is little data on the actual impact of the increased attention towards diabetic foot ulcers and lower extremity amputations in particular. The aim was to determine the incidence of lower extremity amputations among diabetic patients from 1991 till 2000 in the Netherlands.
Materials and Methods: A secondary database containing information regarding all hospital admissions in which a lower extremity amputation occurred for the years 1991 till 2000 was obtained from the Dutch national medical register. As a patient unique identifier was included multiple amputations and hospitalizations for a single individual could be identified. Furthermore, constant age and sex specific diabetes prevalence rates were used to calculate the diabetic population at risk for every year.
Results: In the year 1991 a total of 1687 patients with diabetes was admitted 1865 times for 2409 amputations. In the year 2000 these numbers were 1673, 1932 and 2448 respectively. The overall incidence rates of the number of patients that underwent a lower extremity amputation decreased over the years from 51.0 to 44.5 per 10,000 patients with diabetes ($p<0.05$). For male patients with diabetes the rates remained stable (62.6 versus 64.2) whereas the female population showed a significant decrease (43.4 versus 31.2, $p<0.05$). When the different levels of amputations were considered the rates showed similar trends as for the total population. In the total population the average hospitalization duration was 45.0 days in 1991 and decreased to 36.2 days in 2000. This trend continued to be true for both male patients (42.4 versus 33.8 days) and female patients with diabetes (47.3 versus 39.7 days).
Conclusions: Over the years observed in this study the incidence rates of diabetes related lower extremity amputation in the Netherlands was found to decrease in both the total and female diabetic population. This decrease is supposed to be an underestimation, because an expected increase in the occurrence of diabetes has not been taken into account. Furthermore, the duration of hospitalization decreased in time. Possible explanations may be the growing attention towards the diabetic foot problem by specialized foot clinics and the preventive actions taken by podiatrists.

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Validity of dorsal foot surface monofilament testing for neuropathy in 15,692 diabetic patients.

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Background and Aims: Screening methods for peripheral neuropathy in UK diabetes clinics include assessment of plantar insensitivity to the 10g monofilament (MF) and/or neuropathy disability score (NDS) ≥6/10. We aimed to determine the validity of MF testing on the dorsal site of the diabetic foot compared to these established neuropathy assessments.
Materials and Methods: Over 4 years we assessed 15,692 diabetic patients for peripheral neuropathy in a mass screening programme using: (a) Semmes Weinstein 1g-MF, 10g-MF and 75g-MF at 3 plantar and 1 dorsal site(s) on both feet; (b) NDS.
Results: Neuropathy prevalence, defined by NDS≥6, plantar insensitivity to 10g-MF and dorsal insensitivity to 10g-MF, was 21.3%, 17.1% and 3.8%, respectively. Exact MF score agreement between right and left feet was very high at 93.6% (kappa=0.83, $p<0.0001$) for the dorsal site and 85.7% (kappa=0.77, $p<0.0001$) for plantar sites. However, exact MF score agreement fell to 46.9% (kappa=0.17, $p<0.0001$) comparing dorsal with plantar sites, although this improved to 86.3% (kappa=0.30, $p<0.0001$) using insensitivity to 10g-MF as cut-off. Logistic regression analysis confirmed the strong relationship between NDS and dorsal insensitivity to 10g-MF (odds ratio (OR) = 10.6 [8.8-12.8 95%CI, $p<0.0001$]) and NDS and plantar insensitivity to 10g-MF (OR = 9.1 [8.3-10.0, $p<0.0001$]). The positive predictive value (PPV) of dorsal insensitivity to 10g-MF compared to the NDS≥6 was 71.5%, whereas the PPV of plantar insensitivity was 58.5%. All three neuropathy tests were strongly associated with foot ulcer history: dorsal insensitivity OR=6.05 [4.9-7.5]; NDS≥6 = 4.5 [3.9-6.2]; plantar insensitivity = 4.4 [3.7-5.1] ($p<0.0001$).
Conclusion: Although Semmes Weinstein monofilaments are highly reproducible instruments, the predictive value of the 10g-MF monofilament for defining neuropathy during mass screening is very different between the plantar and dorsal surfaces. Testing of both plantar and dorsal surfaces are recommended, however, in light of close associations with foot ulceration.

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The North Catalonia Diabetes Study (NCDS): diabetic polyneuropathy and foot risk.

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Background and Aims: The diabetic Polyneuropathy (DPN) is the most important predictor of foot ulcer and amputation in type 2 diabetes mellitus (T2DM). It is fundamental to identify the foot risk for an effective treatment

and prevention. It was studied the DPN prevalence and the risk foot to know the situation in North Catalonia Region.
Materials and Methods: The study was performed in three different regions with 92.912 inhabitants. The random sample selected was 307 subjects with T2DM (61.6% men), aged 59.63 ±7.87 years, diabetic evolution (years): 8.6 ±7, HbA1c 7.0 % ± 1.44, BMI 30.01 ± 4.7, and 307 subjects of reference sample matched for sex and age (confidence interval: 95%).
The diagnosis of DPN was performed with major signs and symptoms: two or more of the signs or one sign + symptoms were pathological classified [bilateral affectation of vibration perception thresholds (VPT) with neurothesiometer and graduate tuning fork, pinprick, cold, Semmes-Weinstein Monofilament (SW-MF) 5.07, reflexes, arthrokinesis and the items of modified Neuropathy Symptom Score]. It was also studied the diagnosis by quantitative neurological evaluation with Michigan Diabetic Neuropathy Score (MDNS) and modified scale of NSS+NDS. The foot risk was assessed according to International Group on the Diabetic Foot (table 2).
Results: DPN was observed in 23,1% of T2DM population, by the MDNS was in 28,9% and by using NSS+NDS scale in 20,5%. The technique in primary care using VPT, Ankle Reflexes, SW-MF and NSS, was 22%. In regression analysis, the presence of DPN was positive correlated to age ($p<0.001$), HbA1c ($p=0.001$), diabetes evolution ($p=0.001$), diastolic blood pressure ($p<0.05$), cardiovascular disease ($p<0.05$), HDL cholesterol ($p<0.001$) and also correlated to DPN diagnosis by MDNS>6 ($p<0.001$) and NSS+NDS ($p<0.001$). The diagnosis of DPN by MDNS manifested a sensitivity of 67.1% and specificity 82.5%, by NDS+NSS, sensitivity: 61.4% and specificity: 91.8%. The technique used in primary care manifested a sensitivity of 77,5%, a specificity 94,9% and a correct assessment: 90,8% (Positive predictive value: 85,54%, Negative predictive value: 93,6%), (tab.1). Foot risk, category 2-3, was found in the 10,92% total population of diabetic subjects (tab.2).
Conclusion: The DPN found in this population was considered normal for the age rank among T2DM in primary care. An important average of T2DM patients manifested a foot risk. The study of DPN by MDNS overvalued the DPN an the NDS+NSS underestimates the DPN in subjects with poor neuropathy symptoms. Both methods are not applicable in the Primary Care, and needs to unify diagnosis criteria and easy techniques has to be used.

* Presence of DPN by technique in Primary Care using bilateral affectation and means score of lower limbs with VPT (graduate Tuning Fork) , Ankle Reflex , SW-MF and the modified NSS (Tab. 1)

	Sensitivity	Specificity	% Correct Assessment
* VPT+AR + SW-MF+NSS Primary Care	77,5%	94,9%	90,8%

INTERNATIONAL WORKING GROUP ON THE DIABETIC FOOT CONSENSUS, 1999. RISK SYSTEM CLASSIFICATION (Tab. 2)

RISK PROFILE	RISK CATEGORY	FREQUENCY
Without DPN	0	76,62%
With DPN	1	13,46%
DPN + Peripheral Vascular Disease		
o Deformity in foot, or both.	2	9,64%
Previous ulcer	3	1,28%

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Nuclear imaging in diabetic osteopathy.
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Background and Aims: A focus of Osteomyelitis may underlie a chronic, non-healing diabetic foot ulcer, which may not be detected by an X-ray of the foot. Diagnosis and appropriate treatment of this underlying osteomyelitis is necessary to heal the ulcer. MRI of the foot ($100 per test) is helpful in detecting early osteomyelitis, but nuclear imaging ($20 per test) is a considerably cheaper investigation. Early acute Charcot foot presents as a hot, swollen foot with a history of minimal or no trauma, and the diagnosis is traditionally made on clinical grounds as X-rays of the foot may be normal. We aimed to assess the value of nuclear imaging in the diagnosis of X-ray negative osteomyelitis and early X-ray negative Charcot foot in diabetic patients.
Materials and Methods: All patients who presented to our Podiatry department between April, 2001 and March 2002 who were clinically suspected to have early Charcot foot or osteomyelitis underlying a non-healing foot ulcer (symptoms longer than 3 months duration, despite off-loading and appropriate antibiotic therapy) with normal X-rays were subjected to Nuclear imaging with Technitium99 MDP. Increased tracer uptake in the vascular and soft tissue phase that persisted in the skeletal phase of the bone adjoining the ulcer was taken as positive for osteomyelitis. Increased tracer uptake in the third or skeletal phase in the first 3 tarsal bones in the absence of any evidence of infection was taken as positive for early Charcot foot.
Results: A total of 86 patients (50 M & 36 F) were included in the study. 50/62 patients investigated for suspected osteomyelitis were subjected to bone curettage on the basis of positive bone scan. 46 of these 50 patients had a positive bacteriological bone culture and were then treated with culture specific antibiotics for 12 weeks. None of the 12 patients with a negative bone scan showed any subsequent features clinically or radiologically of osteomyelitis over minimum follow up of 6 months. The specificity was 75% and sensitivity was 100%. 21 out of 24 patients with clinical presentation of acute Charcot foot had a positive bone scan consistent with acute Charcot foot. 15 patients who were screened for osteomyelitis had bone scan evidence of unsuspected incidental early Charcot foot.
Conclusion: Our study has established the value and accuracy of a cost-effective investigation like Technitium99 bone scan in early osteomyelitis and early Charcot foot in diabetic patients with normal radiographs. A positive bone scan could help in the decision to proceed with bone curettage in suspected osteomyelitis and helps to confirm the diagnosis in early acute Charcot foot and will enable prompt therapeutic intervention and offloading.

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Pressure reduction through various premanufactured shoe models with insoles in diabetic foot syndrome to prevent ulceration: a prospective randomised study.
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Aim: A prospective study was performed to answer the question if defined premanufactured models of shoes in combination with special insoles can prevent ulceration in diabetic foot syndrome (dfs).
Methods and Patients: 81 diabetic patients (aged 34-89, mean: 64.2 yr, duration of diabetes 2-54 yr, mean: 18.3 yr; 17 patients with type 1 and 64 patients with type 2 diabetes) with dfs and significant peripheral polyneuropathy without relevant peripheral artery occlusion disease were randomised and were chosen for two kinds of diabetes-adapted shoes (“comfortable shoes“ n = 39 or “semi-orthopedic shoes” n = 42). For each pair of shoes including slippers a special kind of comparable individually manufactured diabetes-adapted foot bedding 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 (esp. among others pedography barefoot with emed®-platform and within in the shoes with emed-pedar®) and at the orthopedic shoe manufacturer.
Results:

Maximum pressure [N/cm2]	barefoot 0year	barefoot 1year	barefoot 2years	in shoe 0year	in shoe 1year	in shoe 2years
„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]	barefoot 0year	barefoot 1year	barefoot 2year	in shoe 0year	in shoe 1year	in shoe 2years
„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 the premanufactured shoes and individually adapted 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. During the two years only 7 patients showed an ulceration (4 recurrency and 3 new). Our low rate of

recurrency of ulceration (21,1%) is much lower than the reported rates in the literature (26% - 87%, in a comparable duration of study: >42%!).
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. With the insoles both shoe models are effective for preventing ulceration in diabetic foot syndrome.

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Prospective trial of percutaneous transluminal angioplasty (PTA) as a primary treatment in diabetic foot ulcer with severe ischaemia.
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Background and Aims: Arterial bypass surgery has proven its efficacy to attain limb salvage in diabetic and severely ischemic foot ulcers. Recently PTA has been proposed as an alternative therapeutic because of the invasiveness and peri-operative risk of the surgical procedure. But its effectiveness in severe ischemic foot is still controversial.Objective : to evaluate the efficacy in terms of limb salvage rate and healing time of PTA as a primary treatment of diabetic severely ischemic foot ulcers, and to identify the factors that determine PTA effectiveness.
Materials and Methods: 32 diabetic patients successively hospitalized in a specialized unit with a C or D stage foot ulcer (grade 2 or 3). Mean age: 67y; type 2 diabetes: 84%; mean diabetes duration : 22y; mean transcutaneous oxygen pression (TcPO2): 15mmHg. PTA was used in any situation in which an interventional radiologist and vascular surgeon agreed that it was possible. Patients were followed until healing or at least 12 months. This cohort was compared with an historical one (n= 27) which had had arterial bypass surgery as primary treatment.
Results: PTA was assessed as feasible in 25 (78%) patients. A mean of 1.6 lesion/patient was treated. Immediate technical failure in 2 cases. Primary poor clinical short-term outcome in 9/25 (36%) leading to 5 secondary bypasses, 4 local worsening without feasible arterial bypass. Good clinical outcome in 13/ 25 (52%). At the end of study: limb salvage rate:68%; death: 20%.The only factor significantly predictor of a primary outcome success (no need of bypass and no major amputation) is the integrity of at least one foot artery (dorsal pedal or common plantar) ($p<0.03$). The number localisation or type of treated lesions, the integrity of lower leg artery are not independent predictive factors. Limb salvage rate (LSR) and healing rate (HR) among patients who could have benefit from a primary surgical bypass (n=23), were not significantly different from those obtained in the historical cohort (primary surgical bypass): LSR=21/23 post PTA compared with 26/27 post primary bypass; HR: 44% and 50% respectively at 6 months, and 86% and 89% at 18 months.
Conclusion: In severe ischemic foot ulcers, PTA may be attempted in $^3/_4$ cases, with effectiveness in half cases. The presence of at least one foot artery is the only significant good prognostic factor. Limb salvage rate, healing rate and time are comparable with those obtained in severe ischemic foot ulcer after bypass grafting.

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Treating infected diabetic foot ulcers: Linezolid is clinically superior to amiopenicillin/β-lactamase inhibitors (Amino/β-LI).
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Background and Aims: Foot infections are a common and serious complication of diabetes, and often lead to amputation. They are predominantly caused by aerobic gram-positive cocci, sometimes in combination with gram-negatives and/or anaerobes. World-wide gram-positive bacteria are becoming increasingly resistant to commonly used antibiotics, especially β-lactams, which are the most frequently used agents. Linezolid is a new oxazolidinone active against virtually all gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). We compared outcomes of treatment with IV or oral linezolid against amino/β–LI (ampicillin-sulbactam IV or amoxicillin-clavulanate po) for infected foot ulcers as part of a large multinational prospective randomized controlled trial.
Materials and Methods: At 45 sites in 8 countries diabetic patients with various types of foot infections underwent a thorough examination, including a vascular assessment, wound curettage cultures, laboratory tests and X-rays. They were then randomized (2:1) to receive either linezolid or the amino/β–LI agent. Vancomycin could be added to the amino/β–LI if MRSA was suspected or cultured. Patients could be treated by the oral or IV route, as an outpatient or inpatient, for 7-28 days, all at the discretion of the investigators.
Results: Among 361 evaluable patients enrolled, 283 (78%) had an infected foot ulcer; 190 were randomized to linezolid, 93 to amino/β–LI. The *overall* cure rate was significantly greater in linezolid than amino/β–LI treated patients (81% vs. 68%, 95% CI 1.9,25.2, $p<0.001$). Initial therapy was oral in 74% and outpatient for 57%, and foot ischemia was present in 43%. The mean duration of total therapy was 17 days in each group. Comparisons of clinical cure rates for the two therapy groups by these parameters are shown in the table.
Conclusion: In this large randomized controlled trial the overall clinical cure rate for infected diabetic foot ulcers was statistically better for linezolid than for the amino/β–LI agents. This was surprising, as the study was only statistically powered to demonstrate equivalence. Of note was that outcomes were similar for patients treated IV vs. oral, as outpatients vs. hospitalized, and for those with and without foot ischemia. Linezolid (oral or IV) appears to be a good choice for treating these infections, especially if resistant gram-positives are suspected or isolated.

Clinical Cure Rate By Therapy

Parameter	Linezolid	Amino/β-LI
Oral therapy	81%	61%
IV therapy	82%	71%
Outpatient therapy	81%	61%
Inpatient therapy	82%	71%
Foot ischemia	82%	70%
No foot ischemia	89%	66%
Duration IV (when used)	8 days	10 days

OP 2
Insulin Therapy

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Both continuous subcutaneous insulin infusion and a multiple daily insulin injection regimen with glargine as basal insulin are better than traditional multiple daily insulin injection treatment.

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Background and Aims: We evaluated, in an open parallel group trial of 1 year duration, the efficacy of two regimens of intensive insulin treatment [1)CSII vs 2)MDI with lispro at each meal plus glargine as basal] in 32 type 1 diabetic patients that had been treated with MDI (regular or lispro insulin before each meal plus NPH as basal insulin) for at least 1 year,.
Materials and Methods: Sixteen type 1 diabetic patients (age 37.7±11.2 yr; 8 M, 8 F; duration of diabetes 19.6±9.2 yr) treated with CSII, receiving lispro at multiple basal infusion rates plus boluses at meals (CSII group) were compared to sixteen type 1 diabetic patients (age 42.9±15.6 yr; 7 M, 9 F; duration of diabetes 14.7±11.1 yr) treated with multiple daily insulin injections with lispro at each meal combined with glargine (glargine group). In all patients, HbA_{1c}, fasting blood glucose, total and HDL cholesterol, triglycerides, insulin requirement, severe hypoglycemic episodes were evaluated every three months both the year before the study and the year during active treatment. We compared the responses to CSII and MDI with glargine by analysing (using a non-paired t test) the differences between measured variables before and after the two treatments.
Results: In the CSII group compared to traditional MDI treatment there was a significant decrease of HbA_{1c} (9.2±1.6% during traditional MDI vs 8.2±1.2% during CSII, p<0.001), fasting plasma glucose (11.9±3.5 vs 8.1±2.8 mmol/l, p<0.001), triglycerides (100.9±41.6 vs 85.5±41.4, p<0.05), severe hypoglycemic episodes (0.37 vs 0.12 per patient/year, p<0.05), insulin requirement (50.4±18 vs 40.1±13.1 U/day, p<0.001). In the glargine group, compared to traditional MDI treatment, there was a significant decrease of HbA_{1c} (8.5±1.3 vs 7.9±1.2%, p<0.001), fasting plasma glucose (12.3±3.9 vs 10.5±3.1 mmol/l, p<0.001), triglycerides (90.6+50.8 vs 77.0±39.3 mg/dl, p<0.05), severe hypoglycemic episodes (0.43 vs 0.18 episodes per patient/year, p<0.05). Insulin dose was unmodified(44.0±11.1 vs 43.1±11.1 U/day, n.s.).There was no significant change in BMI either in the CSII group (24.7±4.2 vs 24.6±4 kg/m^2) or in the glargine group (22.8±2.9 vs 22.9±3 kg/m^2).No significant difference between the two groups was present in the degree of improvement of HbA_{1c}, fasting plasma glucose, triglycerides, severe hypoglycemic episodes. Only insulin requirement reduction was significantly greater in the CSII group than in the glargine group (-10.3±3.3U/day vs -0.9±0.3 U/day, respectively, p<0.001).
Conclusion: Both CSII and MDI with lispro plus glargine improve metabolic control and reduce severe hypoglycemia in type 1 diabetic patients, that are unsatisfactorily controlled on MDI using NPH as basal insulin.

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Co-administration of insulin glargine with oral therapy in patients with Type 2 diabetes allows flexible dosing and improved glycaemic control with reduced hypoglycaemia compared with NPH insulin.

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Background and Aims: Patients with Type 2 diabetes may need insulin if oral antidiabetic drugs (OADs) provide inadequate glycaemic control. Once-daily (morning or bedtime) insulin glargine (LANTUS®) provides 24-hour basal glucose control and facilitates the attainment of target HbA_{1c} levels <7%. The efficacy and safety of combining an OAD (glimepiride) with morning or bedtime insulin glargine versus bedtime NPH insulin was assessed in this multinational, multicentre, randomized study.
Materials and Methods: Insulin glargine data were merged to assess its effect versus NPH insulin, independently of injection timing. A total of 695 patients were randomized; 463 received insulin glargine and 232 NPH insulin for 24 weeks.
Results: There were no between-treatment differences (insulin glargine vs NPH insulin) in: sex; body mass index (28.6 vs 28.9 kg/m^2); duration of diabetes (10.2 vs 9.9 years); duration of previous OAD therapy (8.0 vs 8.3 years); C-peptide levels (1.00 vs 1.07 nmol/L); baseline HbA_{1c} levels (9.1 vs 9.1%); and fasting blood glucose (FBG: 12.1 vs 12.2 mmol/L). Insulin glargine-treated patients were younger versus NPH insulin patients (60.3 vs 61.8 years; p=0.039). HbA_{1c} reductions were greater with insulin glargine versus NPH insulin (1.11 vs 0.83%; p=0.026). Compared with NPH insulin, insulin glargine-treated patients also experienced fewer episodes of symptomatic (57.9 vs 49.7%; p=0.036) and nocturnal (38.2 vs 19.7%; p=0.001) hypoglycaemia. FBG values at study endpoint were the same (16.9 mmol/L; p=not significant [NS]). Insulin glargine-treated patients had lower median daily glucose values versus patients receiving NPH insulin based on an 8-point blood glucose profile (8.9 vs 9.7 mmol/L; p <0.001). Insulin titration led to an increase in insulin dose of 19.7 versus 17.8 IU (insulin glargine vs NPH insulin; p=NS), with final insulin doses of 39.5 versus 36.8 IU (p=NS).
Conclusion: Patients poorly controlled with OADs have better metabolic control with lower HbA_{1c} levels and less symptomatic/nocturnal hypoglycaemia when treated with once-daily, subcutaneous (morning or bedtime) insulin glargine versus NPH insulin. Insulin glargine allows flexible once-daily dosing provided it is administered at the same time each day.

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Incidence of nocturnal hypoglycaemia in patients with Type 2 diabetes is comparable when either morning or bedtime insulin glargine is co-administered with glimepiride.

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Background and Aims: Insulin glargine (LANTUS®) is a long-acting human insulin analogue with no pronounced peak. This study assessed whether there was a difference in the frequency of nocturnal hypoglycaemic episodes in patients with Type 2 diabetes when insulin glargine was given in the morning (06:00–09:00am) or at bedtime (09:00–11:00pm) and with glimepiride (Amaryl®).
Materials and Methods: A total of 624 patients with Type 2 diabetes, poorly controlled with oral antidiabetic drugs (OADs), were treated with individually titrated, once-daily insulin glargine in this open-label, randomized, multinational, multicentre, 28-week study. Insulin glargine doses were titrated to achieve target fasting blood glucose levels 5.5 mmol/L (≤100 mg/dL).
Results: Baseline characteristics in both groups (full-analysis set) were similar. Mean age and duration of diabetes were 62.1 ± 10.2 versus 61.5 ± 9.6 years and 9.5 ± 6.1 versus 10.3 ± 6.8 years in the morning and bedtime groups, respectively. There was no difference in mean daily insulin dose between the morning and bedtime groups from Week 1 to study endpoint (16.1 to 34.7 IU vs 16.1 to 32.4 IU; p=0.15). The number of patients with nocturnal hypoglycaemic episodes during treatment was similar in the morning and bedtime groups (13.0% vs 14.9%; per-protocol analysis, one-sided 95% confidence interval [–100%; 2.84%]). Of those patients who experienced nocturnal hypoglycaemia, 51.3% (morning) and 54.8% (bedtime) experienced one episode only. There was no significant difference (morning vs bedtime) in the incidence of symptomatic hypoglycaemia (42.8% vs 38.1%) and severe hypoglycaemia (1.3 vs 0.7%). All patients achieved a similar reduction of adjusted mean baseline to endpoint HbA_{1c} (morning: 8.82% to 7.18%; bedtime: 8.81% to 7.23%; p=0.42, full-analysis set).
Conclusion: The study shows that similar rates of nocturnal hypoglycaemia, and symptomatic and severe hypoglycaemia, can be achieved with flexible dosing (morning or bedtime) of insulin glargine, whilst maintaining good glycaemic control. Flexibility in insulin injection timing should facilitate treatment adherence in patients with diabetes.

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Lower within-subject variability of insulin detemir compared to NPH insulin and insulin glargine in subjects with Type 1 diabetes.

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Background and Aims: We compared the within-subject variability of the pharmacodynamic and pharmacokinetic effects of the basal insulin analogue insulin detemir (IDet) with that of NPH insulin and insulin glargine (GL) in a double blind, randomised, parallel group design.

Materials and Methods: Fifty-four patients with type 1 diabetes (32 men and 22 women; age 38±10 years (mean±SD); BMI 24±2 kg/m^2; HbA_{1c} 7.5±1.2 %; diabetes duration 18±9 years) participated in four identical study days and received 0.4 U/kg of either human NPH, GL, or IDet s.c. under euglycemic glucose clamp conditions (target blood glucose concentration 5.5 mmol/L). Variability was assessed by comparing the coefficients of variation (CV) based on the four individual measurements of the pharmacodynamic and pharmacokinetic effects of the basal insulin preparations which were recorded for 24 and 28 hours post-dose, respectively.
Results: The within-subject variability in glucose lowering effect was significantly less for IDet than for NPH and GL (2.5 and 1.8-fold lower CV for GIR-$AUC_{0\text{-}24h}$, respectively), see table. Similar findings were also observed for the pharmacokinetic parameters.
Conclusion: This is the first systematic investigation of the variability in the pharmacodynamic and pharmacokinetic properties of insulin detemir, NPH and insulin glargine. The results show that insulin detemir is associated with significantly less within-subject variability than either of the other basal insulin preparations. This suggests that insulin detemir provides a more predictable therapeutic effect than NPH insulin and insulin glargine.

Within-Subject Variability, expressed as Coefficients of Variations (CV) in %

	Insulin Detemir	NPH Insulin	Insulin Glargine
Pharmacodynamics (assessed by Glucose Infusion Rates (GIR))			
GIR-$AUC_{0\text{-}12h}$	27	59*	46*
GIR-$AUC_{0\text{-}24h}$	27	68*	48*
GIR_{max}	23	46*	36*
Pharmacokinetics (assessed by plasma concentrations of insulin (INS), insulin glargine and insulin detemir)			
INS-$AUC_{0\text{-}12h}$	15	26	34
INS-$AUC_{0\text{-}infinity}$	14	28	33

*: p<0.001 compared with insulin detemir (no statistical analyses were performed to compare pharmacokinetic CVs). CVs were determined using an ANOVA model after log-transformation of the parameters.

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Treatment with insulin detemir allows flexible timing of administration in subjects with Type 1 diabetes.
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Background and Aims: The prolonged and predictable time-action profile of insulin detemir (IDet) suggest that this basal insulin analogue could be administered earlier than bedtime and still provide sufficient insulin supply during the night and early morning hours without increasing the risk of nocturnal hypoglycaemia. Therefore, the aim of this trial was to compare the effect of two different administration-time regimens with IDet to that of a traditional administration regimen of NPH insulin (NPH) in subjects with type 1 diabetes.
Materials and Methods: This was a multi-national, 16–week, open, randomised, parallel group trial including 400 subjects with type 1 diabetes (mean age: 40 yrs, duration of diabetes: 15 yrs, BMI: 25.2 kg/m^2, HbA_{1c}: 8.07%). Subjects received twice daily treatment with IDet either morning and before dinner ($IDet_{m+d}$, N=139) or morning and bedtime ($IDet_{m+b}$, N=129) or NPH morning and bedtime (N=132). All groups received insulin aspart at meals.
Results: After 16 weeks of treatment HbA_{1c} decreased ~ 0.4% point in all groups and was comparable between the three treatments, ($IDet_{m+d}$: 7.67%, $IDet_{m+b}$: 7.65%, NPH: 7.73%, p=0.6). Lower fasting plasma glucose were observed with both $IDet_{m+d}$, (9.8 *vs* 11.1 mmol/l, p≤0.006) and $IDet_{m+b}$ (9.1 *vs* 11.1 mmol/l, p<0.001), compared to NPH, while the two IDet groups did not differ (p=0.15). Furthermore, lower within-subject variation in self-measured fasting blood glucose was observed in both IDet groups (SD for $IDet_{m+d}$: 2.5 and $IDet_{m+b}$: 2.6 mmol/l) compared to NPH (SD: 3.1 mmol/l, p<0.001) with no significant difference between the two IDet groups. Ten-point blood glucose profiles were similar during the day but differed at night with lower glucose levels in the $IDet_{m+d}$ group in the period between dinner and breakfast (p=0.043), compared with the two other groups. Glucose fluctuations (area between the individual glucose curve and its mean level) during 24 hours based on continuous glucose monitoring was lower with IDet (both groups combined) than with NPH (46.5 *vs* 54.3 mmol/l * h p=0.04). After 16 weeks, mean body weight (adjusted for baseline and change in HbA_{1c}) was lower with IDet than with NPH ($IDet_{m+d}$: p<0.001, $IDet_{m+b}$: p=0.050) with changes of -0.6 kg ($IDet_{m+d}$), 0.1 kg ($IDet_{m+b}$), 0.7 kg (NPH), respectively. The risk of hypoglycaemia was similar in the three treatment groups (diurnal p=0.52 and during the night p=0.58). Treatment with IDet and NPH gave rise to similar safety profiles.
Conclusion: Treatment with insulin detemir resulted in lower and less variable glucose levels than NPH, and insulin detemir can be administered either morning and dinner, or morning and bedtime, with similar glycaemic control, according to the need of the individual patient.

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Insulin detemir offers improved glycaemic control, less weight gain, and flexible timing of administration compared to NPH insulin.
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Background and Aims: The prolonged and predictable time-action profile of insulin detemir (IDet) should allow flexibility in administration time without increased risk of nocturnal hypoglycaemia. Administration of IDet at fixed times of day, rather than at variable times in association with meals and bed, could potentially provide more stable basal insulin profiles and eliminate the regimen-inherent source of variation. Therefore, the aim of this trial was to compare the effect of two different IDet administration-time regimens to that of a traditional administration regimen with NPH insulin (NPH) in people with type 1 diabetes.
Materials and Methods: This was a multinational, 16-week, open, randomised, parallel group trial including 408 people with type 1 diabetes (mean age 40 yr; duration of diabetes 17 yr; BMI 25.2 kg/m^2; HbA_{1c} 8.60%). Individuals received IDet twice daily either at 12-h intervals ($IDet_{12h}$ n=137) or morning and bedtime ($IDet_{m+b}$ n=139) or NPH morning and bedtime (n=132). All groups received insulin aspart at mealtimes.
Results: After 16 weeks treatment, there was a statistically significant difference in HbA_{1c} between the combined IDet groups and the NPH group (-0.18%; 95% CI; [-0.34;-0.02], (p=0.027)). Although HbA_{1c} was not significantly different between the three groups in the overall analysis (p=0.082), it tended to be lower in both IDet groups; ($IDet_{12h}$ 7.75%, $IDet_{m+b}$ 7.78%) than in the NPH group (7.94%). Lower fasting plasma glucose ($IDet_{12h}$ 9.7, $IDet_{m+b}$ 8.9, NPH 11.2 mmol/l, p<0.001) and lower within-subject variation (SD) in fasting self-monitored blood glucose ($IDet_{12h}$ 3.0, $IDet_{m+b}$ 2.9, NPH 3.5 mmol/l, p<0.001) was achieved in both IDet groups compared to NPH. Glucose fluctuations (area between the individual glucose curve and its mean level) during 24 h based on continuous glucose monitoring tended to be lower with IDet (both groups combined) than with NPH (57.3 *vs* 63.8 mmol/l.h, p=0.065). The risk of night-time hypoglycaemia was 33% lower in the $IDet_{m+b}$ group than in the NPH group (p<0.001), but similar in the $IDet_{12h}$ and NPH groups (NS). The overall shape of night-time blood glucose profiles tended to differ between the three groups (p=0.054); the glucose levels in the $IDet_{12h}$ group being lower than for the two other groups. Weight gain, adjusted for change in HbA_{1c}, was significantly lower in the IDet groups compared with NPH ($IDet_{12h}$ 0.02, $IDet_{m+b}$ 0.24, NPH 0.86 kg, p=0.018).
Conclusion: Treatment with insulin detemir can result in improved glycaemic control and significantly less weight gain compared to NPH. Timing of administration of insulin detemir can be flexible and tailored to the needs of the individual.

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In inadequately controlled patients with Type 2 diabetes, Biphasic Insulin Aspart 30 combined with Pioglitazone provides better glycaemic control than Biphasic Insulin Aspart 30 monotherapy or Pioglitazone/Sulphonylurea combination.

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Background and Aims: This study was designed to confirm the safety and efficacy of combining Biphasic Insulin Aspart 30 and Pioglitazone (BIAsp/Pio) versus Biphasic Insulin Aspart 30 monotherapy (BIAsp) or a combination of Pioglitazone and Glibenclamide (Pio/Glib) in type 2 diabetes subjects inadequately controlled with any kind of sulphonylurea mono- or combination therapy.
Materials and Methods: This open–labelled, parallel group study consisted of 2-week screening followed by 18-weeks' treatment. Key efficacy endpoints included HbA_{1c}, fasting and average 8-point blood glucose profiles, lipid profiles, hypoglycaemic frequency and adverse events.
Results: A total of 246 patients completed the trial. HbA_{1c} at end-of-trial was statistically significantly lower in the BIAsp/Pio group than in the BIAsp and Pio/Glib groups, respectively. For the 8-point blood glucose profiles, average blood glucose and breakfast prandial increment decreased over time, with statistically significantly lower values with BIAsp and BIAsp/Pio groups than with the Pio/Glib group at end-of-trial. No major hypoglycaemic events were reported. Minor hypoglycaemic episodes were few and mainly in the BIAsp group: 1.5 episodes/year; BIAsp/Pio: 0.5 episodes/year; Pio/Glib 0.1 episodes/year. The risk of hypoglycaemic episodes was lower in the BIAsp/Pio group than the BIAsp group, and lower in the Pio/Glib than the BIAsp/Pio group. More patients experienced possibly/probably related adverse events in the BIAsp/Pio group (28%) than in the BIAsp group (20%) and Pio/Glib group (16%). Oedema (mild severity) and weight increase were the more frequently reported adverse events (BIAsp: 1-3%; BIAsp/Pio: 5-8%; Pio/Glib: 1-2%). There were no indications of liver toxicity.
Conclusion: BIAsp/Pio provided an improved glycaemic control compared with BIAsp 30 monotherapy or Pio/Glib combination therapy. We conclude that the best option for type 2 patients who have failed on sulphonylurea mono- or combination therapy is to replace the sulphonylurea mono- or combination therapy with BIAsp + pioglitazone.

95% Confidence Intervals for differences between treatment groups at end of trial (week 18)

	BIAsp/Pio –BIAsp	Glib/Pio – BIAsp	BIAsp/Pio – Glib/Pio
HbA_{1c} (%)	-1.04 ; -0.16	-0.40 ; 0.48	-1.09 ; -0.20
BG Average(mmol/l)	-1.57 ; 0.10	0.38 ; 2.07	-2.81 ; -1.10
BG increment, breakfast(mmol/l)	-1.35 ; 0.60	0.18 ; 2.15	-2.54 ; -0.54
Triglycerides(mmol/l)	-0.43 ; 0.20	0.02 ; 0.66	-0.78 ; -0.13
Total Cholesterol (mmol/l)	-0.08 ; 0.40	-0.08 ; 0.41	-0.25 ; 0.24

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Oral insulin: proof of concept in Type 2 diabetic patients.

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Background and Aims: The objective of this study was to determine the pharmacokinetic (PK) and pharmacodynamic (PD) properties of an oral insulin (OI) formulation with a novel delivery agent (DA) in comparison to s.c. regular insulin (RI). DA is a drug carrier which has been shown to enhance the gastrointestinal absorption of insulin when applied orally as a capsule.
Materials and Methods: Ten male type 2 diabetic patients (mean age 56±9 years [mean±SD], HbA1c 7.0%±1.1%, BMI 28±3 kg/m^2) participated in this 2-period, randomized, cross-over trial. The metabolic effects of the study medication consisting of either OI (300 U insulin combined with 400 mg DA) or 15 U RI s.c. were assessed with the glucose clamp technique (continuous baseline insulin infusion 0.2 mU/kg/min, individual fasting blood glucose concentration as clamp level) over 6h on two separate study days. Plasma insulin (INS) concentrations were determined in regular intervals.
Results: OI exhibited a faster and higher rise in insulin concentrations and showed a faster onset of action than RI ($AUC_{INS\ 0\text{-}1h}$ 2559±1831 vs. 542±296 µU x mL^{-1} x min, $p<0.01$; C_{INSmax} 93±71 vs. 33±11 µU/ml, $p<0.01$; t_{INSmax} 27±9 vs. 161±83 min, $p<0.01$). Accordingly, this trend was mirrored in the PD results ($AUC_{GIR\ 0\text{-}1h}$ 173±86 vs. 27±32 mg/kg, $p<0.01$; t_{GIRmax} 40±16 vs. 255±108 min, $p<0.01$; early $t_{50\%}$ 13±6 vs. 150±87, $p<0.01$). The maximum glucose infusion rates showed no statistically significant difference. Relative bio-efficacy (based on PD results) was as high as 20% (median) in the first hour after application, and 3% over 6h. Respective values for bio-availability (based on PK results) were 13 and 2%. No adverse events were observed with OI.
Conclusion: In conclusion, this is the first glucose clamp study proving that orally applied insulin exhibits a pronounced metabolic effect. In view of the presented PD and PK properties, and the advantages of an oral administration (convenience of administration, high portal insulin concentrations) this oral insulin formulation seems to be a very attractive candidate for meal-related insulin therapy in type 1 and type 2 diabetic patients.

OP 3
Experimental and Clinical Immunology

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Reduced diabetogenic effector cells in the NOD mice lacking interferon regulatory factor-1.

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Background and Aims: Transcription factor interferon regulatory factor-1 (IRF-1) was originally indentified as a protein binding to DNA sequences commonly found in type 1 interferon gene promoters. IRF-1 binds as well to the IFN-stimulated regulatory elements in many IFN-inducible gene promoters, including MHC class I and II, inducible nitric oxide synthase, some caspases, and IL-12/p40. These regulatory functions give IRF-1 major roles in diferentiation of CD8 T cells, NK cells, and NKT cells, development of Th1 immune response, and apoptosis of various cells. We previously established IRF-1-deficient non-obese diabetic (NOD) mice ((-/-) mice) to know the role of IRF-1 in development of type 1 diabetes (Nakazawa et al, J Autoimmun, 2002). In these mice, diabetes and isulitis were completely suppressed, and the percentage of $CD8^+$ splenocytes were significantly reduced compared to wild type NOD mice ((+/+) mice). Spleen cells from (-/-) mice proliferated and showed Th2-deviated cytokine production in response to a murine GAD65-derived peptide. This Th2-deviated phenotype may partially interpret the mechansm of suppretion of diabetes, however, the complete absence of insulitis implicated that reduction of diabetogenic effector cells may be involved as well. In this study, we focused on the effect of genetic disruption of IRF-1 on effector mechanism of type 1 diabetes mellitus in NOD mice.
Materials and Methods: In an adoptive transfer experiment, splenocytes were obtained from the 12-wk-old female mice, and $3.8x10^7$ cells/ mice were intraperitoneally transferred into 6-wk-old female NOD-SCID mice. In flow cytometric analyses, thymocytes and splenocytes were stained with anti-mouse CD3, CD4, CD8, CD24, or CD25 monoclonal antibodies. To induce apoptosis in thymocytes, the 9-week-old female mice were intraperitoneally injected with 50 µg of anti-mouse CD3 monoclonal antibody. The 11-week-old female mice were intraperitoneally injected with 250 mg/kg of cyclophosphamide to induce diabetes.
Results: The recipients from (-/-) mice never developed diabetes (0/8), whereas 75% (6/8) of those from (+/+) mice did by 10 weeks of age (p=0.007, Fisher's exact probability test). Contrary to the donors, very mild insulitis was observed in the recipients from (-/-) mice. (-/-) mice showed significantly reduced percentage of $CD4^-CD8^+$ thymocyte (0.48 ± 0.044 %) compared to the controls (2.0 ± 0.25 %, p<0.05, Mann-Whitney's U test). Impaired sensitivity of apoptosis induced by anti-CD3 antibody in the semimature $CD4^+CD8^-CD24^{high}$ and $CD4^-CD8^+CD24^{high}$ thymocytes of NOD mice, reported by Kishimoto et al (Nature Immunol, 2001), was restored in (-/-) mice. Cyclophosphamide did not induce diabetes in (-/-) mice. Splenocytes from both (+/+) and (-/-) mice contained similar level of $CD3^+CD4^+CD25^+$ cells that may have regulatory function.
Conclusion: The reduction of $CD8^+$ effector T cells, together with an immune deviation to Th2 immune response, may contribute to prevention of developing diabetes in the IRF-1 deficient NOD mice. Active suppression seemed not to be involved in the mechanism.

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Recombinant human interleukin-11 inhibits NF-kB and AP-1 activation in pancreatic islets and prevents diabetes induced with multiple low doses of streptozotocin.

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Background and Aims: Diabetes can be induced with multiple low doses of Streptozotocin (MLD-STZ) in male mice of susceptible strains.This laboratory has reported that MLD-STZ downregulate (P<0.05) the anti-inflammatory T helper (Th)2-type cytokines interleukin (IL)-4 and IL-10 as well as the Th3-type cytokine transforming growth factor (TGF)-ß1 in islets ex vivo of diabetes-susceptible male but not of diabetes-resistant female C57BL/6 mice.The proinflammatory Th1-type cytokine tumor necrosis factor (TNF)-α and interferon (IFN)-γ, in contrast, are similarly upregulated (P<0.05) by MLD-STZ in islets of both genders. Furthermore, MLD-STZ (P<0.001) downregulate the mRNA expression of the anti-inflammatory cytokine IL-11 and its receptor IL-11R only in islets of male mice. Thus, the reduction of anti-inflammatory cytokines may facilitate ß-cell destruction by Th1-type cytokine mediators. IL-11 is a pleiotropic cytokine with potent anti-inflammatory activity. Since IL-11 can prevent spontaneous diabetes in the non obese diabetic (NOD) mouse, we studied the impact of recombinant murine (rm)IL-11 on proinflammatory cytokine profiles in isolated islets in vitro and of recombinant human (rh)IL-11 on both MLD-STZ-induced diabetes and on the transcription factors nuclear factor (NF)-kB and activator protein (AP)-1 in islets ex vivo.
Materials and Methods: C57BL/6 mice were injected intraperitoneally with MLD -STZ - either alone or in addition to a total of 130 µg rhIL-11 given at equal doses for 13 consecutive days. Isolated islets were used for mRNA expression of IL-11 and IL-11R by semiquantitative RT-PCR. Single cell suspensions of islets served for cytokine analyses by flowcytometry. NF-kB and AP-1 activity was measured by the electrophoretic mobility shift assay (EMSA).
Results: rhIL-11 prevented (P<0.001) MLD-STZ diabetes and ameliorated oral glucose intolerance to near normal with time in weeks after treatment. rhIL-11 attenuated (P<0.01) MLD-STZ-induced activation of NF-kB and AP-1, which are involved in proinflammatory gene activation. In vitro, rmIL-11 reduced (P<0.01) the constitutive levels of the proinflammatory cytokines TNF-α and interferon IFN-γ and prevented (P<0.01) their STZ-stimulated increment (P<0.05).
Conclusion: rhIL-11 may protect ß-cells from MLD-STZ damage through upregulation of anti-inflammatory cytokines, which, in turn, counteracts proinflammatory cytokines and NF-kB and AP-1 activation. The ability of rhIL-11 to inhibit inflammatory processes in animal models of type 1 diabetes may justify its application for intervention in individuals at high risk for this disease.

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Dendritic cells-mediated immunomodulation as vaccine to protect against Type 1 diabetes.

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Experimentally Type 1 diabetes (T1D) in NOD mice can be prevented or delayed by blocking or regulating islet beta cell reactive T cells. Dendritic cells (DC) play a key role in the activation of these auto-reactive T cells. Understanding the mechanism by which DC regulate these T cells is crucial to our ability to design effective immunotherapy to prevent T1D. We found DC in NOD mice are similar in numbers to DC in diabetes-resistant strains BALB/c, C57BL/6 and NOR mice. However, NOD DC co-cultured with syngenic T cells showed substantially higher upregulation of costimulatory molecules such as CD40, CD80 and CD86. When activated by LPS and IFN-γ they also secreted higher levels of IL-12p70 than control strain DC. NOD DC showed an increased ability to stimulate T cells compared to control strain DC. T cells interacting with NOD DC had increased expression of the CD69, but not CD25, activation marker. Together these results demonstrate that NOD DC have an inherent bias towards hyper-responsiveness and Th1 polarization, which may contribute to the hyperactivity of _eta cell self-reactive T cells and the loss of peripheral tolerance that leads to autoimmunity in T1D. Therefore, NOD DC are prone to inducing pathogenic T cell responses. We have used several approaches to modulate DC so that they induce regulatory rather than destructive T cell-mediated immune responses. This includes vaccination with mycobacterial preparations from *M.tuberculosis* or *M.bovis*, immunization with self-peptides and autoantigens. These treatments prevented T1D in NOD mice and were found to stimulate the generation of DC from differentiation of a precursor cell population rather than proliferation of existing DC population. These DC induce Th2-like T cells and prevent spontaneous and cyclophosphamide-induced diabetes in NOD mice. In addition, they also prevented adoptively transferred diabetes in NOD.SCID mice. Our studies provide a novel approach to use DC inducing agents as vaccine for the induction of protective/regulatory T cells to vaccinate against T1D.
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Inhibitory macrophage deficiency in Nod mice: a role in Type 1 diabetes pathogenesis?

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Background and Aims: The role of dendritic cells and macrophages in beta cell autoimmune destruction in type I diabetes, remains unclear. NOD mice displays functional dendritic cell abnormalities, which could be involved in self-tolerance breakdown to beta cell antigens. A peculiar set of macrophages, CD11b+Gr-1+ inhibitory macrophages (iMacs), could mature in dendritic cells and is implicated in profound depression of CD8 T-cell functions sometimes observed in treatment with cyclophosphamide (CyP) and during overwhelming infections. iMacs could trigger apoptotic death of T-cells through a secretion of NO, and may be involved in the control of T-cell expansion. The aim of our study was to characterize the phenotype of iMacs in NOD mice and to investigate their possible role in diabetes pathogenesis.
Materials and Methods: iMacs were obtained either (1) after a 6 day-culture of lin- c-kit+ hematopoietic progenitor cells (HPC) with GM-CSF, SCF and TNF-alpha or (2) by purification from spleens of mice treated with low doses of CyP (100mg/kg, three times at three-day intervals).
Results: Compared to HPC from BALB/c and C57BL/6 mice, NOD-HPC were unable to differentiate into iMacs ($p<0.02$). Surprisingly, NOD-HPC could not differentiate into iMacs exhibiting a CD11b+ Gr-1high Ly-6C+ CD31low mature phenotype ($p<0.04$). Simultaneously, iMacs induced by immunosuppressive treatments with CyP were studied. We have previously confirmed that such a protocol increased diabetes incidence in NOD mice ($p<0.003$). Six days after the third injection of CyP, accumulation of iMacs was triggered in spleens, but less importantly in NOD than in BALB/c mice ($p<0.05$). Moreover, the number of iMacs expressing the Ly-6C+ mature phenotype was lower in spleens of NOD-CyP mice ($p<0.0001$). Concerning their suppressive functions, iMacs from NOD-CyP mice secreted similar quantities of NO in response to ConA- or anti-CD3/IL-2-activated T-lymphocytes. However, contrary to iMacs from BALB/c mice, iMacs from NOD mice could not inhibit activated-T-cell proliferations if NO secretion was blocked ($p<0.04$). Finally, iMacs from BALB/c-CyP mice only, but not those from NOD-CyP, are able to delay diabetes transfer in NOD-SCID recipients by T lymphocytes from diabetic NOD mice.
Conclusion: We describe the first characterization of the suppressive macrophage lineage in NOD mice. iMacs from these mice exhibit strong maturation and functional deficiencies. They could not efficiently control T-cell expansions. These abnormalities seem to be implicated in diabetes progression after CyP administration in NOD mice.

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Altered isoform switch in monocyte COX-1 and COX-2 response to lipopolysaccaride in Type 1 diabetes.

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Background and Aims: Monocytes and T-cells infiltrate the islets at the onset of type 1 diabetes (T1DM) and are actively involved in the destructive process. Since activated monocytes express cycoloxygenease-2 (COX-2) which influences prostaglandin-E2 (PGE2) secretion such changes could modulate inflammatory responses. COX-1 expression, however, is thought to be constitutive. The aim of this study was to define the nature of monocyte COX expression both basal and in response to a non-specific antigen stimulation with lipopolysaccharide (LPS) and if COX expression is altered in type 1 diabetes.
Materials and Methods: Isolated $CD14^+$ monocytes from 16 identical twins (discordant for T1DM) and 23 controls were analysed for COX-1 and COX-2 mRNA using quantitative RT-PCR, COX-2 protein expression by intracellular COX-2 staining by FACS and western blotting (WB), as well as functional studies with and without a COX-2 specific inhibitor.
Results: Basal monocyte COX-1 and COX-2 mRNA and protein expression and PGE2 secretion were normal in T1DM. However, following LPS stimulation, monocytes COX-1 mRNA expression significantly decreased in both twins and controls ($p<0.01$). In contrast LPS significantly increased COX-2 mRNA and protein expression in both twins and controls ($p=0.007$, $p=0.0001$ respectively). This LPS induced down-regulation of COX-1 mRNA expression was greater in both diabetes and non-diabetes twins compared to controls ($p=0.02$, $p=0.016$ respectively). While maximum monocyte COX-2 response to LPS was reduced in both twin sets compared to controls on FACS, Western blotting, and quantitative RT-PCR ($p<0.03$, $p=0.01$, $p=0.015$ respectively). Furthermore, LPS dose-response analysis by FACS suggests there was a post-receptor defect in both twin groups, and this defect was functional since a specific COX-2 inhibitor significantly reduced PGE2 production more in both twins compared to controls ($p<0.016$).
Conclusion: We show for the first time that following non-specific antigen stimulation with LPS there is an isoform switch in monocyte COX expression which is altered in T1DM, implicating these innate effector cells in disease pathogenesis. Furthermore, our twin studies suggest that this altered in monocyte isoform switch is genetically determined. This study identifies for the first time a link between bacterial challenge and altered diabetes associated immune responses.

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Thymus tolerance dysfunction in the development of the autoimmune diabetogenic response: a way for a novel type of vaccine/immunotherapy.

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Background and Aims: The thymus is the unique lymphoid organ in which occurs a permanent confrontation between self-antigens and a recently evolved system with original recombination driving random generation of TCR diversity. *IGF2* is the dominant member of the insulin gene family expressed by thymic epithelial cells (TEC) from different species. *INS* transcription also occurs in medullary TEC and thymic dendritic cells, but the concentration of thymic insulin is more than 1,000 lower than IGF-2. *Igf2* transcription is defective in the thymus of more than 80% of diabetes-prone BB (BBDP) rats, suggesting that such defect may be responsible for the absence of central T cell self-tolerance of insulin family in BBDP rats.
Objective: Of this study was to analyse the cytokine profile expression elicited by presentation of IGF-2 and insulin-derived antigens in type 1 diabetic adolescents.
Materials and Methods: Selected antigens were Insulin B:9-23 and the IGF-2 homologous sequence IGF-2 B:11-25 (Neosystem, Strasbourg). These antigens were shown to bind DQ8 with the same affinity and to fully compete for the antigen pocket of this major type 1 diabetes susceptibility allele. PBMCs were isolated from 10 DQ8+ type 1 diabetic adolescents and were cultured in presence of two doses (10 and 50 µM) of Insulin B:9-23 and IGF-2 B:11-25.
Results: (1) The number of IFN-gamma secreting cells was lower after presentation of IGF-2 B:11-25 than Insulin B:9-23 (50 µM) (45.6 ± 11.67 vs. 88.9 ± 28.82 SEM, $p = 0.002$); (2) The number of IL-10 secreting cells was higher after presentation of IGF-2 B:11-25 (1162 ± 208.6 vs. 701.8 ± 160.9 SEM, $p < 0.05$); and (3) The ratio IL-10/IFN-gamma was much higher after presentation of IGF-2 B:11-25 than Insulin B:9-23 (67.36 ± 37.6 vs. 10.2 ± 1.9 SEM, $p = 0.002$).
Conclusion: These data show that, while parameters for DQ8 presentation are identical for both antigens, the cytokine profile elicited by IGF-2 B:11-25 and Insulin B:9-23 are very different. So, this study evidences the different immune responses driven by a thymic self-antigen (IGF-2 B:11-25) and a peripheral 'altered' self-antigen specific of islet ß cells (Insulin B:9-23). The regulatory/immunosuppressive profile driven by IGF-2 B:11-25 constitutes a very valuable basis for further development of an efficient and safe tolerogenic/negative vaccine against type 1 diabetes.
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IgG_4 subclass of human GAD65 antibody is more prevalent in LADA than in Type 1 diabetes in Swedish patients.

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Background and Aims: Pancreatic islet cell autoantibodies are common in both type 1 diabetes (T1DM) and in latent autoimmune diabetes in adults (LADA). Generally, different autoantibodies coexist in T1DM while LADA

often exhibits only one. The most frequently occurring antibody in both T1DM and LADA is directed against GAD65. LADA is known to be a less aggressive form of autoimmune diabetes, maybe due to a different immune response. Autoimmune attack on pancreatic islet cells is associated with a Th1 response. The presence of IgG_4 in contrast, has been proposed to be associated with a Th2 cell response.
Aim: The aim of our study was to compare the GADA IgG_1 and IgG_4 subclass distribution in patients with T1DM and LADA.
Materials and Methods: Newly diagnosed patients with T1DM (n=49; median age 30; range 9-67 yrs) and LADA (n=56; median age 49,5; range 23-80 yrs) between 1995 and 1999 were included. A control group (n=119; median age 30; range 19-65 yrs) consisting of GADA negative blood donors were used to define the limit for positivity by a ROC-curve. Indexes < 0.06 for both GADA IgG_1 and IgG_4 was defined as subclass negative. Total IgG_1, 2 and 4 were measured with standard radioimmunoprecipitating assay, using protein A sepharose. Sera were preincubated with ^{35}S-labelled GAD65 followed by incubation with subclass specific, biotinylated antibodies in streptavidin sepharose to precipitate the GADA subclasses IgG_1 and IgG_4.
Results: The GADA response in T1DM was dominated by the IgG_1 subclass found in 90% (45/49) of the GADA-positive patients in this group, 2% (1/49) of the patients had both IgG_1 and IgG_4. Also in LADA patients the IgG_1 subclass dominated since 80% (45/56; p= NS) of LADA patients were positive and 21 % (12/56; p<0.01) had additional IgG_4, which was significantly higher compared to patients with T1DM.
Conclusion: We concluded that beside the dominance of IgG_1 in both groups the LADA patients more often had GAD65-antibodies of the IgG_4 subclass compared to patients with T1DM. This could indicate a different kind of immune response, maybe an altered balance between Th1 and Th2.

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Cellular immune response to islet cell antigens in latent autoimmune diabetes of adults (LADA).
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Background and Aims: Latent Autoimmune Diabetes in Adults (LADA), which represents 10-30% of T2D, is characterised by the presence of β-cell autoantibodies (AAb) in the face of the lack of dysmetabolic syndrome. As in type 1 diabetes, LADA is considered as the result of a T cell-mediated autoimmune β-cell destruction, although fully comprehensive data are not available yet.
Materials and Methods: In 52 T2D patients without obesity (BMI<28) and 23 matched control subjects (age, HLA), we have studied the reactivity of peripheral blood mononuclear cell (RPBMC) (postive > 3) to 5 islet cell antigens, as well as IL4 and IFNγ secretion (MACS cytokine secretion assay using flow cytometry), presence of AAb (4 tested), HLA genotypes and C-peptide levels.
Results: Patients had at least one AAb+ or one RPBMC+ in 55% of cases vs 4 % of the controls (p=0.00004). Percentage of secreting IFNγ T-cells, especially CD8+, was higher in patients than in controls (2.82+/-1.33 vs 1.44+/-0.52% of lymphocytes; p=0.01). Likewise, percentage of secreting IL4 CD4+T-cells was higher in patients than in controls (2.25+/-1.49 vs 0.58+/- 0.57% of lymphocytes; p=0.003). One AAb or more was present in 29% of patients : anti-insulin (17%), -GAD (13%), ICA (8%) and -IA2 (4%). One RPBMC or more was positive in 29% of patients : insulin (13%), B9-23 peptide (8%), GAD (8%), GAD peptides (6%), IA2 (4%). A positive RPBMC was present in the full absence of AAb in 31% of patients, but associated to at least one AAb in 4% of them, suggesting an inverse relationship between humoral and cellular immunities (p=0.04). Patients AAb+ were younger and had lower C-peptide levels than those AAb- (46.0 +/-8.8 years vs 51.6 +/- 10.2; p=0.03 and 0.37 +/- 0.20 nmol/l vs 0.62 +/- 0.54; p=0.03). Patients with positive RPBMC were older and had higher C-peptide levels than those RPBMC- (52.3+/-10.4 years vs 46.0 +/- 9.2; p=0.03 and 0.68 +/- 0.57 nmol/l vs 0.36 +/- 0.30; p=0.04).
Conclusion: Presence of autoimmunity is frequent in lean T2D. Nevertheless, the inverse association between humoral and cellular immunity suggests two sub-populations among LADA patients. Also, the presence of PBMC proliferative response could reflect a better preservation of residual β-cell function, with therapeutic implications.

OP 4
Diabetic Retinopathy – Pathogenic Mechanisms

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Transgenic mice overexpressing IGF-I in the retina develop diabetic eye disease.
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Background and Aims: Diabetic eye disease (DED) develops in diabetic patients as a complication of chronic hyperglycemia and represents the main cause of vision impairment. The pathogenesis of DED is poorly understood. Animal models are very important as they offer unique advantages to study the mechanisms involved in the development of the disease and to test new therapeutic approaches. Among all the animal models available there is not an appropriate model for DED that mimics all the progressive stages of the human pathology. Here we investigate whether the transgenic mouse over-expressing IGF-I in the retina would be a suitable model for DED.
Materials and Methods: Immunohistochemistry, in situ hybridization, Western and Northern Blot, RT-PCR and ELISA were used to characterize the eye of the IGF-I over-expressing mouse. Fluorescein anigography, vascular corrosion casting and Grifonia simplicifolia were used to study the vascular system of the retina of this animal model.
Results: By in situ hybridization over-expression of IGF-I was localized in the photoreceptors of the transgenic retina. This led to the protein accumulation in the aqueous humor despite no differences in serum IGF-I concentration. The eye of these mice developed with age all pathological changes detected in human DED. Retinal vessels developed the progressive stages of diabetic retinopathy. Thickening of the basal membrane and loss of pericytes were detected as the first hallmarks of non-proliferative retinopathy. Vascular occlusion, venous dilatation and beading, widespread capillary non-perfusion areas and intraretinal microvascular abnormalities (IRMA) were observed. Neovascularization, the main feature of the proliferative stage, was also detected within the retina. Vascular alterations correlated with VEGF increase. Moreover, IGF-I/VEGF molecular signalling pathways were activated in these eyes as shown by increased phosphorylation of PKB/Akt. Over-expression of GFAP in Muller cells was also observed in transgenic eyes as is found in human DED. Transgenic mice also developed cataracts and rubeosis iridis , which may be the cause of secondary glaucoma.
Conclusion: The IGF-I over-expressing mouse presents all the features of DED and therefore constitutes a unique animal model for this human pathology. Future directions are going towards the characterization of the molecular mechanisms involved in the development of this disease and to test new gene therapy approaches in this model.
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Inhibition of connexin 43 expression and gap junction intercellular communication activity by high glucose in retinal pericytes.
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Background and Aims: To investigate the role of gap junction protein, connexin-43 (Cx43), in the maintenance of retinal vascular homeostasis in diabetic retinopathy, we examined whether high glucose condition alters the expression of Cx43 and gap junction intercellular communication (GJIC) activity in retinal pericytes.
Materials and Methods: In human retinal pericytes (HRPs) and bovine retinal pericytes (BRPs) grown for 7 days in normal (5mM) or high (30mM) glucose medium, Cx43 protein level was determined by Western blot analysis. Parallel experiments were performed in human retinal pericytes (HRP) to determine mRNA level by RT-PCR, distribution and localization of Cx43 protein using immunostaining, and GJIC activity by scrape load dye transfer technique. The distribution and localization of Cx43 protein was also determined in pericyte-endothelial cell cocultures.

Results: Western blot analysis of Cx43 protein level in HRPs and BRPs showed significant reduction in Cx43 expression under high glucose condition (69.1±17% of control, p=0.004; 62.3±19% of control, p=0.001, respectively). Cx43 mRNA level in parallel cultures of HRPs grown in high glucose medium also showed significant reduction (71.4±16.8% of control, p=0.02, r=0.9). The relative number of Cx43 „plaques" indicative of Cx43 localization at specific sites of contact between adjacent cells showed significant reduction in high glucose condition (61±10% of control, p= 0.002); similarly, a significant reduction in plaque number was observed in cocultures grown in high glucose medium compared to those in normal medium (59.4±29% of control, p=0.001). Scrape load dye transfer technique used as a functional assay indicated significantly reduced ability of cells to transfer Lucifer yellow through gap junctions in cells grown in high glucose condition (61±13% of control, p=0.001).
Conclusion: High glucose-induced downregulation of Cx43 expression and inhibition of GJIC activity in retinal pericytes may contribute to the disruption of vascular homeostasis in diabetic retinopathy.

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Endothelial nitric oxide synthase gene is associated with diabetic maculopathy in Type 2 diabetes.
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Background and Aims: Diabetic retinopathy remains the major cause of blindness among adults. In addition, diabetic maclopathy (macular edema), which may occur at any stage of diabetic retinopathy, is an important cause of visual impairment. Nitric oxide (NO) synthesized by endothelial NO synthase (eNOS) is important for vascular regulation; in particular it reduces endothelial permeability. In the present study, we examined the eNOS polymorphisms to assess its possible association with diabetic retinopathy and maclopathy.
Materials and Methods: We studied 226 patients with type 2 diabetes: 110 patients without retinopathy, 46 patients with non-proliferative retinopathy (non-PDR), and 70 patients with PDR. Diabetic maclopathy was present in 48 patients. We determined three polymorphisms of the eNOS gene by PCR: Glu298Asp in exon 7, 27-bp repeat in intron 4, and T(-786)C in the promoter region.
Results:
(1) The Glu298Asp polymorphism was not significantly associated with both retinopathy and maclopathy.
(2) The 27-bp repeat polymorphism was not significantly associated with retinopathy, but was associated with maclopathy; „a" allele (4 repeats) was significantly increased in patients with maclopathy compared to patients without maclopathy (18.8% vs 10.3%, p=0.033). Furthermore, logistic regression analysis revealed that, in addition to BMI, systolic blood pressure, HbA_{1c} and insulin treatment, the 27-bp repeat polymorphism had a significant increased risk of maclopathy (odds ratio 4.04, p=0.0009).
(3) The T(-786)C polymorphism was in tight linkage disequilibrium with the 27-bp repeat polymorphism, and the -786C allele was also significantly associated with maclopathy.
Conclusion: From these results, it is suggested that the eNOS gene is a novel genetic risk factor for diabetic maclopathy. Since previous studies reported that the -786C allele is correlated with a reduction in eNOS gene promoter activity and serum NO metabolite levels, the T(-786)C polymorphism may primarily contribute to the development of diabetic maclopathy.

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Relationship between the T786C promoter polymorphism in the 5'-flanking region of the endothelial nitric oxide synthase (eNOS) gene and the onset of the retinal panphotocoagulation among Type 1 diabetic patients with severe diabetic retinopathy.
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Background and Aims: Nitric oxide (NO) synthesized from L-arginine by the constitutive endothelial NO synthase (eNOS) plays a key role in the diabetic retinopathy (DR). Most neoangiogenic effects of VEGF in severe forms of DR are mediated by NO. The variant allele of the polymorphism T786C reduces the eNOS gene promoter activity. To assess whether eNOS 786C promoter polymorphism is implicated in severe DR, a case-control study was performed.
Materials and Methods: 196 unrelated type 1 diabetic individuals with >15 years of diabetes (M/F : 92/104, age : 43.6 ± 12.1 yrs., diabetes duration : 27.9 ± 9.8 yrs., BMI : 24.2 ± 3.5 kg/m^2, HbA1c : 8.67 ± 1.36 %) were recruited. The eNOS T786C polymorphism was analysed by RFLP-PCR using NgoM IV enzyme. Our cohort was classified, using funduscopy and retinal angiograms, into two groups : presence (n=113) or absence (n=83) of severe DR (proliferative or severe preproliferative DR). Only those individuals with <5 microaneurysms were included at the control group, and none had never suffered previous retinal photocoagulation.
Results: genotypes in whole sample and in both study and control groups were in Hardy-Weinberg equilibrium and similar to other Caucasian populations. The genotype distribution was : T786T 31.6 %, T786C 50.0 % and C786C 18.4 %. Allele frequencies were not different between patients (T786 : n= 130 and C786 : n= 96) and control subjects (T786 : n= 92 and C786 : n= 74, p= 0.67). However, the T786C polymorphism was correlated with the time of onset of the retinal panphotocoagulation (19.4 ± 6.79 years of diabetes duration), among patients with severe DR (r^2= 0.46, beta= 0.215, p= 0.022). A multivariate forward stepwise regression analysis confirmed that onset of the retinal panphotocoagulation was independently and significantly determined by the T786C polymorphism (beta= 0.176, p= 0.028).
Conclusion: Among Caucasian type 1 diabetic patients with severe DR, the eNOS T786C promoter polymorphism was correlated with the onset of the retinal panphotocoagulation. This finding is emphasized by a previous association between severe DR and eNOS4 polymorphism.

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Screening for polymorphisms in the rage gene promoter in South Indian subjects with diabetic retinopathy.
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Background and Aims: Interaction between Advanced glycation end products (AGE) and its receptor RAGE have been implicated in the pathogenesis of Diabetic retinopathy. Single nucleotide polymorphisms -374 T/A, -429 T/C and a 63 bp deletion in the RAGE promoter region are known to affect the transcriptional repression of RAGE gene. The aim of this study was to investigate these genetic markers for the first time in a South Indian population with Type 2 diabetes with retinopathy.
Materials and Methods: We studied the prevalence of -374 T/A, -429 T/C and a 63 bp deletion polymorphisms in the promoter region of the RAGE gene in Type 2 diabetic subjects with retinopathy (DR) but without nephropathy (proteinuria < 100mg/day) (n=57),Type 2 diabetic subjects (DM) with duration of diabetes >15 years without any microvascular complications (retinopathy or nephropathy) (n=34) and in control subjects (age >60 years) without diabetes (n=31).Retinopathy was diagnosed using 4 field stereoscopic retinal colour photography using ETDRS grading. The investigation was carried out by PCR-RFLP technique using restriction enzymes *Tsp509 I* and *Alu I*.
Results:

DESCRIPTION	DR (n=57)	DM (n=34)	CONTROLS (n=31)
Clinical characteristics			
Age (years)	58 ± 9	62 ± 8	67 ± 5
HbA_{1C} (%)	8.2 ± 1.6	7.9 ± 1.1	5.9 ± 0.4
Duration of diabetes (years)	20 ± 7	22 ± 5	—-
Genotypic distribution of TA/AA and 63 bp$^-$ in RAGE gene promoter			
TT	37 (64%)	28 (82%)	31 (100%)
TA/AA	15 (26%)	6 (17%)	—-
63 bp$^-$	5 (8%)	—-	—-

Results show that the prevalence of TA/AA and the 63 bp deletion genotypes were higher among Type 2 diabetic subjects with retinopathy compared to Type 2 diabetic subjects without microvascular complications (p=0.09) and non-diabetic controls. The -429 T/C polymorphism was not observed in our study which is in contrast to previous studies from the U.K.
Conclusion: There is an increased prevalence of TA/AA polymorphism in the RAGE gene promoter in South Indian Type 2 diabetic subjects with retinopathy.

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Cerivastatin blocks the AGE-RAGE signaling pathways in microvascular endothelial cells.

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Background and Aims: We previously have shown that glyceraldehyde- and glycolaldehyde-derived advanced glycation end products (glycer-AGE and glycol-AGE), senescent macroproteins formed at an accelerated rate in diabetes, are mainly involved in loss of pericytes, the earliest histopathological hallmark of diabetic retinopathy. However, the effects of these AGE-proteins on angiogenesis, another vascular derangement in diabetic retinopathy, remain to be elucidated.In this study, we investigated whether and how these AGE-proteins elicit changes in cultured endothelial cells (EC) that are associated with angiogenesis.
Materials and Methods: DNA synthesis was determined by measuring thymidine incorporation into cells. Tube formation was quantified by measuring the lengths of capillary-like structures on Matrigel. Angiogenesis-related gene expression was analyzed by quantitative reverse transcriptase-polymer chain reaction. Promoter activity was measured using luciferase reporter gene.
Results: When human microvascular EC were cultured with glycer-AGE or glycol-AGE, growth and tube formation of EC, the key steps of angiogenesis, were significantly stimulated. These AGE increased DNA synthesis and tube formation to about 25 % and 50 %, respectively. The AGE-induced growth stimulation was significantly enhanced in AGE receptor (RAGE)-overexpressed EC, while it was completely blocked by treatments with antisense DNA against RAGE mRNA. Furthermore, AGE increased transcriptional activity of nuclear factor–kB (NF-kB) and activator protein-1 (AP-1) to about 2-fold, and then up-regulated mRNA levels of vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang-2) in EC. Cerivastatin, a hydroxymethylglutaryl CoA reductase inhibitor was found to completely prevent the AGE-induced increase in NF-kB and AP-1 activity, VEGF mRNA up-regulation and the resultant increase in DNA synthesis in microvascular EC.
Conclusion: These results suggest that the AGE-RAGE interaction elicited angiogenesis through the transcriptional activation of the VEGF gene via NF-kB and AP-1 factors. By blocking AGE-RAGE signaling pathways, cerivastatin might be a promising remedy for treating patients with proliferative diabetic retinopathy.

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Food advanced glycation endproducts (AGE) acutely impair endothelial function in patients with diabetes mellitus (DM).

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Background and Aims: In diabetic patients, food AGE intake for several weeks can induce persistent increases of inflammatory mediators linked to vascular dysfunction. But it remained unknown whether food AGE can acutely affect vascular function in vivo.
Materials and Methods: Therefore we studied the acute effect of an AGE-rich beverage (300ml containing 1.8 million AGE-Units and 4.2 µ Mol methylglyoxal derivatives, but no carbohydrates or lipids) on the flow mediated dilation (FMD expressed as % increase in arterial diameter after occlusion compared to the baseline diameter) of the brachial artery and on plasminogen activator inhibitor (PAI)-1 plasma levels (ELISA) as measures of endothelial function in diabetic subjects. FMD was assessed by high resolution ultrasound measurements using the ATL Ultramark 9 HDI, USA, after an overnight fast at baseline and at 90, 150 and 210 min after taking the AGE-rich drink. Forty-four patients with diabetes mellitus from the metabolic ward on regular diet (23 type 1, 21 type 2; m/f: 36/8; age: 50.53, range: 27-72 years; diabetes duration: 15.46, range: 0.1-41 years; HbA1c: 8.6 range: 5.7-14.6%; smokers/nonsmokers: 19/25) were examined in the fasting, nonsmoking state.
Results: The FMD was reduced from 5.34± 2.58% at baseline to 3.99± 2.29% ninety minutes after the AGE intake ($p<0.001$) and recovered to 5.50±2.96% after 150 min (p=NS vs. baseline). The AGE induced FMD decrease was more pronounced in smokers (baseline and 90 min: 5.24±2.35% and 3.34±1.68%) than in nonsmokers (baseline and 90 min: 5.42±2.80% and 4.49±2.59%) ($p<0.05$).
Replacing the AGE-rich drink by 300ml tapwater resulted in no significant FMD differences between baseline and 90min values: 5.22 ± 2.53% and 5.67 ± 2.33% respectively.
FMD changes were accompanied by persistent increases of PAI-1 plasma levels (6.5±3.3 vs. 12.4± 6.1 ng/ml, $p<0.01$)
Conclusion: Already a single ingestion of AGE-rich food can acutely result in arterial endothelial dysfunction. Repeated AGE exposure may transform the transient acute effect of single AGE exposure into chronic dysfunction. This could explain in part why the postprandial state favors the increased precipitation of acute arterial ischemic events.

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Thiamine and benfotiamine are able to correct increased apoptosis and advanced glycation-end products formation in endothelial cells and pericytes cultured under high glucose conditions.

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Background and Aims: High glucose concentrations induce pathological alterations in small and large vessels, the mechanisms of which are not yet fully understood. We previously showed that thiamine and benfotiamine are able to correct delayed replication and increased lactate production due to high glucose in human endothelial cells (HUVEC). The aim of this study was to verify if thiamine and benfotiamine can modify apoptosis, cell cycle and advanced glycation end-products (AGE) formation in HUVEC and bovine retinal pericytes (BRP) grown in high ambient glucose.
Materials and Methods: HUVEC and BRP were cultured in normal (5.6 mmol/l, G5.6) or high (28 mmol/l, G28) glucose concentrations, with or without thiamine 50 (T50) and 100 (T100) µmol/l or benfotiamine 50 (BT50) and 100 (BT100) µmol/l. Apoptosis was determined by ELISA after 3 days of culture, cell cycle by flow cytometry after 48 hours and AGE production by fluorimeter reading after 20 days. Results are mean of 6 exps. ± SD and are expressed as percentage of control (5.6 mmol/l glucose).
Results: Apoptosis was increased in high glucose, both in HUVEC (110.0±6.2, p=0.003 vs G5.6) and BRP (115.9±10.0, p=0.024 vs G5.6), while it was normalized both by thiamine and benfotiamine added to high glucose. In HUVEC: G28+T50 103.9±11.7 (p=NS vs G28), G28+T100 98.6±8.3 (p=0.016 vs G28), G28+BT50 91.4±9.0 (p=0.005 vs G28), G28+BT100 105.4±9.0 (p=0.025 vs G28). In BRP: G28+T50 97.0±10.7 (p=0.016 vs G28), G28+T100 103.4±13.0 (p=0.033 vs G28), G28+BT50 93.9±17.6 (p=0.029 vs G28), G28+BT100 91.7±9.2 (p=0.005 vs G28). Cell cycle traversal was not affected by none of our conditions. AGE production was dramatically increased in high glucose, in HUVEC (232.5±63.4, p=0.025 vs normal glucose), as in BRP (132.9±19.8, p=0.028 vs normal glucose); both thiamine and benfotiamine, when added to high glucose, managed to reduce AGE formation. In HUVEC: G28+T50 162.7±40.7 (p=NS vs G28), G28+T100 158.7±32.9 (p=0.018 vs G28), G28+BT50 136.7±29.2 (p=0.011 vs G28), G28+BT100 137.1±26.5 (p=NS vs G28). In BRP: G28+T50 82.7±32.7 (p=0.046 vs G28), G28+T100 92.8±42.9 (p=0.046 vs G28), G28+BT50 90.4±35.5 (p=0.046 vs G28), G28+BT100 101.1±28.6 (p=0.028 vs G28).
Conclusion: Thiamine and benfotiamine are able to correct increased apoptosis and excess AGE formation due to high glucose in cultured vascular cells. Further elucidation of the mechanisms through which they work could help set the basis for clinical trials to test this vitamin, as a potential approach to the prevention and/or treatment of diabetic retinopathy.

OP 5

Type 1 Genetics, Epidemiology: Prediction and Prevention

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The effect of HLA class II and insulin gene regions on the development of β-cell autoimmunity in a Finnish birth cohort.

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Background and Aims: The effect of various type 1 diabetes risk genotypes - HLA class II, IDDM2 and IDDM12 - on the development of diabetes-associated autoantibodies was analyzed in a birth cohort of infants selected from the Finnish general population (n=65000).
Materials and Methods: The presence of islet cell antibodies (ICA) was used as the primary screening test for β-cell autoimmunity in children genetically at risk and if the child tested positive for ICA, also GADA, IA-2A and IAA were analyzed in all serum samples taken at 3-6 months intervals. All antibody positive children (n=312) identified were compared to a set of controls taken from the same cohort (n=2563). HLA DRB1-DQB1-DQA1, IDDM2 (INS,11p15.5; -2221 MspI C/T) and IDDM12 (CTLA4, 2q33; +49 A/G) genotypes were determined using a PCR-allele specific oligonucleotide hybridization method. GADA, IA-2A, IAA and ICA were analyzed using standard methods.
Results: A higher proportion of children with the DQB1*02/0302 genotype developed diabetes or multiple antibodies than in the 0302/x (x≠DQB1*02, 0301 or 0602) group (45.5% vs. 34.3%, p=0.043). The frequency of the DRB1*0401-DQB1*0302 haplotype was higher, whereas the frequencies of the DRB1*0404-DQB1*0302 and DRB1*0403-DQB1*0302 haplotypes were lower in DQB1*0302/x individuals testing positive for IAA, GADA and/or IA-2A as compared to antibody negative controls (DRB1*0401: 86.4%, 84.3%, 84.3% vs. 50.0%; DRB1*0404: 9.1%, 10.0%, 10.0% vs. 33.1% and DRB1*0403: 0%, 1.4%, 0% vs. 8.5%;p<0.0001, 0.001, 0.0005, respectively). In contrast, the distribution of DRB1*04 subtypes was similar when comparing children carrying ICA only and antibody-negative children. The frequency of the DQB1*0302/*0603 genotype was lower in children with GADA as compared to controls (4.3% vs. 16.8%, p<0.0012). The DR3-DQA1*05-DQB1*02 haplotype was associated with IAA in DRB1*0401-DQB*0302 positive individuals as compared to DR7-DQA1*0201-DQB1*02 (85.4% vs. 63.6%; p=0.03). GADA appeared as the first antibody in children with the DQB1*02/*0302 genotype more often than in those with DQB1*0302/x (11.9% vs. 4.2%, p=0.01). It should be noted that comparisons of HLA genotype frequencies were limited, since all children carried high-risk HLA genotypes. Development of IAA and IA-2A but neither GADA nor ICA was associated with INS C/T polymorphism independently of the HLA genotype (p=0.001, p=0.018). In contrast, CTLA4 +49 A/G polymorphism showed no association with signs of β-cell autoimmunity.
Conclusion: The findings indicate that the DRB1 genotype affects the development of antibodies to biochemically characterized antigens but not the emergence of ICA alone. The DQB1*0603 allele seems to have a specific inhibitory effect on the development of GADA. Insulin gene region influences the appearance of IAA and IA-2A but not that of ICA alone.

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GAD65 and IA-2 autoantibodies in cord blood of children born to healthy mothers are associated with Type 1 diabetes high risk HLA alleles.

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Background and Aims: The etiology and pathogenesis of type 1 diabetes is strongly associated with HLA and the appearence of islet cell autoantibodies (Ab), which are strong markers for the disease. Several gestational risk factors have been implicated in the etiology and we have previously shown that children who later in life developed type 1 diabetes had GAD65Ab or IA-2Ab already in their cord blood. Such Ab primarily represent autoantibodies from the mother. The aim was to test the hypothesis that GAD65Ab and IA-2Ab detected in the cord blood of children born to healthy mothers are associated with type 1 diabetes high risk HLA alleles.
Materials and Methods: More than 15,000 children born in Skåne, Sweden since September 2000 were typed for HLA and analyzed for GAD65Ab and IA-2Ab in the DiPiS (Diabetes Prediction in Skåne) study. In a total of 190 autoantibody positive children, plasma from the mother was also analyzed. Children to mothers with Type 1 diabetes were excluded.
Results: The autoantibody levels in the cord blood exceeded that of the mother. Mothers with autoantibodies below or at the cut-off therefore may give birth to a child with positive autoantibodies. Children with type 1 diabetes HLA high risk DQB1*0201/0302 and middle risk DQB1*0302/X (21.2%) were more likely to be double GAD65Ab and IA-2Ab positive compared to the rest of the children (6.4%, OR=1.29, p<0.01). Mothers positive for GAD65Ab were more likely to give birth to children with genotypes DQB1*0201/0302, 0302/X or 0201/X (p=0.04). IA-2Ab alone were increased in the high and middle risk HLA groups (24.2%) compared to the rest of the children (10%,OR=1.20 , p=0.03). IA-2Ab of the cord blood was associated with the difference in GAD65 autoantibody levels between the mother and the child (p=0.008) as the median GAD65 autoantibody level in cordblood increased from 2.6 in IA-2Ab negative to 3.8 in IA-2Ab and GAD65Ab double positive children (p<0.001).
Conclusion: The presence of GAD65Ab and in particular IA-2Ab in the cord blood of children born to healthy mothers are associated with type 1 diabetes high risk HLA types. Prospective analyses of these children will determine the risk of intrauterine type 1 diabetes autoantibody markers for later development of type 1 diabetes.

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Fasting insulin, fasting glucose and insulin resistance is increased in pre-clinical Type 1 diabetes.

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Background and Aims: Hyperinsulinaemia associated with insulin resistance is recognized in the pathophysiology of pre-clinical type 2 diabetes but not in pre-clinical type 1 diabetes. The aim of this longitudinal study was to investigate whether fasting insulin (FI), fasting glucose (FG) and insulin resistance were associated with progression to type 1 diabetes (T1D) in individuals with islet antibody markers of β-cell autoimmunity.
Materials and Methods: 3773 first-degree relatives of T1D probands were screened for islet antibodies and those confirmed positive for > or = 1 islet antibody were followed with antibody and metabolic testing for a median of 4.0 years. FI, FG and an index of insulin resistance (HOMA-R) were compared between those who progressed (n=18) and did not progress (n=60) to diabetes, including a non-progressor subgroup (n=18) matched for age, sex, islet antibodies and HLA type.
Results: Progressors had greater fasting insulin (12.8 ± 1.6 vs. 8.6 ± 0.6mIU/L, p = 0.019) and fasting glucose (5.4 ± 0.1 vs. 4.8 ± 0.1mmol/L, p = 0.007) at baseline than non-progressors. In addition, insulin resistance was greater in progressors at baseline (3.0 ± 0.4 vs. 1.9 ± 0.1, p = 0.004) and over time (mean 2.9 ± 0.3 vs. 1.9 ± 0.1, p = 0.005) than non-progressors. There was no significant difference in bodyweight percentiles between progressors and non-progressors. Insulin secretion, measured by first phase insulin response to intravenous glucose, was also not significantly different at baseline. These results were confirmed by comparison with matched non-progressors at baseline and over time (Table 1).
Conclusion: Islet antibody-positive relatives who progressed to type 1 diabetes were characterized by increased FI, FG and insulin resistance at least several years before developing T1D. This metabolic profile in an 'at-risk' population for T1D appears to be related to progression and is important in understanding the pathophysiology and prediction of T1D.

Table 1. Comparison of progressors and non-progressors

METABOLIC RESULTS	PROGRESSORS	NON-PROGRESSORS	p
Baseline fasting insulin (mIU/L)	12.8±1.6	6.9±0.6	0.003
Mean fasting insulin (mIU/L)	10.4±1.2	7.1±0.6	0.003
Baseline fasting glucose (mmol/L)	5.4±0.1	4.8±0.2	0.026
Mean fasting glucose (mmol/L)	5.4±0.1	5.0±0.1	0.004
Baseline HOMA-R	3.0±0.4	1.5±0.2	0.002
Mean HOMA-R	2.9±0.3	1.5±0.2	0.002

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Fab of a human monoclonal antibody specifically inhibits GAD65Ab in Type 1 diabetes.

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Background and Aims: Autoimmunity in type 1 diabetes is typically accompanied with the presence of autoantibodies directed to one or more islet cell autoantigens: 65kDa glutamate decarboxylase (GAD65), insulin (IAA), and islet antigen-2 (IA-2 Ab). In recent years it has become obvious that GAD65 autoantibodies (GAD65Ab) in type 1 diabetes patients differ in their epitope specificities from GAD65Ab in non-diabetic individuals. Furthermore it has been suggested that disease-specific GAD65Ab may be associated with the development of type 1 diabetes. A precise epitope analysis of these disease-specific autoantibodies is needed to assess their involvement in the pathogenesis of the disease. However, the conformational nature of the epitopes complicates this analysis. This obstacle could be overcome by the use of Fabs of monoclonal antibodies specific to GAD65 with well-defined specificities.
Materials and Methods: Five recombinant Fabs (rFabs) specific for GAD65 were cloned from human mAb - IgG antibodies (b96.11 and DPD derived from autoimmune disease patients), as well as from murine IgG mAbs (144 and 221-442) to characterize the GAD65 autoantibodies present in the sera of patients with type 1 diabetes. The epitopes recognized by these antibodies are located over the entire length of the molecule to ensure representation of different parts of GAD65; 144 binds amino acid residues 4-22, DP-D: 96-173, 221-442: 221-442, b96.11: 308-365, DP-A: 483-585. The binding of GAD65Ab from sera of type 1 diabetes patients (n=61), type 1.5 diabetes patients (n=44), first degree relatives (n=38), and healthy individuals (n=14) to GAD65 was analyzed by competitive radioimmuno assays with the rFabs to test the hypothesis that competition with Fabs reveals disease-specific GAD65Ab binding specificities.
Results: The median binding of GAD65Ab present in the sera of type 1 diabetes patients to GAD65 was reduced significantly ($p<0.00001$) by rFabs b96.11 (72%), 221-442 (79%), DP-D (80%), and DP-A (84%). No significant competition was observed for rFab 144. The competition pattern in type 1 diabetes patients differed from that in type 1.5 diabetes patients, first degree relatives, and healthy individuals. While 87% of all type 1 diabetes sera were at least partly competed by Fab b96.11, only 34, 18, and 7% of sera of type 1.5 diabetes patients, first degree relatives, and healthy individuals were competed, respectively ($p<0.0001$).
Conclusion: These novel findings with GAD65-specific recombinant Fab fragments support the view that type 1 diabetes is associated with epitope-specific GAD65Ab and suggests that the pathogenesis may be reflected in the associated GAD65Ab epitopes.

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Vaccination with DNA encoding IA2 prevents late-preclinical-stages NOD mice from developing diabetes.

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Background and Aims: Type 1 diabetes mellitus (T1DM) ensues from the autoimmune destruction of insulin-secreting beta cells found in the islets of langerhans.Immunization with plasmid DNA (pDNA) encoding an antoantigen has proven to be an effective approach to protect experiment animals against autoimmune disease. As a new member of the protein tyrosine phosphatase family, insulinoma-associated protein 2 (IA-2) is one of the initial target islet antigens of autoimmune T cell repertoire.We tested whether vaccination with pDNA encoding IA2 could prevent NOD mice treated at early or late preclinical stages from developing diabetes.
Materials and Methods: ***Mice***. Ten early-preclinical-stages (4~5 wk age) and fourteen late-preclinical-stages (10~11 wk age) NOD female mice were housed under specific pathogen-free conditions.
Vaccine preparation. Human IA2 cDNA was inserted into the pEGFP vector. To test expression of the IA2, RIN5F cells were transfected with the recombinant product prepared in Lipofectin and analyzed by Western blot and fluorescent microscope.
Vaccination mice. Five 4~5 wk of age and Eight 10~11 wk of age NOD mice received three intramuscularly injections of 50ug of pIA2 in each quadriceps over 28 days. Control groups were injected with pEGFP vector. Glucose levels were detected every 1~2 weeks.
RT-PCR. Total cellular RNA was prepared from muscle tissue of individual mice. The PCR products were resolving on a 2% agarose gel and detected by ethidium bromide staining.
Histology. Pancreata were prepared for the histology and then stained with hematoxylin and eosin. A minimum of 15 individual islets was scored for each animal to assay the severity of insulitis.
Flow cytometry. Lymphocyte were prepared from the spleens by centrifugation over Ficoll-Hypaque. Cells were stained with FITC-CD4 and PE-CD8 antibodys then measured with flow cytometer.
Results: 1.The pEGFP-IA2 fusion protein was shown to be expressed by transfected RIN5F cells.RNA transcript encoding IA2 also can be found in muscle tissue after vaccination.
2.Among the 4~5 wks age NOD mice group, 2 of 5(40%) NOD mice treated with pEGFP-IA2 developed diabetes while there are 3 of 5(60%) diabetic NOD mice in control group at 32 weeks. There are no significant differences of diabetes incidence in two groups($p>0.05$).
3.Among the 10~11wks age NOD mice group, 1 of 8(12.5%) NOD mice treated with pEGFP-IA2 developed diabetes while there are 4 of 6(66.7%) diabetic NOD mice in control group at 35 weeks. There are significant differences of diabetes incidence in two groups($p<0.05$).
4.The number of $CD4^+$ T lymphocyte, $CD8^+$ T lymphocyte in IA2 treated NOD mice group is significantly less than the control group in 10~11 wk age mice ($p<0.05$). There are no significant differences in 4~5 wk age NOD mice ($p>0.05$).
Conclusions: The results suggest that IA2 can effectively suppress $CD4^+$ and $CD8^+$ T lymphocyte.Vaccination with IA2 can prevent and delay the onset of autoimmune diabetes in late-preclinical-stages NOD mice. This research may reveals new insights into the immune system and open doors for novel methods of type 1 diabetes prevention.

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Genetic and functional evaluation of an Interleukin-12 polymorphism (*IDDM18*) in a large multi-national collection of Type 1 diabetes families.

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Background and Aims: Interleukin 12 (IL-12) drives the differentiation of T-lymphocytes into the Th1-subset, characterized by production of cytokines leading to cell mediated immunity. In the NOD-mouse IL-12 has been shown to play a primary role in Type 1 diabetes induction. Recently, a new susceptibility locus, *IDDM18*, for Type 1 diabetes was identified in Australian and British families. A polymorphism (C1159A SNP) in *IL12B* (the gene encoding the P40 subunit of IL-12) was identified as candidate and evidence for a functional significance of this polymorphism was found. The aims of our study were to seek confirmation of the involvement of the *IL12B* C1159A SNP in Type 1 diabetes susceptibility in a large collection of families, as well as to extend the functional studies.
Material and Methods: We typed a Danish collection of 337 simplex families as well as a large cohort of 795 multi-national Type 1 diabetes sib-pair families, comprising families from Denmark, Sweden, Norway, France and US. Genotyping was performed by a PCR-based RFLP assay, and data were analyzed by transmission disequilibrium testing. IL-12 protein and mRNA levels were measured in LPS and IFN-γ stimulated peripheral blood mononuclear cells from individuals with different genotypes.
Results: Neither in a cohort of 337 Danish simplex families (99 transmissions of allele A (56%) vs. 77 non-transmissions (44%), P=0.10), nor in a cohort of almost 800 multi-national sib-pair families (373 transmissions of allele A (47%) vs. 413 non-transmissions (53%), P=0.15), could significant linkage, in the presence of linkage disequilibrium, to Type 1 diabetes be demonstrated for the C1159A SNP. Data were stratified according to HLA and age at onset, but no subgroup showed significant linkage to Type 1 diabetes. No statistically significant differences in IL-12 mRNA or protein production correlating to genotype of this polymorphism could be demonstrated either, although a trend (P=0.06) for more IL-12 protein being produced in presence of the 1159C allele was found.

Conclusion: The current large multi-national study could not confirm Type 1 diabetes association of the *IL12B* polymorphism. Support for a disease association through functional studies, on mRNA and protein levels, was not achieved either.

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Natural killer cell immunoglobulin-like receptor (KIR) genes in Korean patients with Type 1 diabetes mellitus.

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Background and Aims: Type 1 diabetes (T1D) is a genetic disease influenced by environmental factors. T1D patients have a defect in NK cell mediated functions. NK cell activity is partially controlled through interactions between killer Ig-like receptors (KIR), which belong to polymorphic multigene family in chromosome 19q3.4, and their respective HLA class I ligands. Our study was aimed to investigate associations of KIR, HLA B, C and MICA genes in Korean T1D patients and controls.
Materials and Methods: We typed 14 KIR genes using PCR-SSP in addition to the conventional HLA class I typing (PCR-SSO) in 140 Korean T1D patients and 132 controls. All were typed for HLA DR-DQ and MICA genes earlier.
Results: KIR 2DS5 gene was present at significantly higher frequency in Korean T1D patients compared to healthy subjects (83% vs 39%, $p < 10^{-4}$, OR 7.3). KIR 2DL5 gene was present at significantly lower frequency in T1D patients (41% vs 84%, $p < 10^{-4}$, OR 0.1). 2DL4, 3DL2 and 2DS4 genes were also negatively associated with T1D. When we stratified for HLA class I gene and MICA, there was no significant change in KIR gene frequencies.
Conclusion: We conclude that KIR gene is associated with and may play a role in development of T1D and the differential distribution between T1D vs. controls was not influenced by HLA class I or MICA genes in Korean population.

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Susceptibility effects of variation at the *IDDM2* (Insulin-gene) locus in latent autoimmune diabetes in adults (LADA).

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Background and Aims: LADA is a slowly progressing form of autoimmune diabetes developing in adults (>25 yrs). Though characterised by presence of islet cell autoantibodies, insulin dependence is not inevitable. Our aim was to determine whether the fine structure of the known association between islet autoimmunity and insulin-gene variation in T1D is also observed in LADA. Specifically, we examined the susceptibility effects of the T1D-defined protective (PH) and very protective (VPH) class III haplotypes.
Materials and Methods: 250 antibody-positive (LADA) and 638 antibody-negative (T2D) patients from the UKPDS cohort, plus 297 non-diabetic subjects (ND) were genotyped for -23*HphI* (class I/III) and +1428*FokI* variants by PCR-RFLP. Haplotype frequencies were estimated using the EH and SNPHAP programs. Genotypic associations with age at diagnosis, β-cell function (HOMA%B at diagnosis) and insulin requirement 6 years post-diagnosis were examined in the LADA subjects.
Results: Three major haplotypes were observed, as expected: class I, PH and VPH. Haplotype frequencies differed significantly between groups (LADA, vs. T2D p<0.0001; vs. ND p=0.006), due largely to an excess of class I haplotypes in LADA (79.7% vs 65.9% and 70.2%). The corresponding reduction in class III frequency in LADA principally reflected a deficit of PH haplotypes compared with T2D and ND (13.2% vs 26.2% and 19.7%). In UKPDS subjects, logistic regression confirmed that VPH homozygotes had higher probability of LADA (cf. T2D) than VPH/PH (OR 0.15 (0.03-0.75), p=0.02) or PH/PH (OR 0.22 (0.05-0.93), p=0.04) individuals. In LADA subjects, class I alleles were associated with insulin requirement by 6 years (75.2% in I/I; 21.0% in I/III; 3.8% in III/III, p=0.04) but there was no significant association with age at diagnosis or HOMA%B.
Conclusion: Class I alleles are associated with a more aggressive disease process in LADA as indicated by a higher frequency of patients with insulin requirement. In LADA, unlike T1D, the PH haplotype confers stronger genetic protection than the VPH. Variation within the insulin gene region appears to modulate the course of autoimmune diabetes.

OP 6
Clinical Diabetes

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Telemedicine: the management of diabetes.

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Background and Aims: Diabetes requires life-long clinical follow-up. The Soroka University Medical Center is the sole consulting center serving a population of 700,000 people spread over the southern region of Israel. Many towns and settlements are situated far from the hospital, requiring patients and consultants to travel long distances for follow-up.The aim of this study was to examine the feasibility of performing effective follow- up of patients with diabetes via teleconferencing and to determine patient-satisfaction with this mode of patient care .
Materials and Methods: A telemedicine clinic was initiated at our center using video-conferencing via telephone. Patients from our diabetes clinic living more than 50 kilometers from the Soroka Medical Center, in the towns of Arad, Dimona and the Arava settlements were included. The diabetes clinic and family practice files were pre- prepared and opened at either end. The patient's BP and EKG were transmitted on-line to the diabetes consultant by the family practice clinic.The most recent laboratory results were opened on-line, directly from the central laboratory, at both ends. A regular consultation visit was performed on-line by the diabetologist, with open discussion encouraged between the patient, family physician, clinic nurse and diabetes clinic staff. A summary of the consultation was immediately faxed to the family clinic. A satisfaction questionnaire was sent to all patients who attended. Parameters of metabolic control including HbA1c, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, systolic and diastolic blood pressure prior to initiation of telemedicine followup were compared with the same parameters 3 to 6, and 6 to 9 months following the initial visit.
Results: At the end of the pilot period, 76 visits have been made by 51 patients, 32 men and 19 women with a mean age of 59.2± 9.9 years.Most patients have expressed great satisfaction in the way follow-up was performed, as have the staff of the local family practice clinics involved. From patients' answers , the following advantages are apparent :
• Elimination of long journeys and expense for patient.
• Active involvement of the family physician and local clinic nurse in the consultation.
• Immediate implementation of changes in the therapeutic regimen.
• Facilitation of more frequent visits needed in the initial phase of adaptation of a personal management scheme.
Disadvantages:
• No physical contact between patient and diabetologist.
• The dietician is missing from the consultation.
• The contact with the diabetes nurse educator is not sufficient.
Following the telemedicine intervention, total cholesterol was significantly decreased from 183 ± 36 mg% to 172± mg% (p<0.04), LDL cholesterol dercreased from 103± 288 to 95 ± 26mg% (p<0.015), and systolic blood pressure decreased from146± 26 mm to 139 ± 19 mm (p<0.002).There was a clincally significant decrease in HbA1c from 8.5±1.7% to 8.0±1.8%.
Conclusion: Telemedicine appears to be an acceptable and effective method for the follow-up of patients with diabetes living at a distance from the nearest diabetology service.

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Implementation of the alphabet strategy improves cardiovascular risk estimates in Type 2 diabetes mellitus: the alphabet POEM roject (*P*ractice *O*f *E*vidence-based *M*edicine).

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Background and Aims: UK national guidelines advocate the use of Coronary Heart Disease (CHD) risk calculation to guide therapy. The Alphabet Strategy is a mnemonic-based programme with the following components: ***A*** – ***A***dvice, in particular smoking; ***B*** - ***B***lood pressure control; ***C*** – ***C***holesterol profile and ***C***reatinine control; ***D*** – ***D***iabetes control; ***E*** – ***E***ye screening; ***F*** – ***F***oot examination; ***G*** – ***G***uardian drug usage (aspirin, ACE-inhibitors, and lipid lowering therapy); ***H*** – ***H***eart disease risk score using CHD risk estimates as a surrogate marker of cardiovascular disease. This study assesses the impact of the Alphabet strategy on type 2 diabetes (T2D) care, in particular on CHD risk.
Materials and Methods: Data were collected on 400 consecutive out-patients with T2D from referral (T0) to the most recent follow-up visit (Tfu). Mean follow-up period was 5 years. In those without established CHD 10 year absolute CHD risk was estimated using the Framingham risk function and the UKPDS Risk Engine. Results were analysed using Student's paired t-test and Chi-squared test.
Results:
(T0 vs Tfu):
· *Advice*: Smokers 18.2% vs 15.5%: non- significant reduction, p=0.3.
· *Blood pressure*: Systolic 145.8 vs 140.1mmHg: p<0.0001. Diastolic 82.0 vs 76.5 mmHg: p<0.0001.
· *Cholesterol*: Total cholesterol 5.8 vs 4.9mmol/l: p<0.0001.
· *Diabetes control*: HbA1c% 7.9 vs 8.1: p<0.0001.
· *Eye examination*: 86.5% vs 96.8%: p<0.001.
· *Feet examination*: 69.6% vs 83.3%: p<0.001.
· *Guardian drugs*: Aspirin (28.9% vs 83.3%: p<0.001), ACE inhibitors (31.9% vs 64.3%: p<0.001), statins (16.7% vs 54.9%: p<0.001).
· *Heart disease risk scores*: Framingham 21.1% vs 16.8%: p<0.001. UKPDS 25.2% vs 24.8%:
p=NS. There were significant improvements between age-adjusted risk score (Tadj) and follow-up values (Tfu) (Framingham: 23.7% (Tadj) vs 16.7% (Tfu): p=0.001; UKPDS: 31.2% (Tadj) vs 23.7% (Tfu): p=0.001). The main contributor (approximately 90%) to improved CHD risk score was lipid modification by statin use. A discrepancy was noted between CHD risk estimates as predicted by Framingham and UKPDS: for males 6.6%, for females 0.5%.
Conclusion: Systolic and diastolic blood pressure, cholesterol profile, eye and feet examination, guardian drug usage, and Framingham and UKPDS CHD risk scores improved significantly over a 5 year period. The overwhelming contributor to improvement in CHD risk was lipid profile modification. The UKPDS Risk Engine is, in theory, more accurate than the Framingham risk function since it is based entirely on a diabetic population. The Alphabet POEM project is a novel evidence-based approach to diabetes care audit.

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Characteristics associated with self-monitoring of blood glucose in non insulin treated Type 2 diabetic patients: relationship with long-term metabolic control.

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Background and Aims: The use of self-monitoring of blood glucose (SMBG) in type 2 diabetic patients not treated with insulin is still a matter of debate. In the framework of a nation-wide outcomes research program in type 2 diabetes we identified subgroups of patients showing an increased likelihood of performing SMBG and evaluated the relationship of this practice with metabolic control over 3 years.
Materials and Methods: The study involved 1916 patients not treated with insulin recruited by 101 outpatient diabetes clinics (DOCs) and 103 general practitioners (GPs). At study entry, patients completed a questionnaire investigating SMBG practice and the level of family support for SMBG. Clinical information was collected at six-month intervals for 3 years. The main results were obtained by using a regression-tree technique (RECursive Partitioning and AMalgamation – RECPAM), that permits to identify homogeneous and distinct subgroups expressing a different likelihood of performing SMBG (≥1/week). Within each class identified by RECPAM, the impact of SMBG on long-term metabolic control was assessed by a longitudinal model accounting for physician-level clustering.
Results: Overall, 22% of the patients were on diet alone and 78% were treated with oral agents. Forty-one percent of the patients practiced SMBG with a frequency of at least once a week. Patients with no family support and on diet alone showed the lowest likelihood of performing SMBG (14%) and thus represent the reference category. Patients with higher family support, treated by DOCs and with BMI≤25 showed the highest likelihood of SMBG (OR=13.8; 95% CI 8.6-22.2). Among patients treated by GPs, those reporting frequent hypoglycaemic episodes were more likely to practice SMBG (OR=6.7; 95% CI 3.8-11.9). Among patients with low family support, the likelihood of SMBG was significantly higher among patients treated with oral agents, with higher school education and reporting frequent hypoglycaemic episodes (OR=7.5; 95% CI 4.0-14.1). A final logistic regression model with the RECPAM classes forced in also retained higher income, lower age, longer diabetes duration and female gender as independent correlates of SMBG practice. In none of the RECPAM classes

identified SMBG predicted a better metabolic control over three years of follow-up.
Conclusion: The performance of SMBG among non insulin treated type 2 diabetic patients strongly depends on the interaction between structural, socio-demographic and clinical characteristics. Even among patients with high family support and higher levels of school education the practice of SMBG is not independently related to lower levels of HbA1c over 3 years.

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Tropical calcific pancreatic diabetes is a prediabetic stage of Fibrocalculous Pancreatic Diabetes (FCPD) longitudinal follow up study.

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Background and Aims: Tropical Calcific Pancreatitis (TCP) is an idiopathic, juvenile, non-alcoholic form of chronic Pancreatitis with a unique tropical distribution. While some groups believe that TCP is a pre-diabetic stage of Fibrocalculous pancreatic diabetes (FCPD), others feel that TCP and FCPD are two different entities. The aim of this longitudinal follow up study was to see whether subjects with TCP who were in the non-diabetic stage initially develop diabetes (FCPD) on follow up.
Materials and Methods: Seventy-six TCP patients who did not have diabetes by WHO criteria at baseline, were included in this study. The 53/76 subjects (70%) available for follow-up underwent periodic oral glucose tolerance tests for evaluation of pancreatic endocrine function until they developed diabetes. Pancreatic exocrine function was measured using fecal chymotrypsin. Baseline demographic (age, gender) and clinical (age at onset of pain, body mass index) characteristics were noted. Time to development of diabetes was calculated using Kaplan Meir analysis. Data are expressed as mean ± standard deviation.
Results: Baseline characteristics (age, gender, fasting plasma glucose) of the 53 study subjects who were available for a follow up was similar to the 23 non-respondents.53 subjects were followed for a mean of 6.8 years (range-1- 21). Their mean age at baseline was 27 ± 12 years, 68% were males, and the mean body mass index was 19.5 ± 4.3 kg/m^2. 24 out of the 53 subjects followed-up (45%) developed diabetes while 10(19%) had Impaired glucose tolerance (IGT). Then 34(64%)had abnormal glucose tolerance. The incidence of diabetes in the study cohort was 6.6 per 100 person years of follow up. Subjects who developed diabetes were older (31 ± 12 vs 24 ± 11 years, $p<0.03$), had higher body mass index (21.4 ± 4.6 vs 18.4 ± 3.8 kg/m^2, $p<0.03$) and had lower fecal chymotrypsin (1.4 ± 1.1 vs 3.9 ± 2.9, $p<0.001$) at baseline compared to non-diabetic subjects. Family history of diabetes was more common among subjects who developed diabetes, compared to non-diabetic subjects (63% vs 34%). Median time for development of TCP after onset of pain was 5.2 years and the median time taken for development of diabetes after diagnosis of TCP was 12.5 years. Of the 10 subjects who had undergone surgical procedures (sphincterotomy / stenting / pancreatico jejunostomy / extracorporeal short wave lithotripsy (ESWL) with sphincterotomy + stenting) 9 remained free from diabetes even after a mean follow up period of 14 ± 11 years (9/29 vs 1/24, $p<0.01$, Fischer's exact test).
Conclusion: In TCP, there is a progressive deterioration of pancreatic function with development of diabetes(FCPD) in nearly half and IGT in a third of the patients who were followed. FCPD is merely a later stage in the natural history of TCP. Early decompressive surgery appears to delay or prevent diabetes in TCP subjects.

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Clinical and immunological profile of underweight Type 2 diabetes in north India.

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Background and Aims: While the majority of subjects with type 2 diabetes in developed countries are obese, those from developing countries like India are mostly non-obese, and many of them are underweight. This study attempts to characterize these underweight subjects with type 2 diabetes, especially focusing on insulin resistance, beta cell function and prevalence of antibodies to glutamic acid decarboxylase (GAD).
Materials and Methods: Adult patients (age >30 yrs) of BMI < 18.5kg/m2 with recently diagnosed type 2 diabetes were included. A control group was also recruited: this consisted of normal weight type 2 diabetics (BMI > 18.5 kg/m2). Using the homeostasis model assessment (HOMA), insulin resistance (HOMA-R) and beta cell function (HOMA-B) were calculated. GAD antibody (GAD Ab) positivity was estimated by radioimmunoassay
Results: Eighty three underweight type 2 diabetic patients and twenty two normal weight diabetic controls were studied. The age, sex ratio, duration of diabetes and the prevalence of complications were similar amongst the underweight diabetics and the normal weight diabetic controls. HOMA-B as well as HOMA-R values were lower amongst underweight as compared to normal weight diabetes ($p<0.05$).Twenty one of the eighty three underweight (GAD Ab positive and negative subgroups) and none of the normal body weight type 2 diabetic subjects were positive for GAD Ab. Underweight subjects with GAD positivity had a lower ($p<0.05$) mean age, waist hip ratio, HOMA-B as well as HOMA-R values and had a similar prevalence of complications when compared to GAD Ab negative underweight type 2 diabetics.
Conclusion: A significant proportion (25.3%) of subjects with underweight type 2 diabetes have evidence of islet autoimmunity. Our study shows that beta cell function is reduced in underweight type 2 diabetic subjects regardless of their GAD Ab status. Positivity to GAD Ab was associated with an even lower beta cell function. Insulin resistance in underweight diabetics was less than in normal weight diabetic subjects. This suggests that underweight type 2 diabetes is a distinct clinical entity, and is characterized by beta cell dysfunction. As about 75% of these subjects were GAD Ab negative, underweight type 2 diabetes is a heterogeneous entity of various etiologies, including GAD Ab positivity-associated autoimmunity, occult autoimmune mechanisms, or non immune partial destruction of beta cells. A protective mechanism against total autoimmune destruction of the beta cells and suppression of production of GAD Abs remains to be identified.

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The effect of growth hormone on insulin resistance and atherosclerotic risk factors in obese Type 2 diabetic patients with poor glucose control.

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Background and Aims: Growth hormone (GH) therapy accelerates lipolysis and promotes protein conservation. Thus, we evaluated the effects of GH therapy in combination with diet restriction on lipolysis and anabolism, insulin resistance and atherosclerotic risk factors in obese type 2 diabetic patients.
Materials and Methods: This study included 24 obese type 2 diabetic patients (M:F=12:12, mean age= 53.7¡$^3/_4$7.2 years), with high glucose levels (fasting glucose 192.3 ¡$^3/_4$20.1 mg/dl, HbA1c 9.9¡$^3/_4$2.3%). Among them, 16 obese type 2 diabetic patients were treated with recombinant human GH (rhGH; 1unit/day, 5times/week) and by diet restriction (25Kcal/kg ideal body weight/day) and exercise (250Kcal/day) for 12 weeks. Placebo vials were administered to 8 patients as a control. Anthropometric, and bioelectrical impedance measurements were made to determine total body fat and lean body mass. Computed tomography was used to visualize visceral and subcutaneous fat at the umbilicus level and the muscle area of the mid-thigh, and visceral fat area/subcutaneous fat area ratio (VSR) and visceral fat area/thigh muscle area ratio (VMR) was calculated accordingly. Subjects were monitored for the insulin sensitivity indices (ISI) by insulin tolerance test (ITT).
Results: VSR and VMR and was lower in the GH-treated group than in the controls, but no change of body weight was recorded after GH treatment. The ISI was significantly higher in only the GH-treated group. Levels of total cholesterol, triglyceride, serum free fatty acid, fibrinogen and plasminogen activator inhibitor-1 (PAI-1) were significantly lower after GH treatment. Serum glucose level and HbA1c were unaltered by GH therapy, but were significantly lower after 3 months of GH treatment. Insulin-like growth factor-1 (IGF-1), fasting C- peptide and insulin levels were significantly higher after GH treatment.
Conclusion: This study suggests that GH treatment in obese type 2 DM patients with insulin resistance and uncontrolled blood sugar caused a decrease in visceral fat and an increase in muscle mass, which may result in improvements of ISI, atherosclerotic risk factors and dyslipidemia.

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Hearing function in patients with Type 2 diabetes mellitus.

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Background: The association of type 2 Diabetes Mellitus (DM2) with hearing loss is insufficienty studied. reports range from 0 to 93%. Alterations in audiometric configurations and alterations in the transmission pathways are described with many different characteristics.
Aims: Evaluate the hearing perception, speech reception threshold and auditory brainstem response and their association with years since diagnosis of DM, metabolic control and the presence of other complications such as peripheral neuropathy, retinopathy and nephropathy
Materials and Methods: We carried out a case-control study in 94 patients with DM2, 77 females and 17 males, each diabetic patient was compared with one control non DM group, age and sex-matched. We registered about neurologic or hypertensive diseases previous to the diagnosis of DM2, excessive noise exposure and ototoxic medication. The characteristics of the patients included age, schooling, weight, height, BMI, DM family history, tinnitus, deafness, ototoxicity. Year since diagnosis of DM and age at onset of DM. The objective evaluation included otoscopy, pure-tone audiometry, speech audiometry, auditory evoked potentials (short latency), and fasting glucose in both groups. Albuminuria, retinal examination and the Michigan test for peripheral neuropathy were performed in the study group.
Results: We included 82% female and 18% male in each group, mean age 50.3±5.8 years. Schooling was lower in the DM group (5.4±3.4 years) than in the control group (p<.001). No statistical differences were found for BMI or weight. Fasting glucose170.5±66.6 mg/dl and HbA1c 10.7±2.6%. In the study of hearing function we found significant differences for medium and high frequencies, and speech audiometry (p<.001). Auditory brainstem response, we found differences for V wave and interpeaks I-V (p=.002) in right ear and interpeaks III-V y I-V (p<.001) in left ear. Alteration in hearing function was found associated with age, years since diagnosis of disease, glucose, HbA1c and peripheral neuropathy (p=.003).
Conclusion: DM2 may produce hearing impairment characterized by higher hearing treasholds, worse discrimination and abnormal transmission in central pathways. Those are associated with onset age of DM2, years since diagnosis, metabolic control and peripheral neuropathy. Supported by grant of IMSS.

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Decreased activities of daily living and physical activity related to diabetic vascular complication in the elderly patients with Type 2 diabetes. Two-year follow-up study.

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Background and Aims: General agreement exists regarding the usefulness of enhancing physical activity as a preventive means against development and progression of diabetes. However, it remained to be fully analyzed relationship between activities of daily living (ADL) and physical activity and diabetic vascular complications in the elderly patients with diabetes. The aims of this study were to investigate which risk factors affected ADL and physical activity using baseline data, and to examine whether decreased ADL and physical activity might relate to development and progression of diabetic vascular complication using 2-year follow-up study in the elderly patients with type 2 diabetes.
Materials and Methods: Subjects were 426 outpatients with type 2 diabetes and without dementia, visual loss or serious diseases, consisted of 219 males and 207 females, whose age were 73.4 ±6.7(m ±SD) years old, duration of diabetes 14 ±10 years, BMI 23.3 ±3.4, modality of treatment (diet alone 27%, oral hypoglycemic agent 50%, insulin 23%), diabetic retinopathy 37%, nephropathy 42%, neuropathy 65%, hypertension 45%, hyperlipidemia 32%, and atherosclerotic diseases 29%. ADL score evaluated by the Tokyo Metropolitan Institute of Gerontology (TMIG) index of competence for the elderly people and levels of physical activity evaluated by questionnaire (by Beacke) were measured. Furthermore in 206 patients followed for two years, relationship between changes of ADL and physical activity and development and/or progression of diabetic vascular complication was examined by using chi-square test and regression analysis.
Results: 1) ADL score was almost compared to the score with general population in the elderly in Japan. 2) ADL score and physical activity were declined with aging (p <0.0001). 3) There was significant relationship between ADL score and physical activity (p <0.0001). 4) By using stepwise regression analysis, ADL score significantly declined with higher age, retinopathy, atherosclerotic diseases and decreased physical activity (p <0.0001). Physical activity significantly declined with higher age, female, decreased ADL, pharmacological treatment and atherosclerotic diseases (p <0.0001). Furthermore, 5) during 2 years, ADL and physical activity decreased, but not significantly. 6) Incidences of development and/or progression in diabetic retinopathy, nephropathy and neuropathy were 16%, 25% and 25%, respectively. Diabetic microangiopathic complication (at least one of those) developed or progressed in 46% of subjects, that significantly related to decreased ADL and pharmacological treatment (p=0.007). Incidence of development and/or progression in atherosclerotic diseases was 10%, which significantly related to decreased physical activity (p=0.0002).
Conclusion: Development and/or progression of diabetic microangiopathic complication and atherosclerotic diseases significantly related to decreased ADL and decreased physical activity, respectively. Maintenance or enhancement of ADL and physical activity might prevent development and/or progression of diabetic vascular complication in the elderly patients with type 2 diabetes.

OP 7
Adipocyte Metabolism

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The role of protein kinase B in the regulation of lipid metabolism in adipocytes.

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Background and Aims: Adipocyte lipolysis is under tight hormonal control. Stimulation of adipocytes with isoproterenol leads to increased production of cAMP and activation of protein kinase A (PKA). PKA phosphorylates hormone-sensitive lipase on specific serine residues leading to translocation of the lipase to the triglyceride droplet, activation of lipase activity and lipolysis. Insulin antagonizes lipolysis mainly by inducing a phosphatidyl inositol-3-kinase dependent phosphorylation and activation of phosphodiesterase 3B (PDE3B). PKB has been suggested to be involved in the phosphorylation and activation of PDE3B suggesting, indirectly, a role for PKB in the regulation of lipolysis. The aim of this study was to evaluate the role of PKB in the regulation of lipolysis.
Materials and Methods: Constitutively active PKB (PKBmyr), wtPDE3B and β-galactosidase were over-expressed in mouse 3T3-L1 adipocytes and/or in primary rat adipocytes using an adenovirus expressing system. The adipocytes were then stimulated by insulin, isoproterenol or a combination of the hormones and thereafter analysed. PKB and PDE3B activities were measured in membrane and cytosolic fraction and lipolysis and lipogenesis assays were performed.
Results: In adipocytes overexpressing PKBmyr, basal and isoproterenol-induced lipolysis was reduced by 50% as compared to control cells. A lowering of lipolysis in PKBmyr adipocytes was associated with a 25% increase in membrane bound PDE3B activity. The ability of insulin to antagonize lipolysis in adipocytes overexpressing PKBmyr is presently being investigated. Furthermore, adipocytes overexpressing PDE3B 5-fold showed reduced isoproterenol-induced lipolysis and the ability of insulin to inhibit lipolysis was potentiated as compared to control cells. In addition we have found also that lipogenesis was increased 6-fold and insulin induced lipogenesis was potentiated in primary adipocytes overexpressing PKBmyr as compared to control cells.
Conclusions: The results support a critical role for PKB in the regulation of PDE3B activity and of lipolysis in agreement with previous studies indicating that PDE3B is a substrate for PKB. It has previously been suggested that PKB is involved in mediating glucose uptake and glycogen synthesis. Thus, PKB seems to be involved in several metabolic effects of insulin.

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Leptin impairs insulin signalling in isolated rat adipocytes.

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Background and Aims: Leptin is produced by adipose tissue and serves as mediator in the cross-talk between periphery and the central nervous system to regulate energy balance. Leptin acts mainly in the hypothalamus. However, leptin receptors are expressed in liver, muscle, fat and β cells, suggesting that it acts directly in those peripheral tissues. Although leptin appears to increase insulin sensitivity in non adipose tissues, in isolated adipocytes, leptin impairs most of insulin metabolic effects. Here we investigate the effect of leptin on insulin signalling in rat adipocytes by analyzing the stimulation of MAP kinase and the phosphorylation of GSK3 by insulin and vanadate after preincubation with leptin.
Materials and Methods: Adipocytes from 3-month old Wistar rats were isolated by the collagenase method and incubated for 6h at 37ºC in presence or absence of 50 nM leptin. Cells were further incubated in absence or presence of 16 nM insulin or 1 mM vanadate for 5 min, lysed, and cytosolic extracts were prepared. MAP kinase was determined in anti-erk immunoprecipitates using myelin basic protein as substrate. Phosphorylation of GSK3 was evaluated by western blot with α-phospho-GSK3β (Ser9) antibodies. Insulin receptor phosphorylation and SOCS-3 amount were determined in whole lysates with anti-P-Tyr and anti-SOCS-3 antibodies respectively.
Results: Insulin stimulates MAP kinase 2.8 fold in adipocytes incubated in the absence of leptin whereas only 1.5 fold stimulation was observed in cells preincubated 6 h with 50 nM leptin ($p<0.05$). In contrast, the stimulatory effect of vanadate (≈ 3 fold) was not significantly decreased after adipocyte incubation with leptin. Insulin and vanadate also stimulate the phosphorylation of GSK3 β in control adipocytes (≈ 3 and 2.4 fold respectively; $p>0.05$). However, preincubation of fat cells with leptin causes a 40% decrease of the stimulatory effect of insulin but does not impair the ability of vanadate to stimulate GSK3β phosphorylation. Preincubation with leptin also decreases the stimulation by insulin of insulin receptor β-subunit phosphorylation (more than 4 fold in control cells and 1.8 fold in leptin treated adipocytes) and increases 50% the amount of SOCS-3 in adipocytes.
Conclusions: In isolated adipocytes leptin impairs insulin signalling as manifested by a decreased stimulation by insulin of MAP kinase activity and GSK3β phosphorylation. In contrast vanadate effects on those enzymes are not altered by leptin preincubation. Since vanadate and insulin share the signalling pathway downstream of IRS-1 associated PI3-kinase stimulation, these data suggest that leptin impairs insulin action at an early step of the signalling cascade. Our data show a marked decrease of insulin induced receptor phosphorylation, the first step in insulin signalling, after adipocyte incubation with leptin. Elevated SOCS-3 might mediate in the impairment of the association between insulin receptor and IRS-1. From these data we can speculate that elevated leptin levels, as observed in obesity and during aging, could contribute to induce insulin resistance in adipose tissue acting directly on fat cells.

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Glucokinase and insulin gene expression in adipose tissue leads to increased glucose disposal.

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Background and Aims: Diabetes mellitus is characterized by chronic hyperglycemia, which leads to microvascular, macrovascular and neurological complications. Increased glucose disposal by tissues engineered to overexpress key regulatory genes in glucose transport or phosphorylation may reduce diabetic hyperglycemia. The objective of the present work was to determine whether increased glucose transport and phosphorylation in white adipose tissue (WAT) may reduce diabetic hyperglycemia and study the role of adipose tissue in the control of glucose homeostasis. To this end, transgenic mice expressing the glucose-phosphorylating enzyme glucokinase and insulin in adipose tissue were studied.
Materials and Methods: We generated transgenic mice expressing glucokinase in adipose tissue under the control of the adipocyte lipid-binding protein gene (aP2) promoter (aP2/GK). Transgenic mice expressing insulin in WAT under the control of the phosphoenolpyruvate carboxykinase promoter (PEPCK/Ins) were mated with aP2/GK transgenic mice to obtain double transgenic mice.
Results: Transgenic mice showed levels of GK mRNA in WAT and brown adipose tissue (BAT) about three times higher than in the liver of control mice. Adipose tissue basal glucose uptake was increased in transgenic mice about two folds whereas skeletal muscle basal glucose uptake was similar in control and transgenic mice. Body weight and epididymal fat pad weight were not alterated in transgenic mice compared with controls. Moreover, serum glucose, free fatty acid (FFAs) and triglyceride levels were not affected. Transgenic mice showed increased glucose tolerance and higher whole-body insulin sensitivity. In order to determine whether insulin and GK expression in adipose tissue may reduce diabetic hyperglycemia, double transgenic mice were treated with streptozotocin (STZ). Preliminary results showed that one month after STZ treatment, double transgenic mice were mildly hyperglycemic while STZ-treated control mice were highly hyperglycemic.
Conclusion: Our results indicate that adipose-specific GK expression leads to increased glucose disposal. Furthermore, they also suggest that expression of insulin and GK in WAT may counteract diabetic hyperglycemia.
This study was supported by European Union (EuroDiabetesGene QLRT-1999-00674) and Fundació La Marató de TV3.

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Uncoupling protein-1 (UCP1) expression is controlled by p38 MAP kinase via both transcription and phosphorylation of PPARγ coactivator-1α (PGC-1α).

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Background and Aims: The uncoupling protein-1 (UCP1) is exclusively expressed in brown adipose tissue (BAT), which is a thermogenic organ present in most mammals and is responsible for cold-induced non-shivering thermogenesis. UCP1 is anchored in the inner mitochondrial membrane, where it dissipates the proton gradient generated by oxidative phosphorylation. The resulting partial uncoupling of respiration renders these metabolic reactions less efficient, the net result of which is increased metabolic rate and the release of chemical energy as heat. The final outcome of the uncoupling via UCP1 is the increase of energy expenditure. UCP1 plays a ctritical role in maintaining energy balance in rodents and its function has been linked to type II diabetes. It is clearly established that cold- and diet-induced sympathetic nervous system recruitment of brown fat, with its induction of UCP1 gene expression, is fundamentally a β-adrenergic-mediated process. Nevertheless, although the UCP1 promoter has been studied in multiple species, and a conserved region of the UCP1 promoter was identified as a strong enhancer of brown fat and cAMP-dependent expression, the molecular mechanisms connecting β-adrenoceptors and cAMP to UCP1 gene transactivation are still not well understood. The goal of this study is to investigate signaling pathway from β-adrenergic stimulation to the expression of UCP1 gene.
Materials and Methods: Co-7 cells, HIB-1B brown adipose cells, primary brown adipocytes, and the BAT from C57BL/6J mice were used. Animals were exposed to cold (4 ° C) for 0 - 6 h with or without pre-treatment of p38 MAPK inhibitor SB203580 (50 mg/kg). The expression levels of UCP1 and PGC1α were evaluated with Northern and Western blotting. The activities of UCP1 enhancer and PGC1α promoter were assessed wioth CAT activity.
Results: During cold exposure, p38 MAPK activation and the expression of PGC1α and UCP1 genes were sequentially stimulated. The level of phosphorylated p38 MAPK was increased within a narrow time frame. It was clearly visible and at its peak by 1 h (2.5 fold), and declined over the next few hours, while the level of PGC1α RNA was increased by 1 h and reached its peak at 3 h (15 fold). Meanwhile, UCP1 transcripts were also increased in a time-dependent manner, reaching their maximum at 2 h (4 fold) and beyond. Nevertheless, the cold induction of p38 MAPK activation and the expression of PGC1α and UCP1 expression was completely eliminated by the treatment of p38 MAPK inhibitor SB203580. The PPRE of the UCP1 enhancer and the phosphorylation of PGC1α by p38 MAPK are both essential for the UCP1 transcription. The expression of PGC1α in brown adipose tissue and the PGC1α promoter activity are also controlled by p38 MAPK. Finaly, the treatment of p38 MAPK inhibitor led to the failure of mice to maintain their body temperature under cold exposure.
Conclusion: (1) p38 MAPK phosphorylation, PGC1α expression, and UCP1 transcription are sequentially induced in BAT during cold exposure. (2) The cAMP-dependent induction of UCP1 gene expression in mediated by p38 MAPK. (3) Both expression and phosphorylation of PGC1α by p38 MAPK are necessary for UCP1 transcription.

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Obesity resistance of aP2-*Ucp1* transgenic mice: involvement of lipoprotein lipase and AMP-activated protein kinase in white fat.

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Background and Aims: Transgenic (aP2-*Ucp1*) mice with ectopic UCP1 in white fat are resistant to obesity and they are hypolipidemic. The transgene reduces ATP/ADP ratio, elevates oxygen consumption, decreases fatty acid (FA) synthesis, mitigates lipolysis and induces mitochondrial biogenesis in adipocytes. A hypothesis was tested whether: (i) the hypolipidemic effect of the transgene reflects increased clearence of triacylglycerol by adipose tissue; and (ii) the complex effect of the transgene is mediated by activation of the AMP – activated protein kinase (AMPK).
Materials and Methods: Activity of lipoprotein lipase (LPL) was estimated in the detergent extracts of adipose tissue. ATP and AMP contents (HPLC) and AMPK activity (specific peptide phosphorylation assay) were estimated in adipose tissue of control and transgenic mice. Gene expression was examined quantitative real time RT-PCR. Induction of the phosphorylation of acetyl CoA carboxylase by AMPK was assessed using Western blots.
Results: Specific activity of LPL (related to wet weight of the tissue) was increased by the transgene in a fat depot-specific manner, with a significant increase in the gonadal but not subcutaneous (s.c.) fat. In both fat depots of transgenic mice, ATP/AMP ratio was significantly lower than in control animals. The presence of transgenic UCP1 resulted in approximately a 2-fold increase of AMPK activity in s.c. fat, while in gonadal fat, similar activity was observed in both genotypes. Activation of AMPK by the transgene was associated with increased phosphorylation of its enzyme target, ACC. A significant diminution of PPAR-γ and aP2 mRNA levels was found in s.c. fat of transgenic animals.
Conclusion: Increased LPL-mediated lipid uptake by adipose tissue contributes to hypolipidemic effect of ectopic UCP1 in white fat. The activation of AMPK can explain most of the metabolic changes that occur in the white fat of the aP2-*Ucp1* mice and might represent an important mechanism by which body fat stores are regulated.

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Glucose induces *de novo* fatty acid synthesis in rat skeletal muscle through a SREBP-1c dependent pathway.

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Background and Aims: We have previously shown that Sterol Regulatory Element Binding Protein-1c (SREBP-1c) was expressed and regulated by insulin in rat skeletal muscle. In primary cultures of muscle satellite cells, which form spontaneously contracting myotubes within 10 days, insulin up-regulated glycolytic and lipogenic enzymes through SREBP-1c.
Materials and Methods: The present study was performed using fully differentiated contracting myotubes.
Results: To assess if glucose could regulate SREBP-1c expression and action in these cells, its concentration was increased from 5mM to 25mM in contracting myotubes cultured without serum and insulin. As measured by 2-Deoxyglucose assay, glucose uptake increased by 2-fold within 30min, suggesting acute glucose-stimulated glucose uptake. Time-course experiments showed that glucose, but not mannitol, was able to up-regulate SREBP-1c precursor and nuclear mature proteins by 2 to 3-fold within 30min, the glucose-induced translocation of SREBP-1c mature form being confirmed by immunocytochemistry. Furthermore, within 3 hours, glucose enhanced by 2 to 3-fold the expression of Hexokinase II, fatty acid synthase (FAS) and acetyl-CoA carboxylase 2 (ACC2) proteins, while carnitine palmitoyltransferase 2 (CPT2) remained unchanged. In contracting myotubes, lipogenesis rate from [2- ^{14}C] acetate was 7.64 ± 0.78 nmoles/3h/mg protein under basal conditions, suggesting that de novo fatty acid synthesis occurred. A 3-hour treatment with 25mM glucose or 100nM insulin (24 hours) increased the lipogenesis rate by 55% and 65% respectively, whereas insulin+glucose induced a 90% increase. TOFA (5 µ M), a specific inhibitor of ACC, almost completely prevented lipogenesis under basal and stimulated conditions. Finally, Oil Red O staining of contracting myotubes clearly showed a lipid droplets accumulation 24 hours after exposure to insulin and/or glucose.
Conclusion: We concluded that glucose was able to stimulate the expression of SREBP-1c even more rapidly than insulin, leading to a rapid increase in lipogenic flux in rat skeletal muscle. This is the first evidence that increased intra-muscular lipid accumulation, that is associated with muscle insulin resistance in obesity or Type 2 diabetes, could partly occur from *de novo* fatty acid synthesis by skeletal muscle.

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Inhibition of p38 MAP kinase activity regulates IL-6 secretion and recovers GLUT4 protein expression and glucose transport in insulin-resistant adipocytes.

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Background and Aims: We have recently shown that p38 MAP kinase phosphorylation is increased in adipocytes from Type 2 diabetes patients, and that it seems to have an important role in the downregulation of GLUT4 levels in insulin-resistant states. It is known that intracellular levels of IL-6 in human adipocytes are negatively correlated with insulin sensitivity and

glucose uptake and that p38 can regulate IL-6 in other cell systems. However, it is not known whether activation of p38 and elevation of IL-6 levels may regulate GLUT-4 and glucose transport in adipocytes. In this study we examined the relationship between p38 activation, IL-6 secretion, GLUT4 levels and glucose transport in insulin-resistant adipocytes.
Materials and Methods: 3T3-L1 adipocytes were treated chronically with insulin to induce insulin resistance, in the presence or absence of specific p38 inhibitors. Human adipocytes were isolated from subcutaneous biopsis from healthy and type 2 diabetes patients (BMI range 22-38, insulin range 8-53 microU/mL). Phosphorylation status of p38 and GLUT-4 protein levels were determined by immunoblotting. IL-6 secretion was determined by ELISA and glucose transport was determined using 2-deoxyglucose
Results: Chronic exposure to insulin increased IL-6 secretion by adipocytes, an event associated with a loss of GLUT4 protein levels and diminished insulin-stimulated glucose transport. Inhibition of p38 kinase activity using specific p38 inhibitors prevented the insulin-induced increases in IL-6 secretion as well as the decrease on GLUT4 expression and recovered insulin-induced glucose transport in a dose-dependent manner. Exposure of the cells to IL-6 also decreased GLUT4, however this effect was independent of p38, consistent with p38 acting upstream of IL-6. We have also examined p38 phosphorylation and IL-6 levels in healthy and insulin-resistant human adipocytes. Human adipocytes with low levels of GLUT4 (insulin-resistant) had greater p38 kinase phosphorylation and intracellular concentrations of IL-6 compared to healthy human adipocytes with high levels of GLUT4 suggesting a direct correlation between insulin resistance, p38 activation, IL-6 and GLUT-4 expression.
Conclusion: Our results demonstrate that p38 kinase activation is involved in the regulation of IL-6 secretion by adipocytes and leads to the down-regulation of GLUT4 protein levels and decrease insulin-stimulated glucose transport, consistent with a potential role of p38 MAP kinase in the development of insulin resistance. Thus, inhibition of p38 activity leads to a recovery of GLUT-4 protein expression and insulin-stimulated glucose transport in insulin-resistant adipocytes.

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Transcriptional regulation of human adiponectin gene by TNF-α.
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Background and Aims: Adiponectin, an adipocyte-specific factor, has been shown to play important roles in the regulation of energy homeostasis and insulin sensitivity. A decreased plasma adiponectin level (hypoadiponectinemia) has been shown in type 2 diabetes and obese subjects. Tumor necrosis factor-α (TNF-α) suppresses the secretion and gene expression of adiponectin in adipocytes. The aim of the study is to verify transcriptional regulation of adiponectin by TNF-α.
Materials and Methods: 2.1 kb promoter region of human adiponectin gene was ligated with luciferase reporter plasmid (p2.1AdQ-LUC). (1) Differentiated 3T3-L1 cells transiently transfected with p2.1AdQ-LUC were treated with TNF-α for 48 hrs and luciferase activity was measured. (2) Three gradiently deleted promoter constructs (p1.6, p1.2 and p0.7AdQ-LUC) were created, transfected and treated with TNF-α and pioglitazone. (3) p2.1AdQ-LUC and C/EBPβ expression vector were cotransfected into preadipocytes and treated with TNF-α..
Results: (1) 10 ng/ml TNF-α suppressed luciferase activity to 2% of control and 50% suppression was found at 0.1 through 1.0 ng/ml TNF-α treatment. (2) The shortest promoter (p0.7AdQ-LUC) construct also showed strong suppression (5% of control) by 10 ng/ml TNF-α. Treatment with 10 μM pioglitazone alone stimulated luciferase activity at 3-8 fold in each luciferase construst. However, pioglitazone failed to ameliorate TNF-α-suppressed luciferase activity up to a basal level. (3) C/EBP transfection stimulated luciferase activity at 2-3 fold which was completely suppressed by TNF-α treatment.
Conclusion: Approximately 700 bp length of the promoter region is involved in TNF-α suppression of adiponectin gene transcription. This promoter region contains putative binding elements for C/EBP and SREBP1. Together with the present data, C/EBP may be, in part, a target transcriptional factor to be interfering with TNF-α.

OP 8
Prediction and Prevention of Vascular Events in Diabetes

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Absolute risk of coronary heart disease and carotid atherosclerosis in diabetic patients.
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Background and Aims: A new model estimating the absolute risk of coronary heart disease (CHD) in diabetic patients, based on data of the UKPD-Study, has been recently proposed (UKPDS-Risk Engine, www.dtu.ox.ac.uk/). 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 of the above mentioned model.
Materials 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 fibrilation, 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. Patients were matched into 3 groups according to their level of absolute risk of CHD for the following 10 years (<10%, 10-20%, >20%) and the corresponding mean of each group's IMT were estimated and compared. Results were statistically evaluated by Pearson's correlation coefficient and the ANOVA technique.
Results: The 3 groups of patients and their correspondent IMTs are depicted on Table 1. Table 2 shows the correlations of these means with the estimated absolute risk of the model.

Table 1. Level of risk and carotid - 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= 0.010 , (+) p= 0.020 (ANOVA between groups)

Table 2. Correlation of absolute risk and imt

Carotid – IMT	r	p
ICA	0.328	0.001*
CCA	0.386	0.000*

(*) p<0.01 (Pearson's correlations)

Conclusion: The carotid IMT was positively correlated with the absolute risk of CHD 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.

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The clustering of risk factors for the metabolic syndrome is associated with large artery stiffness in young and apparently healthy adults. *The Amsterdam Growth and Health Longitudinal Study* (AGAHLS).
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Background and Aims: The clustering of risk factors such as hypertension, central obesity and dyslipidaemia (i.e. the features of the

metabolic syndrome) are associated with increased risk for diabetes and cardiovascular events. However, it is not clear to what extent this is due to the impact of the MS on atherosclerosis and arterial stiffness, the major causes of cardiovascular disease and mortality. We therefore sought to investigate, in a 36-year old and apparently healthy population such as the AGAHLS, the relationships between the clustering of risk factors for the metabolic syndrome on the one hand, and carotid intima-media thickness (IMT) and large artery stiffness (i.e. distensibiliity, compliance and Young's elastic modulus of the carotid and femoral arteries) on the other.
Materials and Methods: Arterial properties were assessed by non-invasive ultrasound imaging. Identification of risk factors for the metabolic syndrome conformed with recent definitions and guidelines from the National Cholesterol Education Program (Adult Treatment Panel - III), namely: waist circumference (men102>cm, women>88 cm), triglycerides≥150mg/dL, HDL-C (men<40, women<50 mg/dL), blood pressure (≥130/≥85 mmHg), and HbA_{1c}≥6.2%. Analyses were conducted on 364 (189 women) subjects, which were divided into 4 groups according to the number of risk factors present (i.e. zero, 1, 2 and ≥3). Linear regression analyses with adjustments for gender, mean arterial pressure and height, were used to compare large artery properties between each of these groups and the reference group (group with zero risk factors).
Results: The clustering of risk factors was not associated with carotid IMT. However, the higher the number of risk factors present the stiffer the arteries, i.e. the lower the distensibility and compliance coefficients of the carotid and the femoral arteries, and the higher the carotid Young's elastic modulus (Table). This was specially true among subjects with 3 or more risk factors, thus, with the metabolic syndrome.
Conclusion: The deleterious impact of the clustering of risk factors for the metabolic syndrome on the arterial properties (i.e.stiffness) of young and healthy adults, illustrates the "ticking clock" hypothesis that suggests that macrovascular disease begins in the pre-diabetic state. Moreover, it emphasizes the importance of public health policies directed on the primary prevention (e.g. lifestyle modification programs) of cardiovascular risk factors.

Table. Clustering of risk factors (RF) for the metabolic syndrome and large artery stiffness

		Carotid artery		Femoral artery	
	# RF	β	95%CI	β	95%CI
Distensibility Coefficient	1	-2.46	-3.86; -1.07	-1.20	-2.10; -0.31
(10^{-5}.kPa^{-1})	2	-2.19	-3.95; -0.43	-1.42	-2.53; -0.31
	≥3	-3.70	-5.93; -1.46	-1.72	-3.16; -0.29
Compliance Coefficient	1	-7.66	-13.95; -1.37	-6.05	-11.80; -0.30
(10^{-2}.mm^2.kPa^{-1})	2	-8.10	-16.04; -0.17	-9.85	-17.00; -2.70
	≥3	-10.51	-20.58; -0.43	-11.89	-21.14; -2.63
Young'selastic modulus	1	3.45	0.56; 6.33	-	-
(10^5.kPa)	2	1.75	-1.89; 5.39	-	-
	≥3	7.00	3.38; 12.62	-	-

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Coronary calcification in Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort.
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The Epidemiology of Diabetes Interventions and Complications (EDIC) is a continued observational study of the DCCT cohort in 28 clinics. A major goal of EDIC is to study the development and progression of atherosclerotic disease in type 1 diabetes mellitus (T1DM). Seven to nine years after the closeout of the DCCT and initiation of EDIC, Computed Tomography (CT) of the coronary arteries was performed in 1150 patients with T1DM, 52% male, mean age 43 years, and mean diabetes duration 21 years, to assess the prevalence and degree of coronary calcification (CAC). The CT was carried out in 19 scanning sites, utilizing either Electron Beam CT or multidetector CT by centrally trained and certified technicians. Scans were read in masked fashion at a reading center to quantify coronary calcification, an indication of atherosclerosis. Prevalence was defined as CAC > 0, > 100, and > 200 Agatston units. Data were analyzed to evaluate the influence of prevalent traditional risk factors, prior intensive treatment, and HbA1c on CAC.

Odds Ratio of Selected Risk Factors

	CAC > 0	CAC > 100	CAC > 200
EDIC Mean HbA1c (>8.0 vs <= 8.0)	1.4*	1.2	1.1
DCCT Mean HbA1c (> 8.0 vs <= 8.0)	1.5*	2.3**	2.9***
Smoking (yes vs no)	2.7***	2.6***	3.4***
Hypertension+ (yes vs no)	1.7***	2.2***	2.2**
Hyperlipidemia++ (yes vs no)	1.7***	2.2***	2.4***

*p < .05**p < .01
***p < .001
+Hypertension: systolic bood pressure >= 140 or diastolic blood pressure >= 90 mm Hg, or documented hypertension or using anti-hypertensive agents.
++Hyperlipidemia: LDL >= 130 mg/dl or using lipid-lowering agents.

Summary: After adjusting the odds ratio, for gender, attained age, scanning site, baseline DCCT retinopathy status, and DCCT treatment group, numerous risk factors, EDIC mean HbA1c, DCCT mean HbA1c, smoking, hypertension, and hyperlipidemia were significantly associated with any CAC and some even more so with severe levels of CAC. Prior glycemic exposure appears to contribute to the risk of atherosclerosis in T1DM.

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Predictors of silent myocardial ischemia in patients with Type 2 diabetes mellitus: results from the DIAD Study.
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Background and Aims: Current guidelines for the diagnosis of coronary artery disease (CAD) in diabetes suggest that asymptomatic patients with multiple CAD risk factors may benefit from screening by stress testing. Adenosine-Tc99m-Sestamibi SPECT perfusion imaging (SPECT) is a well-established modality to evaluate patients suspected of having CAD. Moreover, an extensive literature supports the association of perfusion abnormalities by SPECT with adverse outcomes in both diabetic and non-diabetic patients. The Detection of Ischemia in Asymptomatic Diabetics (DIAD) Study is the first prospective, multi-center investigation to establish the prevalence of silent myocardial ischemia in patients with type 2 diabetes (T2DM) and to attempt to define a high-risk clinical profile.
Materials and Methods: Entry criteria included: an established diagnosis of T2DM, age 50-75, no known or suspected CAD, and a normal baseline ECG. 1124 subjects were recruited at 14 clinical sites and randomized to either SPECT and 5 years of follow-up (n=561) or to follow-up alone (no SPECT) (n=563). All subjects underwent a baseline history, physical examination, and laboratory evaluation, in addition to specialized cardiac autonomic testing.
Results: Mean age of DIAD subjects was 61 years (T2DM duration, 9 years); mean body mass index (BMI), 31.0 kg/m^2; 54% were male and 22% from ethnic minorities. 14% were being managed with diet only, 63% with oral agents only, and 23% with insulin. 57% were being treated for hypertension, 24% had albuminuria, and 10% were current smokers. The mean HbA1c was 7.1%, LDL-C 114 mg/dl, HDL-C 50 mg/dl, and TGs 170 mg/dl. SPECT data were available in 522 patients (93% of those randomized to imaging). 113 studies (22%) were abnormal, including 83 perfusion defects and 30 non-perfusion abnormalities (left ventricular dysfunction, transient ischemic dilatation, or ischemic ECG changes). Using bivariate analysis, established and emerging CAD risk factors, such as BMI, smoking, HbA1c, blood pressure, albuminuria, lipid levels, homocysteine, and C-reactive protein were not predictive of an abnormal SPECT (all, p=NS). In contrast, a lower ratio of the maximum to minimum heart rate during and after Valsalva maneuver, indicative of autonomic

dysfunction, was significantly correlated with abnormal SPECT (lowest vs. highest quartile, OR=2.4 [95% CI, 1.2-4.5]).
Conclusion: These data indicate that routine clinical and biochemical features commonly associated with CAD morbidity are not associated with SMI as detected by SPECT, which was abnormal in more than one out of every five asymptomatic patients with T2DM. In contrast, evidence of cardiac dysautonomia was strongly associated with abnormal SPECT. As a result, in the diagnostic pursuit of silent myocardial ischemia in patients with T2DM, risk assessment involving standard clinical data may not be adequate. Ongoing 5-year follow-up in the DIAD Study will serve to assess the association of clinical / biochemical variables and abnormal SPECT with cardiac event rates.

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Both fasting and post-lunch blood glucose are independent risk factors for cardiovascular events in an Italian cohort of Type 2 diabetic subjects.
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Background and Aims: The effect of hyperglycaemia and the role of fasting vs post-prandial blood glucose (BG) in the prediction of cardiovascular events need to be better clarified. We undertook the present study to determine if BG, either fasting or post-prandial, is an independent predictor of cardiovascular events in type 2 diabetic patients.
Materials and Methods: We studied a population of 529 (M=284, F=245) type 2 diabetic subjects for an average follow-up of 5 years at our out-patient clinic. BG was determined fasting (FBG), 2 hours after breakfast, 2 hours after lunch and before dinner. The other variables considered were: gender, age, diabetes duration, smoking habit, body mass index, HbA1c, systolic and diastolic blood pressure, total and HDL cholesterol, triglycerides, albumin excretion rate (AER), fibrinogen and white blood cell count.The first event of acute coronary syndromes, acute cerebrovascular disease,major and minor lower limb amputations for ischemic reasons, revascularization procedures at any site which occurred during the 5-year follow-up was obtained both from clinical records of follow-up visits and from hospital discharge database (ICD9-CM classification) of Piedmont region (4 million inhabitants in North-West of Italy) and used as outcome measure. The comparison between the event and non-event groups was carried out using parametric t-test and non parametric Wilcoxon test for non-normality assumption. Cox proportional hazards models were fitted in order to evaluate the independent effect of each BG measurement, taking into account all the covariates significantly associated with the outcome in the bivariate analysis.
Results: 77 events were recorded in 529 patients (14.5%), 54 of which in men (19%) and 23 in women (9.4%) ($p<0.01$). Patients with and without events significantly differed for fasting, after breakfast and after lunch BG. When models were fitted introducing separately BG measurements and adjusting for gender, age, diabetes duration, total cholesterol, fibrinogen, AER and smoking habits, excess risks were shown for fasting and after lunch BG. The HR (3rd vs 1st tertile) were 2.1 (95%CI: 1.1-4.0) for fasting BG, and 2.6 (95%CI: 1.4-4.9) for BG after lunch. When HbA1c was introduced in the models together with each BG value, fasting BG (HR: 2.0; 95%CI: 1.1-3.8) and BG after lunch (HR:2.5; 95%CI:1.3-4.9) remained significant determinants.Among the other covariates, age, duration of diabetes and AER played the major role.
Conclusion: This study shows that both fasting and post-lunch blood glucose are independent risk factors for major cardiovascular events in a 5-year follow-up in a cohort of type 2 diabetic patients.

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The role of sialic acid in the prediction of coronary heart disease in Type 1 diabetic patients.
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Background and Aims: Sialic acid is a marker of the acute-phase response, since most acute phase proteins are glycoproteins with sialic acid as the terminal sugar of the oligosaccharide chain. It is not known what the role of sialic acid is in the prediction of coronary heart disease (CHD) in type 1 diabetic patients. The main aim is therefore to examine the relationship between sialic acid and 7-year incident coronary heart disease in type 1 diabetic patients.
Materials and Methods: Data from the EURODIAB Prospective Complications Study were analysed. This cohort included 2329 type 1 diabetic patients without CHD at baseline, aged 15-60 years from 14 European countries. CHD at follow-up was defined as physician diagnosed myocardial infarction, angina pectoris, coronary artery bypass graft surgery, and/or Minnesota coded ischaemic ECGs or fatal CHD.
Results: Sialic acid was significantly correlated (Spearman rank test $p < 0.0001$) with glycated haemoglobin (r=0.20), total cholesterol (r=0.28), LDL-cholesterol (r=0.19), non-HDL-cholesterol (r=0.28), fasting triglyceride (r=0.29) and albumin excretion rate (r=0.20). Unadjusted analyses showed that baseline sialic acid concentrations were significantly raised in those who developed CHD, but only in men (2.1 in those with CHD vs. 1.9 mmol/L in those without CHD, t-test $p < 0.001$). In women there was no significant difference (2.04 vs. 2.01 mmol/L respectively). Multivariate models using Cox proportional survival analyses showed that a standard deviation unit increase in sialic acid was significantly associated with CHD (in men) with a hazard ratio of 1.5 (1.1-2.0, p=0.02), adjusted for age, duration, glycated haemoglobin, systolic BP, triglyceride concentration, waist-hip ratio, smoking and albumin excretion rate.
Conclusions: Sialic acid is a strong predictor of coronary heart disease in men with type 1 diabetes, beyond the effect of established risk factors. The sex-difference in the relationship between sialic acid and CHD needs to be further explored.

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C-reactive protein (CRP) is a strong independent predictor of death: association with multiple facets of the metabolic syndrome.
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Background and Aims: The importance of C-reactive protein (CRP) as a predictor of death and cardiovascular events as well as its relation to the facets of the Metabolic Syndrome should be analysed in a cohort of type 2 diabetic patients with a high risk for macrovascular complications.
Materials and Methods: 592 patients at 55 to 74 years of age (311 men, 281 women) examined by duplex ultrasound for cerebrovascular and peripheral arterial disease were followed over a period of 5 years. 315 patients had diabetes (53.2 %). Coronary heart disease was present in 45.3 %, cerbrovascular disease in 21.9 % and peripheral arterial diasease in 39.7 % of cases.
Results: During observation 104 patients died, 72 (69.2 %) due to cardiovascular causes. In multiple logistic regression analysis CRP was the strongest predictor of death and cardiovascular events in the total cohort (RR 3.1 (1.86-5.31)) as well as in the diabetic subgroup (RR 2.7 (1.33-5.57)). In contrast neither the traditional cardiovascular risk factors nor the parameters of diabetic metabolic control were able to improve prediction. Higher CRP was associated with a lower HDL-cholesterol ($p = 0.002$), higher levels of triglyzerides ($p = 0.006$), C peptide ($p = 0.007$) and postprandial glucose ($p = 0.029$) as well as albuminuria ($p = 0.037$) in the diabetic subgroup.
Conclusion: CRP is a better predictor of death and cardiovascular events than traditional risk factors or parameters of metabolic control in diabetic patients with cerebrovascular or peripheral arterial disease. Association of CRP with several factors of the Metabolic Syndrome supports the hypothesis that subclinical inflammation is part of the insulin resistance syndrome.

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BART (By-pass Angioplasty Registry Type I-II Diabetes):1 year follow up results from a prospective study.
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Background and Aims: Previous subset analyses of randomised trials have suggested that percutaneous coronary intervention (PCI) in diabetics with multivessel coronary artery disease (MVD) results in higher mortality than coronary artery bypass grafting (CABG) although registry data can suggest

similar outcomes when diabetics are treated in accordance with physician preference.For this reason we planned a study on prospective registry data analysis to determine outcomes of diabetics undergoing coronary revascularisation according to physician preference.
Materials and Methods: Between January 1998 and December 2001 we prospectively recruited diabetics, undergoing cardiac catheterisation at Cardiology Division of Hammersmith Hospital in London, who had multivessel coronary artery disease defined as narrowings of 50% or more in at least two vessels. After a 1-year follow-up the considered outcomes were in-hospital and 1-year mortality, repeat revascularisation at 1 year and number of revascularised vessels.
Results: Of 9586 patients 1714 patients (17.9%) were diabetics of whom 970 (56.6%) had multivessel disease. CABG was performed in 318 (32.7%), PCI in 351 (36.1%) and 301 (31.0%) were treated medically. Angiographically the number of diseased vessels (mean(SD) was significantly higher in the CABG group 2.94 (0.55) versus the PCI group 2.51 (0.55) and the medically treated group 2.72(0.58) p<0.001. The number of vessels revascularised (mean (SD) in the CABG group 3.15 (0.56) was greater than in the PCI group 1.39 (0.55) p<0.0001. Baseline characteristics were well matched between PCI and CABG for risk factors, co-morbidities, left ventricular function and mean age (62.9). In the PCI group versus the CABG group there were significantly more females (34.8% versus 23.3%), more urgent cases (including those with cardiogenic shock) but less 3 vessel disease.
Inhospital mortality was 1.7% in the PCI group and 3.4% in the CABG group while one year mortality with 98.1% follow up complete was 7.3% for CABG, 9.3% for PCI and 9.8% for medical therapy, p= NS. Rates for repeat revascularisation at one year were 23.0% in the PCI group and 2.0% in the CABG group (p<0.0001).
Conclusion: CABG in diabetics with multivessel disease still requires fewer repeat revascularisations, but PCI in the stent era has improved compared to historical rates and performs well as part of an overall strategy employing both PCI and CABG. Although different from randomised trials evidence, our results suggest that detailed registry data could play an important role in decisional management of high risk diabetic patients

OP 9
Childhood Diabetes

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Challenges in classification of diabetes Type in children: the SEARCH for Diabetes in Youth study.
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Background and Aims: Historically, diabetes (DM) in youth was thought to be almost exclusively T1DM but phenotypes resembling adult-onset T2DM have been increasingly reported in pediatric populations. SEARCH for Diabetes in Youth, a multicenter, population-based registry of diagnosed diabetes in persons under age 20, was established to develop practical approaches to classification of diabetes in youth and to assess the prevalence (year 2001) and incidence (years 2002-2004) of diabetes by type among major U.S. racial/ethnic groups.
Materials and Methods: Cases are ascertained from clinical and administrative databases and active surveillance, and more than 8,000 patients will be invited to participate in a standardized data collection protocol over a 3-year period. Diabetes autoantibodies (DAA: to GAD65, IA-2 and insulin) and fasting C-peptide (FCP) concentrations are measured in prevalent and incident cases. An initial diabetes type is assigned and used to triage participants who are asked to return for stimulated C-peptide testing (SCP) and follow up. Final diabetes type will be assigned at study completion.
Results: In the first 5 months, 618 patients have had a SEARCH visit and an initial assignment to type 1A (DAA positive, FCP < 3.7 ng/ml), type 1 (DAA negative, FCP < 0.8 ng/ml), and type 2 diabetes (DAA negative, FCP ≥ 3.7 ng/ml). In some participants, such assignment was not possible or conflicted with clinical characteristics. As examples, a prevalent case with clinical T2DM (BMI=27, HbA1c=5% with oral medication) had SEARCH-measured negative DAA, but FCP of 1 ng/ml. An obese (BMI=39) prevalent case diagnosed with clinical T2DM had high FCP (2.8 ng/ml) and acanthosis , but positive IA2 antibodies. Under insulin treatment, HbA1c was 7.2%. An incident case had positive GAD65 antibodies, but high FCP (4.1 ng/ml), obesity (BMI= 40) and acanthosis. HbA1C was 7.5%, on insulin and oral medication. The cases were all from minority groups, age ranging from 12 to 18 years and of both genders. Such cases will be followed longitudinally with SCP testing and their biochemical and clinical characteristics will be compared with the biochemical and clinical phenotypes of SEARCH-defined type 1 and type 2 diabetes.
Conclusion: Classification of diabetes type in youth is challenging due to the heterogeneity of childhood diabetes and to probable gaps in our knowledge of diabetes pathophysiology. By longitudinally following a large multi-ethnic cohort of children with diabetes, SEARCH hopes to develop standard definitions of type 1, type 2 and other types (or hybrids) of diabetes, as well as practical approaches to classification of types of diabetes in youth.

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Prevalence of Type 2 diabetes among known cases of diabetes aged 0-18 years in Sweden.
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Background and Aims: The frequent reports of a rising incidence and prevalence of diabetes mellitus type 2 (DMT2) in children and adolescents seen in the world today are worrying. Sweden has the, second to Finland, highest incidence and prevalence of diabetes mellitus type 1 (DMT1) in the world today. The prevalence of DMT2 in children in Sweden is hitherto not known. Body Mass Index (BMI) according to age is rising in Swedish children. To be able to establish an eventual future rise in the incidence of DMT2 in the age group 0-18 years we have made a national retrospective population based case study, detecting all known cases of DMT2 and Maturity Onset Diabetes in the Young (MODY) in Sweden on Dec 31 2001.

Materials and Methods: Sweden has a total population of 8,9 millions. All children in Sweden 0-18 years of age having diabetes are cared for by 42 diabetes teams in paediatric clinics. All cases (n = ~ 6000) were evaluated by their diabetologist regarding age at onset, BMI, occurence of autoantibodies at onset, level of C-peptide at onset, sex, heredity and ethnicity, using a standardised form. The most probable diagnosis, according to the criteria suggested by the American Diabetes Association (Diabetes Care 2000), was made as Type1, Type2, MODY or „other specified diabetes" (i.e. secondary diabetes due to medication or diabetes in connection with syndromes).
Results: Out of ~6000 cases of diabetes 0-18 years we found 31 cases of DMT2, 29 cases of MODY and 25 cases of „other specified diabetes". The rest of the ~ 6000 cases fulfilled the criteria for DMT1. The sex ratio in DMT2 was 2 females in 1 male and in MODY 3 females in 1 male. 50 % of the DMT2 cases had an ethnical background known to have high incidence of DMT2. The diagnosis DMT2 was mainly suspected at onset by the diabetologists based on heredity, obesity and age. Lack of autoantibodies confirmed the diagnosis. MODY diagnosis was established on clinical grounds, heredity, lack of autoantibodies and in a few cases by genetic analysis for specific mutations.
Conclusion: Diabetes mellitus type 2 in the age group 0-18 years in Sweden still is very rare and represents only 0,5% of all cases of diabetes. Whether a rise in the incidence, or not, will occur is continuously under debate. The current background study gives a stable benchmark for future epidemiologic estimations of prevalence and incidence of diabetes mellitus type 2 in children and adolescents in Sweden.

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Prevalence and clinical characteristics of patients with non-Type-1-diabetes in the pediatric age range: analysis of a multicenterdatabase including 20,401 patients from 148 centers in Germany and Austria.
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Background and Aims: While most pediatric patients with diabetes are classified as type-1, other forms of diabetes are increasingly recognised. Our study describes the frequency and the clinical characteristics of patients with type-1 and non-type-1-diabetes diagnosed during the first 2 decades of life based on a longitudinal multicenter documentation.
Patients and Methods: Until September 2002, 20 401 patients (age at onset < 20 y., 10 511 males, 9 890 females) were registered in a total of 148 centers participating in an initiative on quality improvement in pediatric diabetology (DPV-Science). Longitudinal data on the course of diabetes are documented locally and transferred for centralized analysis after anonymization.
Results: 19 796 patients (97 %) were classified as type-1, 130 patients (0.6 %) as type-2, 474 (2.3 %) as "other specific types = type-3" and 1 patient as gestational diabetes. Patients with type-2 diabetes were predominantly female (71.5 %, p<0.0001), significantly older at diagnosis (12.8 versus 8.1 years, p < 0.0001) and more overweight (z-score for BMI: +1.97 ± 1.33 versus +0.46 ± 0.93; mean ± SD, p<0.0001). 43 (35.8%) of type-2 patients received insulin therapy with an average dose of 0.5 U/kg/day compared to 0.83 in type-1 patients. Among patients with "other specific types", the most prevalent diagnosis was CF-related diabetes (85 patients), followed by trisomie 21 (n=57), MODY (n=50), pancreatic disease (n=35), endocrinopathies (n=17), UTS/Noonan-syndrome (n=19), steroid-induced diabetes (n=14), diabetes due to poly-transfusion (n=13) or malignancies (n=12) and DIDMOAD-syndrome (n=13). Connatal diabetes was present in 9, mitochondrial diabetes in 7 and Prader-Willi-syndrome in 7 subjects. In 130 patients classified as type-3, no definitive diagnosis had been established. Age at diabetes onset was 7.0 years in trisomie 21, 10.6 years in MODY and 13.1 in patients with CF-related diabetes. Body-mass-index differed considerably among the patient-groups: z-score averaged +3.5 in PWS, -1.04 in CF-related diabetes, +0.75 in MODY and + 0.63 in trisomie 21. Additional differences were present for insulin therapy and metabolic control achieved. When 17 221 patients with a diabetes onset of < 15 years were analyzed separately, 16 800 (97.5 %) were classified as type-1, 87 (0.5%) as type-2 and 334 (1.9 %) as "type-3".
Conclusion: Despite recent interest in adolescent-onset type-2 diabetes, this form is still rare in Caucasian pediatric patients. After type-1 and type-2-diabetes, the most prevalent diagnosis during the first 2 decades of life is cystic fibrosis (CF)-related diabetes. Age at onset, anthropometric characteristics, insulin requirement and metabolic control differ considerably between patients with type-1 and other types of diabetes.

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Can OGTT-derived indices uncover an overweight threshold for deterioration in adolescent glucose metabolism?
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Background and Aims: Childhood-T2DM epidemic emulates the progression of obesity but typically only the mostly severely obese children are screened. We evaluated OGTT indices of ß-cell function and insulin sensitivity to determine the consequence of wide-ranging overweight on glucose metabolism of adolescents. We also sought to uncover a clinically useful overweight threshold at which ß-cell function and insulin sensitivity decline.
Materials and Methods: Seventy-six healthy overweight children (age=14.2±0.2 yr, BMI =36±0.7, 67% F) referred for overweight management and 13 healthy non-overweight children (age=14.8±0.6 yr, BMI =20.8±2.0, 69% F, 33% AA) underwent a physical examination and 75-gm OGTT with Fasting Insulin (FI), Fasting Glucose (FBG); Insulin Response (Iresp), and Corrected Insulin Response at 30 min (CIR_{30}) to assess ß-cell activity; HOMA and Composite Insulin Sensitivity Index (CISI) to assess insulin resistance obtained. Subjects were categorized as impaired glucose tolerance (IGT) if FBG was ≥110 or a 2hr glucose level was ≥ 140 mg/dl.
Relative BMI (RBMI) was used to estimate percentage of overweight (based on CDC chart 50th percentile for age and gender). Subjects was stratified into 5 relative BMI classes (RBMI: <125, >125<150, >150<175, >175<200 and >200).
Results: Age, gender, FBG were similar among groups, 21% IGT (3 meet T2DM criteria). IGT prevalence greater in groups with RBMI ≥125 (X^2 =29.2, P ≤0.001). Significant differences from the RBMI<125% group occurred in HOMA, Iresp, and CIR_{30} once RBMI exceeded 150%, 175%, and 200% respectively. FI did not detect differences between the most and least overweight groups. Differences in CISI occurred once RBMI exceeded 125% while values remained similar in groups with RMI above 150%.

Relative BMI (n)	Mean BMI	ABG (%)	FBG mg/dl	FI µU/ml	Iresp	CIR_{30}	CISI	HOMA
<125% (13)	20.8±0.5	0	86±3.9	10±10	36±97	1.1±1.7	8.8±0.7	2.2±1.7
>125<150%(11)	27.3±0.4	18	87±4.3	32±11	129±88	2.2±2.0	5.6±0.8*	3.3±2.0
>150<175 (19)	31.3±0.6	37	85±3.2	41±8*	239±67	2.7±1.4	2.3±0.6†*	8.6±1.4†*
>175<200 (22)	36.4±0.5	18	84±3.0	38±8*	341±62†*	4.1±1.4	1.8±0.6†*	8.1±1.3†*
>200 (24)	42.1±0.9	25	86±3.0	33±11	355±60*	5.3±1.3*	1.7±0.6†*	7.0±1.3*

Values reported as means±SE. *P≤0.05 from the <125% group; †P≤0.05 from the >125<150% group.

Conclusion: RBMI is a clinically useful method to evaluate severity of overweight. In our sample, even those adolescents with BMI close or slightly above the 95th percentile (RBMI >125<150) had an increased prevalence of IGT. FBG, FI and CIR_{30} do not reflect the impact of a wide-ranging overweight on glucose metabolism. Changes in Iresp and HOMA occurred at higher levels of obesity. CISI best reflected the progressive influence of overweight on insulin sensitivity of adolescents. Once RBMI exceeded 150%, significant deterioration in CISI had already occurred. Screening for abnormal glucose metabolism is warranted in all adolescents who are >25% overweight.

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Insulin sensitivity deterioration may be the most important factor in the pathogenesis of Type 2 diabetes (T2DM) among adolescents.
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Background and Purpose: The epidemic of T2DM in childhood is clearly associated with the progression of obesity. Whether the major pathogenic factor for developingT2DM is β-cell fatigue or impaired insulin sensitivity in adolescents remains unclear. Accordingly, we evaluated the relationship between body mass index (BMI), relative BMI (RBMI), race and gender

with parameters of β-cell activity and insulin sensitivity derived from glucose and insulin levels during an oral glucose tolerance test (OGTT) in 89 adolescents without a history of diabetes.
Subjects and Methods: Subjects (age =14.2±0.2 yr, 56% African American, 67% female, BMI = 33.5±0.8 kg/m^2) were stratified as having normal glucose tolerance (NGT) if fasting blood glucose (FBG) was < 110 mg/dl, or as having impaired glucose tolerance (IGT) if FBG was ≥110 or a 2hr glucose level was > 140 mg/dl. Relative BMI (RBMI) was used to estimate percentage of overweight (BMI/50th percentile BMI on CDC chart for gender and age), fasting insulin (FI) and indices of β-cell activity [insulin response (Iresp) and corrected insulin response at 30 min (CIR_{30})] and insulin sensitivity [HOMA and Composite Insulin Sensitivity Index (CISI)] were calculated from the OGTT.
Results: The prevalence of IGT was higher in subjects with RBMI > 125 (X^2 = 29.2, P<0.001). A total of 19 (21%) subjects had IGT (3 T2DM, 15 IGT and 1 IFG; 18% of all IGT had a RBMI >125 < 150. With similar FBG between the 2 groups (92±6 mg/dl and 83±1 mg/dl, P=NS) subjects with IGT had higher indices of B-cell activity than NGT subjects (FI : 44±10 vs. 30±4 μU/ml, P=0.02; Iresp: 450±113 vs. 215±26, P=0.02; $CIR_{30;}$ 6±3 vs. 3±0.2, P=0.01), and lower insulin sensitivity (HOMA: 9.3±2 vs.6.3±1, P=0.01; CISI: 1.5±0.3 vs. 3.8±0.5, P<0.001). RBMI was positively correlated with Iresp (r = 0.31, P<0.01), HOMA (r = 0.23, P=0.03) and was negatively associated with CISI (r = - 0.64, P<0.001). Impaired insulin sensitivity as determined by lower CISI was the single predictor of IGT in adolescents (P<0.04). Fifty-four percent of the variance in CISI values (P<0.001) was accounted by RBMI (R^2=0.39), IResp (R^2=0.06), Age (R^2=0.02), BMI (R^2=0.06), and gender (R^2=0.02).
Conclusions: The use of β-cell activity and insulin sensitivity indices derived from OGTT in adolescents are valuable in assessing the effect of overweight on glucose metabolism. Our results indicate that deterioration of insulin sensitivity with increasing overweight represents the most important pathogenic factor leading to impaired glucose tolerance among adolescents. Screening of adolescents who are greater than 25% overweight will allow for early recognition and intervention.

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Influence of sleep and daily activity on heart rate variability in children with beginning vascular complications of diabetes Type 1.
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Background and Aims: Heart rate variability (HRV) has been used to assess cardiac autonomic function noninvasively, understand the patophysiologic mechanisms of heart disease, evaluate therapy, and assess long-term prognosis. The aim of the study is evaluation the heart rate variability in sleeping and awakening children with diabetes type 1 and beginning of chronic vascular complications.
Materials and Methods: The study consisted of 54 children aged 14.3± 3, suffering from diabetes type 1 (5.7± 3 years) and 22 healthy age and sex matched children. The diabetic children were divided into two groups: first – 26 children with beginning of vascular complications (hypertension or simple retinopathy with or without microalbuminuria), second – 28 children without vascular complications and microalbuminuria. Blood samples for determination of glycosylated haemoglobin (HbA_{1C}) and urine collection for estimation of microalbuminuria were done. Time- and frequency-domain HRV indices from 24-hour electrocardiographic monitoring, and 2-hour ECG recordings, obtained in sleep stage (beginning 2 a.m.) and during activity hours (beginning 2 p.m.) in 12-hour intervals were analysed.
Results: There were significant increased in HbA_{1C} level in children with vascular complications as compared to diabetic children without complications (9,6±1,8 vs. 8,2±1,7, p<0,01). Both groups of diabetic children revealed decrease values of HRV indices during 24-hour and 2-hour in daily activity recordings as compared to healthy children. Only in children with vascular complications were observed a decrease on HRV parameters during the sleep stage. There were no differences in HRV indices between diabetic children without vascular complication and healthy group during sleep hours. In children with vascular complications we found a significant decrease in time- and frequency-domain indices during the sleep stage as compared to children without vascular complications. Only in children with diabetes type 1 and vascular complications the high level of HbA_{1C} strongly correlated with decrease values of two bands of spectral power: ultra low and very low frequency.
Conclusion: Diabetes impairs the autonomic nervous regulation depending on slow and fast changes in HRV. Lack of diabetic complications causes a decrease in HRV indices only during activity hours however during the sleep stage these indices have values similar to those observed in healthy children. Despite the fact that diabetes itself reduces HRV indices during daytime the enclosing chronic vascular complications are reflected in the impaired function of the autonomic nervous system that is responsible for slow and fast changes in HRV during sleep hours.

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Selectines in the pathogenesis and diagnosis of early atherosclerotic changes in children and adolescents with diabetes Type 1.
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Background and Aims: Selectines are the group of adhesion molecules, which main role is the tethering of leucocytes to the endothelium. They induce weak and transient adhesion allowing the cells to roll along the vascular wall. That mean, that selectines play part in the earliest stages of the atherosclerotic process. Children with diabetes type 1 are particularly predisposed to early progress of atherosclerosis. The aim of the study was to evaluate levels of E-selecin, L-selectin and P-selectin in children and adolescents with diabetes type 1 and the attempt to answer the question whether selectines, and which of them can be useful in predicting cardiovascular risk in these young patients.
Materials and Methods: We studied 28 children and adolescents with diabetes type 1 aged 15,3 ± 2,6 years, suffering from diabetes 8,3 yrs (4-15), with mean HBA1c – 8,7% (5,6 – 12,3%). In 10 patients we confirmed persistent microalbuminuria. Control group consisted of 15 healthy, slimm children and adolescents, aged 15,4 ± 2,2 yrs. Levels of E-selectin, P-selectin and L-selectin were evaluated by immunoenzymatic methods with use of R&D Systems ELISA kits.
Results: In the study group we found significantly higher level of E-selectin - 83± 25 ng/mL compared to control group: 64± 20 ng/mL (p<0.05). L-selectin level in the study group was 1519± 290 ng/mL and did not differ from control group - 1652± 281 ng/mL (ns). P-selectin level in the study group was 617± 419 ng/mL and was similar to the level of P-selectin in the control group - 669± 295 ng/mL (ns). Correlation analysis showed significant relationship between E-selectin and BMI (r=0,23, p<0,05) and diastolic blood pressure (r=0,24, p<0.05). We did not find any significant differencies in selectines levels taking into consideration metabolic control of the disease or late complications
Conclusion: 1. Young patients with diabetes type 1 have elevated level of E-selectin. 2. E-selectin level correlates with BMI and diastolic blood pressure. 3. Elevated level of E-selectin may confirm endothelial dysfunction in these young patients and can serve as a marker of early atherosclerosis phases.

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Vascular endothelial growth factor (VEGF) level in children and adolescent with diabetes Type 1.
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Background and Aims: Diabetes type 1 is the chronic disease, leading to many acute and late complications. Pathogenesis of the late complications is not fully understood. Lately, growth factors, and especially vascular endothelial growth factor (VEGF) are gaining great interest as potential risk factors of late diabetic complications, mainly nephropathy and retinopathy. The aim of the study was: 1. Evaluation of the level of VEGF in children and adolescents with diabetes type 1. 2. Correlation between level of this cytokine and microangiopathy and metabolic control.
Materials and Methods: 68 young diabetic type1 patients were studied, aged 8-20 yrs (x = 15,54 yrs), suffering from diabetes type 1 from 2,3- 16,5 yrs (x= 7,84 yrs), 34 boys, 34 girls. They were all under control of Outpatient Diabetic Department. All children were divided into two groups: I group- adolescents with diabetes type 1 and the onset of microangiopathic complications, II group – adolescents with diabetes without complications. Control group were healthy children, age and gender matched. Methods: Metabolic control was assessed on the basis of HbA1c level. All children underwent ophtalmology examination, microalbuminuria was evaluated in 24 hours urine samples. VEGF was estimated with use of immunoenzymatic method (RD Systems).
Results: In the study group we found: significantly higher levels of VEGF compared to control group (328,68 ± 251,6 vs 132,19 ± 85,51 pg/ml, p<0,05). We did not find any differences between gender in VEGF level. VEGF increases with diabetes diuration time, and the highest values were found in children with diabetes duration over 10 years, although the

differences did not reach statistical significance. VEGF level was significantly higher in children with poor metabolic control of the disease (406,65 ± 322,11 pg/ml vs 132,19 ± 85,51 pg/ml in patient with good metabolic control, p<0,05). In the group of patients with the onset of microangiopathic complications VEGF level was 449,21± 312 pg/ml and was significantly higher compared to group without complications – 262,93± 183, p<0.05. Patients with simple retinopathy had the highest level of VEGF – 643,44± 395 pg/ml, p<0.05, compared to children without complications.

Conclusion: Assessment of VEGF level can be useful method in prognosis of diabetes microangiopathic complications. As microalbuminuria or retinopathy occur relatively late in diabetic compliations process, evaluation of VEGF may become a prognostic marker of the earliest phases of this complications.

OP 10

Beta Cell Differentiation and Regeneration

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Requirement of glucokinase for compensatory β-cell hyperplasia in response to high-fat diet-induced insulin resistance.

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Background and Aims: Glucokinase plays a crucial role, as a glucose sensor, in the secretion of insulin from individual pancreatic β-cells. Heterozygous β-cell-type glucokinase knockout (*Gck*$^{+/-}$) mice showed impaired glucose tolerance due to decreased insulin secretion in response to glucose, on a normal diet. The prevalence of diabetes has increased markedly in both Western countries and Japan, and the increase can be explained by drastic changes in lifestyle, such as a high-fat (HF) diet. To establish an animal model representative of the current epidemic of human type 2 diabetes, we fed wild-type mice and *Gck*$^{+/-}$ mice a HF diet.
Materials and Methods: We investigated glucose tolerance and β-cell mass in wild-type mice and *Gck*$^{+/-}$ mice on the HF diet.
Results: Although *Gck*$^{+/-}$ and wild-type mice became similarly obese and insulin resistant on a HF diet, *Gck*$^{+/-}$ mice developed severe diabetes due to a lack of compensatory hyperinsulinemia, whereas wild-type mice showed only mild diabetes. Wild-type mice on the HF diet showed a 1.2-fold, 2.0-fold, and 10-fold increase in β-cell mass after 4, 20, and 40 weeks of loading as compared with wild-type mice on the high-carbohydrate diet, respectively. By contrast, *Gck*$^{+/-}$ mice on the HF diet showed only a 2-fold increase even after 40 weeks of loading. Individual β-cell size was not different between wild-type mice on the HF diet and *Gck*$^{+/-}$ mice on the HF diet. Thus, the increased β-cell mass in wild-type mice on the HF diet was due to an increase in number of cells (hyperplasia). On the HF diet, there were significantly more insulin plus BrdU double-positive cells in wild-type mice than in *Gck*$^{+/-}$ mice, and similar results were obtained by PCNA staining. There were no differences in apoptotic reactions among the four mouse groups. Thus, failure of compensatory β-cell hyperplasia in *Gck*$^{+/-}$ mice on the high-fat diet was associated with decreased β-cell replication. Tyrosine kinase pathways including IRS-2, PI 3-kinase, and Akt have reportedly been implicated in β-cell growth, but there were no differences in the expressions of IRS-1, IRS-2, the p85 regulatory subunit of PI 3-kinase, or Akt. Interestingly, however, Pdx1 expression was significantly lower in *Gck*$^{+/-}$ β-cells on the HF diet than in wild-type β-cells on the HF diet.
Conclusion: These results suggest a critical requirement for glucokinase not only for glucose-induced insulin secretion but β-cell hyperplasia in response to HF diet-induced insulin resistance. Our study supports the concept that glucose recognition and glucose metabolic pathways in the β-cell are critical for the increases in β-cell mass seen with insulin resistance.

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Beta-cell differentiation from pancreatic duct cells with heparin-binding epidermal growth factor-like growth factor gene-transduction by injection of adenovirus vector via retrograde trans-pancreatic duct.

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Background and Aims: Pancreatic duct cells or progenitor cells in duct cell lining are considered to be important cell source in beta-cell differentiation and regeneration in the adult pancreas. Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is abundantly expressed in endocrine pancreas cells and primitive duct-like cells, and its expression is regulated in part by PDX-1 which is known to be essential for the pancreatic development. HB-EGF may be involved in differentiation and regeneration in pancreatic endocrine cells.

Materials and Methods: We administered HB-EGF adenovirus vector to male ICR mice (normal mice and diabetic model mice with selective alloxan perfusion) by injection via retrograde trans-pancreatic duct. As a control, we administerd beta-galactosidase adenovirus vector. Whole pancreata were excised and examined by immunohistochemistry at 1, 2, and 8 weeks after the injection. Intraperitoneal glucose tolerance test (IPGTT) was performed at 2 and 8 weeks after the injection.
Results: By immunohistochemical analyses, in the experimental group, cells with double positive for duct cell specific cytokeratin and insulin in duct cell lining, or insulin-positive single cells associated with the ducts, or those forming islet-like cell clusters (ICCs) were detected, and the numbers of these cells were increased compared with those of the control mice. Glucose tolerance in IPGTT of the experimental mice (normal and diabetic) at 8 weeks after the injection was improved and the plasma insulin levels were also increased compared with those of the control mice.
Conclusion: These results indicate that HB-EGF gene-transduction to the adult pancreatic duct cells could promote the beta-cell differentiation and regeneration from the duct cells, and the increase of the beta-cell mass led to the ameliolation of the glucose tolerance in normal and diabetic ICR mice.

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Exenatide (Synthetic Exendin-4) modulates beta cell mass in insulin resistant obese *fa/fa* rats.
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Background and Aims: Exenatide (synthetic exendin-4) is reported to have a number of antidiabetic actions that include glucose-dependent stimulation of insulin secretion, slowing of gastric emptying and suppression of glucagon secretion. The present study evaluated the effect of exenatide on β–cell mass in insulin resistant, non-diabetic, obese *fa/fa* rats independent of the potential confounders of reduced food intake, body weight, and glycemia. For this purpose exenatide-treated (EX) rats were compared with pair-fed (PF) controls that were well matched in regard to these measures.
Materials and Methods: Beginning at 9 weeks of age, 2 groups of weight-matched rats (n=6/group), were fed *ad libitum* and injected s.c. twice daily for 6 weeks with 3 μg/kg exenatide (EX) or saline (CON). A third group (PF) was injected with saline but pair-fed *vs* EX. Insulin sensitivity was assessed in euglycemic hyperinsulinemic clamps performed at 6 weeks, and was expressed as glucose infusion rate/plasma insulin (ISI; insulin sensitivity index). β–cell mass was estimated from immunohistochemically identified areas in multiple representative sections (N=6-8/animal) of whole pancreas.
Results: After 6 weeks, weight gain was reduced in EX and PF rats vs CON (165±7g, 173±4g, vs 246±20g; P<0.01) as was HbA1c (3.0±0.1%, 2.9±0.1%, vs 3.6±0.2%; P<0.01). Total pancreatic mass was not different between treatment groups (EX: 1.93±0.08g, PF: 1.76±0.12g, CON: 1.97±0.04g). ISI was 160% higher in EX vs CON rats (P<0.002), and was 54% higher than in PF animals (P<0.05). Absolute β–cell mass was positively related to HbA1c and body weight, being higher in CON (181.4±24.4 mg/panc; P<0.05) than in EX and PF (115.2±8.1, 133.9± 2.7mg/panc respectively). Fasting plasma insulin levels at 6 weeks correlated with absolute β-cell mass in all groups (CON: 13.0±1.3ng/mL, EX: 5.3±0.4ng/mL, PF: 8.1±1.0ng/mL). In the absence of exenatide, β-cell mass was inversely correlated with ISI (r^2=0.725, P<0.002); animals which were most insulin-resistant had greatest β-cell mass. Adjusted for ISI, EX rats exhibited a 52± 6% increase in β–cell mass vs pair-fed controls (P<0.05), in which HbA1c and body weight was indistinguishable.
Conclusion: When viewed from the perspective of β–cell mass appropriate for a given level of insulin-resistance, *fa/fa* rats treated for 6 weeks with exenatide exhibited increased β-cell mass. These results support a direct trophic effect of exenatide to promote islet neogenesis that is independent of effects on body weight and glycemia in obese *fa/fa* rats.

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Compromised islet PDX-1 expression in glucose intolerance mice.
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Background and Aims: PDX-1, a gene transcription factor present in the duodenum and pancreatic β-cells, is involved in controlling pancreatic development and β-cell function. We have previously shown that short-term high-fat diet compromises β-cell translocation of PDX-1 from nuclear to cytoplasm. Here we have extended the previous findings showing that effect of long-term high-fat diet on the expression of PDX-1 and other islet genes known to be regulated by PDX-1in mice pancreatic islets. To explore the molecular basis of high-fat diet-induced defects of β-cell function, we also used INS-1 cell line to evaluate the effects of fatty acids and high glucose on the expression and regulation of PDX-1.
Materials and Methods: Female C56BL/6J mice were fed with high-fat diet for10-month. The control mice received a standard diet. Body weight, the blood levels of glucose, insulin, glucagon and fatty acids were measured and oral glucose tolerance test performed. Pancreatic islets were isolated in mice after 10-month high-fat diet. INS-1 cells were exposed to palmitate or high glucose for 48h. For western blot analysis, total cell extracts and subcellular fractions were prepared. For Northern blot analysis, total RNA was extracted and the cDNA fragments used as probes for PDX-1, insulin, GLUT2 and glucokinase.
Results: Mice after high-fat feeding for 10 month showed an increased fasting blood glucose (8.3±0.3 vs 4.3±0.16 mmol/l, p<0.05) and insulin levels (852±19 vs 287±49 pmol/l, p<0.05), and had impaired glucose tolerance and secreted less insulin during glucose tolerance testing. High-fat feeding markedly decreased PDX-1 protein and mRNA expression in mice isolated islets, respectively. The reduction of PDX-1 expression was accompanied by decreased expression of insulin, GLUT2 and glucokinase at mRNA levels. Incubation of INS-1 cells with palmitate in the presence of 5.5 and 22 mM glucose induced a dose-dependent decrease in PDX-1 protein expression in total cell lysate and in nuclear extracts. Palmitate also decreased PDX-1 mRNA expression. Treatment of INS-1 cells with 5.5, 11 and 22 mM glucose in the absence of palmitate for 48h induced a translocation of PDX-1 from the cytoplasm to nuclear compared with cells exposed to 3.3 mM glucose. PDX-1 protein expression was significantly reduced in the cells treated with 22 mM glucose. The decreased PDX-1 protein expression was consistent with the reduction of PDX-1 mRNA expression. However, there was no significant glucose effect on palmitate-induced reduction of PDX-1 expression**.**
Conclusion: These data indicate that both hyperlipidemia and hyperglycemia negatively regulate PDX-1 expression *in vivo* animal model and *in vitro* cell lines. This negative control of PDX-1 expression might be an initial event in early-onset islet dysfunction and glucose intolerance.

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Beta-cell regeneration by Reg protein: experiments with Reg knockout and transgenic mice.
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Background and Aims: Reg, Reg gene product (*J. Biol. Chem.* **263,** 2111-2114, 1988), is induced in beta-cells by inflammatory stimulation such as by interleukin-6/glucocorticoids (*PNAS* **98,** 48-53, 2001) and acts as an autocrine/paracrine growth factor for beta-cell regeneration via a cell surface Reg receptor (*J. Biol. Chem.* **275,** 10723-10726, 2000) to ameliorate experimental diabetes (*PNAS* **91,** 3589-3592, 1994; *Endocrinology* **139,** 2369-2374, 1998).
Materials and Methods: *Reg* knockout (KO) mice were generated by homologous recombination using ES cells. Transgenic (Tg) mice expressing *Reg* gene in beta-cells were produced by microinjection of the mouse *Reg* gene under the control of rat insulin II promoter (*Ins-Reg*) into male pronuclei of fertilized mouse eggs. *Ins-Reg* Tg NOD mice were produced by 9 times outcrossing the *Ins-Reg* Tg mice to NOD mice. Proliferation of beta-cells was measured by tritium-thymidine or bromodeoxyuridine (BrdU) incorporation of isolated islets. Induction of islet growth *in vivo* was initiated by intraperitoneal injections of goldthioglucose (GTG).
Results: The *Reg* gene disruption resulted in a null mutation but the KO mice otherwise developed normally. The islets of *Reg* KO mice appeared morphologically indistinguishable from those of the normal control. However, when hyperplastic islets were induced by the injection of GTG, the islet sizes of *Reg* KO mice were significantly smaller than those from the control wild mice. The BrdU incorporation in the isolated islets from *Reg* KO mice was significantly decreased, and the levels of cyclin D1 and phospho-Rb (Retinoblastoma protein) in *Reg* KO islets were greatly decreased compared to those of control mouse islets. The islets of *Ins-Reg* Tg mice appeared morphologically normal and were well stained for insulin. We isolated the islets from *Ins-Reg* Tg and cultured them *in vitro* for 48 h. *Ins-Reg* Tg islets secreted Reg whereas the control wild mouse islets did not. The tritium-thymidine incorporation of islets from *Ins-Reg* Tg

mice was significantly higher than that of non-Tg control mice. The NOD mice carrying the *Ins-Reg* transgene showed a significantly delayed development of diabetes. In 22~24-week-old female mice, the islet volumes in NOD mice carrying the *Ins-Reg* transgene were significantly increased in NOD mice without the transgene (1.98±0.42 μm^3 vs 0.52±0.21 μm^3), whereas the lymphocyte infiltration in islets remained unchanged.
Conclusion: Our results indicate that Reg plays an important role in beta-cell growth/regeneration. Furthermore, the significant delay in diabetes development in the NOD mice carrying the *Ins-Reg* transgene suggests the possible therapeutic use of *Reg* gene and/or Reg protein in diabetes treatment.

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Beta-cell STAT5 activity influences the susceptibility to high-fat diet-induced diabetes in mice.
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Background and Aims: Growth hormone (GH), prolactin (PRL) and placental lactogen are known to stimulate beta cell proliferation and insulin gene expression. Through binding to GH and PRL receptors in insulin-producing cells, these hormones activate "signal transducers and activators of transcription" (STAT) 5a and STAT5b. We have previously shown *in vitro* that STAT5 activation is essential for the GH and PRL-mediated stimulation of beta cell proliferation and insulin gene expression. The aim of the present study was to investigate the effect of altering STAT5 activity in beta cells on diet-induced diabetes in transgenic mice.
Materials and Methods: Two lines of transgenic mice expressing either a dominant negative mutant of STAT5 (STAT5DN) or a constitutively active mutant of STAT5 (STAT5CA) under the control of the rat insulin II promoter were generated. From 5 weeks of age groups of 10 mice were fed either normal chow containing 5% fat or high fat (HF) diet containing 60% fat. The change in body weight, oral glucose tolerance, serum glucose and insulin concentrations were followed for 20 weeks.
Results: The expression of the STAT5 mutants was confirmed *in vitro*. On normal diet the only observed difference in phenotype was a slightly lower weight gain in STAT5DN mice. In response to HF diet both non-transgenic and transgenic mice expressing STAT5DN or STAT5CA mutants became obese. After 16 weeks of feeding the STAT5DN mice showed 40% increase in body weight compared to similar transgenic mice on normal diet, while increases of 21% and 18% in body weight were observed for non-transgenic and STAT5CA transgenic mice, respectively. HF diet further resulted in elevated serum glucose and insulin concentrations and lowered glucose tolerance in both transgenic and non-transgenic mice. After 16 weeks of HF diet a more pronounced increase in serum insulin levels was observed in STAT5DN mice (median=998 pmol/l) than in non-transgenic (median=416 pmol/l; $p<0.1$) and STAT5CA mice (median=321 pmol/l; $p<0.05$). When subjected to oral glucose tolerance tests, HF diet-fed STAT5CA mice showed significantly better glucose tolerance than non-transgenic and STAT5DN mice ($p<0.05$).
Conclusion: These data indicate that impaired STAT5 activity in beta cells results in increased susceptibility to fat-induced diabetes whereas elevated STAT5 activity attenuates fat-induced diabetes in mice suggesting that STAT5 may be a potential drug target for the treatment of type 2 diabetes.

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Diabetes development in T-cadherin deficient mice.
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Background and Aims: T-cadherin is an atypical cadherin that attaches to the plasma-membrane via a GPI-anchor. It lacks the trans-membrane and the intra-cellular signaling domains found in classical cadherins. T-cadherin is normally expressed on the cell surface of a subset of cell types, particularly in the nervous system, where it is able to confer homophilic cell-cell interactions. The aim of this study was to investigate the role of T-cadherin in pancreatic islet function.
Materials and Methods: T-cadherin knock-out mice were generated by standard homologous recombination and breeding into the C57Bl/6 strain. Mice were monitored monthly for development of diabetes by i.p. glucose tolerance tests. Islets were isolated after collagenase digestion by hand-picking. Pancreas and islets were fixed in 2% formalin + 0.2% glutaraldehyde and embedded in Unicryl for immuno-electron microscopy, or fixed in 4% formalin, cryopreserved in 30% sucrose and snap frozen for confocal microscopy. A rabbit T-cadherin antibody for immuno-stainings was developed in house. *In vitro* insulin secretion was performed in RPMI + 1% BSA and 1.67, 22.4 or 22.4mM glucose with 10mM arginine or 30mM KCl for 30 min.
Results: Within the pancreas, T-cadherin is exclusively expressed in the islets of Langerhans and has an atypical cytoplasmic distribution. Homozygous T-cadherin-deficient mice develop non-autoimmune diabetes as they age (Table). Islet architecture is intact, and neither islet nor whole pancreas insulin content is affected. However, *in vitro* insulin secretion in response to glucose is decreased in T-cadherin-deficient islets: 22.4mM glucose stimulation index over basal glucose, SI, 3.5±1.2 (wt) vs. 1.2±0.2 (mutant), $P<0.05$; arginine-SI, 10.7±1.2 (wt) vs. 6.3±1.2 (mutant); $P<0.05$ and KCl-SI, 17.6 ±2.8 (wt) vs. 6.5±1.1 (mutant), $P<0.05$. Preliminary data suggest that T-cadherin is located on or close to the insulin granules, as revealed by confocal and immuno-electron microscopy.
Conclusion: Our work demonstrates that T-cadherin deficiency leads to diabetes in mice. T-cadherin seems to be a novel component of the insulin secretory machinery as *in vitro* insulin secretion is disturbed in knock-out mice islets. The unusual, strictly cytoplasmic distribution of T-cadherin in wild type islets suggests a novel function for a member of the cadherin family of proteins.
Table. Intra-peritoneal glucose tolerance test in 5 month old male normal (wt) and T-cadherin-mutant mice. Blood glucose expressed in mM±SEM. †$P<0.01$, ‡$P<0.001$.

Time (min)	0	15	30	60	90	120	180
wt	3.9±0.3	17.5±1.4	18.7±1.7	14.6±1.9	9.0±0.9	6.7±0.4	5.6±0.3
mutant	3.9±0.6	17.6±1.2	22.0±2	24.2±0.4†	18.6±1.0‡	11.7±0.8‡	6.2±0.

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Transcription factors of beta-cell differentiation and maturation in isolated human islets and the effect of Type 2 and Type 1 diabetes.
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Background and Aims: Several transcription factors (TrF) are involved in the differentiation and maturation of pancreatic beta-cells. No information is currently available as for the expression of these TrF in isolated, adult human islets (Isl), the possible changes in the presence of Type 2 (T2D) or Type 1 (T1D) diabetes, and the relation with Isl physiopathology.
Materials and Methods: Purified Isl were prepared from the pancreas of 3 control multiorgan donors (Ctrl, age: 38±18 yrs, M/F: 2/1, BMI: 25.1 ±0.9 kg/m^2), 3 donors with T2D, (age: 68±3 yrs, M/F: 1/2, BMI: 28.3±0.9 kg/m^2, duration of diabetes: 3.1±1.5 yrs), and 2 donors with T1D (age: 19±7 yrs; M/F: 1/1, BMI: 23.3±6.1 kg/m^2, duration of diabetes: 1.0±0.0 yrs). Isl mRNA expression of various TrF was then measured by semiquantitative RT-PCR after 3-day euglycemic culture, and the results expressed as the ratio over beta-actin.
Results: PDX-1 expression was 0.26±0.05 in Ctrl, and was significantly ($p<0.01$) higher in T2D (0.69±0.03) and T1D (0.49+0.01) Isl. The expression of Nkx6.1 was 0.62±0.08 in Ctrl, and resulted higher ($p<0.05$) in T2D (0.81+0.02) and lower ($p<0.01$) in T1D (0.20±0.01). Nkx2.2 and PAX-6 were similarly expressed in Ctrl (respectively 0.76±0.02 and 0.51±0.06) and T2D (respectively 0.71±0.02 and 0.60±0.01), but significantly less expressed in T1D (respectively 0.32±0.03 and 0.05±0.00, both $p<0.01$). The amount of apoptotic cells (expressed in arbitrary units of optical density, OD) was higher in T2D (1.7±0.3, $p<0.05$) and T1D (1.8±0.6) than in Ctrl (0.9±0.1), and insulin content (μU/islet) was 118±14 in Ctrl, 77±26 in T2D ($p<0.05$ vs Ctrl) and 26±8 in T1D ($p<0.05$ vs Ctrl and T2D).
Conclusion: In conclusion: 1) isolated, adult human pancreatic Isl express a number of TrF involved in beta-cell differentiation and maturation; 2) T2D have increased/normal and T1D have decreased (with the exception of PDX-1) expression of these TrF; 3) in the presence of similar apoptotic rate, the different expression of TrF may contribute to the different insulin mass m T2D and T1D Isl.

OP 11
Nephropathy

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Survival in the Type 1 diabetic patient. A prospective study results. 1990-2000.

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Background and Aim: The optimized multiple therapy improves survival in type 1 diabetic patients with and without overt nephropathy. The main objective of this study was to assess the effect of 2 different therapeutic programs on the survival of type 1 diabetic patients.
Material and Methods: An 11- year prospective study was carried out on 177 ambulatory type 1 diabetic patients (diagnosis age ≤ 30 years old) at the University Hospital. These patients were randomly distributed in 2 groups. Group A: 93 patients undergoing optimized multiple therapy: insulin multiple doses and intensive antihypertensive therapy, hypoproteic diet and hypolipid treatment with polychosanole, only for those who presented hypertension ≥ 130/85 mm Hg, persistent proteinuria ≥ 300 mg/24 hours and total cholesterol ≥ 5,2 mmol/l, respectively. In this group, 34, 4 % (n:32) showed an overt nephropathy with a creatinine mean at the beginning of the study of 197,0 ± 135, 5 µmol/l; Group B: 84 patients undergoing a standard treatment by the family physician. The 29,7 % (n:25) of the patients in this group showed an overt nephropathy with a serum creatinine mean at the beginning of the research of 192,2 ± 125,6 µmol/l. In both groups the mean age, diabetes duration, diagnosis age and the patients distribution by sex, hypertension and smokers in 1990, did not show statistical differences. It was estimated the survival curve (Kaplan Meier methods) and the joint effect of the prognosis factors of survival by the Cox regression model.
Results: The total glycosylated hemoglobin (Hb A1 %) mean (8,1 ± 0,3 vs. 9,9 ± 1,4 % Hb A1), blood pressure (124,3 ± 9,6 / 81,0 ± 8,3 vs. 145,9 ± 10,5 / 92,8 ± 9,5 mm Hg) and total cholesterol (4,9 ± 0,8 vs. 5,9 ± 1,8 mmol/l) in group A showed better results during the study with significant differences compared to group B. During the follow up, 60 patients died, 23 from the optimized multiple therapy and 37 from the standard conventional therapy group. The main causes of death were the renal and cardiovascular ones. The survival curves were significantly different (group A: 74,4 % vs. Group B: 55,9 %, p = 0,006). The Cox multiple regression model determined that the starting serum creatinine, the diabetic duration and quality of the glucemic control, though showing statistical significance, only the glucemic control measured by the HbA1 was the most clinically relevant prognosis variable related to the survival in the diabetic patients studied (RR: 3,2 IC- 95%. 1,7 - 5,9).
Conclusion: The optimized multiple treatment favoured an improvement in the survival of type 1 diabetic patients.

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Microalbuminuria prevalence study (MAPS) in hypertensive Type 2 diabetic patients in Asia.

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Background and Aims: Microalbuminuria (MA) represents the earliest clinical evidence of diabetic nephropathy (DN) and is a marker of increased cardiovascular (CV) morbidity and mortality. Early detection allows the implementation of an individualized and aggressive intervention program. Coexisting hypertension greatly exaggerates the risk. Several recently published studies -IDNT, IRMA 2, RENAAL, MARVAL - have shown that early detection and use of angiotensin receptor blockers (ARB's), together with other established strategies, can prevent or delay the progression of DN to end stage renal disease (ESRD).
Materials and Methods: *Objectives*: a) To assess the prevalence of macro and microalbuminuria in hypertensive type 2 diabetic patients b) To assess the level of blood pressure control in routine clinical practice and c) To record the associated CV risk factors, dyslipidemic status, diabetic control and complications.
Design: Cross-sectional study involving 103 centres (General Practices, General Hospital Outpatient Clinics, Primary Care Centres and Diabetic Clinics) in 10 Asian countries.
Measurements: A random morning urine specimen of consecutive eligible patients was screened first with the Nephur[2]test strip (Roche Diagnostics) to detect macroalbuminuria and hematuria. Negative urine was then tested for MA with the Micral-test (Roche Diagnostics). Supine blood pressures (BP), diabetes and CV complications, and dyslipidemic status were recorded. The prevalence of macroalbuminuria and MA was calculated with a two-sided 95% confidence interval. A multivariate analysis was performed with global predictive model assessed by stepwise logistic regression.
Results: 6802 patients were enrolled between April 2002 and December 2002. Interim results in 2589 patients (38% of the enrolled population) suggest a prevalence of 30.6 % macroalbuminuria (29.7 - 31.5 95% CI) and 33.3% microalbuminuria (32.4 - 34.3 95% CI). Predictive factors for the presence of MA include gender (male, odds ratio = 1.32; 1.07 - 1.64 95% CI), age (>74, odds ratio = 1.92; 1.21 - 3.03 95% CI) and ethnic origin (Malay, odds ratio = 1.45, 1.00 - 2.10 95% CI). The mean Systolic BP and Diastolic BP (mmHg) were 146.4/83.5 above the 130/85 WHO-ISH target. Final results in 6802 patients will be available in May 2003.
Conclusion: If the above results are borne out at study completion, the situation could be alarming. More vigorous effort will be necessary to detect and treat DN at the early stage to prevent ESRD due to hypertensive Type 2 Diabetes Mellitus in Asia.

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Progression from normoalbuminuria to incipient diabetic nephropathy in Type 1 and Type 2 diabetic patients.

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Background and Aims: The aim of this prospective observational study was to compare the rate of progression from normoalbuminuria to incipient diabetic nephropathy in patients with type 1 and type 2 diabetes.
Materials and Methods: We identified two cohorts of Caucasian patients with diabetes and normoalbuminuria (urinary albumin excretion rate (AER) <30 mg/24 hr) and investigated them yearly for the following 10 years. Risk factors for development of incipient nephropathy were evaluated. One cohort with type 1 diabetes (T1) (n=593, 12 lost to follow up, 44 excluded due to antihypertensive treatment at baseline) and another with type 2 diabetes (T2) (n=191, 15 lost to follow up). The remaining patients 537 T1 / 176 T2 were evaluated. Based on AER during follow up patients were stratified into 2 groups: persistent normoalbuminuria (AER <30 mg/24 hr) and incipient diabetic nephropathy (AER ≥30-299 mg/24 hr in 2 out of 3 consecutive samples).
Results: During follow-up 146 T1 / 69 T2 patients (cumulative incidence 33% (95%CI 27 to 37) / 46% (38 to 54) progressed to incipient diabetic nephropathy. The relative importance of each of three selected risk factors (baseline values above median in each cohort for urinary albumin excretion rate (AER >10/>8 mg/24 hr) and haemoglobin A_{1c} (>8.6/ > 7.6%), and presence of any retinopathy at baseline) was analyzed by Cox proportional hazards model, influenced multiplicatively by elevated baseline AER (RR 2.5 (95%CI 1.8 to 3.5) /3.0 (1.8 to 5.0)), retinopathy (RR 1.8 (1.2 to 2.6)/ 1.8 (1.1 to 3.0)) and elevated HbA_{1c} (RR 1.6 (1.2 to 2.3)/1.6 (0.99 to 2.6)). The analysis revealed the following cumulative incidence for the development of incipient diabetic nephropathy during ten years: no risk factors: 12%/23% vs all three risk factors 88/100%.
Conclusion: Type 2 diabetic patients have a higher risk of progression from normoalbuminuria to incipient diabetic nephropathy, but the presence of risk factors have the same impact on the risk in type 1 and type2 patients. The risk of progression to incipient diabetic nephropathy ranged from 12% / 23% (no risk factors present) to 88% / 100% (all three risk factors present) in T1/ T2 diabetic patients during the ten years of follow-up. Such risk estimates can be used to decide on intervention strategies in the individual patients, or when primary prevention studies are designed to identify high risk patients most likely to benefit from intervention.

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Role of immune complexes, oxidized LDL antibodies and total anti-oxidative reserve in diabetic nephropathy.

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Background and Aims: To examine the role of Circulating immune complexes (CIC), adhesion molecules, oxidized LDL, Malondialdehyde (MDA)-LDL and Advanced glycation end-product (AGE)-LDL antibodies, and anti-oxidative reserve in the prediction of overt nephropathy (ON) in patients with type 1 diabetes.
Materials and Methods: The study population is derived from the Pittsburgh Epidemiology of Diabetes Complication (EDC), a prospective follow-up study of 658 childhood –onset type 1 diabetic subjects diagnosed between 1950 and 1980 and first seen as part of the EDC Study in 1986-1988 when their mean age was 28 years and mean duration of diabetes 20 years. In the subsequent 10 years of follow-up, 56 of the 487 subjects without ON, defined as albuminuria >200 µg/min in at least two out of three timed urine samples, at baseline developed ON. For 48 cases, an age-(± 3 years), sex-, and duration-(±3 years) matched control subject was successfully identified giving a study sample of 96 (48 cases, 48 controls). Circulating immune complexes (CIC) as measured by their IgG, IgA, IgM and total cholesterol (TC) as well as Apo B content were assessed. Adhesion molecules and antibodies against modified lipoproteins (such as oxidized LDL, MDA-LDL and AGE-LDL) and oxidative reserve were assessed prior to development of ON.
Results: Univariate analysis showed in subjects who subsequently developed ON, IgG-IC (P=0.0003), IgA-IC (P=0.008), IgM-IC (P=0.015), TC-IC (P=0.02), and apoB-IC (P=0.018) were significantly higher compared to controls. There was no significant difference in the ICAM, VCAM, e-Selectin, oxidized LDL, AGE-LDL and MDA-LDL antibody levels between the cases and controls. Moreover, total anti-oxidant reserve (P=0.012) and retinol (P=0.003) were higher in those subjects who subsequently developed ON than controls. On the other hand, there was no difference in the alpha-tocopherol level, gamma-tocopherol level and the level of thiols between cases and controls. In a multivariate analysis using Cox proportion hazard model that included traditional ON risk factors, IgA-IC (P=0.03), retinol (P= 0.02), HbA1 (P=0.032) and Pulse (P=0.0039) were independent predictors of ON.
Conclusion: These findings on a nested case control analysis raise the possibility that immune complex formation, retinol concentration and antioxidative status may predict those at risk of ON in type 1 diabetes mellitus.

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Improved survival in patients obtaining remission of nephrotic range albuminuria in diabetic nephropathy.

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Background and Aims: To evaluate the impact of remission of nephrotic range albuminuria (NRA) on end stage renal disease (ESRD) and mortality in type 1 diabetic patients with diabetic nephropathy we performed a prospective cohort study.
Materials and Methods: All type 1 diabetic patients with NRA (n=126), who had yearly GFR (^{51}Cr-EDTA plasma clearance) measurement carried out for at least 3 years, were followed from onset of NRA until death or 2000. Nephrotic range albuminuria was defined as persistent albuminuria above 2.5 g/24h, and occurred in 91 men and 35 women, age (mean (SD)) 34 (8) years, duration of diabetes 22 (8) years, follow up time from onset of NRA (median (range)) 8.7 (3.0-20.9) years. All patients but one received antihypertensive treatment. Remission of NRA was defined as sustained albuminuria <0.6 g/24h for at least one year. Remission was induced in 28 patients (22%), 21 predominantly treated with ACE inhibitors, 7 with non-ACE inhibitors. The remission lasted 3.6 (1.0-18.1) years.
Results: At the end of follow up, the composite endpoint of ESRD or death was reached in 21 % in the remission group (2 ESRD and 4 dead) and 59 % in the no remission group (24 ESRD and 34 dead). A Cox proportional hazard regression analysis with sex and age at onset of NRA as fixed covariates and remission as time dependent covariate was performed: obtaining remission was associated with a lower risk of ESRD or death, relative risk (95 % CI) 0.35 (0.16 to 0.80), p<0.012, whereas older age at onset of NRA (per 10 years increase) was associated with higher risk of reaching the endpoint, 1.61 (1.20 to 2.16), p<0.001. As previously shown, the rate of decline in GFR during the whole follow up period was diminished in the remission group as compared to the no remission group (mean (SEM) 3.8 (0.6) vs. 7.5 (0.5) ml/min/year, p<0.001).
Conclusions: Our prospective study suggests that remission of nephrotic range albuminuria in type 1 diabetic patients, induced by aggressive antihypertensive treatment with and without ACE inhibitors, is associated with a slower progression in diabetic nephropathy and a substantially improved survival.

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The Z+2 aldose reductase polymorphism is associated with reduced susceptibility to diabetic nephropathy in Caucasian Type 1 diabetic patients.

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Background and Aims: The Z-2 allele of a $(AC)_n$ dinucleotide repeat in the aldose reductase gene (ALR2) confers increased risk of microvascular diabetic complications, whereas the Z+2 allele has been proposed to confer protection. However, data are conflicting. Therefore, we investigated whether this polymorphism is associated with diabetic nephropathy and retinopathy in Type I diabetes mellitus in a large case-control study and a family-based analysis.
Materials and Methods: 431 Type I diabetic patients with diabetic nephropathy and 468 patients with longstanding Type I diabetes and persistent normoalbuminuria were genotyped for the case-control study. The findings from the case-control study were then further examined in an independent family-based study by standard TDT analysis comprising 102 case-trios and 98 control-trios. The size of the $(AC)_n$ dinucleotide repeat at the 5' end of ALR2 was determined by polymerase chain reaction (PCR) amplification followed by electrophoresis (megaBACE 1000 automated sequencer).
Results: Thirteen different alleles were identified. In the case-control study, the Z+2 allele frequency was significantly higher in the normoalbuminuric diabetic patients than in patients with diabetic nephropathy (0.17 vs. 0.11, p=0.007), suggesting a protective function of the Z+2 allele. No significant increase in the frequency of the putative risk allele Z-2 was found in patients with diabetic nephropathy vs. controls (0.39 vs. 0.36). No association with diabetic retinopathy was found. Although the results of the transmission of the Z-2 and Z+2 alleles in the family based study were consistent with the association study, differences were not statistically significant.
Conclusion: The Z+2 allele of the ALR2 promoter polymorphism is associated with a reduced susceptibility to diabetic nephropathy in Danish Type I diabetic patients, suggesting a role for the polyol pathway in the pathogenesis of diabetic kidney disease. No association of the ALR2 polymorphism with diabetic retinopathy was found.

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Protein kinase C beta-1 gene is a novel susceptibility locus for diabetic nephropathy in Type 1 diabetes mellitus.

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Background and Aims: Abnormal activation of protein kinase C-beta isoforms in the diabetic state has been strongly implicated in diabetic nephropathy. We thus hypothesized that the protein kinase C beta-1 gene (*PRKCB1*), which encodes for both beta-I and beta-II isoforms, may contribute to genetic susceptibility for this microvascular complication.

Materials and Methods: Common DNA polymorphisms in *PRKCB1* were identified and tested for association with diabetic nephropathy. A large case-control study design was employed in which cases were type 1 diabetic patients with advanced diabetic nephropathy (presence of persistent proteinuria or end-stage renal disease (ESRD)) and controls were patients who have remained normoalbuminuric despite >15 years of type 1 diabetes. The family-based transmission disequilibrium test (TDT), which is robust to population stratification, was used to confirm case-control study results.
Results: Nine single nucleotide polymorphisms (SNPs) in *PRKCB1* were identified and tested for association with diabetic nephropathy. Overall allele and genotype distribution of two SNPs in the *PRKCB1* promoter (at positions –1504 and –546) were significantly different between the cases (n = 231) and controls (n = 220) ($P < 0.05$). These associations were strengthened when statistical analyses were focused on individuals with a relatively short period of diabetes (< 24 years) ($P = 0.002$). Specifically, carriers of the 'T' risk allele of –1504 SNP had a significantly increased risk of diabetic nephropathy compared to non-carriers (OR = 2.54, 95% CI = 1.39 to 4.62). Similar results were obtained for –546 SNP with carriers of the risk 'G' allele being at a greater risk of diabetic nephropathy compared to non-carriers (OR = 2.45, 95% CI = 1.37 to 4.38). The estimated frequency of the 'T-G' risk haplotype was also higher in cases than controls. To rule out unrecognized population stratification which may give rise to false positive associations, we performed the family-based TDT. Consistent with the case-control findings, the 'T-G' risk haplotype was preferentially transmitted from heterozygous parents to their offspring with diabetic nephropathy and diabetes duration < 24 years ($P < 0.05$).
Conclusion: Our study provides evidence implicating *PRKCB1* as a novel susceptibility gene for diabetic nephropathy in Type 1 diabetes mellitus.

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Statins versus untreated dyslipidemia on serum creatinine in patients with coronary heart disease and diabetes mellitus with normal renal function.
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Background and Aims: Little is known about the contribution of dyslipidemia in the decline of renal function, and less about the potential of statin treatment to prevent this adverse outcome. We prospectively evaluated the effect of structured care (SC) of dyslipidemia with atorvastatin (strict implementation of guidelines) versus usual care (UC) (physician's standard of care) on serum creatinine (SCr) levels of patients with Coronary Heart Disease (CHD) and Diabetes Mellitus (DM) with normal baseline renal function.
Materials and Methods: From 1,600 consecutive CHD patients randomized to either form of care in the GREek Atorvastatin and Chd Evaluation Study (GREACE), 313 had DM; 161 in the SC arm and 152 in the UC arm. All patients were followed-up for a mean 3-year period. All patients had normal renal function at baseline; serum creatinine (SCr) <115 micromol/l. On-study SCr values (up to 48 months), adjusted for body mass index, gender, and age were compared with those of baseline, using analysis of variance to assess differences over time within and between treatment groups. Both intention-to-treat and compliance-based analyses are reported.
Results: Patients from both groups not treated with statins (n=135) presented a mean increase in SCr levels by 5% (from 98±5 to 103±7 micromol/l, p=0.01), and 16 patients (12%) presented SCr levels > 115 micromol/l. Patients on atorvastatin (n=158, mean dose 23.7 mg/day) presented an 11% reduction in SCr levels (from 97±5 to 86±3 micromol/l, p<0.0001). This effect was more prominent with higher atorvastatin doses (-14%, p<0.0001). Patients from the UC group treated with various statins (n=20) had a mean 4% reduction in SCr (from 95±5 to 91±4 micromol/l, p=0.02). No patient on a statin had a SCr value > 115 micromol/l. After adjustment for all major CHD risk factors, including lipids, SCr reduction correlated with relative risk reduction in all CHD-related events in statin treated patients of both groups (r=0.17, p=0.01) and in structured care patients on atorvastatin (r=0.20, p=0.002).
Conclusion: Dyslipidemia contributes to the decline of renal function over a mean period of 3 years in dyslipidemic patients with CHD and DM, with normal renal function at baseline, not treated with a statin. Moreover, statin treatment significantly improves renal function in these patients. This effect of statins is clinically relevant.

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Hypoglycaemia

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Functional evidence for a central glucoreceptor: glucose and tolbutamide-induced modulation of $[Ca^{2+}]_i$ in hypothalamic neurones.
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Background and Aims: A sub-population of neurones in the ventromedial hypothalamus (VMH) act as a glucose sensor to control counter-regulatory responses by altering neuronal firing rate in response to elevated concentrations of glucose. The mechanisms used by these neurones to sense changes in extracellular glucose are thought to be similar to those used by pancreatic b-cells. We have now analysed the expression of b-cell signal recognition elements in the mouse hypothalamus, and have measured tolbutamide and glucose-evoked changes in intracellular calcium ($[Ca^{2+}]_i$) in hypothalamic cells.
Materials and Methods: Expression of mRNA species encoding b-cell signal recognition elements was detected by RT-PCR amplification of extracts of mouse VMH, mouse primary islets and the MIN6 insulin-secreting cell line. Single cell microfluorimetry was used to determine changes in $[Ca^{2+}]_i$ in fura-2 loaded MIN6 cells and in cells dispersed from the VMH by collagenase digestion.
Results: RT-PCR indicated the expression in the mouse VMH of a number of mRNA species associated with glucose-sensing in b-cells, including the GLUT2 glucose transporter, the pancreatic form of glucokinase, the SUR1 sulphonylurea receptor and the Kir6.2 inwardly-rectifying K^+ channel. In pancreatic b-cells expression of these elements confers glucose-sensitivity by coupling glucose metabolism to depolarisation and Ca^{2+} entry through voltage operated Ca^{2+} channels. As expected, the mRNA for preproinsulin was abundant in islets and MIN6 cells but absent from VMH extracts. Single cell measurements of $[Ca^{2+}]_i$ in dispersed VMH cells suggested that about half of the cells were electrically excitable neurones. Thus, depolarising concentrations of KCl (20mM) produced rapid and sustained increases in $[Ca^{2+}]_i$ in 47 out of 81 cells (58%) in 12 experiments from 4 separate preparations. Tolbutamide (100mM) caused marked and maintained elevations in $[Ca^{2+}]_i$ in a sub-population of these excitable VMH cells (5/81 cells; 6%), suggesting the expression of functional K_{ATP} channels formed from Kir6.2 and SUR1 in these neurones. The tolbutamide-induced changes in $[Ca^{2+}]_i$ in VMH cells were of a similar magnitude and pattern to those observed in MIN6 b-cells. Exposure of the tolbutamide-sensitive VMH cells to elevated concentrations of glucose (20mM) evoked maintained and oscillatory increases in $[Ca^{2+}]_i$, similar to those observed in MIN6 cells and in primary b-cells.
Conclusion: These results suggest that a sub-population of neurones in the VMH recognise and respond to changes in extracellular glucose by coupling glucose metabolism to depolarisation-induced changes in $[Ca^{2+}]_i$ using similar mechanisms to those employed by pancreatic b-cells. The identification of these mechanisms raises the possibility of pharmacological manipulation of the central glucoreceptors.

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Severe hypoglycemia in Type 1 diabetic children and adolescents – assessment of frequency and quest for predisposing factors.
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Background and Aims: Severe hypoglycemia (SH) is a serious complication of type 1 diabetes (T1DM) treatment. Incidence and factors predisposing to SH are not unequivocally established since they can change as a result of application of new treatment methods and differ between diabetes centers. Aims of this study were: 1) to evaluate frequency of SH in children with T1DM; 2) to identify factors associated with SH occurrence.
Methods: 243 type 1 diabetic patients (132 males) aged 2-19 years (mean age 12.8±4.0, mean T1DM duration 4.5±3.7 years) constituted the study population. Patients were treated with human insulin and/or insulin analogue lispro. SH was defined as an episode of unconsciousness or deep conscious disturbances with or without seizures, requiring treatment with glucose or parenteral glucagon. Patients and their caregivers completed a questionnaire concerning the number of SH events during the last 3 years and describing the socio-economic status of the family (retrospective study). Data concerning age, sex, T1DM duration, insulin regimen, daily

insulin doses and HbA_{1c} levels from the last 3 years were obtained from medical documentation. Patients were asked to attend the outpatient clinic within 14 days after any SH that would occur during 18 months following the questionnaire completion; blood samples were collected to determine insulin antibodies (IA) and fasting C-peptide during those visits (prospective study). HbA_{1c} was determined by HPLC (nondiabetic range 4.3-5.7%), C-peptide was measured radioimmunologicaly, IA were determined semiquantitatively by radioimmunoprecipitation (reference range 0-7%).

Results: SH frequency was 14 episodes/100 patients/ year (562.8 patient-years, 80 episodes in 48 patients). Patients, who experienced SH in the last 3 years had longer diabetes duration (median 4.8 years, quartiles 3.4-8.6) than those, who did not have any SH during that period (median 3.2 years, quartiles 1.3-6.3, $p<0.001$). They did not differ in respect to age (median 13.2 vs 13.8 years, NS), sex (males 47.9%% vs 55.9%, NS), insulin regimen (multiple injection/conventional: 43.8%/56.2% vs 54.4% /45.6%, NS), insulin dose (median 0.80 vs 0.76 U/kg/day, NS) and HbA_{1c} (median 8.0 vs 8.1%, NS). Socio-economic status was similar. IA levels determined in 33 patients within 14 days after SH (median 30.6%, quartiles 16.8-39.1) and in 30 of these patients (whose earlier collected blood samples were attainable) also before SH (median 35.9, quartiles 21.6-46.8%) were significantly higher than in patients (N=30) matched for age, sex and diabetes duration, who never in their life experienced any SH episode (median 19.5%, quartiles 13.7-30.1, $p<0.04$ and $p<0.02$ respectively). C-peptide levels were similar in both groups.

Conclusion: 1) Incidence of SH in young type 1 diabetic patients is high, so preventive measures and efforts to minimize the related risk should be continuously undertaken, 2) SH are more frequent in patients with longer diabetes duration, 3) High insulin antibodies may be a marker of susceptibility to SH through a mechanism other than simple imbalance between insulin dose, carbohydrates consumption and physical exercise.

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The impact of ACE activity on cognitive function, symptoms of hypoglycaemia and hormonal counterregulation during hypoglycaemia in normal subjects.

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Background and Aims: We have previously shown a strong relationship between high serum ACE activity and occurrence of severe hypoglycaemia (SH) in type 1 diabetes. The aim of this study was to explore counterregulatory responses and cognitive function in normal subjects with low or high ACE activity during hypoglycaemia.

Materials and Methods: In a balanced single-blinded placebo-controlled cross-over protocol, 8 healthy volunteers with low (mean 20 mU/l) and 8 with high (mean 52 mU/l) ACE activity were subjected to a hypoglycaemic challenge by subcutaneously injected unmodified human insulin. Catecholamines, glucagon, growth hormone, cortisol and symptomatic responses were measured and cognitive function was assessed by the California Computerised Assessment Package (CalCAP) that includes 4 different reaction time tests of different complexity.

Results: The resulting hypoglycaemic stimulus was similar in the two groups (nadir plasma glucose 2.7 mmol/l). There were no significant differences between the groups in counterregulatory responses except for growth hormone that was higher in the group with high ACE activity after normalisation of plasma glucose ($p=0.044$). The high ACE group reported higher total and autonomic symptom scores after normalisation of glucose level ($p=0.049$ and 0.050, respectively). In contrast to the group with low ACE activity those with high ACE activity deteriorated in cognitive performance both in terms of speed and precision. The high ACE group made more errors during maximum hypoglycaemia ($p=0.014$) and had poorer reaction time in the two most complex CalCAP tasks (121 vs. 2 msec. and 114 vs. 16 msec., $p=0.007$ and $p=0.045$, respectively) in the recovery phase compared to the low ACE group.

Conclusion: Normal subjects with high ACE activity more readily develop cognitive dysfunction during moderate hypoglycaemia and recover more slowly than subjects with low ACE activity. This seems not to be explained by differences in counterregulatory responses.

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Responses of counterregulatory hormones and substrates to insulin-induced hypoglycemia in the postprandial as compared to the fasting state in humans.

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Background and Aims: Hormonal responses to insulin-induced hypoglycemia (H) have generally been studied in the fasting (F) state and supine position. No study has, so far, examined responses of counterregulatory hormones to hypoglycemia induced by insulin after ingestion of a mixed meal. Clearly, understanding such responses might be relevant to the prevention of the clinical phenomenon of postprandial (PP) hypoglycemia in T1DM. Aim of these studies was to establish the differences, if any, in physiological responses of counterregulatory hormones and substrates to H in the F as compared to PP in non-diabetic subjects (C) and in patients with T1DM.

Materials and Methods: 11 subjects with T1DM (age 29±2.4 [mean±se] years, diabetes duration 15±2.7, HbA_{1c} 7.2±0.3%) and 10 C were studied on 2 random occasions, in the (F) and (PP) state (mixed meal 450 Kcal, 46% CHO, 22% protein, 32% lipids). Clamped hypoglycemia (44 mg/dl) was induced on both occasions by i.v. infusion of insulin at the rate of 2 mU/Kg/min for 205 minutes and variable infusion of glucose. All subjects were studied in the sitting position.

Results: Data are mean±SE (AUC).

	C			T1DM		
	F-HYPO	PP-HYPO	p	F-HYPO	PP-HYPO	p
GLUCAGON ($ng \cdot L^{-1} \cdot min^{-1}$)	163±25	248±29	0.018	100±9	193±25	0.011
ADRENALINE ($nmol \cdot L^{-1} \cdot min^{-1}$)	3.1±0.4	4.5±0.6	0.037	1.7±0.5	2.9±0.5	0.043
NORADRENALINE ($nmol \cdot L^{-1} \cdot min^{-1}$)	3.2±0.2	3.8±0.3	0.045	2.1±0.3	3.1±0.3	0.005
CORTISOL ($\mu g \cdot dL^{-1} \cdot min^{-1}$)	16.8±2.4	17±2.2	0.721	15.2±2.3	15.7±2.3	0.117
GROWTH HORMONE ($\mu g \cdot L^{-1} \cdot min^{-1}$)	17.8±3.2	11.3±3.7	0.04	26.4±4.2	22±3.5	0.138
PANCREATIC POLYPEPTIDE ($pmol \cdot L^{-1} \cdot min^{-1}$)	159±12	217±22	0.008	136±7	200±15	0.001

Conclusions: The present results demonstrate, for the first time, that responses of counterregulatory hormones to insulin-induced H in the F differ from those in the PP state. Responses of glucagon, adrenaline, noradrenaline, GH and pancreatic polypetide are greater in the PP as compared to the F state both in C and T1DM subjects. The factor(s) involved in the PP modulation of glucagon and other counterregulatory release during hypoglycemia are not known. The aminoacids absorbed with the meal might stimulate glucagon release. In addition, other mechanisms, which are not yet identified, may contribute to stimulate the release of glucagon and other counterregulatory hormones in postprandial hypoglycemia in T1 DM subjects.

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Hypoglycaemia and counterregulatory hormone response after pancreas transplantation: a prospective analysis.

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Background and Aims: Severe and frequent hypoglycaemia and impaired counterregulation are major problems in patients with long standing diabetes. Important aims of pancreas transplantation are not only elimination of hyper- but also hypoglycaemia.

Materials and Methods: In this study 31 type 1 diabetic patients (age 40 ± 1.4 years, diabetes duration 26 ± 1.2 years) after successful pancreas/kidney transplantation (SPK) were examined . We performed an insulin hypoglycaemia test (IHT; i.v. 0.075 IE/kg body weight). Test A was performed 3 ± 0.3 months after SPK , test B was performed 35 ± 6 months after SPK. Glucose and counterregulatory hormones were analysed before and after i.v. insulin (0, 10, 20, 30, 45, 60, 70, 90 and 120 min). Eight healthy volunteers served as a control group . Frequency of hypoglycaemia before and after SPK was assessed by a questionnaire.

Results: All patients showed normal fasting glucose and HbA1c levels at test A and test B(mean fasting glucose: 78 mg/dl vs. 80 mg/dl; mean HbA1c: 4.4% vs 4.7%).The mean nadir of blood glucose during IHT was 38 mg/dl at test A and at test B. Blood glucose nadir in the control group was somewhat lower (33 mg/dl, $p<0.05$). There were no differences in basal

glucagon, epinephrine, norepinephrine, cortisol and growth hormone levels when comparing patients (test A and test B), and healthy controls. A slight stimulation of all stress hormones during IHT was found in test A as well as test B. Compared to the control group the stimulated response of all hormones was significantly reduced in graft recipients in test A as well as test B. Differences between test A and test B were not found. Mild hypoglycaemia was documented prior vs. post SPK: 69 ±7 /year vs.5 ± 1 /year (p<0.001). Severe hypoglycamia (defined as necessity for assistance) was prior vs. post SPK: 4 ± 1 vs. 0 (p<0.001).
Conclusion: Counterregulatory response is impaired despite successful pancreas transplantation. Even three years after normalization of glucose metabolism no improvement in the response of counterregulatory hormones due to hypoglycaemia was observed. However, the incidence of hypoglycaemia is drastically reduced after SPK. Probably endogenous suppression of insulin secretion is sufficient to avoid severe hypoglycaemia.

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Counterregulatory hormone responses to hypoglycaemia in subjects with Type 2 diabetes mellitus (T2DM): a comparison of treatment with sulphonylurea, metformin and insulin.
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Background and Aims: Glucose counterregulatory (CR) failure and hypoglycaemic unawareness frequently complicate treatment of patients with Type 1 DM. Severe hypoglycaemia (HYPO) and CR abnormalities also occur in T2DM. However, little data exists on the effect of specific treatment modalities on CR hormone and symptom response to HYPO in T2DM subjects. In our study, we compared hypoglycaemic CR hormone and symptom responses in T2DM subjects treated with metformin (MET), sulphonylurea (SA), or insulin (INS).
Materials and Methods: We performed hyperinsulinaemic hypoglycaemic clamp studies on 24 subjects with T2DM, 8 on MET (Age 52.8 ± 10.5 years, M:F 6:2, HbA1c* 7.1 ±. 0.7 %, BMI 30.4 ± 3.5 kg/m^2, DM duration 3.5 ± 3.8 years), 7 on SA (Age 56.4 ± 8.9 years, M:F 4:3, HbA1c 7.4 ± 1.2 %, BMI 26.3 ± 3.7 kg/m^2, DM duration 6.5 ± 3.2 years), and 9 on INS therapy (Age 54.1 ± 9.4 years, M:F 4:5, HbA1c 8.4 ± 1.2 %, BMI 29.6 ± 4.9, and DM duration 5.2 ± 2.6 years).[*p<0.05 for HbA1c, MET vs. INS]. A primed continuous insulin infusion (2 – 4 mU/kg/min) was administered for 180 minutes. Glucose levels were lowered to 5.0, 4.4, 3.8, 3.3, 2.8 and 2.4 mmol/l at 30 minutes intervals and CR hormones measured and symptom survey administered at each glucose plateau.
Results: Plasma glucose did not differ except at nadir of 2.8 ± 0.4, 2.5 ± 0.2 and 2.4 ± 0.4 mmol/l in MET, SA and INS treated groups respectively (p=0.04, MET vs. INS). Epinephrine (EPI), norepinephrine (NEPI) and cortisol (CORT) responses to HYPO did not differ between groups. Glucagon (GGN) response to HYPO in INS treated group was significantly diminished compared to those on MET (191 ± 40.8 ng/l vs. 296 ± 121ng/l, p< 0.05) at plasma glucose of 2.8 mmol/l but not SA (221 ± 92 ng/l vs. 296 ± 121 ng/l, p=0.21). GH response to HYPO in MET was significantly lower than SA treated subjects (8.3 ± 7.3 mU/l vs. 19.6 ± 8.8 mU/l, p<0.05) at 2.8mmol/l. The glucose threshold for release of GGN (>mean basal + 3 SD) was 3.54 ± 0.34, 2.86 ± 0.41* and 3.74 ± 0.99 mmol/l in MET, SA and INS treated groups (*p=0.008 SA vs. MET). Glucose threshold for CORT, GH, EPI, NEPI rise did not differ between treatment groups. Glucose threshold for autonomic symptoms was higher in SA compared to MET treated groups 2.88 ± (0.38) vs. 3.34 ± (0.31) mmol/l respectively (p=0.042). Magnitude of autonomic symptoms was lower in SA treated group compared to INS treated group at 3.3, 2.8 and 2.4 mmol/l.
Conclusion: From this study, c-peptide positive T2DM patients treated with SA may be at a greater risk of severe hypoglycaemia due to higher glucose thresholds for glucagon response and onset of autonomic symptoms compared to INS or MET treated patients.

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Hypoglycemia in insulin treated stable Type 2 DM: Diabetes Outcomes in Veteran's Study (DOVES).
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Background and Aims: Hypoglycemia is the major treatment complication of patients with diabetes. The risk of hypoglycemia is increased in intensively treated patients and is the major limiting factor for intensive glucose control. This risk is widely recognized in Type 1, but Type 2 patients are thought to be at lower risk. Studies on hypoglycemia in the Type 2 patients are rare.
Materials and Methods: DOVES was a prospective, observational study of subjects in three Medical Centers. All subjects had stable insulin treated Type 2 DM. All diabetes care was provided by primary care physicians. A total of 344 patients enrolled in the study. The mean age was 65.5 ± 9.7 years. Diabetes duration was 14.7 ± 9.9 years and duration of insulin treatment 8.0 ± 7.7 years. The HbA1c level was 8.0 ± 1.7%. Glucose monitoring was with Accu-Check Complete and values down-loaded at 26, 39, and 52 weeks. Patients maintained logs of hypoglycemic events including symptoms (scale 0-2), possible cause, and BG values. Blood glucose 60 mg/dl or less was defined as hypoglycemia. All subjects completed instruments measuring diabetes knowledge, cognitive function, depression, attitudes, and family support. Diabetes complications, disability, treatment, diet and exercise were also assessed.
Results: A total of 1662 episodes of hypoglycemia were detected during an average follow-up of 41.2 ± 8.6 weeks. 51.2% of subjects had at least one episode. In those with hypoglycemia the median number of episodes was 4. The rate was 0.47 ± 1.04 episodes/4 weeks for the total group and 0.91 ± 1.31/ 4 weeks in those with hypoglycemia. The mean BG for the episodes was 49.7 ± 7.5 mg/dl. Peak times for hypoglycemia were 7AM, 12 noon and 6 PM. Asymptomatic episodes comprised 19.8% of the events, including 8.0% less than 40 mg/dl. Episodes requiring assistance were rare (3.4%). Glucose levels correlated with symptoms (51.6 ± 6.1, 49.6 ± 7.1, and 37.5 ± 10.0 mg/dl for 0,1, and 2 symptom levels, respectively). Over 50% of the episodes could not be related to a cause. Otherwise, missing meals, exercise, dieting, weight loss, medication errors, and treatment changes were mentioned. Patients with hypoglycemia had longer disease duration, higher diabetes knowledge scores, lower BMI and lower HbA1c. Multivariate analysis showed diabetes knowledge, number of oral agents, and number of insulin preparations independently predicted hypoglycemia (higher for knowledge and insulin and lower for oral agents).
Conclusion: Hypoglycemic events are more common in insulin-treated Type 2 DM than usually appreciated. Insulin treatment and diabetes knowledge were associated with increased hypoglycemia and concurrent oral agent use with decreased occurrence.

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Evaluated education and treatment centres for Type 1 diabetes reduce the frequency of severe hypoglycemic events by 50% while improving HbA1c in patients with intensive insulin therapy – 11 year data of the working group for structured diabetes therapy in Germany (ASD).
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Background and Aims: It seemed to be inevitable that the improvement of blood glucose control by means of intensified insulin therapy (twice daily NPH-insulin and mealtime insulin) leads to an increase of severe hypoglycemic events in type 1 diabetes (DCCT 1994). Since 1992 the ASD is involved in the continuous evaluation of the outcome quality of structured diabetes care of its voluntary participating diabetes centers in Germany. Each center presented data (at intervention and at follow-up 12 to 15 months later) of 50 randomly selected patients (of all type 1 diabetes patients who underwent the programme in this center) on an annual public ASD-meeting. The accumulated data of these evaluations were analyzed regarding HbA1c and frequency of severe hypoglycemic events of patients (injection of glucagon s.c. or glucose i.v.) before and after participating in a structured education and treatment programme in an ASD-certified diabetes centre.
Materials and Methods: All 7635 ASD data sets (collected between 1992 and 2002) were included in the analysis: HbA1c and number of severe hypoglycemic events at intervention and at follow-up were calculated. HbA1c was adjusted by calculating relative HbA1c (relative Hba1c = absolute HbA1c / mean normal of healthy subjects ((2 SD upper + lower limit) / 2)).

Results: Mean relative HbA1c at intervention 1,62 vs. 1,44 at follow-up. Number of severe hypoglycaemic events at intervention 0,39 vs. 0,15 events per patient years at follow-up.

rel.HbA1c **	1,0-1,2	1,3-1,5	1,6-1,8	1,9-2,1	2,2-2,4	2,5-2,6
pat (n)	1334	3543	1956	583	169	50
hypo (n) *	571	1561	644	125	47	10
hypo (n) **	236	595	232	67	25	5
hypo (e/p) *	0,42	0,44	0,33	0,21	0,28	0,20
hypo (e/p) **	0,18	0,17	0,12	0,11	0,15	0,10

Relative HbA1c at follow-up **, number (n) of patients (pat), number of severe hypoglycemic events at intervention * and at follow-up **, events per patient year (e/p) at intervention * and at follow-up **

Conclusion: Reducing HbA1c by means of intensive insulin therapy in type 1 diabetes was considered to lead to an increase in severe hypoglycemic events. In contrast, the data show that participation in a structured education and treatment programme for type 1 diabetes in an ASD-certified diabetes centre reduces both HbA1c level and the number of severe hypoglycemic events.

OP 13

Type 2 Genetics - Prediction and Prevention

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Multifactor-dimensionality reduction method uncovers gene to gene interactions among 23 loci in the candidate genes of Type 2 diabetes mellitus.

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Background and Aims: Type 2 diabetes mellitus (T2DM) is a complex genetic disease and the discovery of its genetic cause is one of the greatest challenges ever given to human geneticists. Recently, the multifactor-dimensionality reduction (MDR) method has been developed for detecting and characterizing high-order gene-to-gene interactions in case-control studies with relatively small samples. In this study, we examined the gene-gene interactions among 23 different loci in the 15 candidate genes of T2DM using MDR method.
Materials and Methods: We studied 504 unrelated patients with T2DM and 133 non-diabetic control subjects. We selected 23 polymorphisms among 15 candidate genes that are potentially associated with T2DM. Genotyping was performed by primer extension method. We analyzed the results with MDR followed by conventional statistical methods.
Results: MDR analysis revealed that a two-locus interaction between the UCP2 Ala55Val polymorphism and the PPARγ C161T polymorphism. Among three-locus model, the UCP2 Ala55Val polymorphism, the PPARγ C161T polymorphism, and the FABP3 G5428C polymorphism were expected to interact with each other. After multiple comparison, we found that the combination of the UCP2 55 Ala/Val heterozygote and the PPAR γ 161 C/C homozygote showed a protective effect against T2DM (OR 0.52, 95% CI 0.35-0.79, p=0.0024), and the combination of the UCP2 55Ala/Val heterozygote, the PPARγ 161 C/C homozygote, and the FABP3 5428 G/G homozygote also showed a protective effect against T2DM (OR 0.59, 95% CI 0.39-0.90, p=0.0202).
Conclusions: We demonstrated gene-to-gene interactions among 23 different alleles in the candidate genes of T2DM using MDR method. The identification of genotype combinations attributing to T2DM will provide a new tool for the timely identification of high-risk individuals who might benefit from early intervention to prevent the development of T2DM.

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Genome-wide search for Type 2 diabetes susceptibility genes in African Americans.

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Background and Aims: African Americans are at increased risk for developing type 2 diabetes (T2DM). As with other populations, epidemiological evidence suggests a significant genetic component contributing to this risk. The aim of this study was to identify chromosomal regions linked to T2DM susceptibility in the African American population.
Materials and Methods: A genome-wide scan was performed in 636 African American affected sibling pairs (ASP) with type 2 diabetes from 251 families to identify type 2 diabetes loci in this high-risk population. This represents the first truly large-scale genome-wide scan conducted in African American diabetes families. A total of 390 markers, at average spacing of 9 cM were scored by the Center for Inherited Disease Research as part of the International Type 2 Diabetes Consortium.

Results: Analyses of pedigree structures using Prest indicated that corrections were required for 21% of pedigrees. Non-parametric linkage analyses conducted using Genehunter Plus and ASM and Genehunter Logit determined that that the susceptibility locus of greatest effect is located on chromosome 6q23-27, with a maximum LOD of 2.08 (p=0.002) positioned 163.5 cM from the p telomere. The peak multipoint LOD score increased to 3.00 under a model of heterogeneity (HLOD) using Allegro. In the only other genome-wide scan of type 2 diabetes-related phenotypes in African Americans, using 190 ASP a LOD of approx. 2.0 was detected on 6q for combined impaired glucose homeostasis (Ehm *et al.* AJHG 66:1871-81). Linkage to the same region of 6q has been reported for diabetes and related metabolic phenotypes in other populations, as well as percent body fat in African Americans (LOD 1.5). A second locus of interest lies on chromosome 22q11-q13, LOD 1.28 (p=0.015) at 32 cM. In view of these results, we investigated epistatic and conditioning approaches for these two loci. Increased evidence for linkage at 6q was observed when conditioning on the peak at 22q, with the max LOD rising to 2.97 at 165 cM.
Conclusion: There is strong support that a region of chromosome 6 harbors a diabetogenic gene in African Americans, possibly with pleiotropic effects on other phenotypic components of the metabolic syndrome.

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The heritability of insulin secretion, peripheral and hepatic insulin action and intracellular glucose partitioning among young and elder twins.

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Background and Aims: Type 2 diabetes mellitus (T2DM) has a multifactorial etiology including both genetic and environmental factors. The major pathophysiological mechanisms underlying the development of T2DM are considered to be impaired insulin secretion, peripheral insulin resistance and disproportionately elevated hepatic glucose production. To what extent these abnormalities of glucose metabolism in T2DM are genetically or environmentally determined is currently unknown.
Materials and Methods: Insulin secretion was measured using an intravenous glucose tolerance test. Insulin action was studied using the hyperinsulinaemic, euglycaemic clamp technique with infusion of 3-^{3}H-glucose tracer to measure endogenous glucose production, and indirect calorimetry to measure glucose and lipid oxidation. Interclass correlations coefficients and heritability estimates were calculated for insulin secretion, peripheral and hepatic insulin action and intracellular glucose partitioning among 104 monozygotic (MZ) and 88 same-sex dizygotic (DZ) twins without known T2DM in two age groups (25-34 and 57-66 years.
Results: In the younger twins the interclass correlation for insulin secretion was higher among MZ ($r = 0.73$, $p < 0.0001$) than in DZ twins ($r = 0.44$, $p = 0.06$) giving a heritability estimate of 0.58. In contrast, elder twins MZ and DZ twins had similar correlation coefficients for insulin secretion. The heritabilty of glucose disposal rate (Rd) during the clamp period among younger twins was 0.38, whereas elder MZ had a significant correlation ($r = 0.60$, $p = 0.004$) compared to DZ twins ($r = 0.21$, $p = 0.35$) giving a heritability estimate of 0.78. Similarly, the correlation coefficients for exogenous glucose storage and non-oxidative glucose metabolism were significantly different among elder MZ and DZ twins resulting in heritability estimates of 0.80 and 0.88, respectively. Among younger twins no significant heritability estimates were demonstrated. Hepatic glucose production, glycolytic flux, glucose and lipid oxidation demonstrated similar non-significant heritability estimates among young and elder twins.
Conclusion: The study demonstrated a major genetic component in peripheral insulin resistance and non-oxidative glucose metabolic pathways among elder twins, which was not seen among the younger twins. In contrast, in the younger twins we demonstrated a major genetic component in insulin secretion. Consequently, age may play an important role in unmasking the relative importance of genetic and environmental factors in the etiology of insulin resistance and insulin secretion.

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Prediction of Type 2 diabetes in the Botnia study.

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Background and Aims: A number of risk factors like obesity and a family history of diabetes are known to predict development of type 2 diabetes (DM2). Although it is not known what mediates the inherited risk, a Pro12Ala variant in the PPARγ gene is a strong candidate conferring about 25% of the population attributable risk of DM2. The aim of the study was to evaluate the relative role of the PPARγ Pro12Ala polymorphism and conventional risk factors for the risk of developing DM2 in a large prospective study of high-risk individuals (the Botnia Study).
Materials and Methods: 2313 non-diabetic members from families with type 2 diabetes (1060 males/1253 females; age 45± 14 years, BMI 26± 4 kg/m^2; 1589 NGT, 724 IGT or IFG) were prospectively followed with repeated OGTT every 3 years.
Results: After the mean 6.5-year follow-up the incidence of diabetes was 2.8 % in NGT and 12.7 % in IFG/IGT. Age-adjusted COX proportional hazards regression model revealed that the risk of diabetes was 2.5 (95% CI, 1.4-4.4, p=0.002) times higher in individuals with than without a family history of diabetes, 2.8 (95 CI, 1.9-3.9, p<0.001) times higher in subjects with than without dysmetabolic syndrome (DMS) and 1.75 times lower in carriers of Ala allele compared to Pro allele carriers (CI 95%, 1.1-2.7, p=0.018). Isolated IFG was associated with 3.2-fold (95%, 2.1-4.8, p<0.001) and isolated IGT with a 4.9-fold (95%, 3.3-7.3, p<0.001) increased risk of diabetes. A multiple logistic regression analyses showed that a family history of diabetes (p=0.004), abdominal mass index, AMI = waist circumference/height^2 (p<0.001), DMS (p<0.001), HOMA-IR index (p<0.001), incremental insulin/glucose ratio at 30 min of OGTT = insulinogenic index (p=0.016) and the PPARγ Pro12Ala polymorphism (p=0.021) are independent predictors for the development of diabetes.
Conclusion: A family history of diabetes, glucose intolerance, insulin resistance and β -cell function in addition to the PPARγ Pro12Ala polymorphism predict diabetes in the non-obese middle-aged Botnia population.

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Ethnic differences in the prevalence and determinants of diabetes in Oslo, Norway.

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Background and Aims: A large majority of Norway's migrant population originate from developing countries, especially Asia. High prevalence of type 2 diabetes (T2DM) in this group has been documented worldwide and Norway is no exception. In Norway information on diabetes among immigrants has been scarce and fragmented until the recent Oslo Health Study.To determine the prevalence of Diabetes Type 2 in a multi-ethnic population and study differences in anthropometric and bio -chemical parameters across groups and between diabetics and non diabetics.
Materials and Methods: In 2000 - 2001, a total of 18770 individuals, 45.9% of those invited, participated in the Oslo Health Study. 1931 of the participants originated from Non-Western Countries, in this cross sectional survey, encompassing 5 age cohorts (30-76 years). Participants received a postal invitation with a self administered questionnaire (also translated for immigrants) and thereafter attended a physical examination, including measurements of height, weight, waist- and hip circumference, sitting blood pressure and a blood sample. including blood tests. Two additional questionnaires handed out at the screening, were returned in pre-stamped self-addressed envelopes.
Results: Self reported prevalence of diabetes was highest among subjects from the Indian Subcontinent group (11.6%) and lowest (3.0 %) among those from Western Countries and Eastern Europe (3.1%).In all ethnic groups, the mean Body Mass Index (BMI) was higher among diabetics than non-diabetics. However, important differences were seen between the ethnic groups: the Indian and Mediterranean groups both had high BMI and WHR, but diabetes prevalence was twice as high among the Indian group. Interestingly, while BMI was only marginally elevated in non-diabetic subjects from Sub Saharan Africa (25.7 kg/m2), the diabetics of this ethnic group had the highest BMI (33.2 kg/m2). Non-fasting plasma glucose levels were higher in all the ethnic groups for non diabetics as compared to the Western.
Conclusion: Ethnic differences are apparent both in terms of diabetes prevalence and anthropometric determinants of diabetes. Our findings suggest that important differences in the relationship between obesity and diabetes exist between different immigrant groups, which may be of significance when planning preventive and therapeutic programs.

differences in anthropometric and bio -chemical parameters across groups and between diabetics and non diabetics

	Western Countries (n= 996)	Eastern Europe (n= 261)	Mediterranean (n= 385)	Sub-Saharan (n= 190) (n= 582)	Indian Subcontinent (n= 326)	East Asian
Self reported Diabetes prevalence (p-value chi square test <0.001)	3.0	3.1	4.4	4.2	11.0	4.0
BMI (kg/ m2) (mean values)						
Diabetic 27.28	28.62 ns	30.41*	33.23*	29.22*	25.13ns	
Non Diabetic	25.15	26.68***	27.33***	25.74*	27.09***	23.65***
WHR (mean values)						
Diabetic	0.87	0.91ns	0.90ns	0.89ns	0.94***	0.85ns
Non Diabetic	0.82	0.83*	0.85***	0.84***	0.88***	0.82ns
P-Glucose(mmol/l)						
Diabetic	9.82	6.94*	8.07ns	8.59ns	10.56ns	9.77ns
Non Diabetic	5.19	5.41*	5.34*	5.24ns	5.39**	5.38**

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Higher incidence of Type 2 diabetes in Danish men than women is explained by higher prevalence of unfavourable risk factors in men.

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Aim: To examine the incidence of clinical diabetes, and the association between cardiovascular risk factors and the development of diabetes in a population based study of Danish men and women.
Materials and Methods: A total of 14223 persons randomly recruited from approximately 90.000 persons living in a defined area were examined. The examination took place between 1975 – 77 and included e.g. a self-administered questionnaire, determination of BMI, blood pressure and various non-fasting blood samples. Participants with type 2 diabetes (T2D) defined as self reported T2D or a random non-fasting plasma glucose >11.1 mmol/l were excluded. A follow-up re-examination was performed in 1984-85 and 1993-94.A total of 6154 women and 4733 men were included. Development of T2D was defined as self-reported T2D or a random non-fasting plasma glucose > 11,1 mmol/l at any of these re-examinations. The relation between the development of T2D and the various cardiovascular risk factors were examined by multiple logistic regression analysis.
Results: In women, 152 new cases of T2D were diagnosed corresponding to 2,0 per 1000 person-years. In men, 245 new cases of T2D were diagnosed corresponding to 5,4 per 1000 person-years. In both sexes BMI, systolic blood pressure (s-BP), triglyceride levels, predicted development of diabetes. In men, but not in women, high consumption of alcohol increased the risk of diabetes, whereas high leisure time physical activity decreased the risk. The association between the various risk factors and the risk of developing diabetes was non different between the two sexes: RR of developing diabetes with BMI > 30 kg/m^2 compared to BMI < 20 kg/m^2 was 22,6 (7 – 72, 95% confidence intervals) in women and 16,3 (3,9-66,8) in men; RR of s-BP > 140 despite medical treatment compared to s-BP < 140 without medical treatment was 4,8 (2,8-8,3) in women and 3,7 (0,8-3,0) in men; RR of developing diabetes with non fasting triglyceride >2,0 mmol/l compared to < 1 mmol/l was 10,0 (5,3 – 18,6) in women and 3,7 (2,3 – 6,2) in men. At the examination in 1975-77 a BMI > 30 kg/m^2 was seen in 10% of the women and a BMI < 20 kg/m^2 was seen in 10 %. The corresponding figures were 10,4 % and 3,4 % in men, respectively. In women a s-BP > 140 despite medical treatment was found in 4,6 % and a s-BP < 140 without medical treatment in 63,5 %. The corresponding figures were 3,5 % and 57,2 % in men, respectively. In women a non fasting triglyceride >2,0 mmol/l was found in 16,9 % and a non fasting triglycerides < 1 mmol/l in 30,8 %. The corresponding figures in men were 37,5 % and 14,1 %, respectively.
Conclusion: The present study demonstrate that in a randomly selected Danish population significantly more men than women develop type 2 diabetes. The association between unfavorable risk factors and the development of type 2 diabetes is stronger in women than in men. However, in men the absolute number of persons having unfavourable risk factors are higher than in women, and in women the absolute number of persons having favourable risk factors are higher than in men.

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Effects of the intensive lifestyle intervention (ILS) on risk of diabetes in the diabetes prevention program (DPP).

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Background and Aims: The DPP was a randomized clinical trial comparing ILS ,metformin, and placebo interventions in high-risk adults with impaired glucose tolerance and fasting glucose levels from 95-125 mg/dl (5.3-6.9 mmol/L). Over an average of 3.2 years of follow-up, the ILS reduced diabetes incidence by 55% compared with placebo. We examined the association of changes in weight, diet, physical activity and markers of insulin resistance and secretion on risk of diabetes among ILS participants in order to assess the relative contribution of each.
Materials and Methods: There were 1079 ILS participants at baseline (mean age =50.6 years, BMI = 33.9 kg/m2). Cox proportional hazards models were used to determine the effect of changes in weight, fasting insulin (I0-marker of resistance), and 30 min. D insulin/D glucose ratio (IG ratio - marker of secretion) during an annual oral glucose tolerance test, % of calories from fat and self-reported physical activity (2 questionnaires). Models included baseline age, ethnicity, fasting glucose (G0) and insulin, HbA1c, diet and physical activity. Changes from baseline in weight, diet and physical activity variables, I0 and IG ratio were modeled as time-dependent covariates.
Results: Weight loss was the dominant variable associated with reduced risk of diabetes among the anthropometric or behavioral variables, based on the % variance explained, and was independent of improvements in I0 and IG ratio (table). Weight loss itself was predicted by lower % of calories from fat (at all years, p<0.0001) and increased physical activity (at year 3, p<0.0001) but diet and activity were not associated with lower risk of diabetes beyond the effect of weight loss (table).
Conclusion: We conclude that changes in physical activity and diet were associated with weight loss, but it was weight loss per se that reduced diabetes risk in the DPP ILS participants. Improvements in insulin resistance and secretion were also associated with lower diabetes risk, but did not explain the effect of weight loss.

Cox model hazard ratios for diabetes risk (baseline variables not shown)

Changes in variables during follow-up	Hazard Ratio for diabetes	95% CI	p value	% variance explained
Weight loss (per 5 Kg loss)	0.42	0.35 - 0.51	<0.0001	7.7%
Leisure physical activity (MAQ) (per 5 Met-hrs/week increase)	0.98	0.90 - 1.06	0.59	0.0%
Recreational activity (LoPAR) (per 5 Met-hrs/week increase)	0.99	0.96 - 1.02	0.58	0.0%
% of calories from fat (per 5% decrease)	0.97	0.80 - 1.19	0.79	0.0%
IG ratio (per 10 unit increase)	0.96	0.95 - 0.97	<0.0001 4	1%
Fasting insulin (per 10 IU/dl decrease)	0.74	0.66 - 0.84	<0.0001	1.9%

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Early initiation of thiazolidinedione and lifestyle modification can prevent/delay the onset of Type 2 diabetes mellitus in patients with impaired glucose tolerance.

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Aim: To evaluate the effect of diet, physical exercise, life style modification and early initiation of thiazolidinedione (TZD) in patients with Impaired Glucose Tolerance (IGT) on transformation to Type 2 Diabetes Mellitus (T2DM).
Methods: 213 patients with IGT were randomized into 65 Control group (CON); 72 Intervention group-A (INT-A) with life style modification, diet and physical exercise; 76 Intervention group-B (INT-B) diet, exercise, life style modification with TZD. The mean duration of follow-up was 18 months. The INT-A group received dietary advice, counseling for weight loss, instructions for physical activity and moderate exercise, while the INT-B group in addition received rosiglitazone 4mg/day. At base line the subjects had a mean age of 37.5 ± 4.3 years. 26 patients were excluded (CON 6, INT-A 9, INT-B 11) as they failed for follow-up OGTT done after 12-15 months duration. The cumulative incidence of DM, IGT and normal tolerance (NT) was calculated for each group. Antihypertensive drugs, statins and fibrates were prescribed to all the three groups as and when required.

Results: The incidence of DM in CON group was 32.3%, INT-A 15.3%, INT-B 6.4%. The improvement of IGT to NT was 5.5% in CON group, 48.8% in INT-A and 69.2% in INT-B groups. Significant weight gain and raised BMI was observed in CON group (30.1 ± 3.6 at base line to 33.3 ± 3.8 kg/m^2). The INT-A group achieved desired weight loss and BMI decreased from 29.8 ± 3.4 at base line to 26.9 ± 3.2 kg/m^2, while in the INT-B group there was not much weight loss and the BMI was identical to the base line (30.1 ± 3.2 to 29.9 ± 3.4 kg/m^2).
Conclusion: The transformation of IGT to T2DM can be prevented /delayed by diet control, weight reduction, life style modification and early initiation of TZDs.

OP 14
Insulin Action - Muscle

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Glucose selectively activates PDK-1 and PKC ζ independently of PI 3-kinase and PKB in Soleus muscle from Wistar rats.
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Background and Aims: Hyperglycemia may directly contribute to the development of insulin resistance in Type II diabetes mellitus through alterations in insulin signaling in peripheral tissues. The Ser/Thr kinase Phosphatidylinositol Dependent Kinase 1 (PDK-1) is downstream of Phosphatidylinositol (PI) 3-kinase in the insulin-signaling pathway. PDK-1 activates the AGC (Protein Kinase A (PKA), G (PKG) and C (PKC)) kinases, PKB and PKC ζ. Extracellular Regulated Kinase (ERK), in concert with PDK-1, activates the AGC kinases Mitogen and Stress Activated Kinase (MSK) and Ribosomal S6 Kinase (RSK). We determined effects of acute hyperglycemia on these signal transducers in soleus skeletal muscle.
Material and Methods: Male Wistar and diabetic Goto-Kakizaki (GK) rats (200-250 g) were infused with glucose (5 or 20 mM) for 3 hr. Thereafter, saline or insulin (3 U/kg/hr) was administered by continuous infusion for 20-min. Endogenous insulin secretion was suppressed by continuous infusion of somatostatin (2 μgram/kg/min) throughout the experiment.
Results: Basal and insulin-stimulated PI 3-kinase activity were each reduced 35% ($p<0.05$) in soleus muscle from GK vs. Wistar rats. Additionally, a reduction in basal and insulin-stimulated PKB phosphorylation (65% $p<0.05$ and 30% $p<0.01$) was also observed. In contrast, PKC ζ activity was normal in diabetic GK rats. Glucose infusion did not affect the insulin response at the level of these kinases in either Wistar or GK animals, whereas, basal PKC ζ activity was increased 50 % ($p<0.02$) in Wistar rats. Incubation of isolated skeletal muscle from Wistar rats for 3 hr in the presence of 20 mM glucose confirmed our *in vivo* observations that glucose directly increases PKC ζ activity in a PI 3-Kinase independent manner. This increase in PKC ζ activity was accompanied by a 50 % ($p>0.01$) increase in PDK-1 activity. Interestingly ERK activation was unaltered.
Conclusion: In conclusion, chronic rather than acute hyperglycemia contributes to reduced insulin signal transduction in skeletal muscle. However, acute hyperglycemia specifically increases PKC ζ activity via PDK-1 and this is independent of insulin signaling at the level of PI 3-kinase or PKB. Acute effects of hyperglycemia on PKC ζ and PDK-1 do not interfere with insulin signal transduction.

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PKCζ mediates the inhibitory effects of ceramide on PKB activation.
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Background and Aims: Ceramide has been implicated in the regulation of diverse cellular responses including cell death and insulin sensitivity. Recent evidence indicates that ceramide may regulate these responses by inhibiting the stimulus-mediated activation of protein kinase B (PKB), a key determinant of cell fate and insulin action. There is growing evidence that atypical Protein Kinase C zeta (PKCζ) can be activated by ceramide, and that the kinase may negatively regulate PKB activation. In this study we have assessed the involvement of PKCζ as a mediator of ceramide's inhibitory effect on PKB activity, with a view to ascertaining the mechanism by which PKCζ negatively regulates PKB activation.
Materials and Methods: L6 rat skeletal muscle cells were treated with C2-ceramide (100microM, 2h), and/or insulin (100nM 10min) in the absence or presence of PKC inhibitors prior to assaying PKB activity, and PKB-PKCζ co-immunoprecipitation. PKB activity and phosphorylation were assessed using a synthetic peptide substrate and phospho-specific antibodies, respectively. Recombinant PKB, PKCζ, the isolated PKB PH domain, and a PKB-PH domain in which T34 had been mutated to an alanine, were used to assess the interaction between PKB and PKCζ, and the effect of ceramide on 3-phosphoinositide (PIP_3) binding *in vitro*.
Results: Insulin induced a 15-fold increase in PKB activity, which was lost completely upon prior incubation of cells with ceramide. PKCζ inhibitors and expression of a kinase-dead PKCζ antagonised the inhibitory effect of ceramide on PKB, suggesting that atypical PKCζ is involved in this response. Immunoprecipitation of PKB resulted in coprecipitation of PKCζ

indicating that the kinases interact physically. This association, which requires the PH domain of PKB, was reduced significantly by insulin; a response that was blocked by pre-treatment of cells with ceramide. Under these circumstances, ceramide activates PKCζ, which then phosphorylates the PKB-PH domain on Thr^{34}. This phosphorylation inhibits PIP_3 binding to PKB, and prevents the recruitment and the subsequent activation of the kinase. Conversely, ceramide-activated PKCζ did not phosphorylate a PKB T34A mutant PH domain, or prevent interaction of the latter with PIP_3 *in vitro*.
Conclusion: These findings indicate that ceramide may invoke apoptosis and insulin resistance through its ability to stimulate PKCζ, which blocks PKB activation, thereby suppressing insulin and anti-apoptotic signaling.

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PDK1 interaction with insulin receptor and tyrosine phosphorylation are required for insulin metabolic action.
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Background and Aims: Phosphoinositides-dependent kinase (PDK) - 1 plays a pivotal role in PKB/Akt activation and regulation of glucose metabolism. Insulin induces PDK1 translocation to plasmamembrane where it phosphorylates PKB on Thr308. Recent studies revealed that PDK1 tyrosine phosphorylation is also required for its full activation. However, the kinase(s) responsible for PDK1 tyrosine phosphorylation has (have) not been clearly identified.
Materials and Methods: L6 skeletal muscle cells have been used as a model system. Co-precipitation and tyrosine phosphorylation studies, as well as glucose uptake and glycogen synthase activity assaies have been performed in L6 cell clones stably expressing either human insulin receptor (L6hIR) or a mutant receptor lacking 43 aminoacids at the C-terminus (L6Δ43). Subcellular fractionation studies, in vitro phosphorylation and binding studies were also performed.
Results: Insulin induced PDK1 tyrosine phosphorylation and activation, in L6hIR cells. Treatment with the Src kinase inhibitors, PP1 and PP2, reduced basal PDK1 tyrosine phosphorylation by 50% with no significant effect on insulin-stimulated. In L6Δ43 cells, insulin failed to increase both PDK1 tyrosine phosphorylation and activation, accompanied by lack of PKB Thr308 phosphorylation. This was paralleled by >90% reduction of insulin-stimulated glucose uptake and glycogen synthase activity. Wild-type insulin receptor, but not the truncated variant, co-immunoprecipitated with PDK1 in intact cells. Similarly, purified wild-type receptor, but not the Δ43, was able to directly bind PDK1 in vitro, as assessed by overlay blot, and to phosphorylate it on tyrosine residues. PDK1 tyrosine phosphorylation and binding to IR were blocked upon treatment of the cells with the PI3K inhibitor LY294002. Insulin regulation of PDK1 plasmamembrane translocation was also altered in L6Δ43, compared with L6hIR cells, in spite of similar levels of insulin-induced PI3K activation. Hence, PDK1 was well detectable in the membrane fraction of L6hIR cells upon 2, 5 and 10 min and stable up to 30 min insulin treatment. At variance, in L6Δ43 cells, membrane PDK1 levels were only detectable up to 5 min, while PKB membrane translocation occurred at similar levels in both cell types.
Conclusion. These results indicate that activated insulin receptor directly binds to and tyrosine phosphorylates PDK1. This event might be responsible for the appropriate PDK1 expression at the plasmamembrane and subsequent PKB phosphorylation and transduction of metabolic signals.

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Insulin induces interaction between PKC delta and the juxtamembrane domain of insulin receptor.
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Background and Aims: Certain members of the Protein Kinase C (PKC) family of ser/thr kinases, in particular PKCs βII ,δ and ζ , are activated by insulin stimulation. PKCs βII and ζ are activated via products of PI3 kinase activity. We have reported that insulin stimulation of PKCδ is associated with rapid phosphorylation on tyrosine. In addition, we reported that insulin receptor (IR) may directly interact with PKCδ. Thus, insulin induces a direct association between IR and PKCδ, but not PKCβII or PKCζ. Moreover, the time course of insulin-induced association between IR and PKCδ paralleled that of insulin-induced tyrosine phosphorylation of PKCδ. The purpose of this study was to identify the specific domains of the IR and PKCδ involved in their interactions.
Materials and Methods: In one set of experiments, whole cell lysates were prepared from control and insulin-stimulated (1-5 min) 4-6 day old primary cultures of rat skeletal muscle. Cultures were prepared freshly for each experiment. Purified His-tagged peptides corresponding to the juxtamembrane (JM) and carboxyterminal (CT) domains of IR were added to the lysates. Nickel-conjugated beads were utilized to purify the resulting immunocomplexes. The immunoprecipitates were then subjected to SDS-PAGE and Western blotting with specific anti-PKC antibodies. In another set of experiments, lysates were prepared from L8 skeletal muscle cells stably transfected with chimeras of PKCα/δ regulatory and catalytic domains. These lysates were also probed for binding to the JM and CT domains as described.
Results: Insulin stimulation of skeletal muscle caused a strong increase in PKCδ binding to the JM domain of IR and a slight increase in PKCδ binding to the CT domain. When probed with antibodies to other PKC isoforms activated by insulin, an increase in their binding to either the JM of CT domain could not be detected. Inhibition of PKCδ by treatment with rottlerin (5 μM) , for 7 min before stimulation by insulin, appeared to block induction of PKCδ to the IR domains. The association of the PKCα regulatory/δ catalytic chimera to the IR domains appeared to be similar to that of PKCδ itself, whereas the association of the PKCδ regulatory/α catalytic chimera was not detected.
Conclusion: The results show that PKCδ specifically interacts with IR in response to insulin through distinct domains of each molecule. Moreover, the findings indicate that activation of PKCδ by insulin precedes the induction of association between PKCδ and IR.

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ATM protein kinase participates in GLUT4 translocation by insulin in L6 muscle cells.
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Background and Aims: Ataxia-telangiectasia (A-T) is a disorder characterized by cerebellar ataxia and oculocutaneous telangiectasias. A-T patients also exhibit symptoms of insulin resistance and glucose intolerance and have been shown to have higher incidence of type 2 diabetes. The gene mutated in this disease, *ATM (A-T, mutated),* encodes a protein kinase. Recent studies have demonstrated a role for ATM in an insulin-signaling pathway that controls translation initiation through its phosphorylation of an insulin-responsive protein 4E-BP1. Other recent findings suggest that ATM might participate in cytoplasmic protein transport processes by binding to β-adaptin. The aim of this work is to study the potential linkage between ATM and the translocation of glucose transporter 4 (GLUT4) by insulin in L6 muscle cells.
Materials and Methods: Plasmids encoding wide type ATM (WT-ATM) and dominant negative, kinase-dead ATM (KD-ATM) were from Dr. Michael B. Kastan. L6 muscle cell and GLUT4myc plasmid were provided by Dr. Amira Klip. For colorimetric assay of cell surface GLUT4myc in L6 myotubes, L6 myoblasts were differentiated into myotubes in low-serum medium. 4 days post-seeding, cells were transfected with plasmids by Effectene. Transfected L6 myotubes were serum-starved and treated with or without insulin (100 nM). Cells were then incubated with an anti-myc antibody followed by a secondary peroxidase-conjugated antibody. Optical absorbance was measured after addition of o-Phenylenediamine Dihydrochloride (OPD). OPD assay using L6 myoblasts was essentially the same as described above except that L6 myoblasts were transfected by Lipofectamine. To measure GLUT4myc translocation by immunofluorescence assay, L6 myoblasts transfected by Lipofectamine were serum-starved and treated with or without insulin (100 nM). Cells were then fixed and incubated with an anti-myc antibody followed by a secondary Cy3-conjugated antibody. The cells were then analyzed by confocal microscope to assess the translocation of GLUT4myc to the cell surface.
Results: In L6 myotubes transiently transfected with a plasmid encoding GLUT4myc and a plasmid harboring WT- or KD-ATM, insulin caused nearly 1-fold increase of OPD activity in WT-ATM transfected cells, while addition of insulin in KD-ATM transfected cells only resulted in a 0.2-fold increase of OPD activity which indicates that KD-ATM had an inhibitory effect on translocation of GLUT4 to the cell surface. OPD assay using L6 myoblasts was also performed and similar results were obtained. The amount of GLUT4myc translocated to the cell surface by insulin in cells expressing KD-ATM was dramatically reduced in comparison to cells expressing WT-ATM. Moreover, the effect of ATM on GLUT4 translocation was examined by immunofluorescence analysis using L6 myoblasts transiently transfected with plasmids encoding GLUT4myc, ATM, and green fluorescence protein (GFP). GFP was used to facilitate

recognition of transfected cells. Confocal microscopy data showed that KD-ATM, in contrast to WT-ATM, significantly inhibited GLUT4myc translocation to the cell surface by insulin.
Conclusion: These results suggest that ATM protein is involved in insulin-regulated GLUT4 translocation in muscle cells.

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Potential role of transcription factors MEF2A, MEF2C, MEF2D and NF-kB in the fasting- and contraction-induced GLUT4 mRNA modulation in skeletal muscle.
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Background and Aims: Insulin and muscle contraction have been described as able to modulate GLUT4 gene expression. Additionally, fasting reduces and contractile activity increases GLUT4 translocation to the plasma membrane. The aim of the present study is to investigate, the acute effects of fasting and in vitro contractile activity upon GLUT4 mRNA expression, as well as to determine the role of the transcription factors myocyte enhancer factor (MEF) 2A, 2D, 2C and nuclear factor (NF)- κB in these modulations.
Material and Methods: Soleus muscles were obtained from fed- and 48-hour fasted-rats. Additionally, muscles from 48-hour fasted-rats were preincubated (Krebs Henseleit gazed buffer, pH 7.4, 8 mM glucose) during 40 min; and then electrically stimulated (10-seconds, 100 Hz, once a minute) during 10 min; 20 min later, the muscles were immediately frozen in liquid nitrogen for further analysis. GLUT4 mRNA was analyzed by Northern blotting, and MEF2A, MEF2D, MEF2C and NF-κB mRNAs were analyzed by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Beta-actin was used as internal control of the assays.
Results: Compared to fed-rats, the fasting induced a reduction ($P<0.05$) of 40% in GLUT4 mRNA, which was accompanied by 68% increase ($P<0.01$) in NF-κB mRNA without changing MEF2A, MEF2D and MEF2C mRNAs. In vitro electric stimulation of muscles from fasted rats induced a rapid increase ($P<0.05$) of 40% in GLUT4 mRNA, which was accompanied by 80% ($P<0.001$) and 40% ($P<0.05$) increase in MEF2A and MEF2B, respectively, with unchanged MEF2C and NF-κB.
Conclusions: The results showed that GLUT4 mRNA content in skeletal muscle is acutely modulated by metabolic-hormonal condition (fasting) and contractile activity (electric stimulus). Fasting reduced the GLUT4 mRNA, which seems to involve NF-κB transcription factor. On the other hand, in vitro contractile activity increased the GLUT4 mRNA, which seems to involve MEF2A and MEF2B transcription factors. MEF2C transcription factor seems not to be involved in GLUT4 mRNA modulation in the studied conditions.

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Role of AMP-activated protein kinase in the regulation of cardiac glucose transport.
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Background and Aims: AMP-activated protein kinase (AMPK) represents a master regulator of additional substrate supply to working muscle. Isolated adult rat cardiomyocytes were therefore used 1) to assess the activation of this enzyme and the p38 mitogen-activated protein kinase (MAPK) by hormones, cellular stress and contraction, and 2) to elucidate the implications of this process for insulin-regulated glucose transport.
Materials and Methods: Cardiomyocytes were stimulated by insulin or contracted by field stimulation. Activation of AMPK and MAPK was determined using phospho-antisera.
Results: Leptin and insulin (0-30 min) failed to modify the phosphorylation state of AMPK, but leptin increased STAT3 (signal transducer and activator of transcription) phosphorylation 2fold. Leptin was unable to modify basal and insulin-regulated glucose transport. Cardiomyocytes subjected to osmotic shock exhibited a 30fold increase in AMPK phosphorylation and a 2-3fold activation of glucose transport. A comparable increase in glucose transport was observed in response to contraction of the cardiomyocytes with a much less pronounced activation of AMPK (6fold). Both stimuli produced a 10-15fold increase in the phosphorylation state of p38 MAPK with essentially no effect of insulin on this enzyme. Inhibition of AMPK activation by iodotubercidin eliminated the effects of osmotic shock and contraction on glucose transport. In contrast, inhibition of p38 activity by SB203580 only blocked the contraction-induced glucose uptake with unaltered effects of insulin and osmotic shock.
Conclusion: cellular stress produces a strong activation of AMPK sufficient to activate glucose transport independent of p38 MAPK, whereas contraction regulated glucose transport in cardiomyocytes involves the AMPK/p38 MAPK cascade. Insulin action is completely independent of these kinases suggesting that at least three different pathways contribute to the regulation of cardiac glucose transport.

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Chronic hyperinsulinism demodulates insulin signaling.
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Background and Aims: A crucial issue in the interpretation of hyperinsulinemia is whether its metabolic changes result from the high insulin per se or the underlying insulin resistance. We used cultured myoblasts to study the effect of chronic exposure to normal or high insulin levels on glucose transport and insulin signal transduction.
Materials and Methods: Human cultured skeletal muscle cells (SkMC) were grown in media containing 107 pmol/L (SkMC-L) or 1430 pmol/L (SkMC-H) insulin in presence of normal glucose level (5,5 ±0.7 mM). In our culture conditions myoblasts continued to express alpha-actin and sarcomeric myosin. The 2-DG transport was determined by measuring the 2-DG-3H uptake. The PI3k activation was determined by immunoprecipitation with IRS-1, incubation with 32-P ATP and 32-P PtdIns-3 monophosphate separation by TLC. Genes, proteins expression and phosphorylation were evaluated, respectively, by Northern blot and Western blot.
Results: The rise of medium insulin concentrations from 107 pmol/L to 1430 pmol/L increases the phosphorylation of IR and IRS-1 by about 72% and 100% respectively. Nevertheless, in response to an acute insulin stimulation, phosphorylation of IR and IRS-1 showed a further increase (77% and 106%, respectively) only in control cells, but not in SkMC-H. Interestingly, maximal activation of IR and IRS-1 were similar between SkMC-L and SkMC-H. IR and IRS-1 protein expression measured using appropriate antibodies was similar in both culture conditions. Activation of PI3k results from its binding to phosphorylated IRS-1, thus we have investigated whether the pattern of PI3k activation mirror that of IRS-1 phosphorylation in SkMC-H and SkMC-L. In cells chronically treated with high insulin, PI3k activity was significantly increased (90%) as compared to control cells. After an acute insulin stimulation PI3k activity increased in SkMC-L (97%) but did not increase further in SkMC-H. The inhibition of PI3k activity in SkMC-H after acute insulin stimulation was not due to a change in the total amount of the enzyme detected by immunoblotting. The possibility that incubation with high insulin levels could affect p85 binding to IRS-1 was also tested. After solubilization of cells, activated IRS-1 was immunoprecipitated and probed by immunoblotting with anti-p85. The result obtained shows an efficient binding between these two proteins in both control cells and SkMC-H. Finally we have evaluated the mRNA and the protein expression of SHIP-2 in myoblasts maintained in low or high insulin levels and no differences were found when SkMC-L and SkMC-H were compared. SkMC-H cells showed basal levels of glucose transport similar to that found in control cells. Nevertheless after a further acute insulin stimulation, glucose transport increased (73%) only in SkMC-L.
Conclusion: This study shows that myoblasts cultured in the presence of high insulin concentration develop a demodulation of insulin signalling which is associated with a desensitization of glucose transport, suggesting that hyperinsulinemia could play a role in the insulin resistance which characterizes obesity and type 2 diabetes.

OP 15
Diabetic Retinopathy: Trials and Tribulations

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Initial results of the protein kinase C β inhibitor Diabetic Macular Edema Study (PKC-DMES).

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Background and Aims: The multi-center, multi-national, double-masked, placebo-controlled, parallel PKC-DMES study evaluated 686 patients with DME that was not imminently sight threatening who were randomly assigned to placebo or one of three doses of ruboxistaurin (RBX, LY333531) mesylate, a selective PKC β inhibitor.
Materials and Methods: Eligibility and outcome were assessed at a reading center using stereoscopic fundus photographs taken at 3-6 month intervals. The primary outcome was progression of DME to involve or imminently threaten the macula center or application of focal/grid photocoagulation.
Results: Patients were 55±10.6 years, diabetes duration 16±8.5 years, 18% Type I, and HbA_{1c} 8.9±1.5% (range 5.1-13.1%). Treatment duration was ≥30 months with 45% receiving therapy for 36 months or more. The primary analysis was based on time to occurrence of primary outcome using the intent-to-treat population. At 36 months, Kaplan-Meier event rate estimates were 55%, 51%, 53% and 47% in the placebo, 4, 16 & 32mg groups, respectively (p=0.23 overall, p=0.15 for pair wise comparison of 32mg vs. placebo). When the 18% (55/305) of outcomes based on photocoagulation were excluded, event rates in placebo and RBX groups were 48% & 37%, respectively, a risk reduction of 23% (p=0.046). When subgroup analysis of these patients was conducted based on baseline HbA_{1c} (HbA_{1c} at baseline ≤10%, ≤75th percentile), placebo and RBX (32mg) event rates were 45% and 31%, respectively, a risk reduction of 31% (p=0.019). Treatment effect adjusted for important covariates will be presented. Initial analyses suggest RBX treatment for over 30 months in patients with DME was well tolerated and was not associated with significant adverse events.
Conclusion: Unadjusted analyses did not demonstrate a significant effect of RBX treatment on the primary outcome of DME progression or need for focal laser, although in subgroup analysis excluding patients with very poor glycemic control at baseline, RBX was associated with a risk reduction of 31% in progression of DME.

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Initial results of the Protein Kinase C β Inhibitor Diabetic Retinopathy Study (PKC-DRS).

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Background and Aims: The PKC-DRS was a multi-center, double-masked, placebo-controlled, parallel study that evaluated 252 patients with moderately severe to very severe nonproliferative diabetic retinopathy (NPDR; ETDRS retinopathy severity grades 47B-53E in at least one eye) who were randomly assigned to placebo or one of three doses of ruboxistaurin (RBX, LY333531) mesylate, a selective PKC β inhibitor.
Materials and Methods: Eligibility and outcome were assessed at a reading center using stereoscopic fundus photographs taken at six-month intervals. The primary outcome was ≥3-step retinopathy progression on the ETDRS scale or application of scatter photocoagulation.
Results: Patients were 56±12 years, 68% male, 19% Type 1, 16±7 years diabetes duration, & HbA_{1c} 8.8±1.4% (range 5.7-13.0%). Treatment duration was 36-48 months. At baseline, ETDRS visual acuity was 80±11 letters (~20/25 Snellen), 78% had diabetic macular edema (33% center involved), and retinopathy severity of the more severe eye was level 47 in 45% and level 53 in 55%. The primary analysis was based on time to occurrence of the primary outcome using the intent-to-treat population. At 42 months, Kaplan-Meier event rate estimates were 55%, 57%, 72% & 52% in the placebo, 8, 16 & 32mg groups, respectively (p=0.54 32mg vs. placebo). For progression of ≥2-steps on the ETDRS scale in patients with NPDR in both eyes, 42 month rates were 72% & 49% for placebo and 32mg, respectively (p=0.048), a 32% risk reduction. Sustained moderate visual loss (SMVL; 15 letters for at least six months) occurrence at each visit is outlined in the table.

SMVL at Each Visit (Percent of Patients)

	Visit (Months)				
	12	18	24	30	36
Placebo	15	19	20	22	30
32 mg RBX	5	4	4	11	19
p-value	0.113	0.028	0.027	0.171	0.246

Treatment effects adjusted for important covariates are consistent with results of unadjusted analyses and will be presented. Initial analyses suggest RBX treatment for 36-48 months in patients with diabetic retinopathy was well tolerated and was not associated with significant adverse events.
Conclusion: Unadjusted analyses did not demonstrate a RBX treatment effect on diabetic retinopathy progression; however, a potential beneficial effect of RBX in reducing MVL warrants further investigation.

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Retinopathy is associated with cardiovascular and all-cause mortality, both in diabetic and non-diabetic subjects. The Hoorn Study.

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Background and Aims: To study the association of retinopathy with cardiovascular and all-cause mortality, in diabetic and non-diabetic individuals. Further investigation was directed to the contributing role of cardiovascular risk factors and risk factors for retinopathy.
Materials and Methods: Extensive physical and ophthalmological examinations were performed in a subsample of 625 individuals, aged 50-75 years and stratified for glucose tolerance status, of the original Hoorn Study, a population-based cohort study. Follow-up of mortality until January 2002 was available (median: 10.7 years; 157 deaths, 62 due to cardiovascular causes).
Results: Retinopathy was detected in 85 of the 625 subjects (13.6%). The relative risk of subjects with retinopathy for cardiovascular mortality was 2.1 (95% CI 1.3-3.7) and 1.7 (1.2-2.3) for all-cause mortality. This association was present in subjects with and without diabetes. After adjustment for diabetes, diabetes duration, prior cardiovascular disease, obesity and triglyceride concentrations, still a 1.4-fold (0.7-2.8) higher risk for cardiovascular mortality and a 1.4-fold (0.9-2.1) higher risk for all-cause mortality in subjects with retinopathy remained. Adjustment for other risk factors did not change the estimate.
Conclusion: Subjects with retinopathy have an elevated risk for cardiovascular and all-cause mortality. This association can only partially be explained by risk factors for retinopathy and cardiovascular mortality.

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Risk factors for presence of hard exudates at diagnosis of Type 2 diabetes and after 6 years.

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Background and Aims: Macular field hard exudates (HE), often related to macular oedema, were associated with increased cholesterol in the Early Treatment Diabetic Retinopathy Study (ETDRS). We examined possible

HE risk factors in newly-diagnosed type 2 diabetes using United Kingdom Prospective Diabetes Study data.
Materials and Methods: Biochemical measures and four-field retinal photographs were taken at entry. Photographs were repeated three-yearly with central grading. Risk factors for HE initially and development after six years were determined by logistic regression with stepwise covariate selection of age, systolic blood pressure (SBP), body mass index (BMI), race, gender, cholesterol (total, LDL and HDL), triglycerides and smoking history.
Results: At entry 111 (3.6%) of 3116 with photographs available had HE in the macular field. Women were at lower risk than men (RR 0.51, 95% CI 0.33-0.80) as were those in BMI top third (0.59, 0.37-0.94). Those in SBP top third were at increased risk (1.77, 1.20-2.61). After six years, 163 (8.1%) of a cohort of 2017 without HE at entry developed HE. Top third BMI were at lower risk (0.47, 0.31-0.70), as were those who smoked at entry (0.54, 0.37-0.81). Total cholesterol was not significant when forced into the model (top third 1.44 (0.97-2.15)).
Conclusion: Control of hypertension may reduce HE incidence. Lower HE rates associated with cigarette smoking (possible survivor effect) and increased BMI need further examination. Rising HE risk with higher cholesterol has not been replicated in this low retinopathy prevalence population.

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Clinical risk factors predicting the presence of sight threatening retinopathy in patients with diabetes at presentation to a diabetic retinopathy clinic: a cohort study.
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Background and Aims: Despite evidence that strict control of blood pressure (BP) and blood glucose delays the development and progression of diabetic retinopathy to Sight threatening retinopathy (STR), it still remains a major cause of morbidity in patients with diabetes. Even though studies have highlighted certain risk factors, a clinically modifiable profile predicting STR has not been formulated. Our aim was to identify clinical risk factors for STR at presentation to a diabetic retinopathy clinic.
Materials and Methods: From a large prospective database, haemodynamic and biochemical data were collected (n =929). A multiple logistic regression analysis was done using the S plus statistic package. Analysis was done separately for clinical variables (Systolic and Diastolic BP, Retinal Perfusion Pressure (RPP) and directly calculated variables (Pulse Pressure and Mean Arterial Pressure) to avoid colinearity between related variables.
Results: Of the 929 patients, 20.2% (187) had no evidence of STR and 79.8% (742) had STR. 32% (298) had type 1 DM and 68% (631) type 2 DM. By stepwise logistic regression analysis, important factors for STR were:
For the younger onset: RPP; OR (per 10 mmHg) 1.75[(95% CI) 1.19-2.56; p=0.004], Diastolic blood pressure; OR - 1.59[1.16-2.17; p=0.004], Mean arterial Pressure OR- 1.5 [1.14 – 1.96;p=0.003], total cholesterol ; OR(per 1 mmol)- 1.37[1.06-1.77; p=0.01] and Systolic blood pressure; OR- 1.24 [1.04-1.47;p=0.01] were significant parameters predicting presence of STR. Pulse Pressure; OR per 10 mmHg- 1.16 [0.94 - 1.44;p=0.16] was not significant
For the older onset: RPP; OR-1.49 [1.22 – 1.8; p < 0.001] Diastolic blood pressure; OR -1.36 [1.17-1.59; p< 0.001] and Mean arterial Pressure; OR-1.28 [1.13 – 1.46; p< 0.001] and Systolic blood pressure; OR- 1.13 [1.05-1.23; p=0.01] were significant parameters predicting presence of STR .Total cholesterol was not significant in the older onset group- OR per 1 mmol; 1.14 [0.98 – 1.33;p=0.07]
Conclusion: Our data suggest that for patients with younger onset of diabetes total cholesterol, Retinal perfusion pressure, diastolic blood pressure and mean arterial pressure, and for the older onset of diabetes Retinal perfusion pressure, diastolic blood pressure and mean arterial pressure, are important modifiable risk factors for STR.This highlights the importance of controlling blood pressure and hypercholesterolemia in patients with diabetes who already have retinopathy. These data will help formulate a clinical strategy to reduce progression to STR.

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Markers of inflammation are associated with microvascular complications and cardiovascular disease in Type 1 diabetes - the EURODIAB Prospective Complications study.
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Background and Aims: The pathogenesis of vascular complications in type 1 diabetes is poorly understood, but may involve chronic, low-grade inflammation. We investigated whether markers of inflammatory activity are associated with microvascular complications and cardiovascular disease in type 1 diabetes.
Material and Methods: A nested case-control study from the EURODIAB Prospective Complications Study of 543 (278 men) European individuals with type 1 diabetes diagnosed < 36 years of age. Cases (n=348) were those with one or more complications of diabetes, controls (n=195) were all those with no evidence of any complication. We determined levels of C-reactive protein, interleukin-6 and tumor necrosis factor-α, which were combined in a inflammatory marker Z-score, and investigated their associations with albuminuria, retinopathy and cardiovascular disease by use of ANOVA.
Results: Measures of inflammation were associated with albuminuria, retinopathy and cardiovascular disease. Calculated means (95% confidence intervals) of the inflammatory marker Z-score were -0.15 (-0.22 to -0.07), 0.10 (-0.05 to 0.25), and 0.28 (0.15 to 0.41), p for trend <0.0001, in individuals with normo-, micro- and macroalbuminuria; -0.23 (-0.33 to -0.13), 0.14 (0.02 to 0.25) and 0.20 (0.07 to 0.32), p for trend <0.0001, in individuals with no, non-proliferative and proliferative retinopathy; and -0.28 (-0.39 to -0.18) and 0.06 (-0.08 to 0.20), p <0.001, in individuals without and with cardiovascular disease. These associations were of a similar order of magnitude as the association of HbA_{1c} with vascular complications, and were independent of age, sex, HbA_{1c}, duration of diabetes and systolic blood pressure. In addition, there was evidence that the associations of triglycerides with albuminuria and of HDL cholesterol and body mass index with retinopathy, were, in part (up to 55%), mediated by inflammatory activity. The advanced glycation end product, pentosidine, was associated with albuminuria, but this appeared not to be mediated by inflammatory activity.
Conclusions: We have shown that markers of inflammation are strongly and independently associated with microvascular complications and cardiovascular disease in type 1 diabetes, and may mediate, in part, the effects of triglycerides, HDL cholesterol, and body mass index on vascular complications. These data suggest that strategies to decrease inflammatory activity may help to prevent the development of vascular complications in type 1 diabetes.

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Advanced Glycation Endproducts (AGEs) predict worsening of diabetic retinopathy in Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) participants.
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Non-enzymatic glycation of proteins is considered to be one likely mechanism whereby hyperglycemia causes diabetic complications. Advanced glycation endproducts (AGEs) in collagen from skin biopsies were measured near the end of the DCCT and were found to be associated with diabetic retinopathy (DR) in the same subjects. Four years after DCCT

closeout DR has been assessed again by fundus photography in 85 of 92 DCCT conventional treatment group participants from the skin biopsy study. Significant worsening of DR was defined as 3 step progression on the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale or scatter photocoagulation. Mean ± S.D. of collagen characteristics are presented.

DR worsening

	Yes	No	p
N	24	61	-
Furosine*	1149 ± 220	852 ± 192	0.0000
Carboxymethyllysine*	664 ± 140	550 ± 140	0.001
Pentosidine*	30.4 ± 7.5	26.8 ± 5.3	0.009
Pepsin soluble,* %	4.3 ± 2.1	6.0 ± 2.5	0.0009
Acid soluble,* %	0.4 ± 0.4	0.5 ± 0.3	0.24

*pmoles/mg collagen

In a multivariate logistic regression model with DR progression as the dependent variable, furosine, carboxymethyllysine, HbA1c at DCCT closeout, and diabetes duration were all independent predictors of DR progression ($p < .05$), each explaining 5 – 7% of the total variation. Conclusion: Abnormalities in skin collagen due to glycation predict worsening of DR over 4 years, independent of HbA1c and type 1 diabetes duration. This observation strengthens the view that glycation of tissue proteins contributes to DR.

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Parental insulin resistance and microvascular complications in Type 1 diabetic patients: the GENESIS France Belgium Study.

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Background and Aims: Insulin resistance may be a risk factor for diabetic microangiopathy. We examined through a family-based study which components of insulin resistance are associated with diabetic retinal (DR) and renal (DN) complications.
Materials and Methods: The GENESIS France Belgium Study aimed to search for genetic factors involved in type 1 diabetes (T1D) microvascular complications using a family-based design. Index subjecs were T1D patients (age at diabetes onset < 36, and insulin therapy within 1 year after diagnosis) with DR (simplex / pre proliferative / proliferative), classified as no DN : normoalbuminuria, without ACE-inhibitors and diabetes duration >19 years ; incipient DN (microalbuminuria) ; established DN (clinical proteinuria) ; advanced DN (plasma creatinine > 150 µmol/l or renal replacement therapy). Nuclear families were trios (index + both parents) or pseudo-trios (index + one parent + at least one sibling). In relatives BMI and blood pressure (BP) were measured and we collected personal medical history and all current medications using a structured questionnaire. An insulin resitance score was computed according to BMI (0= BMI<25 ; 0.5=BMI : 25-29.9 ; 1=BMI>30 Kg/m^2), hypertension (0=no ; 1=history of or BP>140/80), history of lipid disorder (0=no ; 1=yes) and history of T2D (0=no ; 1=yes).
Results: We recruited 241 trios and 39 pseudo-trios from 150 index patients with no DN (144 mothers, 126 fathers and 41 siblings), 64 with incipent DN (61 mothers, 59 fathers and 7 siblings), 39 with established DN (32 mothers, 27 fathers and 21 siblings) and 37 with advanced DN (32 mothers, 27 fathers and 16 siblings). Index patients had simplex DR in 120 cases, pre proliferative in 57 and proliferative in 113 cases. T1D was found in 35 relatives.
In index subjects, DN and DR stages were related to systolic BP ($p<0.0001$ and $p=0.015$) but not BMI ($p=0.24$ and $p=0.11$, respectively). In relatives without T1D from index with established or advanced DN, albumin excretion rate (AER) was higher ($p=0.01$ and $p=0.03$, respectively) compared to relatives from index with no DN. There was no difference for AER of non T1D relatives according to the index DR stage. In non T1D relatives, the insulin resistance score was correlated with AER ($p=0.0007$) and was higher in those relatives from index with DN than in those from index with no DN ($p=0.048$). Similarly, the higher this score in non T1D relatives, the more severe the DR stage in index ($p=0.031$). However, the components of the insulin resistance score of non T1D relatives were different according to DR or DN in index. Obesity and hypertension in relatives were associated with DR severity in index, but not with DN while history of dyslipidaemia in relatives was more common in index with DN but not DR.
Conclusion: We conclude that insulin resistance segregates with the risk of both renal and retinal complications : dyslipidaemia in relatives is related to DN and hypertension/obesity to DR.

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Devices

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Evaluation of a non-invasive, continuous glucose monitoring system in patients with diabetes: results of a glucose clamp study with hypoglycemic and hyperglycemic excursions.

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Background and Aims: A completely non-invasive, continuous glucose monitoring system is still considered as the ultimate target of glucose sensor development. We present the data of a newly developed, non-invasive glucose sensor whose glucose measurements are performed on the basis of impedance spectroscopy. Previous evaluations of this system had already demonstrated a reliable performance at euglycemic and hyperglycemic glucose levels. The aim of this study was to evaluate the performance of the sensor, which is as small as a wristwatch, under controlled conditions at hypoglycemia and at rapid changes from hyperglycemia to hypoglycaemia, or vice versa.
Material and Methods: Fifteen patients with type 1 diabetes (age 27±5.7 years (mean±SD), body mass index 23.1±2.3 kg/m^2, HbA_{1C} 6.9±0.8 %) participated in glucose-clamp experiments, each lasting for 8 hours. Their blood glucose (BG) was kept constant by means of a Biostator at three different target concentrations (45, 100, and 200 mg/dl) for at least 30 min at each level. In addition to the Biostator BG measurements, venous BG was measured by a standard laboratory system every 5 – 10 min. Hematology and clinical chemistry laboratory parameters (e.g. hematocrit, sodium, potassium) were measured in regular intervals. Sensors were applied to the subject's wrist. The glucose sensor signals recorded (frequency, impedance) were temperature corrected and calibrated, with reference values taken from each BG level.
Results: An excellent correlation between changes in BG and the sensor recordings was observed. The overall correlation was 0.932 with a standard error of prediction of 20 mg/dl. The Clarke Error Grid analysis of all 15 patients shows 81% of the values being in zone A, 15% in zone B and 4% in zone D.
Conclusion: These data show a reliable performance of this novel, completely non-invasive glucose sensor for continuous glucose monitoring during hypo- and hyperglycemic excursions.

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Therapy adjustments based on CGMS data lower HbA1c with less hypoglycemia than blood glucose meter data alone.

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Background and Aims: The DCCT reported that an increased risk of hypoglycemia was associated with the percent reduction in HbA1c from baseline values. The purpose of this study is to demonstrate that therapy adjustments based on CGMS (Medtronic MiniMed) data improve glycemic control without increasing the incidence or duration of hypoglycemia when compared to therapy adjustments based on self-monitoring of blood glucose (SMBG) alone.
Materials and Methods: In a randomized multi-center study, 128 subjects with Type 1 diabetes were assigned to insulin therapy adjustments based on either CGMS or SMBG values. CGMS values were used as an adjunct to frequent SMBG in the CGMS group. In Week 12, subjects in both groups wore the CGMS for 3-days, these values were used to calculate measures of hypoglycemia (sensor glucose ≤60 mg/dL). Repeated measures ANOVA with post-hoc comparisons were used to test differences in HbA1c and low sensor glucose readings between study groups. Significance was established at P<0.05.
Results: There were no significant differences between age (44.2±11.5 years), gender (65% female), duration of diabetes (20±11.3 years), reported frequency of hypoglycemia (1.9 vs. 2.0 episodes/week), or method of insulin delivery (46% Pump Therapy, 54% Multiple Daily Injections) between the two groups. There were no significant differences in HbA1c between the CGMS and SMBG groups at baseline (9.07±1.07 vs. 8.99±0.97%; P=0.68) and both groups showed significant (P<0.001) and similar (P=0.95) improvement in HbA1c after 12 weeks of study (change=(-0.73%)). However, the CGMS group displayed significantly fewer sensor readings ≤60 mg/dL than the SMBG group (6±7% vs. 11±11%; P=0.05) at Week 12 of the study. The CGMS group also had a significantly shorter duration of hypoglycemia then the SMBG group both at night (43.8±84.4 vs. 94.5±122.6 minutes; P=0.04) and overall (49.4±40.8 vs. 81.0±61.1 minutes; P=0.02).
Conclusion: The DCCT results indicate an increased risk of hypoglycemia with therapy adjustments based on frequent SMBG. Our results demonstrate that CGMS-guided therapy adjustments can be used to quickly reach treatment goals while reducing the incidence and duration of hypoglycemia in patients with Type 1 diabetes.

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The influence of risk factors on hypoglycaemia in Type 1 diabetes assessed by continuous subcutaneous glucose monitoring.

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Background and Aims: Recent studies applying continuous glucose monitoring have indicated that patients with type 1 diabetes are more exposed to hypoglycaemia than known from conventional self-monitoring. The aim of this study was to evaluate the influence of known risk factors on occurrence of hypoglycaemia assessed by continuous subcutaneous glucose monitoring.
Materials and Methods: Seventy-six patients with type 1 diabetes underwent a 6-day continuous subcutaneous glucose monitoring with the MiniMed Continuous Glucose Monitoring System (CGMS). Hemocue blood glucose determinations were used to optimise calibration. Participants completed a detailed diary documenting all meals and snacks, insulin doses and episodes with symptoms of hypoglycaemia. Endpoints were number and total duration of episodes with subcutaneous glucose <3.5 mmol/l for at least 10 minutes and number and total duration of episodes with subcutaneous glucose <2.2 mmol/l for at least 10 minutes. Hypoglycaemic episodes were classified as unrecognised, mild (recognised and managed independently) or severe (needing assistance from others).
Results: During a total valid monitoring period of 372 days, 468 episodes with glucose <3.5 mmol/l were recorded in 74 (97%) subjects and 233 episodes with glucose <2.2 mmol/l were recorded in 60 (79%) subjects. Overall, 15.1% of time was spent at glucose <3.5 mmol/l and 5.9% at glucose <2.2 mmol/l. HbA1c was negatively associated and self-estimated state of awareness of hypoglycaemia tended to be negatively associated with total rate of episodes with glucose <3.5 mmol/l (p<0.001 and p=0.056, respectively). HbA1c and state of awareness were negatively associated with rate of unrecognised episodes with glucose <3.5 mmol/l (p=0.046 and p=0.031, respectively) but neither of the two variables were related with rate of episodes with glucose <2.2 mmol/l (p=0.10 and p=0.16, respectively). Women had a higher rate of recognised episodes with glucose <3.5 mmol/l (p=0.021) but there was no relationship between sex and any other endpoint. Age, duration of diabetes and C-peptide status were not related with rate of episodes with glucose <3.5 or <2.2 mmol/l.
Conclusion: We conclude that conventional risk factors are related with occurrence of subnormal glucose but not with profound biochemical hypoglycaemia assessed by CGMS.

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The Telemetered Glucose Monitoring System (TGMS) alerts patients to hypo- and hyperglycaemia and reduces glycaemic excursions.

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Background and Aims: The TGMS (Medtronic MiniMed) is a multi-component, continuous glucose-monitoring device that alerts patients to hypo- and hyperglycemic events. The purposes of this study were to show that use of these real-time alarms reduces glycemic excursions and to demonstrate the accuracy of the TGMS under conditions of normal daily activity.

Materials and Methods: Subjects wore 4 sensors over a period of 2 weeks. Alarms were turned off during Week 1 and on during Week 2. The alarm settings were 70 mg/dl for hypoglycemic events and 250 mg/dl for hyperglycemic events. Frequency, magnitude and duration of events with alarms turned off and on were compared using Wilcoxon signed-rank test. Sensors were worn for 48 hours and calibrated at least twice daily using the Glucometer DEX (Bayer). Additional self-monitoring of blood glucose (SMBG) to evaluate sensor performance was performed. For analysis, SMBG values were lagged 5 to 10 minutes and paired with pre-calibration sensor values. Agreement of sensor-SMBG pairs and sensitivity and specificity of hypo- and hyperglycemic alarms were analyzed.
Results: This study provided TGMS data for 16 subjects (56% female, 41±15 years old, 75% Type 1 diabetes), wearing 61 sensors, for a total of 105 days of device experience. The median sensor survival time was 47.3 hours. In Week 2 (alarms on), the average duration of a hypoglycemic event was significantly reduced by 41 minutes (p=0.02) and the area under the curve (AUC)-low was significantly decreased by 0.4 mg/dl*day (p=0.01). Conversely, the median number of hyperglycemic events in Week 2 was significantly increased by 1 event (p=0.008), yet the average duration and the AUC-high remained unchanged from Week 1. The sensor readings agreed with paired SMBG values with correlation=0.89, regression=10.05+(X)0.83, and mean absolute difference=18.5±16.6%. Clarke error grid analysis yielded 98.0% of sensor-SMBG pairs within zones A & B. The hypoglycemic alarm effectively distinguished glucose values ≤70 mg/dl with 78% sensitivity and 90% specificity and the hyperglycemic alarm detected sensor values ≥250 mg/dl with 58% sensitivity and 99% specificity. Receiver operated characteristic curve analysis indicated optimal detection of hypoglycemia was achieved with the alarm set only 5 mg/dl higher than the desired detection threshold.
Conclusion: We conclude that the TGMS reliably and accurately alerts users to hypo- and hyperglycemia in real-time, thus reducing the duration and extent of hypoglycemic events. The positive results of this clinical study suggest that once commercially available, the TGMS will have immeasurable value in managing excessive daily variation in glycemic control and may become an optimal means for detecting life-threatening events under high-risk situations.

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Intranasal delivery system enabling effective insulin absorption for the treatment of post-prandial hyperglycemia.
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Background and Aims: To develop a promising insulin delivery system as an alternative to subcutaneous injection, a number of investigations into alternative routes of insulin delivery are being vigorously performed. Especially, several promising pulmonary and buccal insulin delivery systems are at a late stage of clinical development. Our focus is on intranasal delivery, which enables more effective absorption of insulin for the treatment of post-prandial hyperglycemia in diabetic patients. The aim of this study is to investigate the effects of porous spherical calcium carbonate (PS-$CaCO_3$) and specific microcrystalline cellulose (MCC) as powder drug carriers on intranasal insulin absorption in cynomolgus monkeys and healthy humans.
Materials and Methods: Each intranasal insulin formulation prepared by mixing insulin with PS-$CaCO_3$ or MCC was loaded into a capsule. The formulation in the capsule, was administered intranasally using an intranasal administration device (BIOACTIS Ltd.). Serum insulin and glucose levels after administration were evaluated. A four-week repeated dose toxicity study of PS-$CaCO_3$ formulation including examination of local irritability has been conducted in monkeys.
Results: Serum insulin after intranasal delivery (16 IU/monkey) with PS-$CaCO_3$ and MCC attained maximum concentrations of 403.5 μU/mL (at 0.17 hours) and 449.4 μU/mL (at 0.33 hours), respectively, and returned to the endogenous level within 2 hours. Insulin absorption after intranasal administration of each powder formulation was found to be more rapid and shorter in duration than that after subcutaneous administration. Insulin absorption after intranasal administration with MCC was sustained in comparison with that of PS-$CaCO_3$, resulting from the prolonged residence time of MCC formulation in the nasal cavity. In repeated intranasal administration of insulin for 4 weeks, toxicity and local irritability were not observed even when administered at a maximum dose level of 25 IU/monkey. Furthermore, intranasal insulin delivery with PS-$CaCO_3$ and MCC in healthy humans also showed rapid absorption, similar to that seen in the animal studies. The formulations with PS-$CaCO_3$ and MCC achieved relative bioavailabilities of approximately 10 and 20% without any absorption enhancers in healthy humans, respectively, at a dose level of 16 U/body.
Conclusion: Our intranasal insulin delivery system using MCC is proposed as a highly convenient method of treating post-prandial hyperglycemia, and it enables effective insulin absorption, similar to endogenous post-prandial insulin secretion in healthy humans. Additionally, we believe that a pocket size and user-friendly intranasal administration device will reduce the patient's burden of insulin self-medication.

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Inhaled insulin treatment compliance by 46 patients using AERx® iDMS Insulin Diabetes Management System.
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Background and Aims: The objective was to demonstrate the ability of patients to use AERx® iDMS (Insulin Diabetes Management System) to deliver mealtime inhaled insulin doses and explore preliminary data on the importance of compliance for glycaemic control.
Materials and Methods: AERx® iDMS was evaluated in a 12-week, multicentre open trial with 107 type 2 diabetes patients currently taking insulin. Patients were randomised to treatment with inhaled insulin using AERx® iDMS or fast-acting human insulin injections, both before main meals and in combination with bedtime NPH insulin. AERx® iDMS recorded the date and time of each insulin inhalation, insulin units used, and inhalation technique precision. Data from 46 patients (mean age 59.0±7.8, duration of diabetes 11.0±7.6 years) who used AERx® iDMS were reviewed. Compliance was defined as the percentage of prescribed doses taken during the treatment period (83.6±6 days, range 74-100, 235±24 doses, range 186-282).
Results: Mean compliance with inhaled insulin was 93.8±12.3%. Mean percentage of missed doses was 6.2±12.3% including one patient who omitted 78% of doses, or 4.4±17.3% excluding that outlier. Overall, 44 of 46 patients took >80% of prescribed doses (two poor compliers took 22%, 57% of doses). Six patients omitted all doses on ≥1 day. Mean daily insulin dose was 31.5±12 (range 5-59) units. Patients with compliance rates ≥90% (N39) achieved mean 0.77±1.01% decreased HbA_{1c} levels. Six patients whose HbA_{1c} decreased by ≥2% were excellent compliers (≥95%). The HbA_{1c} level increased 0.6% for the patient with the poorest compliance rate (22%). Few patients experienced poor inhalation technique (≤5 doses, <2.5% of doses overall), with only 2 patients experiencing >10 dosing episodes with inadequate extrusion volume.
Conclusion: These preliminary data demonstrate that patients using AERx® iDMS can achieve excellent compliance with a mealtime dosing regimen of inhaled insulin. High rates of compliance with insulin inhalation suggest that AERx® iDMS is an acceptable and convenient system for self-administration of insulin leading to improved glycaemic control. The electronic compliance monitoring feature could in the future provide clinicians and patients with a tool that could give valuable information about patient dosing regimens and compliance and this could also potentially be used to discuss and understand achieved metabolic control.

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Safety in continuous intraperitoneal insulin infusion CIPII via DiaPort®: results from the DiaPort-001-study.
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Background and Aims: The DiaPort-001-Study is a European, multinational, prospective, randomised, crossover, controlled open phase II study comparing efficacy and safety of continuous intraperitoneal insulin infusion CIPII via DiaPort® system (Disetronic, Switzerland), to continuous subcutaneous insulin infusion CSII with Lispro insulin in type 1 diabetic patients. This analysis focuses on device-related adverse events AEs during CIPII.
Material and Methods: DiaPorts® were implanted in 51 type 1 diabetic patients (23 female, 28 male) not sufficiently controlled by CSII. Mean age of the patients was 50 years (min. 24 - max. 73). 12 months of CIPII were compared to six months of CSII. The DiaPort® is a percutaneous titanium port which is connected to an external hand-held insulin pump and delivers insulin into the peritoneal cavity via an exchangeable polyethylene / polyurethane catheter.
Results: In 41,1 patient-years (pt-yr) of CIPII there were no serious device-related adverse events SEAs, and 55 non-serious device-related AEs. In 10

patients these AEs subsequently ended in termination of CIPII, the absolute majority of terminations occurring in the first 6 months of CIPII. The device-related AEs could be classified as external infections, abdominal pain and irreversible under-delivery of insulin (intraabdominal overgrowth of catheters, catheter occlusions or encapsulations): The incidence of infections was 0,58 per pt-yr (leading to 3 terminations of CIPII), abdominal pain 0,44 per pt-yr (leading to 1 termination), insulin under-delivery 0,29 per pt-yr (leading to 6 terminations). 4 patients (8% of all patients) accounted for 14 AEs (25% of all AEs), and 14 patients (28%) for 34 AEs (62%). Concerning all AEs there was no significant difference between male and female patients. Significant differences between sexes could be found in infections (75% in male, p=0,026) and abdominal pain (61% in female, p=0,043).
Conclusion: CIPII using the DiaPort® system is followed by a moderate number of device-related AEs which seem to accumulate in relatively few patients. The most frequent AE is external infection which rarely leads to termination of CIPII, whereas under-delivery of insulin (catheter obstruction) is the least frequent AE, but leads very often to termination of CIPII. Female patients suffer more often of abdominal pain, whereas male patients are more susceptible to external infections. Patient selection, education, and future strategies to improve lifetime of CIPII should be influenced by these findings.

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Sustained safety and accuracy of central IV glucose sensors connected to implanted insulin pumps and short-term closed-loop trials in diabetic patients.
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Background and Aims: Accurate, long-term, continuous online blood glucose monitoring is expected to improve glucose control in insulin-treated diabetic patients. We investigated safety and accuracy of oxygen-based enzymatic glucose sensors, that were implanted in the vena cava superior and connected to implanted insulin pumps as a platform for artificial beta-cell.
Materials and Methods: Fifteen sensors were implanted in 10 type 1 diabetic patients (5M/5F, age : 44-65 years, diabetes duration : 7-50 years) with a cumulated experience of 11.8 patient-years. At the time of present analysis, data per sensor had been collected for an average duration of 287 days (range: 89 - 431). Accuracy of each sensor was assessed by comparing sensor data with fingerstick blood glucose measurements from an average number of 1738 paired-points (range: 599-4524).
Results: No clotting incident or venous trauma occurred at any time. Both average and per sensor correlations between sensor data and fingerstick measurements were tight : r = 0.85, range : 0.73-0.90. Mean absolute deviation of sensor data was 18.1 % (range : 15.4-33.0) and % paired-points in (A+B) zones of Clarke error-grid was 95.8 % (range : 88.7-98.3). Fully-automated 48 hour-closed-loop insulin delivery driven by sensor signal could reduce % time below 70 mg/dl from 18 to 6 and above 240 mg/dl from 17 to 2, vs. sensor data recorded during previous week. Adding manual pre-meal bolus could suppress all deviations outside range : 70-240 mg/dl.
Conclusion: These data show safety and accuracy of long-term blood glucose monitoring using venous sensors and provide favourable perspectives for clinical use of closed-loop systems in type 1 diabetic patients.

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Beta Cell Pathophysiology

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Specific down regulation of insulin mRNA levels in INSIE cells expressing an insulin-targeted siRNA.
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Background and Aim: Elevated levels of circulating glucose are normalized within physiological range by the action of insulin released from β-cells. Concurrently, transcription of several genes involved in glucose metabolism is increased in response to insulin secretion while insulin gene expression is stimulated to replenish cellular stores of the hormone. Recent reports suggest that secreted insulin rather than glucose is the predominant factor modulating gene transcription and β-cell survival in an autocrine feedback loop. However, this hypothesis has been challenged and to date there is no clear consensus on the role of insulin in regulating secretion and transcription of target genes. In order to evaluate the direct contribution of glucose and insulin on β-cell function and survival as well as the potential paracrine effect of insulin on other islet cell types, we have developed a novel RNA interference (RNAi) strategy to suppress insulin levels.
Material and Methods: Two 21-nucleotide insulin hairpin RNA structures with either a 6 or 9-nucleotide loop (siINS6 and siINS9) were cloned into the newly developed RNAi pDLDU6 vector and transfected into INS1E cells along with GFP by lipofectamine. Subsequent to cell sorting using GFP (72 hours post-transfection), the effects of RNAi on endogenous insulin protein levels were analyzed by immunofluorescence and quantified in cellular extracts. Cell viability was measured qualitatively by DAPI staining. Steady state mRNA levels for insulin, GLUT2, GK, PDX1, PAX4, were quantified by real time PCR.
Results: Insulin steady state mRNA levels were inhibited by 80% in INSIE cells co-expressing GFP and either siINS6 or 9. Repression was specific since mRNA levels for GLUT2, GK, PDX1 and PAX4 remained constant. A single point mutation in siINS6 still repressed by 20% insulin mRNA levels indicating that one base pair substitution is not sufficient to completely abolish inhibition. GFP-negative cells stained brightly for insulin while no staining was detected in GFP-positive cells. Total insulin content was reduced by 60% in GFP+ cells. Insulin depletion had no apparent detrimental effect on cell viability over 10 days. Electron microscopy is presently being performed to determine whether these cells contain insulin granules.
Conclusion: Our results demonstrate that the vector-based expression of siRNAs is a powerful tool to specifically repress endogenous insulin levels in INSIE cells without altering viability. More importantly, we can now address the direct contribution of glucose and insulin on β-cell function.

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Lipid synthetic transcription factors SREBPs activate insulin gene promoter.
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Background and Aims: A possible role of de novo lipid synthesis in the lipotoxicity for pancreatic β-cells has not been fully understood. As an initial step to study this issue, potential effects of sterol regulatory element binding proteins (SREBPs), lipid synthetic transcription factors, on insulin gene expression were investigated.
Materials and Methods: Promoter analysis for a rat insulin I promoter was performed using transfection studies in non-β-cells (HEK293 and HepG2 cells) with a luciferase reporter gene system. Expression plasmids encoding nuclear human SREBP isoforms (SREBP-1a, -1c or -2) and rat β-cell-specific factors(PDX1 and BETA2) were co-transfected.
Results: In luciferase assays, any of SREBP isoforms (SREBP-1a, -1c and -2) markedly up-regulated the activity of the insulin promoter (-715 bps containing all known functional *cis*-elements) to the extent equal to that by co-expression of both PDX1 and BETA2, the established β-cell-specific insulin transactivators. In the sequence of the rat insulin I promoter, three

potential SRE sites (SRE1, SRE2 and SRE3) were newly identified. The SRE1 and SRE2 were located adjacent to E1 and E2 box, respectively. Sequential deletion studies supported these three SREs all contributed to the activation. Binding of SREBP proteins to these elements were confirmed by EMSA analysis. Intriguingly, the SREBP-1c activation of the insulin promoter was further enhanced by the presence of BETA2, but not PDX1. Through the extensive mutation analysis for different SRE and E box domains, it was found that especially both E2 box and SRE1 are crucial for the synergistic effects of SREBP/BETA2. In similar studies for rat insulin II and human insulin promoters, activation by SREBP-1c alone and PDX1+BETA2 were also observed, but not SREBP-1c/BETA2 synergism.
Conclusion: Our results demonstrate that SREBPs have potent transcriptional activities for insulin gene promoters in non-β-cells. Synergistic activation of insulin promoter by SREBP-1c/BETA2 suggests potential formation of a complex of these factors with co-activators on the two *cis*-elements. Although these novel effects of SREBPs need to be estimated in β-cells, the transactivity of SREBP-1c for insulin gene could be involved in the complex pathophysiology of β-cell specific lipotoxicity.

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Endogenous insulin is essential to prevent glucose-induced apoptosis in islet β-cells.
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Background and Aims: Insulin and IGF-1 receptors are ubiquitously expressed in most mammalian cells including the pancreatic islet cells. To examine the role of the insulin and IGF-1 receptors in β-cells we and other laboratories have created β-cell-specific knockouts. For example, we have previously shown that β-cell specific insulin receptor (IR) knockout (βIRKO) mice show blunted acute phase insulin secretion and age-dependent hypoplastic islet growth, while mice with a β-cell-specific deletion of the IGF-1 receptor showed no alteration in β-cell mass even up to age 12 months. The aim of the present study was to further understand the role of insulin/IGF-I signaling in regulating β-cell growth, apoptosis and gene expression.
Materials and Methods: We have established SV40-transformed β-cell lines by crossing the βIRKO and wild-type (WT) mice with mice expressing the SV40 'T' antigen on the rat insulin promoter. We have used the cell lines and primary islets derived from the knockouts to examine alterations in insulin/IGF-I signaling, cell growth and apoptosis and evaluated gene expression by Affymetrix arrays and semi-quantitative RT-PCR.
Results: Interestingly, βIRKO cells were observed to grow slowly compared to WT cells, and cell cycle analysis by flow cytometry revealed an apoptotic peak only in the βIRKO fraction (βIRKO 3.1% vs WT 0.5%, n=3, $p<0.05$). Further, insulin and IGF-I were able to rescue serum-starvation-induced apoptosis in WT but not in βIRKO cells (βIRKO 0.5% vs WT 45% rescue, n=3, $p<0.05$). Examination of the IGF-I/insulin signaling pathway, revealed insulin-stimulated tyrosine phosphorylation of IR and insulin receptor substrate-1 (IRS-1) leading to activation of PI 3-kinase and Akt in WT cells which was absent in the mutants. Similarly, glucose also activated PI 3-kinase and Akt following IR/IRS phosphorylation in WT cells, but not in βIRKO cells. Further, glucose-induced apoptosis was greater in the βIRKO cells suggesting that endogenous secretion of insulin normally protects β-cells from apotosis. At the molecular level, glucose failed to increase cleaved caspase 3 in WT cells, corresponding to increased Akt activity, while a robust increase was detected in βIRKO cells, consistent with the absence of Akt activation.
Conclusions: These data indicate that apoptosis is enhanced in β-cells lacking the insulin receptor and suggest that glucose-induced apoptosis is normally prevented by the autocrine anti-apoptotic effects of endogenous insulin. These findings have implications for insulin resistance in β-cells with consequent poor cell growth and anti-apoptotic activity of insulin leading to hypoinsulinemia and hyperglycemia, which in turn worsens the apoptosis and β-cell hypoplasia.

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The role of caspase-3 in diabetes induction.
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Background and Aims: Type 1 diabetes mellitus is an autoimmune disease whereby inappropriately activated T cells target pancreatic insulin-producing beta cells to undergo a highly regulated mode of cell death known as apoptosis or programmed cell death. Caspases are the major molecules involved in apoptosis and caspase-3 is a major effector caspase. Therefore, caspase-3 may play a critical role in immune-mediated pancreatic beta cell destruction during diabetes development.
Materials and Methods: Caspase-3 knockout (KO) mice are used in a multiple low dose streptozotocin (MLDS)-induced diabetes model. Caspase-3 (+/-) and (-/-) mice are injected with streptozotocin (STZ, 40 mg STZ/kg body weight) and tail vein blood glucose levels are monitored weekly.
Results: There is a lower incidence of diabetes in caspase-3 (-/-) mice when compared to littermate controls following MLDS. Histological analysis of mouse pancreata isolated three months post STZ injection showed that the islets of caspse-3 (-/-) mice were intact whereas there were only traces of islets in the pancreata of caspase-3 (+/-) mice. In an attempt to capture earlier STZ-induced apoptotic events occurring in caspase-3 (+/-) and (-/-) mice, the mice were sacrificed 2 weeks post-STZ injection. Pancreatic sections from these mice were stained with terminal deoxynucleotidy transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) to depict apoptotic cells. The results showed that many of the islets of caspase-3 (+/-) mice displayed TUNEL positive nuclei, whereas no TUNEL reactivity was observed in caspase-3 (-/-) pancreata. Islet viability was also examined by other means including hormone staining. Caspase-3 (+/-) islets were observed to have lower levels of insulin staining in their islets compared to caspase-3 (-/-). In addition, the islets from caspase-3 (+/-) displayed an increased proportion of glucagons stained cells. The decreased insulin staining and increased glucagons staining reflects the destruction of the β islet cells and perhaps an attempt in islet regeneration. Furthermore, lymphocyte infiltration was examined on H&E sections of caspase-3 (+/-) and (-/-) pancreata after MLDS. Interestingly, there was more lymphocyte infiltration in the pancreata of caspase-3 (+/-) mice than caspase-3 (-/-) mice after MLDS-induction. On those sections where insulitis was observed, CD3 staining confirmed that the infiltrating cells were indeed lymphocytes.
Conclusion: These data suggest that caspase-3 plays a critical role in mediating islet apoptosis. Thus, therapeutically targeting caspase-3 may have implications in developing strategies to prevent type 1 diabetes.

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Identification of IFN-γ and double-stranded RNA (dsRNA) induced genes in pancreatic β-cells by high-density oligonucleotide arrays.
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Background and Aims: Type 1 diabetes mellitus (T1DM) is an autoimmune disease caused by a progressive destruction of pancreatic β-cells. Both viral infections and local production of the cytokines IFN-γ and IL-1β during insulitis contribute to the pathogenesis of T1DM. dsRNA accumulates in the cytosol of viral–infected cells, and we have previously shown that exposure of purified rat β-cells to dsRNA (tested in the form of polyinosinic-polycytidylic acid, PIC) in combination with IFN-γ results in β-cell dysfunction and apoptosis after 6-9 days. To elucidate the molecular mechanisms involved in PIC + IFN-γ-induced β-cells apoptosis, we presently determined the general pattern of PIC, IFN-γ and PIC + IFN-γ-modified genes in primary rat pancreatic β cells.
Materials and Methods: FACS-purified rat β-cells (a pool of 1.4 x 10^6 cells/condition) were cultured for 6 or 24 hours, either in control condition or in the presence of IFN-γ (1000U/ml), PIC (100 µg/ml) or a combination of both agents. The gene expression profile was analysed in duplicate by high-density oligonucleotide array („Gene Chip", Affymetrix) representing 5000 full-length genes + 3000 EST's. Changes of > 2.5-fold were considered as significant.
Results: Following a 6 h treatment with IFN-γ, PIC or IFN-γ + PIC, we observed changes in the expression of respectively 89, 86 and 165 genes. Following a 24 h treatment with IFN-γ, PIC or IFN-γ + PIC there were changes in the expression of respectively 51, 60 and 189 genes. The PIC and/or IFN-γ responsive genes were clustered in 15 groups, according to the putative biological function of their encoded proteins. After treatment with IFN-γ alone (for 6 and 24 hours), the most frequent changes were observed in β-cell metabolism, protein processing, cytokines and signal transduction, representing respectively 12.7, 13.1 and 12.8% of all modified genes; after 24 hours exposure to the cytokine the MHC-related genes represented 31.4% of all modified genes. Exposure to PIC alone affected preferentially the expression of genes related to cell adhesion (13.9%), cytokines and dsRNA signal transduction (11.4%), transcription factors (13.3%) and

MHC (14.9%). Exposure of β-cells to IFN-γ+PIC led to a combined pattern of the modifications described above. Genes with modified expression and found of special relevance belong to the following groups : metabolism and NO formation (↑GLUT-1, ↑GLUT-2, ↑iNOS, ↑arginase and ↑AS), hormones and growth factors (↓insulin; ↓GIP receptor), cytokines and chemokines (↑IL-15, ↑MIC-1, ↑CINC-1, ↑MIP-3α, ↑Mob-1, ↑TNF-β, ↑RANTES), cell adhesion (↑ICAM-1), transcription factors (↑c-jun, ↑c-myc, ↑IRF-1, ↑IRF-7), defense/repair (↑or↑ hsp-70, ↑Gas-6, ↑MnSOD, ↑HO), ER stress (↑GADD65) and anti-viral responses (↑PKR, ↑Mx3).
Conclusion: We have presently identified several of the key „gene patterns" induced by dsRNA and/or IFN-γ in primary rat β-cells. This allows us to propose a model, to be presented at the meeting, for the signalling pathways leading to β-cell dysfunction and death following exposure to PIC + IFN-γ.

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Overexpression of regenerative, antioxidant and inflammatory genes in adult diabetic GK islets.
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Background and Aims: In the adult GK rat, a spontaneous model of type 2 diabetes, total pancreatic β-cell number is decreased by 60%. This alteration cannot be ascribed to increased β-cell apoptosis but is related to decreased β-cell replication. Moreover, the adult GK pancreas exhibits large islets disrupted by connective tissue. Our objective was therefore to identify genes possibly involved in the β-cell growth phenotype in adult diabetic GK rat. Differential gene expression was evaluated in islets of adult diabetic GK and normal Wistar (W) rats by high density oligonucleotide microarray.
Materials and Methods: Total RNA was extracted from 16 week-old W and GK islets. Biotin-labeled cRNA probes were synthesized and hybridized to Affymetrix RG-U34A oligonucleotide microarrays containing approximately 7,000 rat genes. The arrays were scanned and expression values for the genes were determined using Affymetrix Microarray Suite 5.0 and Affymetrix Data Mining Tool 2.0. Expression pattern of reg in GK pancreas was determined by immunohistochemical analysis.
Results: No difference in expression levels of genes encoding growth factors for β-cells (GH, IGF1, PDGF, HGF, insulin) and the c-Myc transcription factor was found in Wistar and GK rats. By contrast, several reg-related genes were up-regulated in the GK islets (46-, 12- and 11-fold respectively for reg II, reg I and reg III genes). These results were confirmed by real-time PCR. Expression of several stress genes such as glutathione peroxidase (2.7-fold) , thioredoxine interating protein (5.4-fold) and heat shock protein 70 (2.3-fold) were increased in GK islets. Islets from GK rats also display increased expression of inflammatory genes such as lipocalin 2 (72.8-fold), decorin (4.1-fold) and annexins 1 and 2 genes (8.7- and 2.1-fold). To localize the reg-I protein in pancreas we stained sections of GK and W pancreases. Immunoreactivity was observed only in islets. Reg staining was colocalized with staining for insulin but did not seem restricted to the β-cells.
Conclusion: Our data suggest that in GK islets 1) the increased expression of reg family genes whose involvement in β-cell regeneration has been proposed, may be an integral part of the β-cell growth phenotype of GK islets; 2) the lack of increased apoptosis of the β-cells may be related to activation of several stress genes that confer protection against β-cell death; 3) the increased expression of cytokine genes could be related to a local inflammatory response in GK islets; 4) the local inflammatory gene overexpression together with the cytokine environment in GK islets may have an influence on reg gene expression. Our working hypothesis is, that these expression changes reported for the first time in GK islets could represent an acquired adaptation in response to chronic hyperglycemia (glucotoxicity). To learn what genes are more directly involved in the pathology of the GK rats, we are currently analyzing the gene expression profile in young prediabetic GK rats.

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Evidence against direct cytotoxicity of islet amyloid *in vivo*.
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Background and Aims: The islet in type-2 diabetes (TTDM) is characterized by ~70% loss in beta cell mass, increased beta cell apoptosis and islet amyloid derived from islet amyloid polypeptide (IAPP). Some studies suggest that IAPP oligomers (intermediate between IAPP monomers and islet amyloid) cause beta cell toxicity, while others support the notion that islet amyloid per se is cytotoxic.
Materials and Methods: To address this, we studied obese (Avy agouti) mice that were transgenic for human IAPP (TGO, n=46) versus similarly obese non transgenic mice (NTO, n=46) prospectively from age 10-40 weeks by measuring beta cell mass, beta cell apoptosis (TUNEL), islet amyloid and blood glucose.
Results: TGO mice developed diabetes at ~20 weeks of age ($p<0.05$), due to a deficit in beta cell mass (83% by 40 weeks, $p<0.01$). The deficit in beta cell mass was due to an 11 fold increase in beta cell apoptosis ($p<0.01$) in TGO vs NTG mice. TGO mice developed islet amyloid that preceded hyperglycemia, but the extent of islet amyloid did not correlate with the frequency of beta cell apoptosis (10 weeks of age, apoptosis was maximal but islet amyloid minimal; 40 weeks of age, islet amyloid was maximal but apoptosis was minimal) However apoptosis did correlate with the increment in islet amyloid in the preceding 10 week interval ($r=0.83$, $p<0.01$).
Conclusion: These data imply that islet amyloid per se does not cause beta cell apoptosis. However, as the rate of formation of islet amyloid was correlated with the frequency of beta cell apoptosis, these data are consistent with the concept that a precursor of islet amyloid may cause islet amyloid.

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Identification of multiple domains that participate in the fibrillogenesis and cytotoxicity of Human Islet Amyloid Polypeptide (hIAPP).
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Background and Aims: Human islet amyloid polypeptide (hIAPP) is the major component of amyloid deposits found in the pancreas of over 90% of all cases of type-2 diabetes. The presence of islet amyloid has been associated with a loss of β-cell mass and function due to IAPP-mediated apoptosis of β-cells. However, the factors leading to the buildup of amyloid as insoluble fibrous deposits are still unknown. Since the development of amyloid fibrils follows a defined conformational change of soluble IAPP from a random coil to β-sheet, we generated a series of overlapping hexapeptides to target two amyloidogenic regions of hIAPP (residues 20-29 and 8-20) and examined their effects on β-sheet formation and fibrillar assembly.
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. AlamarBlue was added directly to cells, and the fluorescence was measured once daily for a period of 6 days.
Results: Circular dichroism spectroscopy revealed two peptides, SNNFGA and GAILSS, targeting hIAPP 20-29 that were strong inhibitors of β-sheet formation and amyloid aggregation. Negative stain electron microscopy revealed that co-incubation of these peptides with IAPP resulted in the loss of the typical high density and morphology of IAPP fibrils. Peptide SNNFGA not only prevented the conformational change associated with IAPP fibril formation, but results from toxicity assays demonstrate that this peptide was also a strong inhibitor of IAPP-mediated cell death. In a similar analysis of hexapeptides designed to target hIAPP 8-20, we identified two peptides, LANFLV and FLVHSS which were not inhibitory, but rather accelerated fibril formation. Experiments were performed to demonstrate that these peptides can „seed" or initiate fibrillogenesis by full-length IAPP. Data from co-incubation studies illustrate that the addition of these peptides accelerated fibrillogenesis of IAPP compared to IAPP incubated alone. However, neither peptide LANFLV or FLVHSS had any effect on IAPP-mediated cytotoxicity suggesting that neither peptide interacts with the domain involved in IAPP-mediated cytotoxicity.
Conclusion: The IAPP 20-29 domain has been proposed to be the critical amyloidogenic component of the human IAPP molecule. However, the data presented here suggests the presence of an additional domain of hIAPP (8-20) that may play a role in regulating IAPP fibril formation. This data lends support to the hypothesis that interactions between multiple β-sheet domains may be involved in the fibrillogenesis of human IAPP. In addition, we have identified peptide sequences which inhibit normal fibrillar assembly by hIAPP, and which may be used in the future as part of a therapeutic approach to prevent the formation of IAPP amyloid in patients with Type II Diabetes.

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Mechanisms of Vascular Injury in Diabetes

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Multistep resistance at the nitric oxide/cGMP pathway level in vascular smooth muscle cells from Zucker fatty rats: potential role in the pathogenesis of arterial hypertension in insulin resistance.

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Background and Aims: Insulin resistant states show an high prevalence of arterial hypertension: a role could be played by defects in arterial vascular smooth muscle cell (VSMC) function. Nitric oxide (NO) exerts its vasodilating effect by stimulating VSMC guanylate cyclase with production of cGMP.We previously demonstrated that human VSMC express a constitutive NO synthase (cNOS). In the present study, we aimed at investigating whether aortic VSMC from the insulin resistant Zucker fatty rats (fa/fa) show abnormalities in the insulin ability to increase NO synthesis and/or in the NO ability to increase cGMP synthesis: i.e.,defects at the cNOS and/or at the guanylate cyclase levels.
Materials and Methods: In cultured aortic VSMC from fatty Zucker (fa/fa) rats and from lean Zucker (fa/+) rats we measured the ability of insulin to stimulate NO synthesis, detected as L-citrulline production from L-arginine since cNOS produces NO and citrulline equimolecularly, and the ability of the NO donor sodium nitroprusside (SNP) to stimulate cGMP synthesis,measured by radioimmunoassay.
Results: i) NO synthesis (pmol/min/mg protein) after incubation with 2 nmol/l insulin for 5 min rose from 0.07±0.01 to 0.12±0.001(p=0.0001) in VSMC from Zucker lean rats and remained unchanged (0.06±0.001 and 0.06±0.001) in VSMC from Zucker fatty rats; ii) cGMP concentrations (pmol/mg protein) after incubation with 2 nmol insulin for 60 min rose from 0.47±0.01 to 1.08±0.07 (p=0.0001) in VSMC from Zucker lean rats and remained unchanged (1.30±0.07 and 1.32±0.08) in VSMC from Zucker fatty rats; iii) cGMP concentration (pmol/mg protein) after 60 min incubation with 0.1 mmol/l SNP rose from 0.47±0.01 to 1.57±0.03 (p=0.0001) in VSMC from Zucker lean rats and from 1.30 ±0.07 to 1.75±0.1 (p=0.0001) in VSMC from Zucker fatty rats: in particular, SNP induced a 332.83±10.57% increase of cGMP concentrations in Zucker lean rats, and 134.33±6.05% in Zucker fatty rats (p=0.0001). In both groups of rats, the insulin-induced increases of NO and of cGMP were blunted by the phosphatidylinositol 3-kinase (PI-3K) inhibitor wortmannin (200 nmol/l) and the insulin-induced increase of cGMP was blunted by the NOS inhibitor L-NMMA (1 mmol/l).
Conclusion: This study provides one of the first demonstrations of the impairment of the PI-3K pathway of insulin signalling in VSMC in insulin resistant states, and demonstrates that in a classical animal model of insulin-resistance VSMC are resistant to the insulin ability to increase NO (resistance at the cNOS level) and to the NO ability to increase cGMP (resistance at the guanylate cyclase level).These defects could be involved in the complex pathogenesis of arterial hypertension in insulin resistance.

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Atherogenic role of lysophosphatidylcholine in low-density lipoprotein modified by phospholipase A2 and in diabetic patients: protection by nitric oxide donor.

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Background and Aims: Oxidized low-density lipoprotein (LDL) plays a key role in the development and progression of atherosclerosis. During oxidation of LDL, there is extensive conversion of phosphatidylcholine (PC) to lysophosphatidylcholine (lyso-PC), which is catalyzed by phospholipase A2 (PLA2). The aim was to investigate the atherogenic role of lyso-PC in LDL and the protective effect of nitric oxide (NO) donor under diabetic environment.
Materials and Methods: Lyso-PC contents in LDL were measured using electrospray ionization-liquid chromatography/mass spectrometry. Expression of monocyte chemoattractant protein-1 (MCP-1) mRNA and NF-kB-DNA binding activity were determined in human umbilical vein endothelial cells (HUVEC) incubated with native or glycoxidized LDL, LDL modified by venom PLA2 and LDL isolated from diabetic patients and control subjects (n=8, respectively), using Northern blot and EMSA methods. 4-ethyl-2-hydroxyimino-5-nitro-3-hexenamide (NOR3) was used as a NO donor.
Results: Lyso-PC contents were higher in glycoxidized LDL and PLA2-treated LDL compared with native LDL (native LDL, palmitoyl-lyso-PC 4.31±1.09 μg/mg LDL protein, stearoyl-lyso-PC 3.02±0.54 μg/mg LDL protein; glycoxidized LDL, palmitoyl-lyso-PC 10.43±1.36 μg/mg LDL protein, p<0.05, stearoyl-lyso-PC 5.93±0.81 μg/mg LDL protein, p<0.05; PLA2-treated LDL, palmitoyl-lyso-PC 40.80±6.74 μg/mg LDL protein, p<0.01, stearoyl-lyso-PC 39.33±7.66 μg/mg LDL protein, p<0.01). Glycoxidized LDL and enrichment of lyso-PC by PLA2 treatment resulted in up-regulation of MCP-1 mRNA expression through increased NF-kB activity in HUVEC. Palmitoyl- and stearoyl-lyso-PC contents correlated with MCP-1 expression and NF-kB activity, respectively. Moreover, LDL isolated from diabetic patients contained more lyso-PC than that from nondiabetic subjects (nondiabetics, palmitoyl-lyso-PC 4.53±0.14 μg/mg LDL protein, stearoyl-lyso-PC 3.82±0.23 μg/mg LDL protein; diabetics, palmitoyl-lyso-PC 5.68±0.33 μg/mg LDL protein, p<0.01, stearoyl-lyso-PC 4.47±0.11 μg/mg LDL protein, p<0.05). Diabetic LDL induced higher MCP-1 mRNA expression and NF-kB activity in HUVEC. Palmitoyl- and stearoyl-lyso-PC contents correlated with MCP-1 expression and NF-kB activity, respectively (palmitoyl-lyso-PC, r=0.72, p<0.01; r=0.59, p<0.05; stearoyl-lyso-PC, r=0.62, p<0.01; r=0.59, p<0.05). Preincubation with 100μM of NOR3, a NO donor, abrogated increased expression of MCP-1 mRNA and high NF-kB activity induced by PLA2-treated LDL and by LDL isolated from diabetic patients.
Conclusion: We have demonstrated that LDL isolated from patients with diabetes mellitus contained more lyso-PC than that from nondiabetic subjects, and induced greater MCP-1 mRNA expression and NF-kB activity in HUVEC. Our results suggest that lyso-PC contents in LDL play an important role in atherogenesis under diabetic condition, which could be prevented by increased availability of vascular NO.

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Prior activation of the AMP-activated protein kinase by AICAR or exercise induces a cardioprotective effect against ischemic injury of the heart.

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Background and Aims: Cardiovascular disease is the main cause of mortality in patients with diabetes, and risk reduction is an important goal in the treatment of diabetes. Regular exercise is known to reduce ischemic heart disease, and recent animal studies have demonstrated that exercise protects the heart against experimentally induced myocardial infarction 24 hours later. However, the underlying molecular mechanisms are not yet fully understood. AMP-activated protein kinase (AMPK) is an important enzyme concerning glucose metabolism. It is activated in response to conditions of metabolic stress such as ischemia and during exercise. The adenosine analog AICAR (5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside) is a potent activator of AMPK and is able to mimic metabolic changes seen after exercise. The purpose of this study was to determine whether a single bout of exercise or a single injection of AICAR were capable of protecting the myocardium against experimentally induced ischemia 24 hours after the intervention, and to assess whether the AMPK system might be involved.
Materials and Methods: Wistar rats (~ 300 g) were allocated into three groups: an exercise group trained on a treadmill for 1 hour at 30 m/min (5% gradient), an AICAR group subcutaneously injected with AICAR (0.7 mg/g body wt.), and a sedentary control group. 24 hours later hearts were Langendorff-perfused and subjected to 45 min. left main coronary artery occlusion followed by 120 min. reperfusion. Infarct size was determined by tetrazolium staining and expressed as a percentage of the risk zone (I/R%). Isoform specific AMPK-alpha2 activity and phosphorylation (phospho-AMPK (Thr172)) were measured immediately after either 60 min. treadmill run or 30 min. after a single AICAR injection. The amount of glycogen was also determined 24 hours after the intervention.
Results: Infarct size was significantly reduced in the AICAR treated group (I/R%: 17±3% vs. 44±5%, $p < 0.001$, n=10) and exercise group (I/R%: 23±4% vs. 44±5%, $p < 0.05$, n=10) compared to the control group. A single

injection of AICAR or 60 min. of treadmill run resulted in a significant increase in AMPKalpha2 activity and phospho-AMPK (Thr172) expression in the hearts investigated ($p<0.05$, n=11). 24 hours after the intervention the amount of glycogen was increased about 20% in the AICAR treated rats and about 30% in the exercise trained rats.
Conclusion: A single bout of intensive treadmill exercise or a single injection of AICAR significantly reduces the infarct size seen after a coronary artery occlusion 24 hours after the intervention. The AMP-activated protein kinase enzyme system may be an important signal mediator of this protective response, and might thus be a new pharmacological target for reducing cardiovascular mortality.

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Biological actions of C-peptide on cultured human aortic smooth muscle cells.
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Aim: Hyperglycemia and hyperinsulinemia have been known to induce the proliferation of vascular smooth muscle cells (SMCs), which is associated with the acceleration of atherosclerosis in the early stage of diabetes. C-peptide, which is a 31-amino acid peptide that is cleaved in the processing of proinsulin to insulin, was considered to have no biological actions for decades. But recently, several reports revealed that the short-term administration of C-peptide reduces the glomerular filtration and increases glucose utilization in type 1 diabetic patients. C-peptide has a part of insulin-like action mechanisms but the main action mechanism of C-peptide is thought to be different from that of insulin. Here we reporte the novel effects of C-peptide in cultured SMCs.
Methods: Cultured human aortic smooth muscle cells (hSMCs) were purchased from Kurabow (Osaka, Japan). Fourth passage cells were used for the following experiments. 1) The phosphorylation of p42/p44 MAP kinase and Akt; SMCs were stimulated by C-peptide or insulin for indicated peirod. The phosphorylation of p42/p44 MAP kinase and Akt were measured by western blot analysis. 2) The stimulation of Rho/ Rho-kinase; After the stimulation by C-peptide or insulin for indicated period, the translocation of Rho to membrane fraction was measured by western blot analysis. The phosphorylation of myosin-binding subunit of myosin phosphatase (MBS), a substrate for Rho-kinase, was measured by the specific antibody for the phosphorylation of MBS.
Results: 1) C-peptide and insulin significantly increased the phosphorylation of p42/p44 MAP kinase by 5.9 fold and 3.1 fold, 5.6 fold and 3.1 fold, respectively ($P<0.01$). C-peptide significantly decreased the phosphorylation of Akt, whereas insulin significantly increased the phosphorylation of Akt by 2.6 fold. 2) C-peptide signigicantly increased the translocation of Rho to membrane fraction by 2 fold. C-peptide also significantly increased the phosphorylation of MBS. On the other hand, insulin did not affect Rho/ Rho-kinase.
Conclusion: In hSMCs, it has been first reported that C-peptide activates p42/44 MAP kinase and Rho/Rho-kinase pathway, of which mechanisms are different from those of insulin. These results suggest the affirmative association of C-peptide in the development of diabetic macroangiopathy.

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Aminoguanidine ameliorates diabetes-associated atherosclerosis in Apolipoprotein-E KO mice.
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Background and Aims: Diabetes is associated with an increased risk of vascular complications, including atherosclerosis, however the mechanisms underlying this are still not fully understood. Hyperglycaemia is known to contribute toward the non-enzymatic glycation and oxidation of proteins and lipids resulting in the irreversible formation of advanced glycation endproducts (AGE), a process previously shown to be accelerated in diabetes. A previous short-term study showed a reduction in diabetes-associated atherosclerosis after therapy with soluble RAGE, the receptor for AGEs. Aminoguanidine is a compound that blocks the formation of AGEs and has been previously shown to retard the development of diabetic nephropathy and retinopathy. Thus, the aim of this study was to examine the long term effect of aminoguanidine therapy on diabetes-associated atherosclerosis in the diabetic Apo-E knockout mouse.
Materials and Methods: Diabetes was induced in Apo-E KO mice by injection of streptozotocin (55mg/kg) at 6 weeks of age over 6 consecutive days. Diabetic mice received either aminoguanidine (1g/kg/day) or no treatment for 20 weeks. Non-diabetic Apo-E KO mice served as controls.
Results: Diabetes was associated with a 5-fold increase in plaque area when compared to non-diabetic ApoE mice. The increased atherosclerosis was ameliorated by aminoguanidine therapy. A similar increase in CTFG gene expression was observed (measured by real time RT-PCR) in the aorta which was significantly reduced by aminoguanidine. Aminoguanidine therapy had no effect on body weight, or total cholesterol when compared to the untreated diabetic mice.
Conclusion: These findings demonstrate a role for AGEs in the development of diabetes-associated atherosclerosis and suggest a therapeutic role for aminoguanidine in the treatment of diabetes-associated vascular disease. Further studies are required to examine the mechanisms by which AGE influence plaque formation but these may involve the cytokine CTGF.

Data expressed as mean ± SEM. * p<=0.5 vs control group; # p <=0.5 vs diabetic group

	Apo-E Control	Apo-E Diabetic	Apo-E diabetic + Aminoguanidine
Body weight (g)	32 ± 1	21 ± 1*	22 ± 2*
HbA1c (%)	3.3 ± 0.3	13.5 ± 0.3*	12 ± 0.5*
Total Chol (mM)	15 ± 1	38 ± 2 *	32 ± 1*
Total Plaque area (%)	4.0 ± 0.4	23.2 ± 1.4*	12.7 ± 1.1*#
CTGF gene expression (fold induction)	1 ± 0.2	10.47 ± 3.2*	4.79 ± 1.02*#

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Vascular actions of anti-diabetic thiazolidinediones.
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Background and Aims: Diabetes is associated with accelerated development of vascular disease, partially due to the pathological effects of hyperglycemia. Thiazolidinediones (TZDs) are the newest agents for the treatment of hyperglycemia in Type 2 diabetes. The sub-endothelial retention of low density lipoproteins (LDL) through their interactions with vascular proteoglycans has been proposed by Williams and Tabas in 1995 to be a key process in the pathogenisis of atherosclerosis. The proliferation of vascular smooth muscle cells is also a known response to arterial injury. We investigated the direct vascular actions of TZDs on atherogenic properties in vascular smooth muscle cells (VSMC).
Materials and Methods: We investigated the anti-proliferative activity of troglitazone (TRO), rosiglitazone (ROS) and pioglitazone (PIO) towards VSMC derived from the vascular beds used for coronary artery by-pass grafting - the internal mammary (IMA) and radial artery (RA) and saphenous veins (SV). We also investigated the effects of thiazolidinediones on proteoglycan and GAG chain synthesis in human VSMC. Proteoglycans synthesized by IMA VSMC were labelled with ^{35}S-sulphate and ^{35}S-methionine/cystine and analyzed by size through SDS-PAGE gel (4-15% acrylamide) and size exclusion chromatography.
Results: All three TZDs showed inhibitory potency towards cell proliferation with a very similar potency (TRO > ROS ~ PIO) in each vascular preparation. Maximum inhibition was approximately 50% and TRO showed half-maximal inhibition activity between 1 and 3µM. ROS and PIO only showed inhibition at very high concentrations (30 and 100µM). TRO, ROS and PIO all inhibited basal proteoglycan synthesis at 10, 30 and 30µM respectively ($P<0.05$), while SDS-PAGE showed slight reductions in size. All three TZDs also inhibited proteoglycan synthesis stimulated by the atherogenic growth factors PDGF-BB ($P<0.05$) and TGF-β_1 ($P<0.001$) in the same concentrations. When cells were treated with xyloside to investigate GAG chain synthesis, TRO significantly inhibited synthesis while ROS and PIO had no significant affect. SDS-PAGE gels indicated that TRO produced slightly smaller proteoglycans and GAG chains in comparison to control, where as ROS and PIO did not.
Conclusion: The inhibitory potency of clinical TZDs towards proliferation of cells from different vascular sources is dependent upon the individual TZDs and very little on the vascular source.The reduction in proteoglycan size indicates structural modification and potentially reduced binding ability to atherogenic lipoproteins. This data suggests that direct vascular effects may contribute to the anti-atherosclerotic actions of TZDs.

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Structural changes of glycosaminoglycans from human vascular smooth muscle, induced by the PPAR-α ligand, fenofibrate.

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Background and Aims: Fenofibrate, a PPAR-α ligand, is indicated for the treatment of hypertriglyceridemia in patients with Type 1 and Type 2 diabetes. In addition to beneficial effects on lipids, similar agents like gemfibrozil have been shown to reduce heart disease in clinical trials. We investigated whether or not fenofibrate has direct vascular actions on smooth muscle cells (SMCs) and specifically looked for effects on proteoglycan biosynthesis. Modified extracellular matrix secretion by atherogenic growth factors such as transforming growth factor (TGF)-β1 predisposing to increased lipoprotein retention has been proposed as a key event in the atherogenic process. We have previously shown that gemfibrozil has anti-atherogenic effects on proteoglycan structure which may result in less lipoprotein binding.
Materials and Methods: Human SMCs were derived from explants of the internal mammary artery. Serum deprived confluent SMC cultures were treated with fenofibrate (0-50 μM) or the metabolic derivative fenofibric acid (0-300 μM) in the presence or absence of atherogenic growth factors, transforming growth factor (TGF)-β1 and platelet derived growth factor (PDGF). Glucose utilization and lactate production was determined to assess cellular activation. Glycosaminoglycans (GAGs) were assessed by [^{35}S]-sulfate and core proteins by [^{35}S]-methionine/cysteine labeling. Proteoglycans were assessed by the cetylpyridinium chloride (CPC) precipitation method and were sized on SDS-PAGE.
Results: Fenofibrate (50 μM) and fenofibric acid (100 μM) increased glucose utilization by >60%, indicating cellular activation via PPAR-α. Fenofibric acid decreased proteoglycan core protein synthesis by 10% ($p<0.05$), in the presence and absence of TGF-β1 (1 ng/ml). Fenofibrate (50 μM) reduced sulfate incorporation by 21.8% ($p<0.05$), 28.6% ($p<0.05$) and 30.6% ($p<0.01$) under basal, TGF-β1 and PDGF conditions, respectively. The changes in sulfate incorporation were associated with a reduction in size of proteoglycans. Fenofibric acid (300 μM) showed similar reductions in sulfate incorporation with 14.6% ($p<0.05$), 32.6% ($p<0.05$) and 10.6% ($p<0.05$) reduction under, basal, TGF-β1 and PDGF conditions respectively and a decrease in size of proteoglycans. Fenofibrate (30 μM) reduced the relative size of proteoglycans in a time dependent manner, reaching a maximum effect on size after 36 hours of treatment.
Conclusion: We conclude that fenofibrate and its derivative, fenofibric acid, reduce GAG length and speculate that this would lead to reduced LDL binding consequently contributing to a reduction in atherosclerosis.

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Regulation of human vascular smooth muscle proteoglycan biosynthesis by biguanides, sulfonylureas and glitazones.

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Background and Aims: Diabetes is associated with high levels of ischaemic vascular disease leading to strokes, heart attacks, amputations and impotency. Therapy aims to reduce hyperglycaemia with the objective of reducing vascular disease. We are investigating the actions of anti-diabetes drugs in an in vitro model of atherogenesis with a view to understanding and characterising their anti-atherogenic potential. The model is based on the „response to retention“ hypothesis and thus the binding of atherogenic lipoproteins to vascular smooth muscle derived proteoglycans (PG).
Materials and Methods: Experiments were conducted in human vascular smooth muscle cells prepared from saphenous veins excess to cardiac surgery requirements. We assessed ^{35}S-SO_4 incorporation into glycosaminoglycan (GAG) chains and quantitated PG production by the CPC precipitation method. SDS-PAGE was used to determine the apparent size of the PGs. We assessed the effects of drugs on basal and TGFβ (2 ng/ml) stimulated PG production.
Results: The sulphonylureas, tolbutamide (1, 3, 10 μM) and chloropropamide (10, 30, 100 μM) had no effect on ^{35}S-SO_4 into PGs. The biguanides, metformin and phenformin (both 10, 30, 100 μM) caused concentration dependent inhibition of PG synthesis in the absence as well as presence of TGFβ with the effect of phenformin being twice that of metformin at the equivalent concentration. Metformin and phenformin did not reduce the size of the PGs and thus the reduction in ^{35}S-SO_4 incorporation was not due to GAG shortening. Troglitazone (1, 3 10 μM) and pioglitazone (10, 30, 100 μM) also caused concentration dependent inhibition of PG biosynthesis which we have previously associated with GAG shortening.
Conclusion: There is little clinical evidence that sulfonylureas or biguanides reduce vascular disease whereas emerging evidence suggests that glitazones may have direct vascular actions to prevent lipid deposition and atherosclerosis. We have determined that only glitazones cause GAG shortening which results in reduced binding affinity to LDL. We speculate that the intriguing inhibition of PG synthesis by biguanides is due to inhibition of core protein biosynthesis and not GAG shortening. Our results indicate that the in vitro model of atherogenesis based of pharmacologically induced changes in PG biosynthesis may be useful for the evaluation of the vascular actions of new and emerging agents targeting vascular disease in diabetes.

OP 19

Epidemiology and Genetics of Complications in Type 1 Diabetes Mellitus

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Incidence of childhood Type 1 diabetes worldwide. 1990-1999.

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Background and Aims: The WHO Multinational Project for Childhood Diabetes (DiaMond) started in 1990. The objective was to investigate and monitore the patterns in incidence of type 1 diabetes in children aged 15 years and under up to the year 2000. Population-based registries are used to collect standardized data on incidence. The success of this project depends on close cooperation among the participating centers and a standardized approach to data collection. An overview of the observations and trends for the period 1990-1994 within the DiaMond framework was presented in Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J, for the WHO DiaMond Project Group: Incidence of childhood type 1 diabetes worldwide Diabetes Care 23:1516-1526, 2000. Now we are reporting the incidence rates for the years 1995-1999 as well as the trends for the whole period 1990-1999.
Material and Methods: The WHO DiaMond Incidence Data Center located at the Diabetes and Genetic Epidemiology Unit of the National Public Health Institute in Helsinki, Finland, has served as the coordinating center for the DiaMond incidence study. Each of the over 100 participating centers is headed by a local principal investigator who is responsible for data collection and other aspects of a field work. The denominator for the analysis was children under 15 years of age with residency in the study area totaling over 75 million. The numerator comprises over 35000 cases diagnosed with type 1 diabetes over the period 1990-1999 in the WHO DiaMond study areas. Age-standardized incidence rates were calculated per calendar year and 100,000 individuals at risk as well as for the whole study period. Age adjustment was done over 5-year intervals (0-4, 5-9 and 10-14). The 95% CI's were estimated assuming the Poisson distribution of the cases. An average yearly change (%) was estimated for each center assuming exponential trend. Also the male-to-female excess incidence ratio together with the corresponding 95% CI was calculated.
Results: The overall age-adjusted incidence rates of type 1 diabetes vary from 0.1/100 000 in China to 40.5 in Finland, i.e. the variation in the world remains very large. Relative levels of incidence in various countries have remained the sameThe observed average yearly trends also exhibit a high degree of variation and are negative for some centers (for example -2.84% for Gafsa (Tunisia) and -1.08% for Puerto Rico (USA)).
Conclusion: The global pattern of the incidence of type 1 diabetes mellitus is now covered in a more detailed way. The trends in individual countries may vary markedly partly due to a relatively short monitoring period. Although the incidence is on average increasing worldwide, the growth is not necessarily uniform. WHO DiaMond data project allows to monitor such trends in various populations from all over the globe.

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A 24-year follow-up of the incidence of Type 1 diabetes in northern Finland: evidence for the existence of hot and cold spots.

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Background and Aims: Our previous observations have indicated that there are differences in the incidence of type 1 diabetes in 0-14-year-old children between various communities in the province of Oulu in Northern Finland. However, over short time periods these differencies seemed not to be temporally stable, a fact probably influenced by the relatively small number of newly-diagnosed cases and people living in this area. To find out whether there are true and persistent local differences in the incidence rates we have now collected incidence data from all municipalities in the province of Oulu during an extended time period of 24 years (1978-2001).
Materials and Methods: The incidence rates are based on the registry of the National Social Insurance Institution and the diabetes registry at the Department of Pediatrics, University of Oulu.
Results: The average total population aged 0-14 years in the province of Oulu in 1978-2001 was 80,314 and the mean annual incidence of type 1 diabetes was 37.2/100,000 in this population. A gradual increase in the incidence was observed over time (31.5/100,000 for 1978-1985, 37.1/100,000 for 1986-1993 and 42.9/100,000 for 1994-2001). A hot spot comprising two municipalities (average population aged 0-14 years 3,831) in the southern part of the province was found, and also a cold spot comprising three municipalities in the northwestern part of the province (average population aged 0-14 years 2,787) was confirmed. The mean annual incidence of type 1 diabetes among children aged 0-14 years in the hot spot area was 53.3/100,000 (95% CI 38.4–68.2) and 22.4/100,000 (95% CI 11.1–33.8) in the cold spot area.
Conclusion: This study based on incidence data over 24 years suggests that there are local differences in the incidence of type 1 diabetes in Northern Finland. Further studies on genetic and environmental factors are needed to explain these variations.

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High incidence of both autoimmune and non-autoimmune diabetes in Swedish county of Kronoberg 1998-2001 with new WHO diagnostic criteria and serological classification.

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Background and Aims: Reports of increasing incidence of diabetes mellitus (DM) led us to estimate the current incidence of DM in our region, according to the new diagnostic criteria based on fasting blood glucose (FBG) and with diabetes classification by serology.
Materials and Methods: All new cases in the adult population, >/= 18 years, of Kronoberg were registered prospectively for 3 years, May1998 – April2001. Autoantibodies, ICA and GADA, and C-peptide were analyzed for classification of diabetes type. Diagnosis of diabetes was defined according to the criteria adopted by WHO 1998 of FBG values of >/=6,1 mmol/L on at least 2 occasions, or one single value of >/= 11,1 mmol/L. All 25 primary health care centers and the two county hospitals participated. The population of Kronoberg was 177 149 on dec 31, 1999. 138 292 were >/= 18 years.
Results: 1694 new cases were registered. The total incidence of DM was 408/100 000 inhab+year. Blood samples were available from 1655 cases or 97,7%. Of these 105, 6,4 % had autoimmune (Ai) DM, that is were positive(pos) to at least one antibody. 1550, 93,6 % had nonautoimmune (NonAi), antibodyneg DM. Of the 105 antibodypos 95, 90,5%, were pos for GADA; 75, 71,4%, were pos for ICA and 65, 61,9%, were pos for both GADA and ICA. 30, or 28,6%, were pos for only GADA and 10, 9,5%, were pos for only ICA. The incidence of Ai DM was 25,5/100 000 + yr, and of NonAi DM 374 / 100 000 + yr. If the group of antibodyneg with low C-peptide, < 0,25 nmol/L is grouped together with the antibodypos to estimate the incidence of Type 1 DM, the incidence is 28,4/100 000 + yr. The remaining antibodyneg with a normal or high C-peptide, >/= 0,25 nmol/L then represent Type 2 DM with an incidence of 371/100 000 + yr. If the pediatric cases of the 3 years, registered in the pediatric department and adding up to 50, all classified as Type 1, are added to the Type 1 DM group, and calculated for the whole population, the incidence of Type 1 DM, age 0-105, was 29,4 /100 000. Median body mass index (BMI) for the AI group was 26,0 kg/m2(range 16,0-46,4); for the NonAi group 28,0 kg/m2 (range 15,5-62,6). The Ai group had a median C-peptide of 0,64 nmol/L, the NonAi group 1,20nmol/L.
Conclusion: The incidences of both autoimmune and nonautoimmune diabetes registered are higher than previously reported from Scandinavia. Both new diagnostic criteria and a true increase in incidence are likely contributors to the high incidence. A good ascertainment of cases in the geographic area might also contribute. Neither group of adult newly diagnosed diabetics have a median BMI under 26 kg/m2. The nonautoimmunes have a median C-peptide level at diagnosis above the upper reference limit, and the autoimmunes have a median C-peptide level well within the reference interval. These later findings suggest a connection between the high incidence of diabetes and the documented increased BMI in the general population. 85% of autoimmune diabetes is diagnosed above age 40, and clinical parameters are not sufficient to accurately classify diabetes type.

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Was the increase in the incidence of IDDM seen in black youngsters in the 1990's secondary to misclassification?

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Background and Aims: It has been suggested that the increase in the incidence of insulin dependent diabetes mellitus (IDDM) reported in Black youth in the early 1990's across the US was the result of inclusion of children with type 2 diabetes (non-autoimmune). Consistent with this hypothesis, we previously reported an increase in the prevalence of obesity, a characteristic of type 2 diabetes, during that period in youngsters diagnosed with IDDM.
Materials and Methods: In order to explore this issue further, we evaluated the absence of conventional β-cell autoantibodies (ICA on human pancreas, insulin, GAD65A, IA-2A and IAA) in a group of Blacks (B) and Whites (W) < 19 years of age diagnosed with insulin-treated diabetes between 1980 and 1998 from the Children's Hospital of Pittsburgh IDDM Registry. Statistical analysis included chi-square test.
Results: The table shows the percentage without antibodies by race and period.

Period Race	1980-84 (n=16B/17W)	1985-89 (n=32B/35W)	1990-94 (n=21B/26W)	1995-98 (n=43B/43W)	p-value
Blacks (n=112)	12.5	25	42.8	43	0.22
Whites (n=121)	5.8	11.4	23	9.3	0.28
Total (n=233)	9.1	17.9	31.9	24.6	0.07

Overall, the prevalence of being negative for β-cell autoantibodies showed an almost significant trend towards an increase over time, from the early 1980's to early 1990's, with a slight decrease in the late 1990's. Blacks had a higher prevalence of absent antibodies than Whites which reached statistical significance in the last period (p=0.014). Insulin deficiency as denoted by ketosis was present in 51% of those without antibodies (37% of Whites and 55% of Blacks).
Conclusions: These data suggest that non-type 1A diabetes may have possibly accounted for part of the increase in the incidence of IDDM seen in Black children more than Whites in the early 1990's.

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Sexual dimorphism in the risk for developing insulin treated diabetes among relatives to diabetes patients diagnosed between 15 and 34 years of age.

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Background and Aims: Type 1 diabetes results from destruction of b cells in the endocrine pancreas. Since the patient is unable to produce sufficient amounts of insulin daily injections with insulin are required. In contrast to the well established observations of an approximately 10% risk for diabetes among first-degree relatives to diabetic children, the risk for diabetes among first-degree relatives to patients with adult type 1 diabetes has not been determined. It has previously been observed for childhood onset type 1 diabetes that the risk to develop type 1 diabetes for offspring to type 1 diabetic fathers is higher than for offspring to type 1 diabetic mothers. The protective effect of the mother with type 1 diabetes is not understood and thus we wanted to test the hypothesis that the protective effect was reduced with increasing age of the index case. We also wanted to determine if the life-time risk for insulin treated diabetes may increase with increasing age at onset.
Materials and Methods: It was possible to test these hypotheses by sending a questionnaire to all patients in the Diabetes Incidence study in Sweden (DISS) registry. The DISS registry registers all patients between 15 and 34 years of age when diagnosed with diabetes in Sweden. In the present study we analyzed in total 4466 patients, reported to DISS 1982 - 1993. Comparison of frequencies between groups were made using chi-square tests. Analysis of survival times was carried out using the LIFETEST procedure in the SAS computer program package. The product-limit or Kaplan-Meier method was used as recommended. The log-rank test was used to test whether the observed difference between the survival curves was due to random variation or not. Confidence intervals were calculated as 95% confidence intervals as described.
Results: Among the 3087 index patients treated with insulin 17.8% (95%, CI 16.5-19.2) had a first-degree relative (excluding offspring) treated with insulin, the frequency being higher among female (19.8%) than male (16.5%, p<0.02) patients. A total of 10.7 % had a parent treated with insulin. The prevalence of insulin treated diabetes was higher among parents to female (12.5%) than to male (9.5%), insulin treated index patients (p<0.004). A similar difference was observed using lifetable analysis (p<0.003). The frequency of insulin treated diabetes among fathers was higher if the index case was diagnosed between 25 -34 (7.8%) than between 15-24 years of age (4.6%, p<0.003). Among insulin treated index patients 8.4% had a sibling with insulin treated diabetes the risk for siblings to develop insulin treated diabetes being 2.7% (2.3-3.1%) by 14 years of age.
Conclusion: We suggest that a female in the 15-34 age group need more susceptibility genes to develop diabetes than males and hence might carry more diabetic genes and therefore more of their relatives also develop diabetes**.**

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Diet, growth and the risk of Type 1 diabetes in childhood.

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Background and Aims: Rapid growth, larger body size and more frequent dietary intake of certain nutrients have been associated with increased risk of Type 1 diabetes. Given a positive association between the body size and energy intake there is a need to simultaneously consider the influence of both factors for diabetes risk.
Materials and Methods: Case-control study using detailed semi-quantitative food frequency interviews regarding dietary intake one-year prior diabetes diagnosis in 99 newly diagnosed cases 7-14 years old at diagnosis, and 180 age and sex matched controls from Stockholm area. Dietary data was transformed into energy and nutrient intake per day using the Swedish Food Data Bank. Growth data was retrieved from prospectively recorded charts from Child Health Care Centres and schools. Combined analysis of dietary and growth information was possible for 66 matched sets of cases and controls. Odds ratios were calculated using conditional logistic regression. High level of exposure was defined as above the 66th percentile of the distribution in cases and controls.
Results: Average intake of energy, protein, fat and carbohydrates was higher (p<0.01) among the cases compared to the controls, as was mean weight-for-age standard deviation score (SDS) from birth up to one-year prior diagnosis (p=0.02). High intake of energy (OR=3.84; 95% CI 1.61-9.16) and high mean weight-for-age SDS (OR=2.18; 95% CI 1.04-4.55) were both associated with increased risk of diabetes, and remained independent risk factors after adjustment for each other. There was a crude association between increased diabetes risk and high intake of protein, fat and carbohydrates that disappeared after adjustment for the energy intake. However, high intake of disaccharides, (OR=3.52; 95% CI 1.29-9.62), especially saccharose, (OR=2.63; 95% CI 1.15-6.04) was associated with increased diabetes risk even after adjustment for the energy intake and average weight-for-age SDS. Higher intake of milk, bread and candy was associated with increased risk of diabetes, but only the latter remained significant after adjustment for the energy intake (p=0.02).
Conclusion: A higher energy intake one year prior to the diagnosis and a higher average weight-for-age up to one year before the diagnosis were independent risk factors for childhood diabetes. Over-nutrition of children may by different mechanisms explain part of the increasing incidence of childhood onset Type 1 diabetes.

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EURAGEDIC; European consortium for the genetics of diabetic nephropathy: strategy and first results.

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Background and Aims: Microvascular lesions and accelerated atherosclerosis are the major causes of morbidity and early mortality in diabetes. Epidemiological and familial studies suggest that genetic factors influence the risk of developing both, micro- and macrovascular complications in diabetic patients. The objective of the European consortium EURAGEDIC is the identification of genes and pathways involved in the pathogenesis of diabetic complications, in particular diabetic nephropathy,thereby providing new means for early diagnosis and prevention of these complications.
Materials and Methods: Our strategy is based on large-scale case/control and intra-familial association studies of candidate genes for diabetic nephropathy. A total of 5152 DNAs have been assembled from 3 different European populations (French, Danish and Finnish with 1718 samples in each collection). These include: a) type 1 diabetic patients with nephropathy (n=1588) and without nephropathy (n=1356) for case/control studies, b) 600 trios (i.e. case or control proband plus both parents) for intra-familial association studies (n=1472) and c) non diabetic controls from each population (n=736). The study of one hundred candidate genes (functional, positional and/or derived from animal models) is ongoing. The overall strategy includes identification of polymorphisms by direct sequencing of each gene in 47 pooled DNAs (188 chromosomes) from non diabetic controls and from cases and controls from each population. Algorithms and computer programs to estimate allelic and haplotype frequencies from the genotype data on pooled samples are used to select non redundant SNPs which determine the most frequent haplotypes. Selected SNPs are genotyped by MALDI-TOF mass spectrometry in the cohorts described above.
Results: SNP identification and selection are completed for over thirty genes. Genotyping of 3 variants in the ACE and PON2 genes, for which previous association studies with diabetic nephropathy showed conflicting results, has been performed in the case/control cohort. We computed a test of association for each marker to compare cases (DM with nephropathy) with controls (DM without nephropathy), the test is for all three countries but allows for the strength of the association to vary between countries. The OR (odds-ratio) is presented together with the 95% confidence interval. Preliminary analyses show some modest support for an association of diabetic nephropathy with ACE (p=0.042 OR=0.90 CI 0.81-0.99).
Conclusion: We have studied a number of candidate genes for diabetic nephropathy in a large pan-european cohort. Our results show some evidence for an association of the ACE gene with diabetic nephropathy. It is noteworthy that the confidence limits are small reflecting the substantial body of data and stressing the importance of large multi-center cohorts.

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A genome-wide linkage scan on lipid and lipoprotein levels in the Québec Family Study.

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Background and Aims: Studies investigating the genetics of blood lipids and lipoproteins have clearly established that genetic factors contribute to these phenotypes. Although the molecular bases of several monogenic dyslipidemic states have been identified, genes underlying the variation in the population at large remain to be found. The aim of the present study was to identify chromosomal regions harboring genes influencing lipid and lipoprotein levels in a population-based study.
Materials and Methods: A genome wide linkage scan for plasma cholesterol and triglyceride as well as high-density (HDL-C) and low density lipoprotein cholesterol (LDL-C) concentrations was performed in 927 subjects enrolled in the Québec Family Study. A maximum of 508 pairs of siblings from 223 families were available. A total of 443 markers spanning the 22 autosomal chromosomes with an average intermarker distance of 7.2 centimorgans were genotyped. Linkage was tested using both sibpair- and variance components-based linkage methods. Prior to genetic analyses, the phenotypes were adjusted for age, gender and body mass index (BMI) using a stepwise multiple regression procedure. The interpretation of linkage was considered as highly suggestive ($p \leq 0.0023$; $LOD \geq 1.75$) or significant ($p \leq 0.0001$; $LOD \geq 3.0$).
Results: The strongest evidence of linkage with the variance components-based methods was found on chromosome 12q14.3 between marker D12S334 and HDL-C (LOD = 4.06). This locus provided highly suggestive evidence of linkage with the sibpair linkage method as well. Chromosomal regions harboring QTLs with significant evidence of linkage for LDL-C included 1q44, 15q26.2 and 19q13.2. In the case of triglycerides, three markers located at 2p14, 11p13 and 11q24.1 provided highly suggestive evidence of linkage with both sibpair- and variance components-based methods. Tests for total cholesterol levels yielded significant evidence of linkage at 15q26.2 and 18q22, but these results were inconsistent between linkage methods.
Conclusion: This genome wide linkage scan reveals that there are several loci influencing lipid and lipoprotein levels in normolipidemic subjects. Promising candidate genes were located in the vicinity of the genomic regions showing evidence of linkage.

OP 20
Nutrition

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Dietary *trans*9*cis*11 conjugated linoleic acid (CLA) increases insulin resistance and markers of oxidativ stress and inflammation in men with abdominal obesity.

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Background and Aims: We have recently shown that dietary *trans*10*cis*12 conjugated linoleic acid (CLA) aggravates the metabolic syndrome in prediabetic men. Despite *cis*9*trans*11CLA is the predominant isomer in the human diet, metabolic effects are unknown in obese humans. In addition, *cis*9*trans*11CLA is found in dietary supplements marketed as weight loss agents widely used by obese subjects. The present aim was to investigate the effects of *c*9*t*11CLA on peripheral insulin sensitivity, lipid peroxidation and lipid metabolism in prediabetic men.
Materials and Methods: In a randomised, double-blind controlled study, twenty-five abdominally obese, non-diabetic men were randomized to either 2.5g/d of *cis*9*trans*11CLA (representing 1% of energy intake) or placebo (olive oil). Before and after 3 months supplementation we assessed insulin sensitivity (M/I-ratio, hyperinsulinemic euglycemic clamp), lipid metabolism, body composition, urinary 8-iso-prostaglandin (PG)F_{2a} (isoprostanes) and 15-keto-dihydro-$PGF_{2\alpha}$, reliable and clinical relevant markers of *in-vivo* oxidative stress and inflammation, respectively.
Results: All subjects completed the study. Compared to placebo, *c*9*t*11CLA decreased insulin sensitivity (-15%, $p<0.05$) and increased 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ excretion (50%, $p<0.01$ and 15%, $p<0.05$, respectively). The increased insulin resistance was independent of changes in fasting serum lipids, glycemia, BMI and abdominal fat, but was abolished when changes of 8-iso-$PGF_{2\alpha}$ was adjusted for. The changes in oxidative stress were significantly correlated with changes in insulin sensitivity from baseline to 3 months ($r=-0.43$, $p<0.05$). There were no differences between groups in BMI, body composition, serum lipids or glucose levels.
Conclusion: In contrast to the beneficial metabolic effects in animals, in abdominally obese men, *c*9*t*11CLA increased insulin resistance, lipid peroxidation and proinflammatory prostaglandins without impairing fasting glucose or lipid concentrations. This is the first study of *c*9*t*11CLA in obese humans and further studies are warranted, considering *c*9*t*11CLA is the major dietary from of CLA. The close association between induced oxidative stress and insulin resistance is interesting and support our previous hypothesis that fatty acid-induced oxidative stress might be primary to insulin resistance.

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Chromium picolinate supplementation increases insulin-stimulated Akt phosphorylation *in vivo* in skeletal muscle from subjects with Type 2 diabetes.

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Background and Aims: Chromium picolinate (CrPic) has been shown in recent human and animal studies to improve carbohydrate metabolism. However, the mechanism of action is not known. To evaluate a potential cellular mechanism operative *in vivo* in human subjects, we assessed the effect of CrPic supplementation to alter the cellular signaling involved in insulin action and glucose uptake in skeletal muscle from human subjects randomized to receive CrPic supplementation vs placebo.
Materials and Methods: CrPic supplementation is currently being evaluated in two cohorts of subjects with type 2 diabetes; one cohort treated with sulfonylureas only (Glipizide GITS) and the other cohort treated with dietary instruction only. After baseline assessment of carbohydrate metabolism and glycemic control, both groups have been randomized to receive CrPic (1000 µg daily) or placebo. Hyperinsulinemic, euglycemic clamp studies were obtained on all subjects prior to randomization and at the end of study. In the latter cohort of subjects receiving nutritional therapy only (n=8), skeletal muscle biopsies (vastus lateralis) were obtained at the zero and 30 min post-insulin time point during the pre-randomization (baseline) euglycemic clamp for assessment of intra-cellular signaling. Repeated biopsies (zero and 30 min post-insulin) were obtained during the euglycemic clamp conducted at the end of the treatment phase (3 months). The skeletal muscle tissue was processed and assessed for protein content for insulin receptor substrate (IRS) proteins, PI-3 kinase, Akt, insulin receptor (IR), glucose transporter (Glut-4), and actin levels by Western Blot. In addition, phosphorylation for IRS1/2, IR, and Akt were assessed pre- and post-insulin stimulation.
Results: Subjects randomized to CrPic had a mean increase in insulin sensitivity of 8.9%, whereas the placebo group had a mean decrease of 3.6%. There were no changes observed in skeletal muscle protein content (normalized for β-actin) for IR, IRS-1, PI-3 kinase, Akt, or Glut-4 at the end of study compared to baseline for either group. In addition, there appeared to be no difference in pre- or post-insulin-stimulated IR or IRS-1 phosphorylation for either group compared to baseline. However, insulin-stimulated Akt phosphorylation in the skeletal muscle was significantly increased at the end of study for those subjects randomized to CrPic vs placebo (180±23 ASU vs 105±17 ASU, respectively, $p<0.01$, mean±SE).
Conclusion: A potential *in vivo* mechanism by which CrPic may alter insulin action in human skeletal muscle is by increasing insulin-stimulated Akt phosphorylation.

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Selenium antioxidant activity may impair glucose metabolism in rats.

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Background and Aims: Redox sensitive pathways in glucose metabolism (glucose uptake, oxidation, storage and synthesis) are thought to be regulated by reactive oxygen species (ROS). Our recent observation that alpha-lipoic acid, considered to be a powerful antioxidant, acts as a mild pro-oxidant in muscles, increasing glucose uptake, and causing mitochondrial uncoupling and inhibition of glycogen synthesis support this assumption. Thus, intervention with antioxidants may lead to deleterious effects on cellular physiology. We further hypothesize that physiological levels of ROS production may be important in the regulation of biochemical pathways involved in glucose metabolism. Therefore, not only elevated oxidative stress may alter glucose metabolism but also nutritional antioxidants that downregulate cellular ROS.
Materials and methods: Selenium was selected as a dietary antioxidant. Sprague-Dawley rats were fed high-selenium diet (2microg/g/day) for three weeks, and ROS production capacity using Dihidrodichlorofluorecine, was measured. Selenium content and glucose uptake using 14C 2-deoxy-D-glucose were evaluated ex-vivo in isolated soleus muscles. In addition, an in vivo oral glucose tolerance test was performed.
Results: High selenium diet increased intra-muscular selenium contant by 40% ($P<0.01$). Selenium supplementation lowered intra-muscular ROS levels by 27% ($P<0.01$) indicating a powerful antioxidant effect. Glutathione-peroxidase activity remained unchanged suggesting that selenium lowered muscle ROS by other, yet to be investigated, anti-oxidative mechanisms. Selenium supplemention abolished the in vitro stimulatory effect of insulin on glucose uptake. In isolated muscles from experimental animals incubated with the oxidant t-butylhydroperoxide (TBH) the inhibitory effect of selenium was reversed. The addition of TBH to muscles reversed the selenium induced suppression of insulin action. Selenium-supplementation significantly increased blood glucose levels at 30, 60 90 min following on OGTT ($P<0.05$), indicating a decrease in tissue uptake of glucose with no effect on insulin levels.
Conclusion: An increase of selenium muscle content generated an antioxidant effect that down regulated ROS production in muscles below baseline levels resulting in impairment of muscle tissue ability to utilize glucose. The antioxidant mechanism by which selenium facilitated decreased muscle production of DCF-sensitive ROS was independent of GPX. Nutrients that modulate ROS appear to have profound effect on glucose metabolism.

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Time course of oxidative stress status in the postprandial and postabsorptive phases in Type 1 diabetes mellitus.

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Background and Aims: Postprandial changes in glucose and lipids are associated with cardiovascular risk in diabetes, but the pathophysiological changes mediating this relationship have not been fully identified yet. We aimed to investigate the role of oxidative stress (OS) by describing its evolution during the fasting, postprandial and postabsorptive phases in T1DM.

Materials and Methods: Twenty-three T1DM patients on intensive insulin treatment (12 m/11 f; mean ± SD age 42 ± 9 y; duration DM 17 ± 8 y; HbA_{1c} 7.7 ± 0.8 %; daily insulin 50 ± 13U; BMI 23.8 ± 2.2 kg/m^2) were hospitalised for 1 day in the metabolic ward. They all received a standard breakfast (870 kcal, 61 energy% as fat, 28% as carbohydrate) and 3 hours later lunch (670 kcal, 46 energy% as fat, 28% as carbohydrate) and did not modify their habitual insulin regime. Blood samples were taken at fasting (F), just after the post-breakfast glucose peak (BP) (identified by continuous subcutaneous glucose monitoring, GlucoDay ®), 3-h postbreakfast (B), just after the post-lunch peak (LP), just after the post-lunch dale (LD) and 5 hours after lunch (L). OS status in blood was monitored by measuring total antioxidant capacity (TAC), antioxidants, peroxides and malondialdehyde (MDA) as a measure of lipid peroxidation in vivo. Significance of changes was analysed by repeated measures ANOVA.
Results: Glutathione increased from 6.52 ± 1.20 µmol/g Hb at F to 7.08 ± 1.45 at BP, remained high in B and LP and started to decrease in LD reaching the lowest value at L (5.93 ± 1.52, p = 0.01 when compared to F and p = 0.005 for the overall change over time). Plasma protein-thiols increased from 3.00 ± 1.33 µmol/g protein at F to 3.23 ± 1.43 at B and further to 3.59 ± 1.40 at L (p = 0.048 vs F). In contrast, ascorbate decreased gradually from 44 ± 17 µmol/L at F to a minimum of 39 ± 19 at LD followed by a return to 42 ± 15 at L (p = 0.015). Similarly to retinol, α-tocopherol decreased from 11.7 ± 3.0 µg/mL at F to 10.9 ± 2.3 at BP, remained low till LD and returned to 11.2 ± 2.5 at L (p = 0.005). TAC only decreased significantly in L and uric acid only decreased from 3.58 ± 1.30 mg/dL at BP to 3.42 ± 1.14 at B (p = 0.01) but then increased in LP and LD to values higher than F (3.83 ± 1.23, p = 0.01) and returned to baseline at L. Peroxides did not change, but MDA increased gradually from 1.02 ± 0.36 µmol/L at F to a maximum of 1.14 ± 0.40 at LP followed by a significant decrease to 0.92 ± 0.29 at L (p = 0.028). Although men had higher MDA, uric acid and TAC, the time course of all the parameters was the same in both sexes.
Conclusions: These results indicate that, except for glutathione and thiols, which parallel the changes in glycemia, there is a decrease in all antioxidants and an increase in lipid peroxidation in the postprandial phase with a return to fasting values in the postabsorptive phase. It is noteworthy that the time course of these changes differed between the individual antioxidants.

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A high intake of marine n-3 fatty acids increases insulin resistance in Type 2 diabetes.
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Background and Aims: Fish oil supplements are widely recommended. However, recommendations have been questioned for subjects with type II diabetes because of discrepant effects on metabolic control. Discrepancies could result from heterogeneity of type II diabetes and/or varying dosage of marine n-3 fatty acids. Here we investigated the effects of high dosage on insulin sensitivity and secretion in a relatively homogenous and well-defined group of type II diabetic subjects.
Materials and Methods: Twenty-six non-smoking subjects (13 M, 13 F) with type II diabetes treated by diet alone, and some additionally by metformin, but not by insulin, participated in a 9-week double-blind randomized study. Subjects were randomized by minimization to a Fish Oil (FO) group, n=12, or to a Corn Oil (CO) group, n=14. The FO group ingested 16.3 g/d fish oil containing 6.4 g PUFA (6.0 g/d n-3 FA (1.9 g/d EPA, 3.1 g/d DHA)). The CO group ingested 16.5 g/d corn oil containing 8.7 g PUFA (8.5 g/d n-6 FA). Insulin sensitivity was assessed from infused glucose during the last 40 min of 2 h isoglycemic hyperinsulinemic (40 mU/m^2/min) clamps. Insulin secretion was assessed by C-peptid-glucagon tests. Median (25-75 percentile) variables at baseline were: age 58 y (55-66), diabetes duration 3 y (2-5), BMI 29.5 kg/m^2 (27.8-30.8), weight 86.0 kg (72.6-94.5), lean weight (LW) 54.1 kg (48.6-67.1), HbA1c 6.8 % (6.4-7.5), fasting glucose 7.8 mmol/l (6.9-8.7).
Results: Phospholipid content of n-3 FA increased by median 89 % and n-6 FA decreased by 25 % in the FO group. Contents were stable in the CO group (- 8 % and + 3 % respectively, p<0.0001 for difference between groups). Fasting blood glucose after 9 weeks had increased non-significantly in the FO group (+ 0.5 mmol/l vs + 0.1 mmol/l in the CO group, p<0.3). Neither did weight, HbA1c, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides nor leptin change significantly between groups. After 9 weeks of intervention the glucose infused during the isoglycemic clamp decreased in the FO group by - 0.32 mg/kg LW/min, i.e. by 8 % but increased in the CO group by 1.21 mg/kg LW/min, i.e. by 39 %, p<0.05 for difference between groups. Conversely, insulin secretion increased in the FO group (delta C-peptide value + 0.42 nmol/l vs. - 0.05 nmol/l in the CO group, p<0.05).
Conclusion: A high intake of marine n-3 fatty acids increases insulin resistance in type II diabetes. These findings should be considered when recommending marine n-3 fatty acid supplementation to type II diabetic subjects.

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Energy requirements of morbidly obese female patients are misestimated in 30 % of cases.
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Background and Aims: We studied a cohort of obese female patients before and one year after gastric bypass. In stable weight conditions, before surgery, we noticed a substantial difference between calculated and measured energy expenditure rate. We followed evolution of this difference in parallel of evolution of metabolic parameters with body weight loss.
Material and Methods: We studied 107 morbidly obese female patients (mean age 39.7 ± 1.0 y, mean body weight 121. 1 ± 1.6 kg, BMI 46.1 ± 0.6 kg/m^2). We performed blood sample analyses for fasting glycemia, insulin, FFA and leptin. We measured energy expenditure by indirect calorimetry and we compared it to the predicted value using the Harris-Benedict formula.
Results: The range of variation from the predicted energy expenditure was wide (from – 468 kcal/day to +496 kcal/day). In 69 % of cases, the measured value matched the predicted value ± 10 %. In 16 % of cases the measured value was more than 10 % higher than the predicted value. In 15 % of cases the measured value was more than 10 % lower than the predicted. In an ANOVA factorial analysis the characteristics of the three groups showed no differences of body weight but significative statistical difference for age (p=0.01), fasting plasma glucose (p=0.04), insulin (p=0.07), FFA (p=0.03) and leptin over fat ratio (p=0.02). In a multiple regression analysis with energy expenditure deviation from predicted value as an independent variable the mentioned parameters except age were still significantly correlated. Indeed, the patients with the highest energy expenditure deviation from predicted are those with a metabolic profile suggesting insulin resistance. The evolution after remarkable weight loss following gastric bypass surgery showed a substantial correction of this deviation between predicted and measured energy expenditure rate in parallel with an improvement of metabolic parameters.
Conclusion : In about 30 % of cases of morbidly obese female patients the energy expenditure rate can be substantially different (from – 468 kcal/day to +496 kcal/day) from the value predicted by Harris-Benedict formula probably because of altered metabolic parameters. This should be taken into account when we estimate energy requirements for dietary prescription.

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Improvement of glycaemic control and plasma lipid levels by chronic low glycemic index diet in Type 2 diabetes.
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Background and Aims: In a previous study chronic low glycemic index (LGI) diet was found to decrease total fat mass and plasma lipids in a group of non-diabetic subjects. Therefore, we aimed to determine whether a chronic LGI diet might have beneficial effects on total fat mass, lipid metabolism and/or insulin resistance in type 2 diabetic patients.
Materials and Methods: Twelve type 2 diabetic men (fasting plasma glucose of 8.7<0.7 mM, HbA1c of 7.6<0.4 %, BMI of 31.2<1.5%) accepted to participate in this study. Patients were randomly allocated to 2 periods of 4 weeks of a diet rich in LGI or high glycemic index (HGI) carbohydrates separated by a 2-week washout interval, in a crossover design. The LGI diet resulted in lower postprandial plasma glucose and insulin profiles and areas under curves than after the HGI diet.
Results: At the end of 4 weeks of either LGI or HGI diet, the 7-day dietary records demonstrated equal daily total energy and macronutrients intake.

Body weight and total fat mass measured by dual-energy X-ray absorptiometry were comparable after the two dietary periods. Four-week LGI diet induced improvement of plasma glucose control (fasting plasma glucose: –1.1 mM, $p<0.05$, HbA1c: -0.5% $p<0.05$). The same diet increased whole body glucose utilization measured by the euglycemic hyperinsulinemic clamp technique (3.50<1.24 vs 5.86<0.85 mg/kg/min, HGI vs LGI $p<0.05$). These modifications were associated to a decrease in fasting plasma cholesterol: -1 mM ($p<0.05$), triacylglycerols ($p<0.05$), free fatty acids ($p<0.05$) and apo B. There was no difference in total fat mass or in RNAm quantities of genes implicated in its regulation between the 2 dietary periods.
Conclusion: Only 4 weeks of a LGI diet were able to improve glycemic control and glucose utilization without any modification in body weight or total fat mass in type 2 diabetic patients. This study demonstrates the clinical utility to use LGI diets in type 2 diabetic subjects.

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A cross-sectional study of dietary patterns with glucose intolerance in populations of West African origin: Cameroon, Jamaica, and African-Caribbeans in the UK.
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Background and Aims: Type 2 diabetes is linked to multiple cardiovascular risk factors and in its aetiology is subject to a variety of environmental influences, including levels of physical activity and diet. Diet has been postulated to influence dysregulation of insulin and insulin-like growth factor action. The effect of this dysregulation on consequent cardiovascular disease may well occur long before the diagnosis of type 2 diabetes. Here we report differences in nutrient intake and associations with glucose intolerance among populations of similar (African) origin, yet with differing rates of diabetes and cardiovascular disease.
Materials and Methods: Habitual dietary intake was estimated with a quantitative FFQ, developed specifically for each country. Random samples of men and women aged 24-74 years drawn from urban Cameroon (n = 1045), rural Cameroon (745), Jamaica (857) and African-Caribbeans from Manchester, UK (244) underwent a standard 75g glucose tolerance test. Habitual dietary intakes and estimates of BMR were calculated using age and sex specific equations.
Results: The prevalence of type 2 diabetes was lowest in rural and urban Cameroon (0.6% vs. 1.3%), and highest in Jamaica (11.6%) and UK (12.6%). Total energy intake was highest in rural Cameroon, intermediate in urban Cameroon and Jamaica, and lowest in UK subjects. Reported intakes of total dietary fat, and dietary fat sub-types (saturated and polyunsaturated), fibre, starch, carbohydrates and protein followed similar trends ($P<0.0001$). Within each site, there were no differences between percentage total energy from fat and carbohydrate in subjects with impaired glucose homeostasis compared to normoglycaemic subjects. However between sites, subjects with impaired glucose homeostasis had the highest percentage total energy from fat (after adjusting for BMR) in rural Cameroon (43.7 (43.1, 44.3)), and lowest in Jamaica (30.8 (29.8, 31.8) and Manchester UK (33.6 (31.2, 34.2)); $F = 82.3$, $P<0.0001$. Percentage total energy from carbohydrates was highest in Jamaica (59.6 (58.4, 60.8)) and UK (55.4 (2.4, 58.5)) and lowest in rural Cameroon (41.9 (38.7, 45.13)) after adjusting for BMR; $F = 63.7$, $P<0.0001$. Similar trends were observed for percentage total energy from protein. In a multivariate logistic regression analysis, controlling for site and BMR, each unit rise in percent energy from protein was associated with a 50% increase risk of type 2 diabetes (OR = 1.50 (1.24, 1.80)). This was independent of an increase in total energy from carbohydrate (OR = 1.24 (1.05, 1.48)) and total fat (OR = 1.22 (1.01, 1.47)).
Conclusion: The independent influence of dietary factors according to migration on type 2 diabetes prevalence within this genetically similar group, is further evidence for the profound effects of lifestyle in modifying an individual's predisposition to impaired glucose handling. This has important implications for managing diabetes prevention programs in at risk populations worldwide.

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Contraction-mediated muscle capillary recruitment and metabolic responses *in vivo* are resistant to TNFα.
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Background and Aims: Exercise and insulin each stimulate glucose uptake by muscle *in vivo*. The cellular mechanisms differ and insulin-mediated glucose uptake is wortmannin-sensitive, while that due to contraction is not. Both exercise and insulin also result in capillary recruitment *in vivo* that may contribute to glucose uptake. It is not known whether there is any overlap between insulin- and exercise-mediated capillary recruitment. Recently, we have shown that that acutely administered TNFα *in vivo* inhibits insulin-mediated capillary recruitment and approx. 50% glucose uptake in muscle, the latter effect shown by others to be absent in incubated muscle. Since TNFα is elevated in many insulin resistant states and over-expression of TNFα in adipose tissue and muscle of animals and humans may contribute to the development of insulin resistance, we now assess whether contraction-mediated hemodynamic and metabolic changes are affected by TNFα.
Materials and Methods: Whole body glucose infusion (GIR), femoral blood flow (FBF), hindleg vascular resistance (VR), hindleg glucose uptake (HGU), 2-deoxyglucose uptake into muscles of the lower leg (R'g) and hindleg metabolism of infused 1-methylxanthine (1-MX) a measure of capillary recruitment were determined. Two groups were studied with and without infusion of TNFα: one-leg field stimulated (2 Hz, 0.1 ms at 30V) and saline-infused control anesthetized rats. Previous data from euglycemic insulin-clamped (3 $mU.min^{-1}.kg^{-1} \times 2h$) have been included for comparison.
Results: Contraction increased FBF, HGU and 1-MX values. TNFα (0.5 $\mu g.kg^{-1}.h^{-1}$) totally blocked the 3 mU insulin-mediated increases in FBF and 1-MX, and partly blocked the increases in GIR, HGU and R'g. None of the increases due to twitch contraction were affected by TNFα.
Conclusion: We conclude that muscle capillary recruitment and glucose uptake due to muscle contraction under twitch stimuli at 2Hz are resistant to inhibition by TNFα, unlike the effects of insulin. These findings may have implications for circumventing muscle insulin resistance resulting from increased TNFα expression. The resistance of contraction-mediated capillary recruitment to the effects of TNFα implies that the cellular pathways by which capillaries are recruited by contraction and insulin are divergent.

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TNFα and IL-6 differentially affect insulin action in vivo.
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Background and Aims: Recent studies show that interleukin-6 (IL-6) is released from contracting skeletal muscle and acts in a "hormone like" manner to mediate metabolic processes. Specifically IL-6 increases lipolysis and fatty acid oxidation and it may play a role in glucose homeostasis by increasing hepatic glucose output to meet the glucose requirement of muscle. Hence, it has become of interest to those studying insulin resistance and type 2 diabetes. We have previously shown that TNFα selectively inhibits the haemodynamic effects of insulin but not that of exercise to increase muscle capillary recruitment and bulk limb blood flow *in vivo*. Insulin-stimulated muscle glucose uptake was also inhibited by approx. 50% by TNFα. Thus our aim in the present study was to compare the effects of IL-6 and TNFα on physiologic insulin *in vivo*.
Materials and Methods: Blood pressure (BP), blood glucose, plasma free fatty acids (FA), whole body glucose infusion (GIR), femoral blood flow (FBF), hindleg vascular resistance (VR), hindleg glucose uptake (HGU), 2-deoxyglucose uptake into muscles of the lower leg (R'g) and hindleg metabolism of infused 1-methylxanthine (1-MX) a measure of capillary recruitment were determined. Five groups were studied: euglycemic insulin-clamped ($3\ mU.min^{-1}.kg^{-1} \times 2h$) with and without 3h (1h before and 2h during insulin) infusion of TNFα, or IL-6 and saline-infused control anesthetized rats.

Results: Insulin alone at steady state plasma levels of 638 ± 84 pM required a GIR of 10mg.kg^{-1}.min^{-1} for euglycaemia , and increased HGU, R'g, FBF and 1-MX (P<0.05, for each), but had no effect on BP. TNFα (0.5 μg.kg^{-1}.h^{-1}) alone had no effect on BP, blood glucose, HGU, plasma insulin, FBF or 1-MX, but increased FA. TNFα totally blocked insulin-mediated increases in FBF and 1-MX, and partly blocked GIR, HGU and R'g, but did not affect plasma insulin. IL-6 (0.5 μg.kg^{-1}.h^{-1}) alone had no effect on BP, but increased FBF, HGU, R'g, 1-MX, FA (10-fold), plasma insulin (2.5-fold) and increased blood glucose from 5 ± 0.2 to 6 ± 0.2 mM. IL-6 decreased GIR due to insulin by 30% but had no effect on insulin-stimulated R'g or 1-MX, both of which were inhibited (50 and 100%, respectively) by TNFα under identical conditions.
Conclusion: The two cytokines have markedly differing effects *in vivo*. Whereas TNFα has strong anti-insulin action to inhibit insulin-mediated increases in the haemodynamic parameters of capillary recruitment and limb blood flow and thereby to decrease muscle glucose uptake, the only anti-insulin activity of IL-6 involves marked increase in plasma free fatty acids. IL-6 either alone or because of the increased release of insulin, increases capillary recruitment, limb blood flow, muscle glucose uptake and probably hepatic glucose output. Within the diabetes context, elevated plasma levels of IL-6 may be beneficial, unlike TNFα.

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Exercise-induced stimulation of glycogen synthesis is reduced in obese, insulin resistant subjects. A study using 13C-magnetic resonance spectroscopy.
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Background and Aims: Insulin resistance is a central feature in type 2 diabetes as well as in obesity. In muscle, glucose uptake is not only stimulated by insulin, but also by exercise, which raises the possibility that exercise may increase glucose uptake normally in skeletal muscle of insulin-resistant subjects. To test this hypothesis, we applied 13C-Magnetic Resonance Spectroscopy (MRS), which enables continuous, non-invasive measurement of glycogen synthesis rate in skeletal muscle in humans. The effect of a short bout of exercise on insulin-induced glycogen synthesis in skeletal muscle in obese subjects was studied. Because we hypothesised that exercise may change blood flow and thereby increase substrate to the target cells, we also measured blood flow responses to exercise in a separate experiment.
Materials and Methods: Four groups of subjects (n=5) were studied (lean: age 20.2 ± 1.8, BMI 21.4 ± 1.2; obese young: age 24.6 ± 2.2, BMI 30.8 ± 3.0, five elderly obese: mean age 56.8 ± 5.1, BMI 36.2 ± 3.8 and 5 elderly lean: age 57.4 ± 3.9, BMI 22.8 ± 3.4) and underwent a euglycemic hyperinsulinemic clamp (430 pM/m2/min insulin, infusion of 20% glucose, 30% enriched with 1-13C-glucose) for 150 min, with simultaneous measurement of glycogen in gastrocnemius muscle. After baseline measurements, all subjects performed acute exercise of the calf muscle (two 1-minute periods of single-legged toe lifting separated by 1 minute of rest). MRS measurements were subsequently continued for at least 50 min. On a separate day blood flow was measured at baseline, during insulin and before and after exercise was measured using strain-gauge plethysmography in the exercised leg, the control leg and the right forearm.
Results: Obese subjects had lower glycogen synthesis rate (98.9 ± 15.7 vs 168.2 ± 20.7 μmol/kg muscle/min in lean, p=0.016). Exercise increased the rate of glycogen synthesis rate substantially, in lean from 168.2 ± 29.0 to 458.2 ± 91.0 μmol/kg muscle/min (percent increase 272 +/- 49. Exercise-induced increase in the rate of glycogen synthesis in obese was substantially reduced (from 98.9 ± 20.5 to 177.7 ± 39.1, percent increase 180 ± 30% (p<0.01 vs lean)). A strong correlation (r = 0.69; p= 0.003) was found between the whole body glucose uptake (clamp) and insulin stimulated glycogen synthesis rate. Also a strong correlation (r = 0.52; p= 0.01) was found between whole body glucose infusion rate and the increase in glycogen synthesis in response to to exercise.
After exercise, calf blood flow increased strongly but returned to baseline values within 30 minutes. No correlation was found between blood flow and glycogen synthesis rate.
Conclusion: These results indicate that in humans, a short bout of exercise has an acute effect on the glycogen synthesis rate. This increase in glycogen synthesis rate is reduced in obese subjects.
These results, suggest that the insulin- and exercise-mediated glucose uptake share a common pathway and that insulin resistance is associated with a decreased ability of exercise to stimulate glucose uptake.

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Effects of acquired obesity on liver fat accumulation and insulin resistance. Studies in monozygotic twins concordant and discordant for obesity.
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Background and Aims: Fat accumulation in the liver and intra-abdominally is associated with reduced insulin sensitivity, but it is unclear whether these associations are confounded by genetic factors. We determined which fat depots are influenced by acquired obesity and how they relate to insulin sensitivity independent of genetic effects, by studying monozygotic (MZ) twin pairs.
Materials and Methods: Eighteen pairs of MZ twins aged 24-27 yr (BMI 20.0-33.9 kg/m^2), with intrapair differences in body weight ranging from 0.1 to 24.7 kg, were identified from a population-based FinnTwin16-sample. Body fat% was determined by dual energy x-ray absorptiometry (DEXA), abdominal subcutaneous (s.c.) and intra-abdominal (i.a.) fat by magnetic resonance imaging, and liver fat (LFAT) by proton spectroscopy. Fasting insulin and whole body insulin sensitivity (euglycemic clamp) were determined as markers of insulin sensitivity.
Results: Intrapair differences in body weight correlated significantly with intrapair differences in BMI (r=0.97, p=0.001), DEXA (r=0.83, p=0.001), s.c. fat (r=0.97, p=0.001), i.a. fat (r=0.79, p=0.001), and LFAT (r=0.51, p=0.029).
Along with our previous studies, liver fat correlated with fasting insulin (r=0.41, p=0.014) in these twin individuals. Correcting for pairwise (mainly genetic) similarities, intrapair differences in fasting insulin correlated with intrapair differences in fat depots as follows: BMI r=0.60, p=0.008, DEXA r=0.39, p=0.13, s.c. fat r=0.54, p=0.021, i.a. fat r=0.56, p=0.016, LFAT r=0.15, p=0.56. The respective correlations for whole body insulin sensitivity were: BMI r=-0.68, p=0.002, DEXA r=-0.80, p=0.001, s.c. fat r=-0.72, p=0.001, i.a. fat r=-0.55, p=0.012, LFAT r=-0.16, p=0.53.
Conclusion: Acquired obesity increases body fat, liver fat, fasting insulin concentrations and whole body insulin resistance. When genes are controlled for, insulin resistance is correlated with the size of fat depots other than liver fat. This implies that genetic factors regulate the association between liver fat and insulin resistance.

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Insulin resistance of whole-body protein metabolism in subjects with BMI>30.
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Background and Aim: We have shown that insulin causes protein anabolism in lean subjects and propose that adiposity interferes with this action., in parallel with insulin resistance of glucose metabolism.
Materials and Methods: Whole-body ^{13}C-leucine and ^{3}H-glucose kinetics were measured in 15 lean subjects (BMI<25; 10 men , 5 women) and 14 obese (OB) subjects (BMI>30; 5 men, 9 women) during the hyperinsulinemic, euglycemic, isoaminoacidemic clamp. During the clamp, plasma amino acid (AA) concentrations were maintained by adjusting the infusion rates of a commercial AA solution, based on fluorometric measurements of plasma branched-chain amino acids (BCAA) every 5 min.
Results: All the indices of adiposity were higher in the OB subjects, but fat-free mass (FFM) was not significantly different from the lean. OB had higher fasting plasma insulin, and with the same infusion rate of insulin (40 mU/m^2.min), their plasma insulin rose to higher levels (775 ± 70 pmol/L in the OB vs 564 ± 25 in the lean, P<0.05). Plasma BCAAs were maintained at target levels, while most of the other AA remained within 15% of postabsorptive values. The infusion rates of AA required to maintain postabsorptive levels of BCAAs were significantly lower in OB, (0.23 ± 0.01 vs 0.42 ± 0.02 mL/kg.h, P<0.0001) as were those of glucose (3.22 ± 0.20 vs 7.79 ± 0.45 mg/kg.min, P<0.0001) and were positively correlated (R=0.55, *P*=0.003) suggesting insulin resistance of protein metabolism. In the postabsorptive state, leucine flux was significantly higher in the OB subjects, due to both higher protein breakdown and synthesis, while leucine oxidation rates were not different from those of the lean subjects, thus resulting in a similar negative leucine balance in both groups (-0.55 ± 0.04 vs -0.50 ± 0.03 μmol/FFM.min in the lean). During the clamp, leucine flux increased to the same levels in both groups. In the lean subjects, this was mainly due to an increase in protein synthesis, since breakdown was partly suppressed and oxidation was not changed. These changes led to a marked

switch from a negative to a positive net leucine balance (0.34 ± 0.02 µmol/FFM.min). In contrast, in the OB, leucine oxidation was increased during hyperinsulinemia, with only a slight increase in synthesis and a similar suppression of breakdown as in the lean subjects, resulting in neutral net leucine balance (0.01 ± 0.04 µmol/FFM.min, $P<0.00001$ vs lean). BMI, % body fat, and waist circumference were all negatively correlated with net protein balance (R= -0.84, -0.76, -0.77 respectively, $P<0.0001$) during hyperinsulinemia.
Conclusions: Insulin resistance of protein is present in obesity, concurrent with that of glucose, affecting both the suppression of protein breakdown and stimulation of synthesis by insulin. The abnormalities are present in the postabsorptive state as well, with increased breakdown and synthesis in the phase of hyperinsulinemia. Infusion rates of AA during a clamp can serve as an index of resistance of protein metabolism to insulin.

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Whole body de novo lipogenesis and adipocyte lipogenic markers after short term carbohydrate overfeeding in lean and obese humans.
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Background and Aims: We have previously observed that carbohydrate overfeeding increases whole body net de novo lipogenesis (DNL) and upregulates the expression of lipogenic genes in adipose tissue. This suggests that adipose DNL contributes significantly to the disposal of excess carbohydrate. In the present study, we assessed whether these adaptations to carbohydrate overfeeding are altered in obese patients.
Materials and Methods: Eight healthy obese subjects (4 males, 4 females, age 29±1 y, BMI 30±0 kg/m^2) and 11 healthy lean subjects (5 males, 6 females, age 27±1 y, BMI 21±0 kg/m^2) were studied after 4 days of either an isocaloric diet (I:100% energy requirement based on 1.6 times resting energy expenditure, 50% carbohydrate) or a carbohydrate overfeeding (O:175% of energy requirement, 71% carbohydrate). Whole net DNL was measured over 5 hours during ingestion of 3.25g glucose/kg fat free mass (FFM) by indirect calorimetry. At the end of the test, a biopsy of gluteal subcutaneous adipose tissue was obtained to measure mRNA levels of sterol regulatory element binding protein (SREBP)-1c and fatty acid synthase (FAS) as markers of adipose lipogenic genes.
Results: Compared to lean subjects, obese subjects had similar fasting and post-glucose glycemia, but higher plasma insulin levels after both dietary intervention. Post-prandial suppression of plasma free fatty acid concentrations was impaired in obese subjects after overfeeding (0.16±0.03 vs 0.40±0.05 mmol/l, P=0.001). Lipid oxidation after glucose ingestion was decreased after overfeeding in both group, but was higher in obese subjects than in lean subjects (I vs O; lean: 90±12 vs 5±2; obese: 167±32 vs 29±8 mg/kg FFM/5hours). Glucose oxidation after glucose ingestion was increased in both group after overfeeding (lean: 7.6±0.2 vs 10.1±0.3; obese: 5.9±0.3 vs 8.7±0.4 mmol/kg FFM/5hours). Significant increase in net DNL was found in both group after overfeeding (I vs O; lean: 35±9 vs 156±21; obese: 12±6 vs 64±11 mg/kg FFM/5hours). Compared to lean subjects, obese subjects had smaller net DNL after overfeeding (P <0.0001). Overfeeding increased SREBP-1c mRNA by 25% and 43% and FAS mRNA by 66% and 84% in adipose biopsy of obese and lean subjects respectively. FAS mRNA levels after overfeeding were significantly lower in obese subjects (P=0.0008).
Conclusion: A 4-day carbohydrate overfeeding stimulates whole body DNL and increases adipose markers of DNL in both lean and obese subjects. This indicates that adipose DNL occurs after overfeeding. Whole body net DNL and FAS mRNA levels after overfeeding were significantly lower in obese subjects, suggesting that stimulation of adipose DNL may be less effective as a means to dispose of excess carbohydrate in obesity. The possible consequences on glucose homeostasis during longer period of carbohydrate overfeeding remain to be investigated.

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Intestinal microsomal triglyceride transfer protein is raised in diabetic patients. A mechanism for the production of atherogenic postprandial lipoproteins.
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Background and Aims: The abnormal postprandial triglyceride-rich lipoproteins may be an important cause of the increased atherosclerosis in diabetic patients. Microsomal triglyceride transfer protein (MTP) is central to the assembly of the apo B48-containing lipoproteins in the intestine and VLDL in the liver. Insulin has been shown to suppress MTP mRNA expression in HepG2 cells and we have shown an increase in MTP mRNA and activity in the intestine of diabetic animal models with a good correlation between MTP mRNA and MTP activity. The aim of the present study was to measure intestinal MTP mRNA in diabetic and control subjects and to examine the relationship between MTP mRNA and postprandial lipoproteins.
Materials and Methods: Intestinal biopsies were collected from 10 type 2 diabetic and 10 control subjects undergoing routine gastroscopy. Ethics committee approval and informed consent were obtained. Patients with neoplastic disease and those on statin or fibrate drugs were excluded as were patients with evidence of small bowel disease, renal failure, liver disease or untreated thyroid disease. In the week following the intestinal biopsy all subjects were given a high fat test meal and blood taken at 4 and 6h postprandially. MTP was measured by the RNase protection assay. Lipoproteins were isolated by ultracentrifugation and apo B48 and apo B100 measured by density gradient gel electrophoresis
Results: Patients were of similar age and BMI as controls. Mean HbA1c for the diabetic patients was7.7±1.2%. Plasma cholesterol and triglyceride were not significantly different between diabetic patients (4.7±0.4 and 1.7±0.39mmol/l) and controls (5.3±1.0 and 1.9±1.0mmol/l). Chylomicron Apo B48 and in the diabetic patients fasting and 4h were 3.4±1.8 and 10.3±4.4 compared to 5.6±3.7 and 9.24±5.3µg/ml plasma for control subjects. The postprandial increment was significantly greater in the diabetic patients compared to controls ($p<0.03$) Apo B100 fasting and 4h in the diabetic patients were 6.1±4.6 and 19.4±10.4 vs 8.6±6.2 and 18.3±12.2µg/ml plasma in control subjects. MTP mRNA for the diabetic patients was 17.2±5.0 vs 7.9±3.5 amol/µg total mRNA for controls ($p<0.03$). In the diabetic patients there was a significant positive correlation between MTP mRNA and chylomicron apo B48/cholesterol ($r=0.76$, $p<0.01$) and apoB100/cholesterol ($r=0.50$, $p<0.05$) while there was no correlation in the control subjects. There was no correlation between triglyceride/apoB48 or apo B100 and MTP mRNA in either diabetic or control subjects
Conclusion: This study, which is the first to report MTP expression in the human intestine, demonstrates an increase in MTP in diabetes which results in the production of a potentially atherogenic cholesterol-rich lipoprotein particles.

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Intravascular metabolism of a chylomicron-like emulsion in patients with lipoatrophic diabetes.
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Background and Aims: The intravascular metabolism of chylomicrons, the lipoproteins that carry the absorbed dietary lipids in the circulation for storage mainly in the adipose tissue consists in lipolysis by lipoprotein lipase in the endothelial surface of the blood vessels and uptake of the resulting chylomicron remnants by the liver. Here, this metabolism was evaluated in diabetic lipoatrophy, a disease in which there is nearly absence of adipose tissue and insulin resistance.
Materials and Methods: The plasma kinetics of intravenously injected chylomicron-like emulsions labeled with ^{3}H-triglycerides (^{3}H-TG) was evaluated in three female patients (age: 22, 24 and 27 years) with lipoatrophic diabetes, wherein there is nearly absence of adipose tissue, and compared with 7 healthy females. ^{3}H-TG traces the chylomicron lipolysis by lipoprotein lipase and ^{14}C-CE follows the remnants removal of chylomicron remnants from the plasma was determined.
Results: The fractional clearance rate (FCR, in min^{-1}) of ^{3}H-TG was 0.016, 0.036 and 0.041 in the 3 patients and 0.071±0.011 in controls. FCR of ^{14}C-CE was 0.021, 0.014 and 0.011 in the patients and 0.024±0.015 in the controls. Therefore, FCRs of both emulsion labels were pronouncedly smaller in patients than in controls, indicating that lipolysis and remnant removal were diminished. The smaller the FCR^{3}H-TG in the patients the greater the patient insulin resistance. One of the patients was studied under three three-week periods of different caloric intake levels. When she lost weight (7% body weight), insulin resistance and the fasting triglyceridemia diminished but the emulsion catabolism unexpectedly did not improve, but when she gained weight (6%) the emulsion catabolism deteriorated as

expected, together with increase in both insulin resistance and fasting triglyceridemia.
Conclusion: The metabolism of chylomicrons tested by the emulsion method is impaired in diabetic lipodistrophy and the caloric intake influences this metabolism and the lipid profile in those patients.

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A comparison of the efficacy of pioglitazone plus sulphonylurea with metformin plus sulphonylurea over one year in patients with Type 2 diabetes.
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Background: To date, no long-term trials comparing pioglitazone (Pio) added to a sulphonylurea (SU) with the established Metformin (Met) +SU combination have been performed. This study compared the efficacy and tolerability of the two combination therapies over one year in patients with type 2 diabetes.
Materials and Methods: In this European multicentre, double-blind study, patients with an HbA_{1c} level of 7.5% to 11.0% and a stable dose of an SU for >3 months at greater than 50% of the maximum recommended dose were randomly allocated to receive either pioglitazone 15-45 mg (n = 319) or metformin 850-2550 mg daily (n = 320) for 52 weeks. The primary efficacy endpoint was the change from baseline HbA_{1c} after 52 weeks of treatment.
Results: In total, 639 patients were treated, 319 with Pio+SU and 320 with Met+SU. Mean HbA_{1c} was reduced from baseline to Week 52 by 1.21% in the Pio+SU group and 1.36% in the Met+SU group. Reduction in FPG from baseline at Week 52 was similar in both groups: 2.2 mmol/L with Pio+SU and 2.3 mmol/L with Met+SU (p=NS). Hypoglycaemic episodes were the most frequent event in both groups, with a slightly higher incidence in the Met+SU group (14.1% vs 10.7%). Incidence of oedema/peripheral oedema in the Pio+SU group was 6.9% (n = 22), higher than with Met+SU (1.6%; n = 5). In the Pio+SU group, one patient was withdrawn prematurely and one patient had a dose interruption due to oedema, but no patients had dose reductions because of oedema and there were no cases reported as serious. Diarrhoea occurred more commonly with Met+SU (12.5% vs 2.5%) and weight gain was reported more frequently in the Pio+SU group (3.8% vs 0.6%). A mean weight gain of 2.8 kg over the 52 weeks of the study was observed in the Pio+SU group compared with a mean reduction of 1.0 kg in the Met+SU group. Treatment with Pio+SU results in effective glycaemic control over a period of 52 weeks similar to Met+SU.
Conclusions: Although Glycaemic control was comparable between the two interventional groups there appeared to be a trend toward greater durability of effect with Pio+SU compared to Met+SU but this remains to be confirmed in studies of longer duration.

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Pioglitazone treatment reduces hepatic fat content, enhances hepatic insulin sensitivity, and increases plasma adiponectin concentration in patients with Type 2 diabetes.
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Background and Aims: The effect of pioglitazone on plasma adiponectin concentration, endogenous (primarily hepatic) glucose production (EGP), and hepatic fat content was studied in 11 type 2 diabetic patients (age = 52±2 y, BMI = 29.6±1.1 kg/m^2, HbA_{1c} = 7.8±0.4%).
Materials and Methods: Hepatic fat content (magnetic resonance spectroscopy) and basal plasma adiponectin concentration were quantitated before and after pioglitazone (45 mg/day) for 16 wk. Subjects received a 3h euglycemic insulin (100 mU/m^2 per min) clamp combined with 3-[^{3}H] glucose infusion to determine rates of EGP and tissue glucose disappearance (Rd) before and after pioglitazone.
Results: Following pioglitazone treatment, hepatic fat content decreased from 21.3±4.2 to 11.0±2.4% ($p<0.01$), and plasma adiponectin increased from 7±1 to 21±2 µg/ml ($p<0.0001$). Pioglitazone reduced fasting plasma glucose (10.0±0.7 to 7.2±0.6 mmol/l, $p<0.01$), plasma triglyceride concentration(138±16 to 107±17 mg/dl, $p<0.05$), and HbA_{1c} (7.8±0.4 to 6.5±0.3%, $p<0.01$) despite increased body weight (83.0±3.0 to 86.4±3.0 kg, $p<0.01$). When pre and post-pioglitazone treatment results were analyzed collectively, plasma adiponectin concentration correlated negatively with fasting plasma insulin ($r=-0.50$, $p<0.02$), fasting plasma glucose ($r=-0.59$, $p<0.005$), HbA_{1c} ($r=-0.61$, $p<0.005$), and fasting plasma triglyceride (r=-

0.49, p<0.02) concentrations. Pioglitazone improved Rd (5.2±0.5 to 6.6±0.6 mg/kg-min, p<0.005) and insulin-mediated suppression of EGP (0.23±0.04 to 0.05±0.02 mg/kg-min, p<0.01) during the 3h insulin clamp. Plasma adiponectin concentration correlated negatively with hepatic fat content (r=-0.60, p<0.05) and insulin-mediated suppression of EGP (r=-0.80, p<0.004), and positively with Rd before (r=0.68, p<0.02) pioglitazone treatment; similar correlations were observed between plasma adiponectin levels and hepatic fat content (r=-0.65, p<0.03) and Rd after (r=0.70, p=0.01) pioglitazone treatment. EGP was almost completely suppressed after pioglitazone treatment; taken collectively, plasma adiponectin concentration, before and after pioglitazone treatment, still correlated negatively with the suppression of EGP during the insulin clamp (r=-0.65, p<0.001).
Conclusion: Pioglitazone treatment in patients with type 2 diabetes causes a 3-fold increase in plasma adiponectin concentration. The increase in plasma adiponectin is associated with a decrease in hepatic fat content and improvements in hepatic and peripheral insulin sensitivity. The increase in plasma adiponectin concentration following thiazolidinedione therapy may play an important role in reversing the disturbance in hepatic fat mobilization and improving hepatic/muscle insulin resistance in patients with type 2 diabetes.

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Rosiglitazone combined with insulin preserves islet β cell function in adult-onset latent autoimmune diabetes (LADA).
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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, affects about 10% NIDDM subjects,being more prevalent than classic type 1 diabetes. Pilot trials in LADA patients have showed higher C peptide when treated with insulin versus sulfonylureas. There is no clinical data so far to indicate that thiazolidinediones are beneficial to β cells in LADA. Rosiglitazone possesses anti-inflammatory properties in vitro and in vivo. We hypothesize that administration of rosiglitazone in combination with insulin could preserve islet β cell function in LADA.
Materials and Methods: Glutamic acid decarboxylase antibody (GADA) was screened in patients initially diagnosed type 2 diabetes. LADA patients, defined as GADA-positive and with a fasting C peptide of 300pmol/L or more, were selected and randomly assigned to receive subcutaneous insulin alone (n=6) or rosiglitazone combined with insulin (n=6) to compare the changes of islet β cell function. At entry and 1 year after treatment, blood was drawn to determine plasma glucose, HbA1c and C peptide at fasting and 2 hours after taking 75 g glucose without medication. GADA and C peptide were measured with RIA. Daily insulin dosage, ankle edema and liver function were monitored.
Results: After 1 year treatment, LADA patients in rosiglitazone combined with insulin group had higher postprandial C peptide than those in subcutaneous insulin group(1695.0±838.4 vs 782.1±307.6 pmol/L,P<0.05). During the 1 year observation, fasting and postprandial C peptide levels in patients treated with insulin alone had a tendency to decline (from 619.6±569.9 to 359.5±204.5 pmol/L and 1400.6±1019.5 to 782.1±307.6 pmol/L respectively), while those in rosiglitazone combined with insulin group stayed steady (from 656.4±382.3 to 648.7±375.3 pmol/L and 1370.7±921.2 to 1695.0±838.4 pmol/L respectively). The linear stepwise regression analysis showed that body mass index (BMI) at entry and different treatment regime were positively correlated with C peptide after 1 year, of which the latter factor contributed more. Daily insulin dosage in the combined group were reduced from 17.3±7.1 U/day to 13.2±2.5 U/day, but increased from 19.3±9.0 U/day to 25.3±9.4 U/day in insulin alone group, suggesting rosiglitazone rather than insulin played a key role in the changes of islet β cell function. HbA1c and plasma glucose levels were not statistically different at entry and after treatment. Serum alanine amine liver transaminase (ALT) was not elevated and no ankle edema or other side effects observed in both groups.
Conclusions: We found, for the first time to our knowledge, that rosiglitazone combined with insulin could preserve β cell function in LADA patients.

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Effects of metformin and rosiglitazone monotherapy on insulin-mediated hepatic glucose uptake and their relation to visceral fat in Type 2 diabetes.
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Background and Aims: Impaired hepatic glucose uptake has been implicated in the hyperglycemia of type 2 diabetes. The defect appears to involve the first steps of glucose uptake and metabolism in the liver, eventually leading to decreased glycogen synthesis.
Materials and Methods: We examined the effects of metformin (2 g/day) and rosiglitazone (8 mg/day) on insulin-mediated hepatic glucose uptake and its relation to subcutaneous fat, visceral fat, and whole-body insulin-mediated glucose metabolism - in a double-blinded, placebo-controlled study involving 30 newly diagnosed type 2 diabetic patients. Glucose uptake was measured before and after 26 weeks of treatment using positron emission tomography with [18F]-2-fluoro-2-deoxyglucose during euglycemic hyperinsulinemia; fat depots were identified by magnetic resonance imaging.
Results: At 26 weeks, fasting plasma glucose levels were significantly decreased after either rosiglitazone (-0.9±0.5 mmol/l) or metformin treatment (-1.1±0.5 mmol/l) in comparison with placebo. Both drugs enhanced whole-body glucose uptake, although only the changes induced by rosiglitazone were significant (+38%, p=0.01). Following either metformin or rosiglitazone, insulin-mediated visceral fat glucose uptake per mass unit was higher than with placebo (p<0.01), whereas neither agent had any significant effect on subcutaneous fat glucose uptake. Both rosiglitazone and metformin treatment were associated with an increase in hepatic glucose uptake, which reached statistical significance in men only. In the whole dataset, changes in hepatic glucose uptake were negatively related to changes in HbA_{1c} (r=0.43, p=0.01), and positively associated with changes in visceral fat glucose uptake (r=0.48, p<0.01).
Conclusion: We conclude that both metformin and rosiglitazone monotherapy increase hepatic glucose uptake in type 2 diabetes; direct drug actions, better glycemic control, and enhanced visceral fat insulin sensitivity are likely determinants of this phenomenon.

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Metabolic effects of changes in visceral fat accumulation in Type 2 diabetes.
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Background and Aims: We have previously shown that in type 2 diabetic subjects (T2DM) visceral fat accumulation (VF) is associated with worse glycemic control through a decrease in peripheral insulin sensitivity and an enhancement of gluconeogenesis. However, these associations have not been verified prospectively.
Materials and Methods: 27 T2DM subjects (age=54 ± 2 yr; BMI=29 ± 4 kg/m^2; fasting plasma glucose FPG=8.3 ± 0.4 mM; haemoglobin A1c=8.4 ± 0.2 %) were randomized to a 4-month treatment with a thiozolidinedione (TZD, n=6 rosiglitazone, n=11 pioglitazone) or placebo (n=10) and underwent measurement of 1) fat-free mass (^3H_2O technique), 2) subcutaneous (SC) and visceral (VF) abdominal fat area (by magnetic resonance imaging), 3) insulin resistance (IR, euglycemic insulin clamp), 4) endogenous glucose output (EGP, by [^{3}H]glucose infusion technique), and 5) gluconeogenesis (GNG, by the ^2H_2O method).
Results: TZD treatment resulted in the expected reduction in FPG (-1.7±0.4 vs 0.8±0.8 mM of placebo, p<0.005). In the TZD-treated group, there were modest decrements in VF (-14cm^2, p<0.01) and increments in SC (38 cm^2, p<0.001) and total body fat (3.3kg, p<0.001). TZD treatment was also associated with a significant improvement in both peripheral (-19%) and hepatic (-21%) IR. The improvement in peripheral IR was not correlated with the changes in VF (ΔVF). In contrast, after adjustment for sex, age, treatment, ΔSC, and Δ body weight, ΔVF was positively related to ΔGNG (partial r = 0.54, p = 0.01) and to ΔEGP (partial r = 0.39, p = 0.03). The relationship between glycogenolysis and VF was not statistically significant.
Conclusion: In patients with established type 2 diabetes, even a small decrease in VF is strongly and independently associated with a decrease in gluconeogenesis.

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LY307161 SR, a long-acting GLP-1 analog, improved glycemic control in patients with Type 2 diabetes.

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Background and Aims: LY307161 SR is a sustained-release formulation of a dipeptidyl peptidase IV-resistant analog of glucagon-like peptide-1. The aim of this study was to evaluate the glucose-lowering effects of 4 doses of LY307161 SR compared with inactive solution (saline) after 4 weeks of treatment in patients with type 2 diabetes previously treated with diet or oral antidiabetic agents. Safety, tolerability, and long-term glycemic control were evaluated after 12 weeks of treatment.
Materials and Methods: In this single-blind, Phase 2 study, a total of 182 patients (129M:53F, age 57.5±9.0 years [mean±SD]) were randomly assigned to 12 weeks of treatment with LY307161 SR by once-daily subcutaneous injection. Patients received LY307161 SR at daily doses of 0.5 mg, 1.0 mg, 2.0 mg, or 3.0 mg. A total of 21 patients received saline for 4 weeks prior to the 12-week treatment period with LY307161 SR.
Results: Treatment with LY307161 SR (LY) for 4 weeks resulted in significant, dose-dependent decreases in blood glucose (mean of the average change from baseline of the fasting and peak 1- to 4-hour postprandial glucose concentration) between treatment groups.

	Blood Glucose Change (mmol/L) after 4 Weeks				
Dose (mg)	**Saline**	**LY 0.5**	**LY 1.0**	**LY 2.0**	**LY 3.0**
All patients	-0.20	-0.44	-1.23	-1.67*†	-1.96*†
Diet/metformin subgroup	-0.78	-0.32	-2.18†	-1.96†	-2.95*†

Significantly different from Saline (*) and LY 0.5 (†)

Various subgroup analysis indicated that the metformin or diet-alone treated patients demonstrated a greater treatment effect. There were no significant differences in glycemic control parameters between treatment groups at 12 weeks. The mean HbA1c-lowering for patients on diet or metformin ranged from 0.6% to 0.9% at doses of 1 to 3 mg. No confirmed hypoglycemia was reported. Injections of LY307161 SR led to the frequent occurrence (69.2%) of mild injection site reactions (ISRs) after 4 weeks. Low-titer antibodies to LY307161 were detected only in a subset of patients who had ISRs during the study. In patients with ISRs, lower drug levels suggest reduced exposure to LY307161 SR over 12 weeks.
Conclusions: This study confirmed the beneficial therapeutic effect of LY307161 SR in patients with type 2 diabetes, but treatment was confounded by limited long-term tolerability at the injection sites.

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Tesaglitazar (GALIDA™) improves the metabolic abnormalities associated with insulin resistance in a non-diabetic population.

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Background and Aims: Insulin resistance (IR), an important underlying feature of type 2 diabetes, is associated with abnormalities in both glucose and lipid metabolism. These abnormalities are major components of the metabolic syndrome and are associated with an increased risk of cardiovascular disease. This study examined the effects of tesaglitazar (GALIDA™), a novel balanced PPARα/γ agonist, on the glucose and lipid abnormalities associated with IR in non-diabetic subjects.
Materials and Methods: The efficacy and safety of tesaglitazar (0.1, 0.25, 0.5 and 1mg) were compared with placebo in a 12-week study (SIR) in 390 non-diabetic subjects (23% women) with manifestations of IR as defined by plasma TG >1.7mmol/L and waist/hip >0.9 for men, >0.85 for women. Mean baseline characteristics (range) were: age 50 yrs (29-77), BMI 31 (21-41), TG 3.0mmol/L (1.7-7.2). Plasma LDL-C and LDL particle size and concentration were measured using NMR and grouped according to particle size: pattern A=mean particle diameter >20.5nm; pattern B (atherogenic)=mean particle diameter ≤20.5nm.
Results: Significant dose-dependent reductions in fasting TG, TC and FFA and an increase in HDL-C were seen with tesaglitazar. There was a decrease in non-HDL-C, and a dose-dependent significant increase in LDL particle size. Of the 62 patients who received tesaglitazar 1mg and had pattern B at baseline, 79% (30/38) switched to pattern A. Significant dose-dependent decreases in fasting insulin, glucose (FPG 5.71 to 5.39mmol/L at 1mg dose) and Homeostasis Model Assessment (HOMA) were also seen. There were no dose-dependent adverse events.
Conclusion: Tesaglitazar was well tolerated and showed significant and dose-dependent improvements in lipid and glucose metabolism and insulin sensitivity. The beneficial effects on cardiovascular risk factors (decrease in TG and TC, increase in HDL-C and LDL particle size) indicate a potential long-term effect on risk reduction for atherosclerosis. Tesaglitazar may also have the potential to delay progression to diabetes. Its clinical potential is now being evaluated in patients with type 2 diabetes.

Placebo[#] corrected change from baseline %

Dose (mg)	TG	HDL-C	TC	FFA	Ins	HOMA
0.1 (n=60)	-10	4	-1	-17	-9	-6
0.25 (n=70)	-16***	1	-4	-18*	13*	-18***
0.5 (n=58)	-27***	11**	-3	-21**	-16**	-22***
1.0 (n=65)	-37***	16***	-8***	-40***	-35***	41***

*p<0.05; **p<0.01; ***p<0.0001; #placebo group, n=137

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Improved β-cell survival in a Type I diabetes rat model after treatment with a β-cell selective potassium channel opener.

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Background and Aims: Type I diabetes results from an immune-mediated destruction of the β-cells. Reduction in β-cell mass during the diabetogenic process will increase the functional stress on the remaining β-cells. This may render them more susceptible to apoptosis, necrosis and autoimmune destruction. It is hypothesized that suppression of insulin secretion by administration of a β-cell selective potassium channel opener, NN414, can induce metabolic "rest" in the β-cells thereby reducing β-cell death resulting from metabolic stress. Furthermore, treatment with NN414 may reduce the antigenicity of the β-cells thereby reducing their autoimmune destruction.
Material and Methods: Diabetes was induced in 24-33 day-old BioBreeding Diabetes Resistant (BBDR) rats by combing RT6 depletion with PolyI:C treatment. The rats were randomized into 3 groups that were treated orally every 8 hrs from day 2-19 with 40 mg/kg NN414, 40 mg/kg diazoxide or vehicle. Blood glucose was measured daily before dosing, and when the blood glucose level exceeded 20 mM a supplementary insulin treatment was given 3 times daily. At day 21 the rats were fasted for 5-6 hrs, and an intravenous glucose tolerance test (IvGTT) was conducted to asses β-cell function. The rats were subsequently sacrificed and their pancreas removed for histological assessment of insulitis and remaining β-cell mass. Insulitis was scored on a scale from 0 to 4 where 0 indicates normal islets and 4 indicates end-stage destruction with no remaining β-cells. Blood samples were taken weekly for determination of the plasma drug concentration and the HbA_{1c} level.
Results: Treatment with $RT6^+$-depleting antibody and poly(I:C) induced diabetes (defined as blood sugar > 20 mM) in 86% of the rats during the 3-week trial. Average time to diabetes onset was 13±3 days in all three groups. Among NN414 treated rats 54,5% (12/22) had an average insulitis score below 3.0 whereas 18,2% (4/22) rats in both the vehicle group and the diazoxide group had an average insulitis score below 3.0 (p=0.01). The presence of functional β-cells in these rats was confirmed by a significant glucose-induced C-peptide response (increase in plasma C-peptide > 100 pM) during IvGTT. There was no difference in $\%HbA_{1c}$ values among the three groups.
Conclusion: This study demonstrate that β-cell rest induced by treatment with the potassium channel opener, NN414, improves β-cell survival and function, suggesting that therapy with NN414 may be used to rescue remaining β-cells in recent onset type I diabetic patients.

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Diabetes in Pregnancy

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Altered levels and function of the endogenous PPARgamma activator 15deoxydelta^{12,14}prostaglandinJ_2 in placental tissue from pregestational and gestational diabetic patients.

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Background and Aims: 15deoxydelta^{12,14}prostaglandinJ_2 (15dPGJ_2) is the highest affinity endogenous ligand for PPARgamma, a nuclear receptor highly expressed in placental tissue. 15dPGJ_2 has potent antiinflammatory properties, and inhibits NOS activity in different cell types. Diabetes mellitus alters the tissular expression and activity of NOS. Aiming to understand the importance of 15dPGJ_2 as a modulator of nitric oxide levels in control and diabetic placenta, we evaluate the levels of 15dPGJ_2, the nitric oxide production and the capability of 15dPGJ_2 to modulate nitric oxide concentrations in term placental tissues from control, pregestational and gestational diabetic women.
Materials and Methods: Term placental tissues from controls (n=12), Type I pregestational diabetic women (PGD, n=9) and gestational diabetic women (GD, n=11) were obtained by cesarean section. 15dPGJ_2 levels were measured by a commercial EIA kit. Placental explants were cultured during 3 h in the presence of 15dPGJ_2 $2x10^{-6}$M. After incubation nitrates were reduced to nitrites by nitrate reductase and the nitrite levels, index of NO production, were measured by the Griess reaction.
Results: 15dPGJ_2 was detected in placental tissues from controls (18±2 pg/mg), and was found reduced in GD placenta (11±1 pg/mg, p<0.05), and more importantly in PGD placenta (7±1 pg/mg p<0.002). Nitrate/nitrite levels (nmol/mg) were increased in placental tissues from GD (6±0.5 p<0.05) and PGD (9±0.9 p<0.001) when related to controls (4±0.3). In healthy placental tissues, 15dPGJ_2 additions highly reduced nitrate/nitrite levels (1±0.3 p<0.001). Differently, no effect of 15dPGJ_2 on NO production has been detected in GD placenta (7±1). Nitrate/nitrite levels in PGD placenta were reduced by 15dPGJ_2 (4±0.7 p<0.01), and the addition of BADGE, a PPARgamma antagonist, enhanced nitrate/nitrite levels over their initial value (14±1p<0.01).
Conclusion: 15dPGJ_2 levels are reduced in placental tissues from GD and PGD women, probably altering the functions mediated by its receptor PPARgamma. 15dPGJ_2 modulates NO production in placental tissue from healthy patients, but an alteration of this modulatory pathway is detected in placenta from GD patients. In PGD placenta, 15dPGJ_2 decreases nitrate-nitrite concentrations, a mechanism that seems to be mediated by PPARgamma. The important reduction of 15dPGJ_2 levels in this tissue may be involved in the increased nitric oxide levels in placenta from PGD patients.

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Long-term consequences of total caloric restriction during pregnancy on glucose homeostasis.

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Background and Aims: Increasing epidemiological evidence links low birth weight to an increased risk of developing adult diseases including type 2 diabetes, hypertension, and cardiovascular disease. Altered β cell development in utero has been proposed as a key factor for the later occurrence of dysregulations of glucose homeostasis in adulthood. We investigated the long-term effects of the hypercaloric diet on glucose metabolism from offspring undernourished mothers during pregnancy.
Materials and Methods: After wearing, male offspring from undernourished (UN) and control (C) mothers, were divided into two postnatal nutritional groups: standard diet or a hypercaloric diet. Intraperitoneal glucose tolerance test (2g per kg body weight) and intraperitoneal insulin tolerance test (1U per kg body weight) were carried out in both groups before and after standard or hypercaloric diet. Weights and food intake of all offspring were measured for eight weeks. Immunohistochemical and morphometrical studies of islets were carried out.
Results: Offspring from UN mother were significantly smaller at birth (UN 4.56±0.06gr, C 7.10±0.09gr, p<0.05) and after the wearing (UN 58.59±1.55gr, C 82.24±5.45gr, p<0.05) than offspring from C mothers. Differences were found in group fed with hypercaloric diet. Before diet, morphometrical studies showed (UN vs C) individual β-cell area(um^2) 106.78±9.70 vs 114.82±7.95; β-cell/islet (%) 67.55±6.31 vs 51.18±2.50, p<0.05; β-cell/pancreas (%) 4.29±1.56 vs 4.65±1.14. Weight in offspring from UN mothers was significantly high from the first week of hypercaloric diet treatment. After this diet, morphometrical studies showed (UN vs C) individual β-cell area(um^2) 151.64±19.60 vs 101.02±3.87, p<0.05; β-cell/islet (%) 76.09± 4.89 vs 84.66±1.08; β-cell/pancreas (%)11.54± 4.32 vs 3.51± 0.59, p<0.05. Before and after diet, the glucose-AUC wasn´t significantly different. Before diet, insulin AUC were significantly different (UN 36.49±7.25 vs C 20.08±2.99); this difference is still present after diet treatment.
Conclusion: Environmental factors as hypercaloric diet can amplify the metabolic abnormalities in offspring induced by fetal undernutrition. Development of profound adult hyperphagia and weight gain as a probably consequence of fetal programming

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Abnormal free fatty acid suppression in pregnant gestational diabetic women: the role of insulin resistance and insulin secretion.

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Background and Aims: Gestational diabetes (GDM) is a common complication of pregnancy and is associated with insulin resistance and high free fatty acid (FFA) levels. We investigated the suppressibility of FFAs to a glucose load in GDM women during pregnancy and related this to insulin sensitivity(SI) and first phase insulin release (FPIR).
Materials and Methods: 18 Caucasian women with GDM and 18 BMI and age matched control pregnant women underwent an intravenous glucose tolerance test(0.3g/kg) in the third trimester (mean 34 weeks). Glucose, insulin, FFAs were measured serially. SI and FPIR were calculated using the Areas method. Results were analysed using paired t tests and ANOVA.
Results: The two groups were well matched for age (GDM vs C: 32.6 vs 32.8 yrs) and BMI (31.6 vs 31.5 kg/m^2). Kg (glucose disappearance constant) was reduced in the GDM women (1.77±0.08 vs1.34±0.09min^{-1},p<0.01). SI was not significantly different (1.41±0.15 vs 1.00±0.12min^{-1}per mU/l) but FPIR was significantly reduced in the GDM subjects (7.03±1.22 vs12.9±1.52mU/mmol, p=0.001). Fasting FFAs were not different in the two populations(0.558 vs 0.519mM). The suppression curves of the GDM women showed a significantly reduced rate of suppression, with a lower 'Kffa'(FFA disappearance constant - 1.7±0.23vs 2.3±0.25min^{-1},p=0.05). Kffa correlated with Kg (r=0.49,p=0.002) and FPIR (r=0.42,p=0.01), although the latter correlation was present only in GDM subjects (r=0.60, p=0.01 vs NS in controls). Closer inspection of the results revealed two populations within the GDM group: those requiring insulin during pregnancy (n=7) had markedly reduced FPIR (4.01vs15.68mU/mmol,p=0.002) and Kffa (1.01 vs 2.68 min^{-1}, p=0.02) compared to their matched controls, whilst those not requiring insulin(n=11) had similar FFA suppression curves and Kffa (2.20 vs 2.05min^{-1},NS), and did not have a significant reduction in their FPIR(8.17 vs11.23mU/mmol, NS). In order to investigate whether the reduced suppression of FFAs in insulin requiring GDM women was due solely to altered lipolysis, glycerol levels were examined. Although there was a trend towards slower lipolysis in the GDM women (Glycerol disappearance constant: 1.30vs 2.00 min^{-1}), this was not statistically significant. In order to identify if the decreased Kffa was due to altered sensitivity of the adipocytes to insulin, the change in Kffa per incremental insulin release was calculated. There was no significant difference between the GDM and control women (1.58±0.22 vs 1.31 ±0.20min^{-1}per mU/L)
Conclusion: The hyperglycaemia of GDM is due to beta cell insufficiency. The GDM women demonstrated impaired suppression of FFAs compared to their matched controls and this difference was wholly due to a minority of GDM subjects with significant beta cell dysfunction. In these subjects a reduced FFA uptake may contribute to the impaired FFA suppression- as the suppression of lipolysis was not significantly different from controls. Since GDM and control women had similar suppression of serum FFAs per unit of insulin released, the difference in FFA kinetics observed in GDM may be predominantly due to their relative lack of insulin rather than a manifestation of resistance to it.

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Pre-eclampsia in women with Type 1 diabetes is associated with reduced maximal vasodilatory capacity late in pregnancy.

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Background and Aims: Diabetes is associated with increased incidence of pre-eclampsia especially in patients with elevated urinary albumin excretion, high blood pressure and poor glycaemic control. The pathophysiological mechanism is unknown but a vascular dysfunction in diabetic women prone to pre-eclampsia has been hypothesized. the aim of the present study was to investigate vascular function early and late in pregnancy in women with type 1 diabetes and test a potential association with subsequent risk of pre-eclampsia.
Materials and Methods: Eighty-five consecutive pregnant women with type 1 diabetes for more than 10 years were enrolled and vascular function investigated in gestational weeks 10 and 28 by ultrasonic measurements of flow-associated endothelium dependent and nitroglycerin-induced endothelium independent dilatory capacities of the brachial artery. Haemoglobin A1c, s-von Willebrand factor (a potential marker of endothelial dysfunction), s-lipoprotein(a), s-uric acid, s-creatinine, and s-total cholesterol were also measured together with blood pressure.
Results: Fourteen women developed pre-eclampsia. They were at gestational week 10 characterized by elevated urinary albumin excretion (189 (range, 6-3452) vs. 8 (1-310) mg / 24h, p<0.001), higher blood pressure (122 (SD, 12) / 75 (6) vs. 111 (11) / 69 (8) mmHg, p<0.01), and higher haemoglobin A1c (8.2 (1.3) vs. 7.1 (0.9) % p<0.001) than women not developing pre-eclampsia. However, no differences were found in flow-associated (107.0 (5) vs. 106.5 (5) % (percent of baseline diameter), NS) and nitroglycerine-induced (117.2 (7) vs. 122.9 (10) %, p=0.13) dilatory capacities, or in s-von Willebrand factor (1.67 (0.7) vs. 1.34 (0.6) kIU / L, p= 0.09). Serum lipids and s-creatinine were also comparablel but s-uric acid was higher (0.20 (0.05) vs. 0.16 (0.03) mmol / L, p<0.0001) in women developing pre-eclampsia. In week 28 seventy-two women were investigated. Still no difference in flow-associated dilatory capacity was seen (106 (7) vs. 106 (6) %). However, nitroglycerine-induced dilatory capacity was at this point impaired in women developing pre-eclampsia (108,8 (9) vs. 116,9 (9) %, p<0.05). No significant differences were found in the other variables except for s-uric acid (0.26 (0.03) vs. 0.20 (0.04) mmol / L, p<0.01).
Conclusion: Pre-eclampsia in women with type 1 diabetes is associated with reduced maximal vasodilatory capacity late in pregnancy and such reduction may precede development of pre-eclampsia. The reduction does not seem to be caused by endothelial dysfunction.

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TNFα and diabetes-induced embryopathic stress.

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Background and Aims: TNFα is suggested to act as a mediator of diabetes-induced embryopathic stimuli. However, we recently observed that TNFα might act as a protector of embryos exposed to teratogens, possibly, via restoration of the transcription factor NF-κB signaling in embryonic cells. This study was performed to evaluate the input of TNFα in the process of diabetes-induced embryonic maldevelopment and whether NF-κB may be involved in the response of embryos to diabetes-induced teratogenic stimuli.
Materials and Methods: TNFα$^{-/-}$ (B6;129S-Tnf^{tm1Gkl}) and TNFα$^{+/+}$ (B6129SF2/J) female mice were injected with streptozotocin (STZ)immediately after detection of a vaginal plug (day 1 of pregnancy). The blood glucose level (BGL) was measured twice: 2 days after STZ injection and then on days 4, 8 or 9 of pregnancy. Females having a BGL>10 mmol/l at both tested time points were considered as diabetic. Intact females and STZ-injected females having a BGL < 10 mmol/l were used as controls. On days 4 and 8 of pregnancy, females were tested for the presence of blastocysts or implantation sites in the uterus, respectively. 18 day fetuses were examined for structural anomalies using Wilson 's sectioning technique. NF-κB DNA-binding activity was evaluated by EMSA in nuclear extracts isolated from the head of 11day embryos. GT-2 method or Fisher's exact test were used to analyze results statistically.
Results: Two main effects may be attributed to the involvement of TNFα in the response to diabetes-induced embryopathic stress. The first one is a drastically decreased pregnancy rate (approximately, 12%) in the group of TNFα$^{+/+}$ diabetic females having a BGL > 19mmol/l and tested on days 18 and 8 of pregnancy (10 pregnant out of 84 mated females and 6 pregnant out of 47 mated females, respectively). In their TNFα$^{-/-}$ counterparts this index was 35% (13 out of 50) and 40.5% (17 out of 40), respectively, and it was, approximately, 55% in non-diabetic TNFα$^{-/-}$ and TNFα$^{+/+}$ females. The second effect is an increased resistance of TNFα$^{+/+}$ embryos to diabetes-induced teratogenic stimuli. Indeed, the incidence of embryos with anomalies such as micro- and anophthalmia, cleft face, eventration of abdominal wall was near to the zero level in non-diabetic TNFα$^{-/-}$ and TNFα$^{+/+}$ females, reached, approximately, 24% in the above mentioned group of TNFα$^{+/+}$ females and was as twice as higher (approximately, 50%) in their TNFα negative counterparts. We also observed that the level of NF-κB complex formation was clearly lowered in samples obtained from embryos of diabetic TNFα$^{-/-}$ mice having a BGL in the range of 26.6-28.9 mmol/l.
Conclusion: Results of this study suggest that TNFα may act to prevent the occurrence of fetuses with gross structural anomalies in diabetic mice.

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A shift between selective and universal screening of gestational diabetes: preliminary results about 2039 women.

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Background and Aims: Before September 2001, we used to screen gestational diabetes in women meeting one or more of the following criteria: family history of diabetes; body mass index > 27 kg/m2, > 35 years of age; previous pregnancy with gestational diabetes, preeclampsia, malformation, macrosomia; current pregnancy with hypertension, macrosomia, glycosuria, weight gain of at least 12 kg at the end of the second trimester. The aim of the study was to determine the impact of the shift in October 2001 to a universal screening.
Materials and Methods: A 2-h 75-g oral glucose tolerance test was performed between the 24th and the 28th weeks of gestation. Gestational diabetes was defined as glucose value > 5.2 mM (fasting) or > 7.8 mM (2h) or both.
Results: Between October 2001 and September 2002, 2039 pregnant women were consecutively followed-up. In fact 1474 were effectively screened and 220 had gestational diabetes (10.8 % of the population and 14.9 % of the screened women). If the previous selection criteria had been considered, 71 out of the 220 women with gestational diabetes would not have been identified. During October 2000-September 2001, 1948 women had been followed and 162 had had gestational diabetes (selective screening). The determinants of gestational diabetes during the period 2001-2002 were previous pregnancy with gestational diabetes (Odds ratio 10.1 [CI 95% 4.0-26.4] p < 10-4), with macrosomia (5.3 [2.4-11.5] p < 10-4), with malformation (5.3 [1.7-15.8] p < 10-3), family history of diabetes (1.8 [1.3-2.6] p 30 years of age (2.0 [1.5-2.7] p < 10-4), Pakistan or Indian origin (2.5 [1.3-4.8] p < 10-2), current twin pregnancy (3.2 [1.2-8.3] p < 10-2), and third trimester echographic macrosomia (2.5 [1.5-4.2] p < 10-3).
Conclusion: Compared with our previous selective screening, 27 % additional women with gestational diabetes were identified with universal screening. This result is underestimated because only 72 % of the pregnant women were really screened and we should further motivate the women to effectively undergo the screening test. The major determinants of gestational diabetes found here are previous pregnancy with gestational diabetes or macrosomia or malformation, family history of diabetes, > 30 years of age, twin pregnancy and Pakistan or Indian origin.

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Gestational diabetes mellitus and lesser degrees of pregnancy hyperglycemia are associated with increased risk of spontaneous preterm birth.

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Background and Aims: Gestational diabetes mellitus and lesser degrees of glucose intolerance have been associated with several perinatal complications that are more common among preterm infants such as: respiratory distress syndrome, hypocalcemia, hypoglycemia, and

preeclampsia. Four studies have examined the relationship between pregnancy hyperglycemia and preterm birth, however, results have been inconsistent. We investigated the risk of spontaneous preterm birth (SPB) across a spectrum of pregnancy glucose category.
Materials and Methods: We identified a multiethnic cohort of 45,447 pregnancies among women aged 15-19, without recognized diabetes, that underwent a screening 50-g, 1-h oral glucose tolerance test (OGTT) between 24-28 weeks of gestation at the Northern California Kaiser Permanente Medical Care Program between January 1996 and June 1998. Computerized hospitalization and laboratory records were searched to identify women's age, ethnicity, plasma glucose values, infant gestational age at delivery and pregnancy related procedures and complications. Pregnancies with infants born at < 37 weeks after a c-section or induced labor were excluded. SPB was defined as an infant born at < 37 weeks of gestation with at least one of the following: spontaneous labor, preterm premature rupture of membranes or incompetent cervix. Glucose tolerance status was categorized as: normal screening (NS) [1-h plasma glucose <140 mg/dl], abnormal screening (AS) [1-h plasma glucose ≥140 mg/dl with normal diagnostic 100-g, 3-h OGTT], Carpenter Coustan (CC) [plasma glucose measurements during the diagnostic OGTT met the CC thresholds but were lower than the National Diabetes Data Group (NDDG) thresholds], and gestational diabetes mellitus (GDM) by the NDDG criteria.
Results: 1,163 SBPs occurred. The age-adjusted incidence of SPB was: 2.3% in NS, 3.2% in AS, 4.2% in CC and 4.1% in GDM. In a logistic regression model adjusted for age, ethnicity, preeclampsia, abruptio placenta and birthweight for gestational age, pregnancies with AS, CC, and GDM had significantly higher risk of SPB than pregnancies with NS [(RR (95% CI): 1.4 (1.2-1.6), 1.7 (1.2-2.4), and 1.9 (1.5-2.5), respectively].
Conclusion: The risk of SPB increased with increasing levels of pregnancy glycemia and this association was independent of perinatal complications that could have triggered early delivery.

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Obesity increases the risk of congenital heart defects in women with gestational diabetes mellitus.

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Background and Aims: Recent reports have identified maternal obesity as a risk factor for congenital malformations in the offspring of women with diabetes mellitus. In the present study we assessed the influence of pre-gestational body mass index (BMI) on the risk of congenital heart defects (CHD) in the offspring of women with gestational diabetes mellitus (GDM).
Materials and Methods: Using data from the Spanish Collaborative Study of Congenital Malformations, a hospital-based case-control study and surveillance system, we compared the prevalence of CHD in children of mothers with GDM (n=279) with the prevalence of CHD in children of mothers with normal glucose tolerance (NGT, n=6,694). We stratified mothers in both groups into 3 pre-gestational BMI categories (<24, 24-29, ≥30 Kg/m^2) and calculated the ORs and 95% C.I. for CHD using EPI-INFO 6. Logistic regression analyses were performed in all maternal BMI strata to control for confounders including maternal age, maternal educational level, maternal ingestion of alcohol, and use of illicit drugs.
Results: CHD were present in 49 infants of 279 mothers with GDM (9 conotruncal, 34 non-conotruncal, 6 vascular) and 764 infants of 6,694 mothers with NGT (131 conotruncal, 569 non-conotruncal, 64 vascular). The risk of all types of CHD was significantly higher in the infants of mothers with GDM compared with infants of mothers with NGT (OR=1.65; 95% CI 1.19-2.30; P=0.002). After stratifying the two groups by pre-gestational BMI, no difference in infant rate of CHD was observed between women with GDM and non-diabetic controls with BMIs <24 kg/m^2 (OR=1.23; 95% CI 0.76-1.98; P=0.38) and with BMIs 25-29 kg/m^2 (OR=1.72; 95% CI 0.89-3.25; P=0.08). However, offspring of mothers with GDM and a BMI ≥30 kg/m^2 had a significantly higher risk of CHD than the children of obese mothers with NGT (OR=3.56; 95% CI 1.62-7.74; P=0.0003). The analysis of CHD cases by pathogenetic categories showed that women with GDM and BMI ≥30 kg/m^2 had significantly higher risks than their non-diabetic controls for conotruncal (OR=3.92; CI 1.00-14.26; P=0.016) and non-conotruncal defects (OR=3.78; CI 1.50-9.35; P=0.001). Risk of these defects in the lower BMI strata and risk of vascular CHD in all BMI categories were not significantly different between the two groups. Within the GDM group, there was a significantly greater risk of CHD in infants of obese mothers compared with infants of normal weight mothers (OR=2.82; CI 1.19-6.65; P=0.008). The rate of CHD in infants of women with NGT was not affected by maternal pre-gestational BMI.
Conclusion: Infants of obese women with GDM have a significantly increased risk of CHD. In our cohort, increased pre-gestational BMI did not affect the rate of CHD in infants of women with NGT.

OP 24
Pathogenesis of Microvascular Disease

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Increased plasma adiponectin concentrations in patients with Type 1 diabetes insulin-dependent: association with microangiopathy.

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Background and Aims: Adiponectin, secreted by adipocytes, seems to play a causative role in the development of insulin resistance which is implicated in diabetic microangiopathy risk. We tested association between plasma adiponectin levels and microangiopathy in type 1 diabetic patients.
Materials and Methods: We compared plasma adiponectin levels of 126 type 1 diabetic patients (T1D) without renal complications, followed prospectively during 9 years, to that of 360 non diabetic control subjects, age (44 ± 9 vs 39 ± 12 years), sex (172M/188F vs 76M/50F) and BMI (24.3 ± 3.7 vs 23.0 ± 3.0 kg/m2) matched. Plasma adiponectin was determined by RIA (Linco, St Charles, MO, USA; sensitivity 1 ng/ml, intra and inter assay CV 4.4 % and 9.9 % respectively). T45G polymorphism was genotyped by Molecular Beacon technique.
Results: Plasma adiponectin levels were higher in T1D than in healthy controls : 31.3 ± 13.8 vs 18.3 ± 8.8 µg/ml ($p < 0.001$). This was not explained by the frequency of GG genotype: 1.7 vs 1.8 % in T1D and controls respectively. Adiponectinemia was higher in females than in males in T1D and controls groups: 36.2 ± 13.8 vs 28.1 ± 13.7 and 22.2 ± 9.0 vs 14.0 ± 6.3 µg/ml respectively. Plasma adiponectin concentration was negatively correlated with BMI in controls ($p<0.01$) but not in T1D patients. In T1D, no correlation between adiponectin levels and HbA1c at entry or during the study was observed. Adiponectin levels at entry in the study were higher in patients with preproliferative (39.8 ± 13.6, n = 19) or proliferative retinopathy (37.7 ± 15.5, n = 10) than in patients having no retinopathy (29.8 ± 12.9, n = 69) or with simplex retinopathy (27.0 ± 13.0, n = 28) ($p = 0.0041$). However, adiponectin levels of patients who developped preproliferative or proliferative retinopathy during the follow up were not different from subject without complication. There was no difference beetwen adiponectin levels of patients developing or not nephropathy during the follow up.
Conclusion: Adiponectin levels in type 1 diabetes patients are not related with BMI nor with diabetes control but seem to be a marker of the severity of diabetic microangiopathy.

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Retinal angiogenesis is mediated by an interaction between the angiotensin Type 2 receptor, VEGF and angiopoietin.

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Background and Aims: There is evidence that angiotensin II, vascular endothelial growth factor (VEGF), angiopoietins and their cognate receptors participate in retinal angiogenesis, a hallmark feature of proliferative diabetic retinopathy. We investigated whether angiotensin type 2 (AT2) receptor antagonism (AT2-RB) reduces retinal angiogenesis and alters the expression of VEGF/VEGF-R2 and angiopoietin-Tie2.
Materials and Methods: Retinopathy of prematurity (ROP) was induced in Sprague Dawley (SD) rats by exposure to 80% oxygen from postnatal (P) days 0-11, followed by 7 days in room air. ROP shams were in room air from P0-18. A group of ROP rats received the AT2-RB, PD123319, by miniosmotic pump (5mg/kg/day) from P11-18 (angiogenesis period).
Results: The abundance of the angiotensin type 1 (AT1) and AT2 receptors was evaluated in SD retina at postnatal days 1,7,14,21 and 90. Evaluation of the retinal status of the AT2 receptor indicated that this receptor, as assessed by real-time PCR, immunohistochemistry and in vitro autoradiography, was present in the retina, was more abundant than the AT1 receptor in the neonatal retina and was increased in the ROP model. AT2-RB with PD123319 reduced retinal new vessel formation. Gene expression for VEGF and VEGF-R2 revealed an increase in the ROP model which was localised to blood vessels, ganglion cells and the inner nuclear layer and were decreased by PD123319. Angiopoietin2 and Tie2, but not angiopoietin1 mRNA were increased with ROP, and angiopoietin2 was reduced with PD123319.
Conclusion: The present study has identified a potential retinoprotective role for AT2 receptor inhibition possibly mediated via interactions with VEGF and angiopoietin dependent pathways. These findings extend the evidence for a pivotal role of the renin-angiotensin system in retinal disorders.

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Impaired angiogenesis response to limb ischemia in genetically Type 2 diabetic mice is corrected by gene therapy with tissue kallikrein or activated Akt-B kinase.

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Background and Aims: Human type II diabetes is associated with microvascular complications leading, between the others, to limb muscle microangiopathy, predisposition to develop peripheral ischemia, and reduced angiogenesis compensatory response to the ischemic event. We used a genetic murine model of type II diabetes, the c57bl/ks-Lepr<db> mice, to investigate the therapeutic potential of pro-angiogenic and anti-apoptotic gene therapy approaches to rescue the ischemic diabetic limb adductors.
Materials and Methods: Ischemia was induced in 5 month-old diabetic mice and in their wild type controls (c57bl/ks-Lepr<db/+) by coagulating the upper part of left femoral artery. Immediately thereafter, 10^8 plaque forming units (p.f.u.) of an adenoviral vector carrying the gene for human tissue kallikrein (TK) or for activated Myr-Akt-B kinase were injected (in a 10 µL of injection volume) into the ischemic adductor muscles. Hindlimb blood flow was measured by color laser doppler (Lisca) at 30 min from surgery and at weekly intervals thereafter up to 2 weeks. Clinical outcome was evaluated at 1 and 2 weeks. Then mice were sacrificed and their adductor muscles processed for immuno-histochemical analysis of capillary and arteriole densities. Apoptosis of endothelial cells (ECs) and myocytes was recognized by TUNEL staining and quantified.
Results: We have found that under basal conditions untreated diabetic adductors present rarefaction of both capillaries (596±51 versus 752±49 cap/mm^2 in non-diabetics, $P<0.05$) and arterioles (8.9±1.8 versus 17.9±0.24 art/mm^2 in non-diabetic mice, $P<0.05$). In addition, the neovascularization reparative response to ischemia that is present in non-diabetic mice (capillary density increased to 1036±54 cap/mm^2 and arteriole density increased to 14.6±2.9 art/mm^2,$P<0.05$ vs not ischemic for both comparisons) is negated to the diseased strain ($P<0.05$ vs not ischemic for both comparisons), thus producing a deficit in its blood flow recovery ($P<0.05$ versus not diabetics) and compromising clinical outcome. Gene therapy with TK normalized capillary and arteriole denisity (1033±66 cap/mm^2 and 36.6±6 art/mm^2, $P<0.05$ for both comparisons vs. untreated) and a similar effect was induced by Myr-Akt-B gene transfer (data not shown). In addition, Myr-Akt-B reduced EC and myocyte apoptosis in ischemic diabetic muscles (10±9 versus 30±28 TUNEL-positive EC/1000 cap and 11±9 versus 27±16 TUNEL-positive myocytes, $P<0.05$ for both comparisons).
Conclusions: Our data suggest that a form of microangiopathy similar to the human diseases compromises the post-ischemic recovery of murine diabetic muscles. Angiogenesis gene therapy with TK or Myr-Akt-B could be used in the attempt to restore the proper healing response to peripheral ischemia.

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Prophylactic gene therapy with human tissue kallikrein ameliorates post-ischemic recovery of diabetic limbs.

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Background and Aims: Diabetic macro and microvascular disease causes tissue hypoperfusion. This deficit together with failure to mount an adequate angiogenic response explains why vascular occlusion evolves more severely among diabetics. Recently, we demonstrated that local gene therapy with human tissue kallikrein (hTK) is able to prevent microangiopathy in skeletal muscles of type 1 diabetic mice. The present study was designed to assess if prophylactic hTK angiogenic therapy would protect diabetic limbs from the consequences of supervening ischemic insult.
Materials and Methods: In streptozotocin (STZ)-induced diabetic mice, hTK gene was delivered to the left adductor muscle 2 weeks before operative occlusion of ipsilateral femoral artery. Hindlimb blood flow

recovery was analysed sequentially up to 14 days. At necroscopy, microvessel density and endothelial cell (EC) proliferation and apoptosis were quantified in skeletal muscles. *In vitro*, we tested if kinins, the hTK biologic end-products, suppresses EC apoptosis caused by incubation in high glucose medium.
Results: Hemodynamic recovery after femoral artery occlusion was impaired in diabetic mice. In fact, femoral blood flow remained persistently reduced at 14d, a time sufficient for non-diabetic to regain preoperative levels (0.74±0.03 vs. 0.98±0.04, $P<0.05$). In diabetic muscles, ischemia-induced native capillarization was abrogated and arteriogenesis attenuated. EC proliferation was not altered in diabetic muscles. However, the effort to build up new vessels to restore proper perfusion was frustrated by a dramatic increase in EC death by apoptosis at late stages of repair. The effect was even more evident when normalizing the number of apoptotic ECs by capillary density (37±20 vs 4±3 apoptotic ECs/1000 cap in contralateral normoperfused muscles, $P<0.01$). Prophylactic gene therapy made diabetic animals capable of a robust reparative response to supervening ischemia. Capillarization was increased by 40% in Ad.hTK-pre-treated ischemic muscles, whereas the response was virtually absent in adductors given saline- or Ad.Luc . HTK drastically reduced apoptosis at EC and myocyte level. Diabetic mice given preventive hTK gene transfer displayed accelerated perfusion recovery compared with other groups ($P<0.05$ vs. either saline or Ad.Luc). Prophylactic intervention restored the figure observed in non-diabetic controls. Exposure to high glucose induced apoptosis of cultured EC, while BK reduced this effect ($P<0.05$).
Conclusion: Results indicate that uncontrolled apoptosis jeopardizes post-ischemic healing in diabetes. Prophylactic gene therapy with hTK improves the biology of diabetic ECs, by enhancing their proliferative potential and making them more resistant to death, and thereby facilitates reparative angiogenesis. These discoveries disclose new therapeutic options for the treatment of diabetic complications.

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Effects of angiotensin II receptor inhibition on insulin-induced endothelial dysfunction in humans.
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Background and Aims: In healthy humans, experimentally-induced mild hyperinsulinemia (~120 pM) impairs flow-mediated endothelium-dependent vasodilation (FMD) in large conduit arteries by increasing oxidant stress. In in vitro systems, insulin (I) up-regulates angiotensin II-receptor 1 (ATR1) in endothelial and vascular smooth muscle cells, and this in turn can augment superoxide anion generation by NADP(H)-oxidase which is a target of ATR1 action. We therefore hypothesized that ATR1 blockade (ATR1-B) could prevent the detrimental effect of I on FMD.
Materials and Methods: In 8 healthy (age: 23.3±0.4 y/o; BMI: 22.4±0.5 kg/m^2) subjects we performed 4-hours euglycemic low dose I (~85 pM) clamps on two separate occasions, preceded by the oral administration of either an ATR1 blocker (valsartan: 160 mg) or placebo (Plc). FMD was assessed by high-resolution echo-doppler in the common femoral (FA). Endothelium-independent vasodilation was assessed with sequential sublingual administration of a sub-maximal (15 μg/m^2 of body surface area) and a maximal (300 μg) dose of glyceryl-trinitrate (GTN). Vascular reactivity was assessed before treatment and at the end of the I clamp and is expressed as average % increase over resting arterial diameter over 8 minutes (FMD) and over 5 minutes (GTN).
Results: I-mediated glucose disposal was superimposable in the two occasions (15.8±1.7 vs 14.7±1.2 μmol/min/kg, with Plc and ATR1-B respectively), but the aldosterone to renin ratio in plasma was lower after ATR1-B (0.0064±0.002) than after Plc (0.011±0.03), documenting effective inhibition of ATR1. During the Plc study, I caused a fall in FMD (2.8±0.4 to 1.0±0.4%, $p<0.01$). During ATR1-B study, there was a significant preservation of FMD in the FA (2.5±0.5% $p<0.05$ vs Plc) while GTN-induced vasodilation was superimposable to the Plc study (3.2±0.6 vs 3.4±0.7% at the sub-maximal dose, and 4.7±0.5 vs 5.2±1.0% at the maximal dose, ATR1-B vs Plc study, respectively).
Conclusion. ATR1-B by valsartan can prevent, at least partially, the detrimental action of I on FMD of the FA. ATR1-B seems to act selectively on endothelium, because no effects are detectable in endothelium-independent vasodilation. Further studies are needed to elucidate whether ATR1-B specifically stops the mechanism(s) put in motion by I to cause endothelial dysfunction.

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Hyperglycaemia-induced changes in MAPK and Akt signaling pathways and hypoxia-inducible factor-1a level in cerebral microvascular endothelial cells.
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Background and Aims: Hyperglycaemia-induced microvascular damages have been implicated in the development of diabetic complications including neuropathy, retinopathy, and stroke. The effects of high glucose concentration and hypoxia-reoxygenation induced oxidative stress were investigated on the activation of several members of MAPK superfamily, Akt, and HIF-1a in cerebral microvascular endothelial cells (CMVECs) forming the blood-brain barrier.
Materials and Methods: This study was carried out (i) on isolated cerebral microvessel fractions derived from parietal cortex of dogs suffering from alloxan-induced diabetes for 5-6 weeks, (ii) on primary cultures of rat CMVECs, and (iii) on monolayers of immortalized rat CMVEC line GP8. Rat CMVECs were treated with DME medium containing either high (30 mM) or normal (5 mM) glucose concentration for up to 10 days in order to characterize time-dependent changes. In some experiments the cells were challenged with hypoxia (1% O2 for 4-16 h) alone, or combined with reoxygenation (for 2-8 h). Cell extracts were analysed using phosphorylation-specific ERK1/2, Akt, p38, and nitro-Tyr antibodies, and HIF-1a specific antibody. Luciferase reporter plasmid contruct served to analyse HIF-1a activity during hypoxia and reoxygenation in GP8 cells. Production of ROS and NO was assayed in vitro in GP8 cells cultured in 96-well dishes using fluororescence tracers CM-H2DFDCA and DAF, respectively. VEGF expression was assessed by Northern blot to evaluate the effect of HIF-1a level.
Results: Alloxan-induced diabetes decreased Akt and ERK1/2 activity in canine brain capillaries, and these changes could be restored with insulin treatment. High glucose concentration resulted in significantly ($P<0.01$) elevated ROS production and slightly increased NO release by GP8 cells. Elevated glucose concentration decreased the basal level of HIF-1a in rat CMVECs, and hypoxia resulted in a smaller increase of HIF-1a during hyperglycaemia, than in cells treated with 5 mM glucose. Similarly, hypoxia produced more pronounced increases in the activity of Akt and ERK1/2 pathways in CMVECs treated with normal than high glucose medium. Both basal intensity and hypoxia-induced increase of nitro-tyrosine in GP8 cells were slightly higher after 5 mM, than 30 mM glucose. Novel N-Gene hidroximic derivatives were tested, and some found effective, on hyperglycemia-induced depression in signaling pathways.
Conclusion: Hyperglycaemia decreased ERK1/2 and Akt activity in CMVEC both in vivo and in vitro. Hypoxia induced lower increases in HIF-1a expression in cells treated with high glucose level, and this finding was in accordance with the level of tyrosine nitrilation of proteins. Data suggest that reactivation of the signaling pathways may represent a rational therapy for diabetic endothelial dysfunction.

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Effects of p38 MAP kinase inhibition on vascular reactivity in experimental diabetes.
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Background and Aims: In addition to a variety of cellular functions, p38 MAPK transduce signals that modulate activity of vasoactive factors such as nitric oxide (NO) or prostanoids. Increased vascular and renal p38 activity has been recently documented in diabetes. However, the pathophysiological consequences of those findings remain unclear and possible role of p38 in alterations of vascular reactivity in diabetes has not been studied. The aim of these studies was to determine effects of p38 inhibition with SB202190 (SB) on vascular reactivity in control and diabetic rats.
Methods: Aortic rings and renal arteries (n=4 per treatment) were harvested from control (C) and moderately hyperglycemic streptozotocin

diabetic rats (D) 3 to 4 weeks after induction of diabetes. KCl or endothelin-1 (ET-1) preconstricted vessels were relaxed with increasing doses of the endothelium-dependent (ED) vasodilator acetylcholine (ACh). Prior to inducing relaxation vessels were incubated with 25 μM SB, inhibitor of p38, or vehicle for 30 minutes. The dose response curves were utilized to calculate the dose of ACh necessary to elicit 50% response (ED50).
Results: ACh induced significant vasodilation both in C and D aortic rings. In KCl-, although not in ET-1-preconstricted vessels the vasodilator response was greater in D as compared to C (0.08±0.01 vs. 0.20±0.01 μM ACh, p<0.05). With either contracting compound, addition of SB impaired the vasodilatory effect of ACh in D, but not in C (data in KCl-preconstricted vessels are shown: C-vehicle, 0.20±0.01, C-SB, 0.20±0.02 μM ACh, n.s.; D-vehicle, 0.08±0.01 D-SB, 0.15±0.02 μM ACh, p<0.01). SB on its own relaxed renal arteries both in C and D. This effect was greater in C (C-vehicle, 28±5, C-SB, 68±4%, p<0.05.; D-vehicle, 3±3 D-SB, 55±4%, p<0.05; p<0.05 C-SB vs. D-SB). ACh-induced vasodilation of renal arteries was similar in C and D. In contrast to aortic responses, incubation with SB resulted in enhanced ACh-induced vasodilation both in C and D (C-vehicle, 0.31±0.02 C-SB, 0.20±0.03 μM ACh, n.s.; D-vehicle, 0.33±0.02 D-SB, 0.17±0.03 μM ACh, p<0.01, p=n.s. C-SB vs. D-SB).
Conclusion: p38 inhibition attenuated ED vasodilation in the diabetic, but not in normal rat aorta. p38 inhibition per se induced vasodilation and further enhanced ED vasodilation in the renal artery both in C and D vessels. However, the renal artery vasodilator effect of p38 inhibitor was weaker in D. These data indicate differential roles of p38 signaling in the control of vascular tone in large conduit and renal arteries and their complex alterations in diabetes.

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Ethnic differences in microvasculature in diabetes: a possible explanation for the greater vulnerability of people of Black African descent to vascular target organ damage.

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Background and Aims: People of Black African descent with diabetes have a greater risk of vascular target organ damage than would be anticipated for any given blood pressure level. This may be due to differences in microvascular structure and function.
Materials and Methods: Men and women aged 40-64 from the general population; 100 Europeans, 88 African Caribbeans without diabetes, and 49 Europeans and 66 African Caribbeans with known diabetes, were studied. Skin microcirculation was assessed using laser Doppler fluximetry. Average flux over 8 points was measured on the dorsum of the foot at room temperature and compared to average flux from a region maintained at 42°C for 30 minutes. Peak hyperaemic response was assessed following release of a 3-minute arterial occlusion. Echocardiographic intra-ventricular septal (IVS) thickness was measured as a proxy for macrovascular target organ damage.
Results: Maximum microcirculatory response to heating was lower in volunteers with diabetes compared to those without (Age-adjusted mean; 129.8 (25^{th}, 75^{th} percentile; 122.3, 138.4) vs. 151.7 (140.6, 161.8) arbitrary units (au) respectively; p=0.005). Within the diabetic population, maximum microcirculatory response was attenuated in the African Caribbeans compared to Europeans (118.6 (102.3, 125.8) vs. 151.7 (138.8, 156.3) au; p=0.018). This ethnic difference persisted after adjustment for age, sex, blood pressure, and weight (β (regression coefficient for African Caribbeans vs. Europeans; ± SE.) -0.234± 0.105; p=0.028). Peak response following arterial occlusion was attenuated in those with diabetes (31.9 (30.4, 33.7) vs. 41.7(39.1, 44.0) au; p<0.001), and attenuated in African Caribbeans with diabetes (26.5 (25.7, 27.1) vs. 37.5 (36.9, 38.0) au; p=0.002). This difference was not altered by adjustment for standard risk factors. There was no diabetes/ethnicity interaction in these measures. There was an inverse association between IVS and maximal microcirculatory response (β -0.408 ± 0.142; p=0.004), which persisted after adjustment for age, sex, blood pressure and weight (β -0.241±0.117; p=0.04). There was no ethnic difference in diabetic control (HbA_{1C} in African Caribbeans 7.67±0.26 vs. 7.80±0.29; p=0.8).
Conclusion: Microvascular response to temperature and arterial occlusion was attenuated in African Caribbeans compared to Europeans with diabetes. These changes could not be accounted for by standard risk factors and we believe represent changes in microvascular structure and function that could account for increased target organ damage in African Caribbeans. The inverse association between heated response and IVS supports this.

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Dissociation of protein kinase C activities in the endo- and perineurium in diabetic mice transgenic for human aldose reductase and the effects of aldose reductase inhibitor.

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Background and Aims: Alterations of protein kinase C (PKC) activity are considered to play a major role in the pathogenesis of diabetic neuropathy. However, it is still unclear whether the polyol pathway hyperactivity is related to the changes of PKC activity. To clarify this issue, we examined PKC activities separately in the endoneurial and vessel-rich perineurial tissues in diabetic mice transgenic for human aldose reductase (Tg) and the effects of aldose reductase inhibitor (ARI). We also evaluated the expressions of PKC isoforms by Western blot analysis.
Materials and Methods: Tg and littermate control mice (Lm) were made diabetic by streptozotocin (i.p. 200 mg/kg) at 8 wks of age and treated with ARI (fidarestat 4mg/kg/day, per os) or placebo for 12 wks. Non-diabetic Tg and Lm were used for comparison. At the end of experiment, following the measurement of motor nerve conduction velocity (MNCV), peripheral nerves were excised and immediately prepared into fractions of endoneurial and perineurial tissues. These samples served for the assay of sorbitol concentrations, PKC activities and Western blot analysis on the isoforms of PKC.
Results: Both diabetic Tg and diabetic Lm showed reduced body weight and elevated blood glucose levels to similar extents. ARI-treatment did not influence these values. MNCV was reduced 26 % in diabetic Tg compared to non-diabetic Tg ($p<0.01$). ARI treatment prevented this reduction. Sorbitol levels were increased in diabetic groups in both endoneurial and perineurial tissues compared to non-diabetic groups, and the levels were 3-5 times greater in the former than in the latter. Compared to non-diabetic state, the increase was 6.4 fold in the endoneurium and 5.1 fold in the perineurium in diabetic Tg, whereas diabetic Lm showed a 3.5 fold increase in the former and 1.8 fold in the latter. Average PKC activity of the membrane fraction was significantly reduced by 38 % in the endoneurium of diabetic Tg ($p<0.01$ vs non-diabetic Tg) but not so in diabetic Lm ($p=0.076$ vs non-diabetic Lm). ARI treatment normalized the decreased PKC activity in diabetic Tg. By contrast, the membrane PKC activity in perineurial tissues was increased in both diabetic Lm and diabetic Tg ($p<0.01$ vs both non-diabetic Tg and Lm) and there was no significant difference between these groups. These values were all corrected by ARI treatment. Western blot analysis revealed that expressions of PKC α in the endoneurial membrane fraction were decreased by 67 % in diabetic Tg and 37 % in diabetic Lm compared to their non-diabetic groups ($p<0.01$ for both). By contrast, there were increased expressions of PKC βII in the perineurial membrane fractions in diabetic Lm and diabetic Tg compared to non-diabetic groups ($p<0.01$ for both). The elevated expressions were reverted to normal by ARI treatment.
Conclusion: These findings indicate that an increased polyol pathway is associated with decreased PKC activities in the endoneurium and increased PKC activities in the perineurium. The alterations of PKC activities are considered to be mainly due to depressed PKC α expressions in the endoneurium and augmented PKC βII expressions the perineurium.

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Neuroprotective effect of AT-1015, a novel 5-HT_{2A} antagonist, in experimental diabetic neuropathy.

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Background and Aims: Impaired nerve blood flow and oxidative stress play an important role in the etiology of experimental diabetic neuropathy. AT-1015 is a novel 5-HT_{2A} antagonist that has been reported to improve impaired nerve blood flow and nerve conduction deficits in diabetic rats. In addition, AT-1015 exhibits hydroxyl radical scavenging activity in the H_2O_2/UV system (ED_{50}=1.1mM) and improves impaired endothelium-dependent vascular relaxation in diabetic rats. In this study, we therefore investigated the long-term effect of AT-1015 on neuronal and schwann cell function in diabetic rats by analysis of changes in gene expression.
Materials and Methods: Streptozotocin-induced diabetic rats were administered the vehicle, AT-1015 (3mg/kg) or insulin for 18 weeks. During the experimental period, HbA_{1c} and motor nerve conduction velocity (MNCV) were monitored. At the end of the experiment, mRNA expression of MnSOD, catalase and bcl-2 in dorsal root ganglia (DRG), and cycline D1 and nerve growth factor (NGF) in sciatic nerve was analyzed by quantitative RT-PCR analysis.
Results: AT-1015 had no effect on the elevated HbA1c level, whereas insulin normalized it. Diabetic rats showed a 20% reduction of MNCV ($p<0.01$) by wk 18, but it was normalized by both AT-1015 and insulin treatment from wk 3. In diabetic rats, expression of MnSOD and catalase mRNAs in the DRG was decreased to 75% of normal in wk 3 ($p<0.05$), but recovered to normal by wk 18. In contrast, bcl-2 expression in the DRG was increased by 1.5-fold above the normal level ($p<0.05$) and cyclin D1 expression in the sciatic nerve was decreased to 70% of normal ($p<0.05$) in wk 18. Both AT-1015 and insulin treatment were able to normalize bcl-2 and cyclin D1 expression in wk 18. In addition, AT-1015 increased NGF mRNA expression in the sciatic nerve by 1.9-fold compared with untreated diabetic rats ($p<0.05$).
Conclusion: These findings indicated that AT-1015 protected peripheral neurons from increased apoptotic stress caused by hyperglycemia, such as oxidative stress. In addition, AT-1015 might also have restored the proliferative activity of schwann cells and promoted the production of neurotrophic factors. From these results, AT-1015 may have a neuroprotective effect and support the regeneration of axons in the peripheral nerves of patients with diabetic neuropathy through restoration of nerve blood flow and antioxidative activity

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Nuclear factor kappa B inhibition improves nerve function in diabetic rats.

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Background and Aims: Impaired nerve perfusion contributes to the neuropathic changes in experimental diabetes. Elevated advanced glycation and oxidative stress are implicated in vascular complications. One potential mechanism whereby these stressors act is via activation of the transcription factor, nuclear factor kappa B, (NFkB). This hypothesis could apply to neuropathy, although this has not been direct tested. The aim was to ascertain whether NFkB is involved in the adverse neurovascular changes in streptozotocin-diabetic rats by examining the effects of NFkB inhibitors.
Materials and Methods: Streptozotocin-diabetic rats were treated for 2 weeks after 6 weeks of untreated diabetes. The drugs used were pyrrolidine dithiocarbamate (PDTC, 40 mg/kg i.p.), which is a powerful NFkB inhibitor that also has antioxidant properties, and N-alpha-tosyl-L-lysine chloromethylketone (TLCK, 5mg/kg i.p.), which is a serine protease inhibitor that blocks the activity of IkappaB alpha protease, thus potentiating endogenous NFkB inhibition. Measurements were made on nerve conduction velocity (NCV) and nerve blood flow. Sciatic nerve frozen sections were examined immunohistochemically for NFkB staining. A further investigation, using TLCK alone, examined the relaxant responses of the gastric fundus, in vitro, following stimulation of the nitrergic autonomic innervation.
Results: Diabetes caused a 23.2±1.3% reduction (±SEM; $p<0.001$) in sciatic motor NCV. This was 77.0±7.4% and 90.7±7.9% corrected by PDTC and TLCK treatment, respectively (both $p<0.001$). Saphenous nerve sensory NCV was 21.0±1.2% decreased ($p<0.001$) by diabetes; PDTC and TLCK treatments caused 80.9±4.5% and 81.5±4.6% improvements ($p<0.001$), respectively. Sciatic nerve blood flow was 48.0±3.0% reduced ($p<0.001$) by diabetes: perfusion was completely restored by TLCK and 84.2±10.8% improved by PDTC ($p<0.001$). Immunohistochemical staining of sciatic nerve sections for the NFkB p65 subunit showed diffuse low level staining across endoneurium and epi / perineurium. This was elevated by diabetes, particularly in the epi / perineurium and associated blood vessels where there was a 4-fold increase in staining intensity ($p<0.01$): PDTC reduced staining intensity by 97.6% ($p<0.01$). TLCK was without effect on NFkB p65 staining, in keeping with an indirect action via inhibitory mechanisms. Maximum relaxation of 5-hydroxytryptamine precontracted gastric fundus strips to electrical field stimulation was 42.5±9.0% reduced by diabetes ($p<0.001$). TLCK treatment partially (55.1±14.8%; $p<0.05$) corrected this deficit.
Conclusion: The data support the hypothesis that NFkB activation makes an important contribution to experimental somatic and autonomic diabetic neuropathy, and could be a potential new therapeutic target.

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Evaluation of orally active poly (ADP-Ribose) polymerase inhibitor in the model of early diabetic neuropathy.

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Background and Aims: Oxidative stress is involved in diabetes-induced functional, metabolic and morphological changes in peripheral nerve, but the mechanisms are unknown. ROS-induced poly(ADP-ribose) polymerase (PARP) activation depletes NAD^+ and high-energy phosphates, and activates protein kinase C, NF-κB and other transcription factors. This, in turn, upregulates expression of numerous genes including those implicated in diabetic complications e.g., endothelin-1, inducible nitric oxide synthase, cyclooxygenase-2. Thus, PARP activation triggers multiple pathogenetic mechanisms downstream from oxidative stress. It has been reported that PARP-deficient mice do not develop early diabetic and diabetes-like neuropathy (DN). This study was designed to assess if established functional and metabolic deficits of early DN can be reversed by a short-term treatment with orally active PARP inhibitor.
Materials and Methods: Control (C) and streptozotocin-diabetic (D) rats were treated with the potent, specific, orally active inhibitor PJ34 (30 mg kg^{-1} d^{-1}, in the drinking water) for 2 weeks after 2 weeks without treatment. We and others previously reported that rats with 2-week duration of STZ-diabetes have established functional and metabolic changes of early DN. Sciatic endoneurial nutritive blood flow (NBF) was assessed by microelectrode polarography and hydrogen clearance and nerve metabolite concentrations spectrofluorometrically, by enzymatic procedures. Griffonia simplicifolia isolectin B4 and anti-S-100-antibody have been used for immunolocalization of poly(ADP-ribose) in endothelial and Schwann cells, respectively.
Results: Intense poly(ADP-ribose) immunostaining localized in both endothelial and Schwann cells of sciatic nerve, was observed in D, but not in 3 other groups. Blood glucose concentrations were similarly elevated in D and D+PJ34 vs. C. Sciatic motor and digital sensory nerve conduction velocities (MNCV, SNCV) were reduced in D vs C (Mean±SEM, 44.6±1.0 and 30.6±0.5 vs 58.5±1.7 and 39.2±1.3 m s^{-1}, $p<0.01$ for both), and essentially corrected by PJ34 (57.1±1.2 and 38.3±0.4, $p<0.01$ vs. D for both). Of interest, NBF showed a modest (17%) increase with PJ34 treatment [C:15.09±0.39; D: 7.57±0.48 ($p<0.01$ vs. C) and D+PJ34: 10.06±0.48 ml $min^{-1}100$ g^{-1}, $p<0.01$ vs D], and vascular conductance only a trend to an increase ($p>0.05$ vs. D). Free mitochondrial and cytosolic NAD^+/NADH ratios, assessed from the glutamate and lactate dehydrogenase systems, as well as phosphocreatine (PCr) concentrations and PCr/Creatine ratios were decreased in D and essentially normalized in D+ PJ34. Nerve glucose, sorbitol and fructose concentrations were similarly increased in D and D+PJ34. PJ34 did not affect any variable in C.
Conclusion: Short-term PARP inhibitor treatment can reverse functional and metabolic abnormalities of, at least, early DN. *Complete* normalization of NBF is not necessarily required for correction of either MNCV or SNCV provided that the therapeutic agent (or combination therapy) can restore peripheral nerve energy state via direct action on non-vascular nerve elements, like Schwann cells in this particular case.

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Erectile dysfunction in diabetic patients: results of 4 years of screening in patients population.

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Background and Aims: The Erectile Dysfunction is a pathology that interests many patients; in United States it has been estimated that from the 20 to 30 million persons are affected. From the Massachusets Male Aging Study (MMAS) to all main international studies made in the general population have shown an increase of prevalence of pathology in the subgroup of diabetic patients. Studies carry out in diabetic patients have shown discordant data: McCullock in 1980 prevalence of 35%, Brunner of 49% in diabetic type 1. Klein stratifying the data for age has shown prevalence of 1% between the range 21-30 years old and of 47% in patients with advanced age to the 43 years, always in patients type 1. Nathan in patients type 2 of age comprised between the 55 and 74 years has found prevalence of 71%. In Italy in 1996 Fedele has shown the presence of Erective Dysfunction in 36% of the interviewed patients; the prevalence varied from 63% in the diabetic patients type 2 to 74% in diabetic the patients type 1 and increased with age, presence of bad metabolic control, smoke, Diabetic Neuropathy, Peripheral Vasculopathy, Diabetic Retinopathy, Diabetic Nephropathy. Scope of our study has been to verify this data in our outpatients population.
Materials and Methods: We have screened for Erectile Dysfunction in 4 years 2019 (438 Type 1 and 1581 Type 2) diabetic patients afferent to clinic for the screening and diagnosis of complication of the diabetes using the International Index of Erectile Function-5 (IIEF-5).
Results: 1428 (70.7%) patients have positive score of Erectile Dysfunction (IIEF-5≤21). Between positive and negative (IIEF-5>21) to screening we have found significant differences about age, duration of diabetic disease and anthropometrical indices (BMI, Waist Circumference and Waist/Hip ratio). Smokers and former smokers had a significant reduced score regarding patients never smoking. Also metabolic control as higher HbA1c, Dyslipidaemia and Microalbuminuria influenced in significant way score of IIEF-5. Presence of complications increase in significant way the probability to be positive to IIEF-5; moreover if complications added the score significant reduced. Subgroup of diabetic patients type 2 has shown that when metabolic syndrome risk factors (Dyslipidaemia, Hypertension, Microalbuminuria, Visceral Obesity) increased the score significant reduced.
Conclusion: We have demonstrated that diabetic patient is particularly at risk to develop Erectile Dysfunction especially when to increase of presence of the complications and others risk factors, and that in the diabetic patient type 2 the presence of the Metabolic Syndrome is a serious risk factor for Erectile Dysfunction like it is for the cardiovascular events, strengthening hypothesis that to the base of both pathologies, there are a common mechanism. If it is true, in diabetic patient type 2 with Erectile Dysfunction must make a screening for cardiovascular disease.

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Physician perception of neuropathy in a large Type 2 diabetes population (GOAL A1C study) confirms underdiagnosis of neuropathy in everyday clinical practice.

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Background and Aims: In the GOAL A1C (Glycemic Optimization with Algorithms and Labs At Po1nt of Care) study, a large number of patients with Type 2 diabetes (target enrolment, n=14,000) will be treated in a predominantly primary care practice (PCP) setting (89% in PCP offices and 11% in endocrinology clinics).
Materials and Methods: As part of the evaluation, patients will be screened for the presence of significant neuropathy (3.61 monofilament testing) and clinical symptoms. Prior to screening, physician perception of whether patients had neuropathy will also be assessed with a simple survey.
Results: To date, data are available for 3563 patients; 39% of patients were diagnosed with neuropathy by inability to perceive the 3.61 monofilament. Overall, patients with neuropathy differed significantly from those without, in that they were older, predominantly male, had a longer duration of diabetes, had slightly higher HbA_{1c} values, had more foot ulcers, had more 'pins/needles' sensations in their feet and wore more custom footwear (Table). The majority of patients without neuropathy (92.4%) were identified correctly by treating physicians. However, 62.4% of patients who were positive for neuropathy were not identified as such. Interestingly, 7.6% of patients without large fibre (5.07 monofilament) findings of neuropathy were identified as having neuropathy by physicians, suggesting the presence of small fibre neuropathic disease. Physicians also missed the diagnosis of neuropathy in 39% of patients with severe neuropathy when the ability to feel the 5.07 monofilament was used to discern the absence or presence of severe neuropathy (insensate feet).
Conclusions: Neuropathy is a commonly underdiagnosed complication of diabetes in PCP. This underscores the importance of routine testing for the presence of neuropathy on a regular basis.

	Without neuropathy (n=2184)	With neuropathy (n=1379)
Patient characteristics		
Age (years)*†	55 ± 12	59 ± 12
Male (%)	46	55
Duration of diabetes (years)*†	7.8 ± 5.8	9.2 ± 7.0
HbA_{1c} (%)*‡	8.8 ± 1.5	8.9 ± 1.6
Clinical symptoms		
'Pins/needles' (%)†	15.1	36.0
Foot ulcers (%)†	1.2	6.5
Wore custom footwear (%)†	4.4	8.9
Physician perception of neuropathy (%)†		
Yes	7.6	37.6
No	92.4	62.4

*mean ± SD; †p <0.0001, ‡p <0.05, patients with neuropathy vs those without neuropathy

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Dermal nerve depletion and angiogenesis in diabetic neuropathy.

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Background and Aims: The pathogenesis of diabetic neuropathy particularly painful neuropathy is poorly understood. Pathophysiological studies suggest a role for vascular factors and sympathetic denervation in painful diabetic neuropathy (PDN). However, we lack detailed mechanisitic studies at the molecular level in support of this contention.
Materials and Methods: Diabetic patients with painful (n=13) and painless (n=18) neuropathy (matched for severity of neuropathy), and diabetic patients without neuropathy (n=8) were compared to healthy controls (n=8). Skin sympathetic function was assessed using laser Doppler flowmetry on the pulp of the great toe, at rest and after three different vasoconstrictor stimuli. Endothelium-dependent and independent vasodilatation was also evaluated using iontophoresis of acetylcholine and sodium nitroprusside (100mcA, 60 seconds) on the dorsum of the foot. Subsets of each group underwent 5 mm punch skin biopsies of the dorsum of the foot. Immunolocalisation of the endothelial marker CD31, pan-axonal marker PGP9.5, and the angiogenic cytokine VEGF-A was performed on routinely processed biopsies. The density of microvessels and nerve endings was quantified in the papillary dermis.
Results: The vasoconstrictive response to sympathetic stimulation did not differ between control subjects and diabetic patients without neuropathy and painless neuropathy, but was significantly reduced in patients with painful compared to painless neuropathy ($p<0.05$). Both endothelium-dependent ($p<0.05$) and endothelium-independent ($p<0.05$) microvascular responses were significantly reduced in patients with painful compared to painless neuropathy. The skin biopsies demonstrated a significant reduction in PGP9.5 +ve nerve endings in all diabetic subjects ($p<0.01$) even those without neuropathy. VEGF positive microvessel density was significantly increased in all diabetic patients compared to control subjects ($p<0.05$). The highest microvessel density was observed in patients with painless neuropathy and the lowest in those with painful neuropathy. VEGF positive nerve twigs were observed in the papillary dermis of diabetic patients but not in healthy controls.
Conclusion: Our functional data suggest a selective sympathetic impairment in PDN. However, immunostaining with the pan-axonal marker PGP 9.5 demonstrated severe loss of all types of nerve endings in all diabetic patients. Furthermore, dermal angiogenesis was observed in all groups of diabetic patients but appeared to be less pronounced in those with painful neuropathy.

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Loss of epidermal small nerve fibres reflects severity of neuropathic pain.

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Background/Objective: People with diabetes often report painful symptoms in the absence of objective clinical abnormalities. The genesis of painful peripheral neuropathy is unclear with few structural studies having been performed. As such, treatment is often unsatisfactory due to a paucity of information. The minimally invasive technique of skin biopsy makes the harvesting and examination of pain transmitting epidermal small nerve fibres (ESNF) possible. Using this technique, we have previously shown that epidermal nerve fibres are reduced in our diabetic baboon colony compared with non-diabetic controls. However we were unable to associate this finding with the presence or absence of pain in these animals. The aim of this study is to determine whether:
1) differences in ESNF density and morphology exist between people with or without neuropathic pain and,
2) ESNF density is associated with severity of symptoms.
Methods/Design: We studied patients attending the Diabetes Centre for complication assessment or review of painful neuropathy. Subjects with or without pain were matched for age, duration of diabetes, glycaemic control and degree of sensory loss. To harvest epidermal small nerves, a sample of skin 10cm above the lateral malleolus was collected using a 3mm punch biopsy. The tissue is fixed in 2% paraformaldehyde/lysine/periodate (PLP) and fifty micron frozen microtome sections were stained with the panaxonal marker PGP 9.5. Epidermal small nerve fibres were counted in a blinded fashion using a confocal microscope at x40 magnification. Sensation was measured as vibration perception threshold (VPT) using the Biothesiometer (Bio-medical Instrument, Newbury, Ohio). The severity of pain was graded 0-10 on a 10cm Visual Analogue Scale (VAS).
Results: To date, 23 samples have been analysed. Overall there is significant reduction in ESNF density in people with pain compared to those without, 9.4 vs 2.0 fibres/3mm ($Z=2.5;P=0.01$), even when degree of large fibre sensation was stratified by VPT ($Z=2.3;P=0.01$). Moreover, as fibre density decreased, the grading of pain increased (t-trend=3.23;$P=0.0046$). By contrast, VPT is less predictive of pain ($P=0.51$).
Conclusion: These results suggest that loss of small nerve fibres is a feature of diabetic neuropathic pain and reflects the degree of pain reported by patients. Fibre loss can occur without objective clinical abnormalities. Quantifying ESNF is an exacting and time-consuming procedure, but it provides a structural correlate for painful neuropathy.
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Alterations of Lipids and Lipoproteins in Diabetes - Impact of Therapies

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The prevalence of dyslipidaemia in Type 1 diabetic patients is underestimated and influenced by gender and overweight.

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Background and Aims: Dyslipidaemia is not a classical hallmark of type 1 diiabetes (T1 DM) and is considered to be essentially a consequence of bad metabolic control. The new international lipid targets could however show another picture important to consider in this population exposed to increased cardiovascular risk. The aim of this work is to determine traditional lipid parameters in a large unselected group of T1 DM patients attending a university out-patient clinic and to study factors influencing these parameters.
Materials and Methods: LDL-cholesterol, HDL-cholesterol and triglycerides (TG) (<3mmol/l,>1 mmol/l,<2mmol/l) were assayed with recognized methods in 500 consecutive patients and compared to the newest European criteria for normolipidaemia. The cohort 279 men (group A) and 221 women (group B) has a mean age of 41.2 y (sd:11.7), a mean duration of T1DM of 19.4 y (sd:10.9), a mean HbA1c of 7.85 % (sd:1.2) and a mean BMI of 25.5 kg/m2 (sd:2.5). Since the mean BMI is higher then 25 a subdivision is made in both genders between normal weight (BMI<25, ANO and BNO) and overweight (BMI=or>25,AO and BO) patients. Anova and Fisher exact tests were used for statistics, p<0.05 considered significant.
Results: The prevalence of high LDL was 40.6%, of low HDL 7% and of high TG 7.8% in the total population. There were no significant differences between groups A and B (resp.41.6%, 9%, 7.9% and 39.4%, 4.5% and 7.7%).
When BMI was taken into consideration the prevalence of dyslipidaemia was significantly higher in overweight men: AO: 54%, 12.7%, 12.6% versus ANO: 28.5%, 5.1%, 3% (p<0.001,p<0.05,p<0.01) despite an identical level of metabolic control. Anova shows increasing levels of LDL (p<0.0001), decreasing HDL (p<0.001) and increasing TG (p<0.01) with increasing BMI. In overweight women no such differences could be disclosed:BO:43%,5.6%,10.3% versus BNO: 36%,3.5%,5.3% (NS) When overweight men (AO) were compared to overweight women (BO) only the prevalence of high LDL-choleterol was significantly different: OR=2.139 (CI:266-3.613),p=0.006.
Conclusion: Using the new lipid targets for the general population, nearly 40% of the T1DM patients have elevated LDL levels. This risk factor is more pronounced in overweight men, where low HDL and high TG are observed as compared to normal weight males is an additional finding.

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Apolipoprotein B, apoA-I, triglycerides and glucose - a powerful combination of cardiac risk predictors.

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Background and Aims: Risk of dying from an acute myocardial infarction (AMI) is related to high total cholesterol (TC) and high low density lipoprotein-cholesterol (LDL)-C. Hypertriglyceridemia (HTG) may also increase this risk. Total apoB, one molecule per atherogenic very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) or LDL particle, can be used to indicate cardiac risk. ApoA-I in high density lipoproteins (HDL) may be protective. Patients with the metabolic syndrome or type 2 diabetes often have normal or low LDL-C. The correlation between TG, TC, apoB and apoA-I and predicting the risk of dying from an AMI has already been described. The aim of this study was to include glucose in the analysis of risk of dying from an AMI.
Materials and Methods: In the prospective AMORIS (Apolipoprotein-related MOrtality RISk) study we examined 57794 males and 45735 females, age range 40 to 80+ years. Subjects were investigated mainly in non-acute, open-ward conditions. Lipids and glucose were measured by enzymatic methods; apoB and apoA-I were measured by immunoturbidimetric methods. Univariate, multivariate and Receiver Operating Characteristics (ROC) analyses were performed.
Results: After a mean follow up of 98 months, 1048 males and 490 females died from AMI. TG, TC, apoB, apoB/apoA-I and glucose were all related to increased risk of fatal AMI, in both genders (p <0.0001). ApoA-I was associated with a reduced risk (p <0.0001). ApoB/apoA-I showed the most significant correlation with risk of dying from an AMI; AUC was 0.62 for males and 0.66 for females, as determined by ROC. Adding glucose values to apoB/apoA-I increased the AUC to 0.65 and 0.70 for males and females; age-adjusted values were 0.81 and 0.88, respectively (all ROC values p <0.0001). In those with the highest tertile values for both apoB/apoA-I and glucose, the relative risk ratios versus those with lowest values were 11.9 for males and 3.2 for females (both p < 0.0001). Highest risks were obtained in people with either isolated or combined HTG, especially in those with an apoB/apoA-I-ratio > 0.9 for males and > 0.8 for females. Those subjects also had the highest glucose values.
Conclusion: In this large population study, apoB/apoA-1 ratio in combination with TG and glucose were the best biochemical predictors of cardiac mortality. ApoB, HTG and hyperglycemia are all features of type 2 diabetes and the metabolic syndrome. International targets and guidelines for the measurement of glucose, triglycerides and cholesterol already exist in clinical practice. We recommend that these be extended to include apoB and apoA-I, and especially the apoB/apoA-1 ratio, in order to identify those patients at particular risk of dying from an AMI. New therapies that address these major cardiovascular risk factors would offer a major advance in the treatment of type 2 diabetes and metabolic syndrome.

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Direct measurement of plasma oxidized LDL levels in Type 2 diabetic patients before and after insulin therapy.

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Background and Aims: The oxidative conversion of low density lipoproteins (LDL) to oxidative low density lipoproteins (oxidized LDL) is now considered to be an essential step in the atherogenic process. An increased susceptibility of LDL to oxidation has been shown in type 2 diabetic patients. However, so far only susceptibility of LDL to oxidation has been assessed, which is an indirect method, and no direct measurement of plasma oxidized LDL levels have been performed, in type 2 diabetic patients. Moreover, the effect of insulin treatment on oxidized LDL levels remains unknown.
Materials and Methods: With a recent sandwich ELISA assay, using a monoclonal antibody reacting specifically against oxidized phostatidylcholine and a peroxidase labeled anti-human apoB monoclonal antibody, we measured directly plasma levels of LDL oxidized particles in 46 type 2 diabetic patients, under oral hypoglycemic agents, and in 33 age-matched controls. Moreover, in 23 subjects among the 46 type 2 diabetic patients, a second measurement of plasma levels of LDL oxidized particles was performed 3 months after the introduction of insulin therapy.
Results: Type 2 diabetic patients had, compared to controls, significantly increased levels of oxidized LDL (71.3± 23.6 vs. 54.3 ± 13.1 U/l, p<0.0001) and an increased oxidized LDL/LDL cholesterol ratio (22.2 ± 5 vs. 16.7 ± 4, p<0.0001). In multivariate analyses, performed in type 2 diabetic patients, plasma LDL oxidized level was independently associated with LDL-cholesterol (p<0.001) and LDL(triglyceride[TG]/apoB) (p=0.01), when oxidized LDL/LDL cholesterol ratio was associated with LDL(TG/apoB) (p=0.008) and plasma TG (p=0.02). Three months after insulin treatment, oxidized LDL particles were significantly reduced (oxidized LDL/LDL-chol ratio: 20.5 ± 5.3 vs 24.6 ± 5.3 U/mmol before insulin, p<0.0001). The reduction of oxidized LDL after insulin was positively correlated with reduction of plasma TG (p<0.05) and of LDL-TG (p<0.05).
Conclusion: 1) plasma oxidized LDL levels, assessed by direct measurement, are significantly increased in type 2 diabetes. 2) The increase of plasma oxidized LDL levels is associated with TG-enrichment of LDL 3) Insulin therapy significantly reduces plasma oxidized LDL levels in parallel with a reduction of plasma and LDL triglyceride levels.

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Pioglitazone in combination with insulin results in changes in lipid subspecies and subparticle profiles in patients with Type 2 diabetes.
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Background and Aims: Dyslipidemia in type 2 diabetes is commonly associated with increased triglycerides, decreased HDL, and increased small LDL subparticles. A higher proportion of small, dense LDL particles tends to be linked directly to a higher risk for cardiovascular events. Two relevant LDL phenotypes have been determined: pattern B with small dense particles and pattern A with large, more buoyant particles. We evaluated whether treatment with pioglitazone (PIO) in combination with insulin resulted in changes from baseline in the lipid subspecies and subparticle profiles.
Materials and Methods: Blood samples were obtained from patients with type 2 diabetes mellitus who participated in a randomized double-blind clinical study to examine the effects of PIO in combination with insulin. Samples were obtained from patients at Baseline, Week 12, and Week 24. A total of 82 subject sets (40 at 30 mg PIO, 42 at 45 mg PIO) were randomly chosen from a blinded subject list. In this LOCF analysis, missing values at Week 24 were replaced by corresponding values from Week 12. Missing values at Week 12 were not replaced. Samples for each subject set were analyzed for LDL subparticle profile.
Results: There were **statistically significant increases in average and peak LDL particle sizes** compared to baseline. The overall shifts in LDL particles subclasses (large, intermediate, small) from baseline were also statistically significant. The percentage of large (A) LDL particles increased, while the percentage of small (B) LDL particles correspondingly decreased. **These changes were statistically significant**.

Lipid Subspecies Parameters	Baseline (n = 82)	Δ from baseline Week 12 (n = 71)	Week 24 (n= 82)
Average LDL particle size (Å)	259.7	3.0*	2.4*
Peak LDL particle size (Å)	258.3	3.1*	2.2*
LDL Large A (%)	46.58	10.73*	8.31*
LDL Small B (%)	31.83	-8.45*	-6.10*

*$P < .01$ compared to baseline.

Conclusions: The results of this analysis demonstrate that treatment with PIO in combination with insulin significantly increases average and peak LDL particle size and significantly shifts LDL subclass (large, intermediate, small) category distribution. This shift is primarily observed in **significant increases in large (A) LDL** particle percentages and **significant decreases in small (B) LDL** particle percentages.

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Fenofibrate and lipoprotein size in Type 2 diabetes, evaluated by NMR and comparison to gradient gel electrophoresis (GGE) determinations.
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Background: Several epidemiologic studies indicate that those individuals whose LDL particles are small and dense are at greater coronary risk than are those with an equivalent amount of LDL cholesterol, but in particles that are larger and more buoyant. The greater presence of small dense LDL particles in people with diabetes compared to those without diabetes, has been suggested as one of the factors contributing to the increased incidence of coronary artery disease in diabetes. Recently we demonstrated (Diabetes Atherosclerosis Intervention Study, DAIS) that treatment with fenofibrate reduced the progression of angiographically-evaluated coronary artery changes in 418 men and women with type 2 diabetes, and increased GGE measured LDL size. The beneficial angiographic changes were in part attributable to the LDL size increase (*Circulation* – in press). NMR has also been used to assess lipoprotein particle size.
Aims: 1] to evaluate, by NMR, the sizes of LDL, VLDL and HDL in type 2 diabetes, 2] to determine the effect of fenofibrate treatment on these and 3] to compare the GGE-determined and NMR determined-LDL sizes.
Methods: Weighted average lipoprotein particle sizes were calculated from subclass levels measured by NMR. The LDL particle sizes determined by NMR were compared to those determined by GGE. Samples were obtained from a subset of the DAIS population (105 placebo treated; 113 fenofibrate treated).
Results: In accord with GGE measurements, fenofibrate increased the size of LDL particles assessed by NMR. It also decreased the size of both VLDL and HDL.

	Average Diameter Placebo (n=105)		Average Diameter Fenofibrate (n=113)		*p**
	Baseline	On-treatment	Baseline	On-treatment	
VLDL (nm ± SD)	56.41 ± 11.69	58.57 ± 11.08	56.58 ± 10.89	53.10 ± 10.63	<0.0001
LDL (nm ± SD)	20.57 ± 0.77	20.67 ± 0.84	20.48 ± 0.72	20.98 ± 0.60	<0.010
HDL (nm ± SD)	8.83 ± 0.32	8.78 ± 0.38	8.78 ± 0.26	8.60 ± 0.31	<0.0001

p placebo on-treatment vs fenofibrate on-treatment

Comparing the sizes of LDL measured by GGE to those measured by NMR, showed both to be highly correlated both at baseline ($LDL_{(NMR)}$ = 9.10 + 0.46 $LDL_{(gel)}$ r=0.71;p<0.0001) and at the end of the treatment period ($LDL_{(NMR)}$ = 10.24 + 0.42 $LDL_{(gel)}$ r=0.68; p<0.0001), but the NMR approach systematically gave average diameters that were smaller than those obtained by GGE.
Conclusions: 1] Fenofibrate treatment increases the size of LDL particles in type 2 diabetes. 2] It also decreases the size of VLDL and HDL particles. 3] NMR determinations of LDL diameter correlate to those measured by GGE, however LDL diameters by NMR were smaller than those by GGE.

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Early benefit from structured care with Atorvastatin in patients with coronary heart disease and diabetes mellitus. A subgroup analysis of the GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) Study.
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Background and Aims: Patients with coronary heart disease (CHD) and diabetes mellitus (DM) benefit from statin treatment, however the effect of atorvastatin on such patients has not yet been investigated. We prospectively evaluated the effect of structured care (SC) of dyslipidemia with atorvastatin (strict implementation of guidelines) versus usual care (UC) (physician's standard of care) on morbidity and mortality of patients with CHD and DM.
Materials and Methods: From 1,600 consecutive CHD patients randomized to either form of care in the GREek Atorvastatin and Chd Evaluation Study (GREACE), 313 had DM; 161 in the SC arm and 152 in the UC arm. All patients were followed-up for a mean 3-year period. In the SC group patients were treated with atorvastatin to achieve the National Cholesterol Education Program (NCEP) low-density lipoprotein cholesterol (LDL-C) treatment goal of <100 mg/dL. Primary endpoints were all-cause and coronary mortality, coronary morbidity (non-fatal myocardial infarction, revascularization, unstable angina, and heart failure), and stroke.
Results: In the SC group 97% (n=156) of the patients were on atorvastatin (10-80 mg/day, mean 23.7 mg/day) throughout the study and the NCEP LDL-C treatment goal was reached by 93% (n=150) of the patients. Only 17% (n=26) of the UC patients were on long-term hypolipidemic drug treatment and 4% (n=6) of them reached the NCEP LDL-C treatment goal. During the study, 46 out of 152 (30.3%) CHD patients with DM on UC experienced a major vascular event or died vs 20 out of 161 (12.5%) patients on SC; relative risk reduction (RRR) 58%, p<0.0001. RRR for all-cause mortality was 52%,p=0.049, coronary mortality 62%,p=0.042, coronary morbidity 59%,p<0.002, and stroke 68%,p=0.046. Event rate curves started deviating from 6th treatment month and RRR was almost 60% by 12th month. RRRs remained at that level until the end of the study, time at which they became statistically significant. The cost/life-year gained with SC was $US 6,200.
Conclusion: In CHD patients with DM, SC of dyslipidemia with atorvastatin, to achieve the NCEP LDL-C treatment goal, reduces all-cause and coronary mortality, coronary morbidity and stroke by more than one-half within a 3-year period, in comparison to UC. Clinical benefit is

manifested as early as the 6th treatment month, but becomes statistically significant by the 3rd year of treatment.

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Reduction of coronary events by simvastatin in non-diabetic coronary heart disease patients with and without metabolic syndrome: subgroup analyses of the Scandinavian Simvastatin Survival Study (4S).

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Background and Aims: Metabolic syndrome (MS) is associated with increased risk of coronary heart disease (CHD) and progression to type 2 diabetes.
Materials and Methods: Post-hoc subgroup analyses were performed on data from 4S to evaluate the effect of simvastatin (SIM) on the risk of recurrent CHD events in nondiabetic CHD patients with and without MS. Because of their known high risk, patients with previously diagnosed diabetes or fasting plasma glucose (FPG) >6.9 mmol/L were excluded. In 4S, 4444 hypercholesterolemic patients with CHD were randomized to placebo (PBO) or SIM 20 mg (37% titrated to 40 mg) for 5.4 years. For subgroup analyses, MS was defined as 3 or more of the following NCEP ATP III criteria: 1) triglyceride ≥1.7 mmol/L, 2) HDL-C <1.0 (men) or <1.3 (women) mmol/L, 3) FPG 6.1-6.9 mmol/L, 4) hypertension and/or blood pressure ≥ 130/≥ 85 mmHg, and 5) BMI ≥30 kg/m^2 (surrogate for waist circumference).
Results: Of 4154 evaluable patients, 836 (20%) were classified as MS, with 387 and 449 in the SIM and PBO groups, respectively. In both patient subgroups, treatment with simvastatin lowered LDL-C by 37% and significantly ($p \leq 0.05$) reduced the relative risk of total and coronary mortality, coronary events, and revascularizations (Table). MS patients on placebo had a higher incidence of all 4 outcomes than non-MS PBO-treated patients. Consequently, MS patients treated with simvastatin had a numerically greater absolute risk reduction than non-MS patients.
Conclusion: Thus, CHD patients with or without MS realize substantial benefit from treatment with simvastatin.

	Relative Risk (95% CI)	
	MS	Non-MS
Total Mortality	0.61 (0.40, 0.93)	0.69 (0.55, 0.88)
Coronary Mortality	0.46 (0.27, 0.80)	0.57 (0.43, 0.76)
Major Coronary Event	0.58 (0.44, 0.76)	0.70 (0.60, 0.81)
Revascularization	0.53 (0.37, 0.77)	0.67 (0.55, 0.80)

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Effects of ezetimibe added to on-going statin therapy on the lipid profile of hypercholesterolemic patients with metabolic syndrome.

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Background and Aims: Patients with metabolic syndrome (MS) often present with dyslipidemia (elevated low-density lipoprotein cholesterol [LDL-C] and triglycerides [TG], and low high-density lipoprotein cholesterol [HDL-C]) that places them at high risk for coronary heart disease (CHD). Previous studies in hypercholesterolemic (HC) patients have shown that greater reductions in LDL-C can be achieved when statins are co-administered with the cholesterol absorption inhibitor, ezetimibe (EZE).
Materials and Methods: We examined EZE's LDL-C-lowering efficacy in patients with metabolic syndrome (MS) in a post-hoc analysis from a study of HC patients already on statin monotherapy and not at their National Cholesterol Education Program target and randomized to placebo (Pbo) or 10 mg EZE for 8 weeks. Patients were classified as having MS if they met 3 or more of the following criteria: TG≥1.7 mmol/L; HDL <1.0 (men) or <1.3 (women) mmol/L; type 2 diabetes and/or fasting serum glucose ≥6.1 mmol/L; hypertension and/or blood pressure ≥130/≥85 mmHg; and waist circumference of >102 (men) or >88 (women) cm.
Results: Of the 769 evaluable patients, 342 (44%) had MS at baseline, with 182 and 160 in the statin + Pbo and statin + EZE arms, respectively. The effects of EZE on lipid levels were consistent between the total study cohort and patients with MS. In MS patients, adding EZE to statin reduced LDL-C by 25% compared with a 4% decrease for those treated with statin + Pbo ($p<0.001$). Relative to Pbo, EZE also reduced TG ($p<0.001$) and non-HDL-C ($p<0.001$).
Conclusion: In summary, the addition of EZE to ongoing statin therapy led to significant improvements in the CHD risk profile of HC patients with MS.

MS Patients

Lipid Variable	Statin + Placebo Mean Baseline ± SE	LS** Mean % Change ± SE	Statin + EZE Mean Baseline ± SE	LS** Mean % Change ± SE
LDL-C* (mmol/L)	3.4 ± 0.05	-4 ± 1	3.4 ± 0.08	-25 ± 1
TG* (mmol/L)***	2.0 ± 0.06	-6 ± 2	2.0 ± 0.08	-15 ± 2
Non-HDL-C* (mmol/L)	4.3 ± 0.08	-4 ± 1	4.4 ± 0.08	-23 ± 1
HDL-C (mmol/L)	1.2 ± 0.03	1 ± 1	1.2 ± 0.03	3 ± 1

*p<0.001 Statin + Pbo vs. Statin + EZE;
**Least square mean percentage change from baseline;
***values are medians

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Epidemiology of Insulin Resistance, Complications and Mortality in Type 2 Diabetes Mellitus

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Insulin resistance, proinsulin and coronary heart disease. A population-based, follow-up study 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 852 men in Uppsala, Sweden, aged 70 years at baseline with a follow-up of up to 7.5 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, 0.76, CI, 0.64-0.91), total cholesterol (HR, 1.22, CI, 1.05-1.41), smoking (HR, 1.41, CI, 0.99-2.01) and hypertension (HR, 1.97, CI, 1.42-2.74) predicted CHD. Intact proinsulin (HR, 1.23, CI, 1.03-1.46) was independent of these risk factors and M/I when added to the model while M/I turned non-significant (HR, 0.87, CI, 0.70-1.06).
Conclusions: Insulin resistance, i.e. low insulin sensitivity, predicts subsequent CHD. Proinsulin makes this association non-significant. Proinsulin is a strong and statistically significant predictor for CHD independent of conventional risk factors.

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Metabolic syndrome is associated with fatal cardiovascular disease in men and non-fatal cardiovascular disease in women. The Hoorn Study.

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Background and Aims: Obesity and insulin resistance are generally believed to be the culprit in the metabolic syndrome, a high-risk state for diabetes and cardiovascular disease. Alternative definitions of the metabolic syndrome have been proposed by the European Group for the Study of Insulin Resistance (EGIR), and the Adult Treatment Panel III (NCEP). We studied the concordance of these definitions and their predictive value for fatal and non-fatal cardiovascular disease in Dutch men and women.
Materials and Methods: In the Hoorn Study, a cohort study in the general population, which started in 1989, 2484 men and women of 50 to 75 years old participated. For the present study we excluded patients with known diabetes and with plasma glucose values ≥ 7.0 mmol/l, subjects with self reported history of cardiovascular disease, and subjects with missing information on morbidity (available until 2000) or any of the variables of the definitions of the metabolic syndrome. Thus the study population for the present analyses consisted of 618 men and 750 women. The EGIR definition: upper quartile of fasting insulin AND at least 2 of these risk factors: fasting plasma glucose ≥ 6.1 mmol/l; triglycerides > 2.0 mmol/l or HDL < 1.0 mmol/l or treatment; blood pressure ≥ 140/90 or treatment; waist circumference ≥ 94 cm for men and ≥ 80 cm for women. The NCEP definition: at least 3 of the following: fasting plasma glucose ≥ 6.1 mmol/l; triglycerides ≥ 1.7 mmol/l; HDL cholesterol < 1.0 mmol/l for men and < 1.3 mmol/l for women; blood pressure ≥ 130/85 mmHg (or medication); waist circumference > 102 cm for men and > 88 cm for women.
Results: Among men, 19 % had the syndrome according to the EGIR definition, 20 % according to the NCEP definition, and 10 % according to both. Among women, 17 % had the syndrome according to the EGIR definition, 26 % according to the NCEP and 12 % according to both. For men, the age-adjusted relative risks (hazard ratios) of fatal and nonfatal cardiovascular disease were 1.86 (0.95-3.64) and 1.38 (0.89-2.15) respectively for the EGIR definition, and 2.37 (1.24-4.52) and 1.53 (1.00-2.34) respectively for the NCEP definition. For women, the hazard ratios for fatal and non-fatal cardiovascular disease were 0.95 (0.38-2.38) and 1.46 (0.88-2.44) respectively for the EGIR definition, and 0.90 (0.38-2.15) and 2.01 (1.27-3.20) respectively for the NCEP definition.
Conclusion: In Dutch men and women without diabetes of CVD at baseline, the NCEP definition of the metabolic syndrome was a slightly better predictor for fatal and non-fatal cardiovascular disease. For men, the strongest associations were observed with fatal cardiovascular disease, whereas for women the strongest associations were found with non-fatal events.

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Estimations of the clinical benefit of optimal blood glucose, blood pressure and lipid control in Type 2 diabetic patients with the metabolic syndrome.

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Background and Aims: Appropriate management of multiple coronary heart disease (CHD) risk factors is known to reduce CHD events in general populations. Our objective is to estimate the proportion of CHD events potentially averted with appropriate management of particular risk factors in a sample of newly diagnosed type 2 diabetic patients with the metabolic syndrome (MetS).
Material and Methods: The study sample was derived from 4,293 subjects enrolled in ADOPT, a randomized, double-blind study comparing the durability of glucose lowering and preservation of pancreatic beta-cell function of rosiglitazone compared to metformin or glyburide/glibenclamide in patients with drug-naive, recently diagnosed type 2 diabetes mellitus. Patients aged 30-74 years, with the MetS as defined by the National Cholesterol Education Program - Third Adult Treatment Panel were included in the analysis. Baseline risk factor data and Framingham risk score were used to estimate the risk of developing CHD (myocardial infarction and coronary heart disease death) within a ten-year period. We then estimated CHD events preventable by modelling the benefit of achieving optimal glycemic, blood pressure and lipid control in newly diagnosed type 2 diabetic patients with MetS.
Results: 1,573 men and 1,371 women 30 to 45 years old among the study participants exhibited MetS and had information on Framingham risk factors. Three hundred and thirty eight participants with the MetS who were missing information on the Framingham risk factors were excluded. Applying Framingham risk estimates to this population, 290 (18.4%) men and 204 (14.9%) women will experience CHD events over the next decade. Incremental benefit was expressed as the estimated number of CHD events averted due to control of the following risk factors: 1) HbA1C to < 7% (assumes control to 'non-diabetic' status) 2) blood pressure (BP) to <130mmHg systolic and <85 mmHg diastolic, 3) HDL-cholesterol to ≥45 mg/dl in men and ≥50 mg/dl in women, and 4) LDL-cholesterol <100 mg/dl. The number and proportion of CHD events averted is displayed in the table below:

Risk Factor (s) Controlled	Estimated Number of CHD Events Averted Men (%)	Women (%)	Total (%)
A. Normal HbA1C	101 (34.8 %)	93 (45.6%)	194 (39.3%)
B. BP <130 mmHg systolic and <85 mmHg diastolic	76 (26.2 %)	31 (15.2%)	107 (21.7%)
C. HDL-C ≥45 mg/dl in men and ≥50 mg/dl in women	42 (14.4 %)	29 (14.2%)	71 (14.3%)
D. LDL-C to <100 mg/dl	120 (41.4 %)	52 (25.5%)	172 (34.8%)
E. A + B + C	175 (60.3 %)	126 (61.7%)	301 (60.9 %)
F. A + B + C + D	224 (77.2 %)	148 (72.5%)	372 (75.3%)

Our analysis suggests that up to seventy five percent of CHD events may be preventable with aggressive control of CHD risk factors in this sample of newly diagnosed type 2 diabetic patients with the metabolic syndrome.

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Subclinical cardiovascular disease in impaired fasting glucose and Type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA).

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Background and Aims: Glucose intolerance increases the risk of clinical cardiovascular disease (CVD). Nonetheless, the relation between glucose intolerance and subclinical CVD is less clear.
Materials and Methods: We assessed subclinical CVD in impaired fasting glucose (IFG) and type 2 diabetes (T2DM) in participants in MESA, a multicenter study examining the determinants of subclinical CVD in a multi-ethnic population. The study included 6811 subjects (2612 whites, 1902 African Americans, 1497 Hispanics, and 800 Chinese Americans) recruited in six clinical centers in the United States between 2000 and 2002. Subjects were aged 45-84 years and had no history of clinical CVD. Subclinical CVD was evaluated by measuring coronary artery calcium by computed tomography, common and internal carotid intima-media wall thickness (IMT) by ultrasound, and ankle/brachial blood pressure index (ABI).
Results: Subjects with IFG and T2DM had increased prevalence and higher levels of coronary calcification, increased common and internal carotid IMT, and higher rates of peripheral vascular disease. This increase was not explained by other risk factors and was observed in each ethnic group (Table)

Table

	Normal	IFG	Diabetes	p
Age (yr)	62±0.1	65±0.4	65±0.3	<0.001
Sex (%F)	55	42	48	<0.001
Coronary Calcium (present %)	48	59	62	<0.001
Coronary calcium Score (Agatston Units)†*	77±1	88±1	114±1	<0.001
Common carotid IMT (μm)*	863±2	881±7	898±6	<0.001
Internal carotid IMT (μm)*	1055±8	1101±25	1190±19	<0.001
ABI (%<0.9)	2.4	4.4	5.9	0.024

mean±SE;
p values are by ANOVA for continuous variables and chi-square for categorical variables;
analyzed after log transformation;
* adjusted for age, sex, ethnicity, lipids, blood pressure, and smoking.

Conclusions: Both IFG and T2DM are associated with increased subclinical CVD. Early intervention in subjects with IFG should be explored for its potential to decrease CVD morbidity and mortality.

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Adult thresholds for obesity may not define metabolic risk in children (The EarlyBird Diabetes Study).

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Background and Aims: Adult BMI thresholds for overweight and obesity relate to health risk. Paediatric cut-offs for overweight and obesity, corresponding to an adult BMI of 25 and 30, respectively, have been proposed by the International Obesity Task Force. However, the centile equivalents proposed for children have been derived statistically - not according to risk. Insulin resistance, not fatness itself, poses the metabolic risk. This study sought to establish whether cut-points defining overweight and obesity in children embrace the same thresholds for insulin resistance or triglycerides as in adults.
Materials and Methods: EarlyBird is a prospective cohort study that aims to monitor the emergence of insulin resistance in a normal population. Measures of insulin resistance (IR-HOMA), triglycerides and BMI (or centile equivalent in children) were made in 300, randomly selected healthy children (mean age 4.9y) and their parents (mean age mothers 33.4 y, fathers 36.4y).
Results: 1) Insulin resistance in both mothers and fathers rose significantly according to category of BMI. A similar pattern was observed in the children, but levels of insulin resistance were much lower for equivalent 'fatness'. Thus the back-transformed means of log HOMA-IR for normal (BMI < 25), overweight (BMI 25.0 - 29.9) and obese (BMI ≥ 30) mothers were 1.23 (n= 92), 1.75 (n= 67) and 2.78 n= 39), respectively; for fathers 1.29 (n= 68), 1.65 (n= 115) and 3.15 (n= 51) and for children, using the centile equivalents, 0.69 (n= 244), 1.01 (n= 44) and 1.24 (n= 12). 2) In mothers and fathers, BMI accounted for 41% and 28% of the variance in insulin resistance, respectively (p< 0.001). In children, BMI accounted for only 6% of the variance in girls (p= 0.005) and just 2% in boys (p= 0.06). 3) In parents and children, triglyceride levels also rose with increasing category of BMI, but again were lower in children of equivalent 'fatness'. The back-transformed means of log triglycerides for normal, overweight, and obese mothers were 0.71 (n= 94), 0.90 (n= 67) and 1.00 mmol/l (n= 40), respectively; for fathers 0.87 (n= 68), 1.25 (n= 117) and 1.66 mmol/l (n= 51) and, for children, 0.57 (n= 244), 0.63 (n= 44) and 0.78 mmol/l (n= 12). 4) In mothers and fathers BMI accounted for 12% and 17% of the variance in triglycerides, respectively (p< 0.001). In girls, no relationship was found between BMI and triglicerides (p= 0.43) and in boys, it explained just 7% of the variance (p< 0.001).
Conclusions: The proposed BMI thresholds for overweight/obesity in children are poor predictors of insulin resistance and triglycerides in five-year-olds and, by implication, of metabolic risk. Furthermore, BMI tracks poorly from childhood to adulthood so that undue emphasis on weight may stigmatise the fat child unnecessarily. It is now important to establish whether insulin resistance, together with other markers of metabolic risk, track from childhood to adulthood, before extrapolating risk from adults to children.

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Mortality in people with Type 2 diabetes.

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Background and Aims. There are few longitudinal studies of mortality in people with Type 2 diabetes in the UK. The aim of this study was to compare mortality among people with Type 2 diabetes in England and Wales relative to those without diabetes.
Materials and Methods: A retrospective cohort study (follow-up period 1992-1999) was conducted using the General Practice Research Database. We identified patients aged 40-79 years of age. Those with a first record of diabetes after the age of 35 years of age or a code for diabetes and evidence of regular use of oral hypoglycaemic agents were classified as having Type 2 diabetes. For each patient with diabetes two non-diabetic subjects matched for age, sex and general practice of registration were randomly selected from the database to provide a reference population. Cox proportional hazards survival regression analyses were used to compare mortality in diabetic and non-diabetic patients, adjusting for age, smoking, BMI and hypertension.
Results: We identified 25013 type 2 patients (13890 men and 11123 women), and 49958 non-diabetic controls. There were 3812 deaths in men and 2789 in women with diabetes. The hazard ratio (HR) for people with diabetes indicated that mortality in patients with type 2 diabetes is more than double those without diabetes (HRs 2.07, 95% CI 1.96-2.20 and 2.77, 95% CI 2.57-2.98 for men and women respectively). Men with Type 2 diabetes had significantly higher mortality than female counterparts (HR 1.3, 95%CI 1.2-1.4, females as reference). The HR in males aged 40-49 was 3.8 (95% CI 2.4-5.9) reducing to 1.8 (95% CI 1.6-1.9) in those aged 70-79 years. In women the HR was 7.0 (95% CI 4.8-9.6) in those aged 40-49 reducing to 2.5 (95% CI 2.3 – 2.8) in those aged 70-79.
Conclusions: The study provides robust estimates of the absolute and relative mortality associated with diabetes in England and Wales. Its strengths are that it is a population based sample, its size and the fact that it is a national sample. It also uses a reference group who have no record of diabetes rather than using the general population.

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Trends in mortality in elderly patients with Type 2 diabetes mellitus.

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Background and Aims: To assess changes in death rate and its predictors in 3 cohorts of elderly type 2 diabetic patients.
Materials and Methods: Study subjects were 3 cohorts of patients aged over 65 years when their type 2 diabetes was diagnosed. Cohort A encompassed patients diagnosed between January 1, 1970 and December 31, 1974, cohort B patients diagnosed between January 1, 1980 and December 31, 1984 and cohort C patients diagnosed between January 1, 1990 and December 31, 1994. We examined trends in overall and cause-specific mortality as well as their predictors in these 3 cohorts.
Results:

Table 1-Predictive factors, overall and cause-specific mortality in study cohorts

	Cohort A (1970-1974)	*Cohort B (1980-1984)*	*Cohort C (1990-1994)*	*Cohort A vs. B*	*Cohort A vs.C*	*Cohort B vs. C*
n	323	232	346	-	-	-
Age at death (years) [b]	71.43± 6.12	73.5± 5.89	74.65± 6.12	P < 0.05	P < 0.001	P < 0.05
Sex: M[a]	144 (44.6)	102 (44)	147 (42.5)	NS	NS	NS
BMI (Kg/m^2)	28.57± 5.54	29.25± 4.8	29.29± 5.09	NS	P=0.08	NS
Smoking[a]	36 (11.14)	34 (14.65)	96 (27.74)	NS P	< 0.05	NS
Hypertension[a]	211 (65.32)	194 (83.62)	222 (64.16)	P < 0.001	NS	P < 0.001
Cholesterol >200mg/dl[a]	122 (37.77)	87 (34.91)	103 (29.76)	NS	NS	NS
Evolution of DM[b](years)	10.45± 7.24	10.09± 7.06	9.31± 2.91	NS	P<0.01	P=0.059
Fasting glycemia(mg/dl)[a]	178.3± 37.37	176± 38.9	166.6± 40.67	NS	P < 0.001	P < 0.01
Deaths of all causes[a]	320 (93.49)	188 (81.03)	51 (14.01)	P < 0.0001	P < 0.001	P < 0.001
Cardiovascular deaths[a]	225 (70.03)	125 (66.48)	31 (60.78)	NS	NS	NS

[a]Data are n(%), P was calculated with χ^2 test. [b]Data are means ± SD, P was calculated with t-test.

Mean age at death was increased significantly in subjects diagnosed between 1990-1994 vs those diagnosed between 1980-1984 ($p<0.05$) and extremely significant vs. those diagnosed between 1970-1974.Woman had mean age at death similar to men ($p>0.05$) in all cohorts. Mortality from cardiovascular causes varied insignificantly between the three cohorts. Mortality from infectious causes and from acute metabolic complications was lower in patients diagnosed in 1990-1994 in compare to those diagnosed in 1970-1974 ($p<0.05$), while deaths from malignant neoplasms increased significantly ($p<0.05$). The prevalence of smoking was increased in subjects diagnosed in 1990-1994 in compare to those diagnosed in 1970-1974 ($p<0.05$). Fasting plasma glucose was significantly lower in patients diagnosed in 1990-1994 than in the two previous periods.
Conclusion: Mean age at death increased significantly in the cohort diagnosed between 1990-1994 in compare to the cohorts diagnosed between 1980-1984 and between 1970-1974. Mean age at death was similar in both sexes. Mortality from cardiovascular causes varied insignificantly between the three cohorts.

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Women with diabetes are at increased risk of colorectal cancer and endometrial cancer.

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Background and Aims: Several dietary and behavior risk factors of type 2 diabetes, including obesity, high glycemic and/or high fat diet, and low levels of physical activity, are also risk factors for colorectal cancer and endometrial cancer. We investigated whether women with type 2 diabetes are at higher risk of colorectal cancer and endometrial cancer than women without diabetes in a large U.S. managed care population (Kaiser Permanente).
Materials and Methods: A retrospective longitudinal cohort study design was used to investigate the incidence of colorectal cancer and endometrial cancer among 33,118 adult women with recognized diabetes. Subjects included type 2 diabetic members of the Kaiser Permanente Northern California Diabetes Registry who completed a survey in 1994-7. The reference population included a random sample of Kaiser members (n= 18,901) without recognized diabetes who completed a survey in 1993 or in 1996. Data on incidence of colorectal cancer and endometrial cancer were obtained from the Kaiser Cancer Registry.
Results: The mean age was 60.4 years (SD 15.5). The study cohort was comprised of 60.9% non-Hispanic whites, 11.3% non-Hispanic blacks, 11.3% Hispanics, 11.7% Asians, 3.0% „other“ ethnic groups, and 1.8% with missing ethnicity data. Women with diabetes were more likely to be older, obese, less educated, from U.S. minority ethnic groups, and less likely to smoke and consume alcohol than women without diabetes ($p<0.001$). During a 5-year follow-up 271 colorectal cancer cases and 260 endometrial cancer were identified. The age-adjusted incidence rates of colorectal cancer were 0.62 per 1,000 person-years (p-yrs) in diabetic women and 0.34 /1,000 p-yrs in women without recognized diabetes. The age-adjusted incidence rates of endometrial cancer were 1.06/1,000 p-yrs in diabetic women and 0.35 /1,000 p-yrs in women without recognized diabetes. In proportional hazards models adjusted for age, obesity, self-reported ethnicity, education, smoking, and alcohol consumption, diabetes remained significantly associated with increased risk of colorectal cancer [RH= 1.8 (95%CI= 1.3-2.6)], and endometrial cancer [RH= 1.9 (95%CI= 1.3-2.8)].
Conclusion: Type 2 diabetes is associated with increased risk of colorectal and endometrial cancer independently of obesity. Screening for colorectal and endometrial cancer is warranted in women with diabetes. More work is needed to assess whether behavioral interventions aimed at reducing the incidence of diabetes also reduce the incidence of these two types of cancer.

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Other Hormones

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Adiponectin expression in human non-adipose cells.
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Background and Aims: Adiponectin, an adipocytokine known to be down-regulated in obesity-linked disorders, is considered to be a potential key mediator of insulin sensitivity. Furthermore, adiponectin is reported to be exclusively produced by adipocytes. In this study, we looked for adiponectin gene expression in different human cell types such as myotubes, fibroblasts, HepG2, coronary artery endothelial and smooth muscle cells.
Materials and Methods: Adiponectin mRNA expression was assessed by real-time RT-PCR. Adiponectin protein was visualized by immunoblotting and quantified by radioimmunoassay (Linco Research, St. Charles, MO, USA).
Results: Adiponectin mRNA was present but ranged at the detection limit in all cell types tested. Surprisingly, after exposition to an adiponectin (~1 µg/ml)-containing HEK293 cell culture supernatant, adiponectin mRNA expression was enhanced in all cell types except HepG2 and reached significant levels in human myotubes and coronary artery endothelial cells. In myotubes, the highly consistent increase in mRNA (90-fold over control, $p < 0.001$, $n = 8$) was paralleled by a significant rise in intracellular adiponectin protein (8-fold over control, $p < 0.05$, $n = 4$). Furthermore, adiponectin expression reached levels (29.7 ± 6.7 ng/mg cell protein, $n = 4$) that were detectable by immunoblotting.
Conclusion: We show here that adiponectin gene expression is inducible in non-adipose cells. Furthermore, it seems that adiponectin is able to induce its own gene. This opens the possibility that circulating adipose tissue-derived adiponectin induces local adiponectin expression levels necessary for the maintainance of target cell insulin sensitivity.

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Adiponectin may be a new therapeutic target for the metabolic syndrome.
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Background and Aims: Adiponectin is a novel adipose-specific secretory protein, which we identified in human adipose cDNA project. Plasma concentration of adiponectin is decreased in the subjects with the metabolic syndrome such as type 2 diabetes and cardiovascular disease. In vitro, adiponectin enhances glucose uptake in myocytes and suppresses proliferation of vascular smooth muscle cells. Therefore, the decrease of adiponectin may play some role in the development of the metabolic syndrome. In this study, we aimed to clarify the physiological function of adiponectin in vivo and to investigate the effect of synthetic PPARgamma ligands on adiponectin.
Materials and Methods: 1. We generated adiponectin knockout (KO) mice. To disrupt the mouse adiponectin gene we replaced exon 2, containing the translation initiation sites, with the neomycine-resistance gene. We analyzed mice backcrossed to C57BL/6J for five generations. Supplement of adiponectin in vivo was performed by adenovirus producing full-length adiponectin. We used apoE-deficient mice as the atherosclerotic model animal. 2. We used thiazolidinediones (TZDs) as the synthetic PPARgamma ligands. Adiponectin promoter assays were performed using the Dual-Luciferase Reporter Assay System.
Results: 1. KO mice exhibited low levels of fatty acid transport protein 1 (FATP-1) mRNA in muscle and high levels of TNF-alpha mRNA in adipose tissue, but their insulin sensitivity was normal under regular diet in comparison with the wild-type mice. When mice were fed with high-fat/high-sucrose (HF/HS) diet, KO mice exhibited severer insulin resistance with reduced IRS-1-associated PI3-kinase activity in muscle. Adenovirus mediated production of adiponectin reversed the decreased muscle FATP-1 and increased adipose TNF-alpha mRNA, and insulin resistance in KO mice. The structure of the arterial wall was morphologically normal in KO mice. KO mice fed with regular diet revealed severe neointimal thickening and increased proliferation of vascular smooth muscle cells by the mechanical injury. Adenovirus mediated production of adiponectin suppressed neointimal thickening in KO mice and reduced atherosclerosis in apoE-deficient mice. 2. TZDs remarkably activated the adiponectin promoter and increased the mRNA levels and protein concentrations of adiponectin in 3T3-L1 adipocytes. Administration of TZDs increased the mRNA levels and plasma concentrations of adiponectin in mice. Administration of TZDs significantly increased plasma adiponectin levels in human.
Conclusion: Adiponectin may be a key molecule in the development of the metabolic syndrome. Therefore, pharmacological interventions to increase plasma adiponectin levels should be useful for the treatment of metabolic syndrome.

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Gastric inhibitory polypeptide dose-dependently stimulates glucagon secretion in healthy human subjects.
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Background and Aims: In the isolated perfused pancreas, gastric inhibitory polypeptide (GIP) has been shown to enhance glucagon secretion at basal glucose levels, but in healthy humans no glucagonotropic effect of GIP has yet been reported. Therefore, we studied the effect of GIP on glucagon secretion in healthy subjects under normoglycaemic conditions.
Materials and Methods: Three different doses of GIP (7, 20, and 60 pmol/kg body weight) and placebo were injected intravenously into 10 healthy subjects, each administered on separate occasions in the fasting state. Venous blood samples were drawn twice in the basal state and after 1, 3, 5, 10, 15, 20, and 30 min for glucagon and GIP (specific radioimmunoassays). 31 further healthy subjects (16 male, 15 female, 42 ± 11 yrs., BMI: 24.4 ± 2.7 kg/m^2) were studied with the intermediate dose of 20 pmol/kg. Increments (Δ) in glucagon concentrations were calculated as differences between 10 and 0 min. Statistics: Repeated-measures-ANOVA and Duncan's post hoc tests.
Results: Following the administration of placebo, and of 7, 20, and 60 pmol GIP/kg b.w., GIP rose to peak concentrations of 11 ± 3, 39 ± 6, 99 ± 12, and 390 ± 48 pmol/l, respectively ($p < 0.0001$). GIP dose-dependently stimulated glucagon secretion ($p = 0.019$) with a maximal increment observed after 10 minutes. Incremental glucagon concentrations ($\Delta_{10-0\ min}$) were 0.1 ± 0.7, 1.4 ± 0.5, 2.4 ± 0.5, and 3.4 ± 0.8 pmol/ l for placebo and for 7, 20, and 60 pmol GIP/kg b.w., respectively ($p = 0.017$). Likewise, after the injection of 20 pmol GIP/kg bw in 31 healthy subjects, glucagon concentrations significantly increased over baseline from 7.5 ± 0.5 to 9.3 ± 0.7 pmol/l ($p = 0.0082$).
Conclusion: Glucagon secretion is dose-dependently stimulated by GIP at basal glucose concentration. The absence of a glucagonotropic GIP effect in previous studies using hyperglycaemic conditions may highlight the dependency on normal fasting glucose values. Our results underscore typical differences between the incretin hormones GIP and the glucagonostatic incretin GLP-1.

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Effects of glucose changes and of regulatory peptides on the expression of GLP-1 receptor and glucokinase in GT1-7 cells and in slices of rat hypothalamus.
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Background and Aims: Coexpression of glucose transporter isoform GLUT-2, glucokinase (GK) and glucagon-like peptide-1 receptor (GLP-1R) genes in hypothalamic neurons implied in feeding behaviour, may suggest a role of these molecules in glucose sensing prior to the onset of a state of fullness. Thus, increased glycemia after meals may be recognized by these hypothalamic neurons because the high-Km of GLUT-2 and GK and the glucose-dependent effect of GLP-1. In an attemp to get better insights on this matter we tested the effects of the glucose concentration and of regulatory peptides on the expression of GK and GLP-1R genes and of GK activities in rat hypothalamus.
Materials and Methods: The transcriptional regulation of GLP-1R and GK were analysed in hypothalamic GT1-7 cells by transient transfection of vectors in which the promoters of these genes were fused to the luciferase gene. Transfected cells were incubated in the presence of 2.8, 5.5 and 20mM glucose and/or 10 nM peptides. Glucose phosphorylating activities were determined with a radiometric assay. Hypothalamic slices were

prepared with a tissue chopper and incubated in presence of 2.8, 5.5 and 20 mM glucose and/or 10 nM peptides. After incubation the central (in which is included the VMH) and lateral (LH) areas were isolated.
Results: The promoter activity of GLP-1 receptor gene transfected in GT1-7 cells was decreased about 20% when concentration of glucose was reduced from 5.5 to 2.8 mM. GLP-1 downregulated (20%) the transcription of its own receptor at 5.5 mM glucose. However, another anorexigenic regulatory peptides (leptin and insulin) increased the promoter activity of GLP-1R and the orexigenic peptides NPY, galanin, and orexin B did not or had a light effect on the activity of GLP-1R promoter. Glucose did not affect the GK promoter activity nor GK enzyme activity in transfected GT1-7 cells. Nevertheless GK enzyme activity changed as a function of glucose concentration in rat hypothalamic slices, especially when the data were compared between central and lateral locations. Thus, in LH area GK enzyme activity was decreased 65% at 20 mM glucose, while in central area was increased about 50% at low glucose concentrations.
Conclusions: Our results indicate that the anorexigenic peptides insulin and leptin increase, and the orexigenic ones NPY, galanin and orexin B did not modify or had light effects on GLP1R promoter activity, which might contribute to a state of fullness mediated at least in part through the GLP-1 receptor. By contrast, lack of glucose-induced or neuropeptides effects on GK transcriptional or GK activity in GT1-7 cells as compared with hypothalamic slices, may be explained because the latter constitutes a better physiological model since that keeps tissue arquitecture and functional connections implied in feeding behaviour. Thus, the distinctive pattern of GK activities between central and lateral hypothalamic areas should be conditioned by the action of glucose responsive and glucose sensitive neurons present in such locations and its potential role in glucose sensing.

OP 29
Liver Metabolism

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Adenovirus-mediated expression of dominant-negative JNK in liver reduces insulin resistance and ameliorates glucose tolerance in diabetic animal models.
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Background and Aims: The c-Jun N-terminal kinase (JNK) pathway is known to be activated by certain stress such as reactive oxygen species and various cytokine signals. Whereas oxidative stress is provoked in the hyperglycemic conditions and levels of tumor necrosis factor-a and/or free fatty acids, all of which are known as potent JNK activators, are increased in obesity-induced insulin resistance. JNK is indeed known to be activated in diabetic animal models. Also, it was recently shown that the disruption of JNK pathway (in whole body) ameliorates insulin resistance in ob/ob mice, suggesting that JNK activation is involved in development of insulin resistance in diabetes and/or obesity. In this study, we examined the effects of modification of the JNK activity in liver on insulin sensitivity/resistance and glucose tolerance in normal and diabetic animals.
Materials and Methods: Adenoviruses expressing wild type or dominant-negative type JNK (Ad-WT-JNK and Ad-DN-JNK) and control adenovirus Ad-GFP were prepared. Adenovirus was given to mice via cervical veins. Two weeks later, insulin resistance was evaluated by euglycemic-hyperinsulinemic clamp using stable isotopes and gas chromatography/mass spectrometry. Phosphorylation of serine307 of IRS-1 and serine473 of Akt in liver was examined by Western blotting using specific antibodies.
Results: Ad-WT-JNK-treated C57BL6 mice, in which the increase of JNK expression was observed in liver but not in muscle, revealed 15% reduction in glucose infusion rate (GIR). In contrast, when Ad-DN-JNK was injected to C57BL/KsJ-db/db diabetic mice, the insulin resistance in those mice was markedly ameliorated: GIR was 2-fold higher than Ad-GFP-treated control mice. Also, nonfasting blood glucose levels were markedly reduced in the Ad-DN-JNK-treated mice: from 400-500 mg/dl to 200-250 mg/dl. Thus, the modification of JNK activity in the liver gave a huge impact on whole body insulin resistance and glucose tolerance. In consistent with these observations, IRS-1 phophorylation at serine307 and Akt phosphorylation at serine473 in liver were decreased by treatment with Ad-DN-JNK and increased with Ad-WT-JNK.
Conclusion: JNK activation in liver appears to play a pivotal role in causing insulin resistance and glucose intolerance and may therefore be a potential therapeutic target for diabetes.

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PI 3-Kinase/Akt pathway participates insulin-induced down-regulation of the insulin receptor substrate (IRS)-2 expression in the liver.
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Background and Aims: The liver is the major insulin-target organ responsible for control of glucose homeostasis in the fasted state. Decreased IRS-1 and IRS-2 protein levels have been found in patients with insulin resistance such as obesity or type 2 diabetes and in animal models of insulin resistance in multiple insulin-sensitive tissues, including the liver. It is possible that the decrease in IRS-2 expression, and to a lesser extent in IRS-1 expression, in the liver contributes to the abnormal glucose homeostasis. Although the regulation of IRS-1 has been well studied, little is known about the mechanisms by which IRS-2 protein level is controlled in normal or abnormal states, such as insulin resistance or diabetes. To clarify the mechanism of the effects of insulin on IRS-2 expression, we have examined the potential signaling pathway of which insulin lead to the regulation of IRS-2 expression.
Materials and Methods: We used Fao rat hepatoma cells treated with insulin to study the mechanism of the effects of insulin on IRS-2 expression. Effect of insulin on IRS-2 expression was examined by Western blot, Northern blot, and EMSA (electrophoretic mobility shift assay) in the absence and/or presence of a MEK inhibitor, a PI 3-kinase inhibitor, a proteasome inhibitor, a dominant-negative form of Akt using adenovirus system, a protein synthesis inhibitor, or an inhibitor of RNA synthesis.

Results: Both IRS-1 and IRS-2 protein levels were decreased by insulin in Fao cells. The decrease in IRS-1 protein occurs via proteasomal degradation without any change in IRS-1 mRNA, whereas the decrease in IRS-2 protein is associated with a parallel decrease in IRS-2 mRNA without changing IRS-2 mRNA half-life. The decrease in IRS-2 mRNA and protein by insulin is blocked by PI 3-kinase inhibitor, LY294002, but not by MEK inhibitor, PD98059. Inhibition of Akt by overexpression of dominant-negative Akt also causes complete attenuation of insulin-induced decrease in IRS-2 protein and partial attenuation of its mRNA down-regulation. Some nuclear proteins bind to an insulin response element (IRE) like sequence exist in IRS-2 gene promoter with an insulin-dependent manner in vitro, and the binding is again blocked by PI 3-kinase inhibitor. Reporter gene assay shows that insulin suppresses activity of both human and rat IRS-2 gene promoter through the IRE in a PI 3-kinase dependent manner.
Conclusion: Decrease of IRS-2 expression is likely due to a repression of IRS-2 gene transcription by insulin via a process mediated by the PI 3-kinase/Akt pathway and by some nuclear proteins binding to the IRE sequence in IRS-2 gene promoter. These new findings should help to clarify the role of IRS-2 and insulin resistance in liver.

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Tumor necrosis factor and anisomycin induce serine phosphorylation of IRS-1 by a redox-sensitive p38MAPK mediated cross-talk with ErbB2/ErbB3 receptors.
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Background and Aims: Insulin resistance is associated with several diseases including chronic infection, obesity and type 2 diabetes. The pro-inflammatory cytokine TNF was implicated as the mediator of insulin resistance in these pathological states. Previously we have demonstrated that transactivation of ErbB2/ErbB3 by TNF and additional cellular stressors like anisomycin (AN), triggers a PI-3 kinase (PI3K) cascade which induces serine phosphorylation of IRS proteins and leads to their dissociation from the insulin receptor, resulting in the termination of insulin signal. In the current study we have attempted to identify the cellular pathways which lead to ErbB2/ErbB3 transactivation by TNF and AN and have focused on the role of stress-activated kinases.
Results: Treatment of rat hepatoma Fao cells with SB203580, a specific p38MAPK inhibitor, suppressed the stressors-induced tyrosine phosphorylation of ErbB2/ErbB3 and the PI3K activity associated with the ErbB receptors, while MEK1, PKC, PI3K and mTOR inhibitors had no effect. SB203580, in contrast to the specific PI3K inhibitor Wortmannin, did not abolish the stressors-induced PI3K activity in cell free system. In addition, the p38MAPK inhibitor had no effect on the induction of ErbB2/ErbB3 tyrosine phosphorylation by the ErbB3 natural ligand NDF. These findings support the notion that p38MAPK activation by stress stimuli mediates ErbB2/ErbB3 transactivation and downstream stimulation of PI3K. TNF and AN are known to modulate the cellular redox state and to induce the generation of reactive oxygen species, therefore we have tested the effect of several antioxidants on ErbB2/ErbB3 transactivation. NAC, GSH and DPI inhibited TNF and AN-induced, but not NDF-induced, ErbB2/ErbB3 tyrosine phosphorylation. Moreover, they attenuated the stressors-induced p38MAPK activation, suggesting that the cellular redox state regulates p38MAPK activation and p38MAPK-mediated ErbB2/ErbB3 transactivation. Since activation of ErbB2/ErbB3 mediates the phosphorylation of IRS proteins, we have next examined the effect of SB203580 and NAC on IRS-1 function. Both agents reduced IRS-1 phosphorylation on several serine residues, as demonstrated with specific phosphoserine antibodies. In addition, they improved the ability of IRS-1 to interact with the insulin receptor. Moreover, SB203580 and NAC partially reversed the stress-induced impairment of insulin-dependent IRS-1 tyrosine phosphorylation and its association with PI3K.
Conclusion: Taken together, our findings suggest a novel mechanism for cellular stress-induced insulin resistance. Under stress stimuli, intracellular redox-state alterations stimulate p38MAPK. This stress-activated kinase contributes to ErbB2/ErbB3 transactivation that induces serine phosphorylation of IRS-1, culminating in insulin signaling impairment. Our findings point to potential sites for therapeutic intervention using either antioxidants or kinase inhibitors to correct insulin-resistance.

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Inhibition of insulin-dependent expression of the glucokinase gene by IL-1β, in primary cultured rat hepatocytes.
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Background and Aims: Pro-inflammatory cytokines, such as IL-1β, have profound effects on glucose and lipid homeostasis. Furthermore, recent studies have demonstrated a close relationship between increased pro-inflammatory cytokine production and insulin resistance. In the liver, glucokinase (GK) is a key enzyme in the control of glucose utilization, its expression being strictly dependent upon insulin action. In this work, we have investigated the influence of IL-1β on the expression of the GK gene induced by insulin, in primary cultures of rat hepatocytes.
Material and Methods: Hepatocytes were obtained by collagenase perfusion of livers and cultured, for 19 h, in 199 medium supplemented with 10% FBS, 1 µmol/l T_3 and 1 µmol/l dexamethasone; 2 h before insulin and IL-1β additions, hepatocytes were deprived of FBS and hormones. GK mRNA was analysed by Northern blot. The transcription rate of the GK gene was determined by nuclear run-on assays. In signalling experiments, proteins and their phosphorylated forms were detected by Western blot analysis.
Results: Treatment of cultured hepatocytes with IL-1β, for 4 h, caused a dose-dependent reduction in GK mRNA levels induced by 10 nmol/l insulin. The maximal inhibition (about 88 %) was obtained with 3.1 nmol/l IL-1β (101.3 ± 0.78 vs. 12.45 ± 4.40 arbitrary units, for hepatocytes incubated without and with IL-1β, respectively; $p< 0.001$, $n=4$), the calculated EC_{50} value being 0.14 ± 0.004 nmol/l. The decrease in insulin-induced GK mRNA levels caused by IL-1β occurred without significant changes in the half-life of GK mRNA (51.4 vs. 51.5 min, for hepatocytes incubated without and with 1 nmol/l IL-1β, respectively). In nuclear run-on transcription assays, IL-1β (1 nmol/l) caused an 85 % reduction in the rate of GK gene transcription induced by 10 nmol/l insulin. The inhibitory effect of IL-1β (1 nmol/l) on insulin-mediated GK expression was achieved without significant changes in the phosphorylation states of p42/p44 mitogen-activated protein kinases (p42/p44 MAPK), protein kinase B (PKB) or p70 S6-kinase (p70 S6-K), as compared to those observed in hepatocytes incubated with insulin alone (10 nmol/l). Furthermore, the IL-1β effect was not modified by the presence of 10 µmol/l SB 203580, a specific inhibitor of p38 mitogen-activated protein kinase (p38 MAPK), in the incubation medium.
Conclusions: IL-1β reduces, in a dose-dependent manner, insulin-induced GK mRNA levels in primary cultured rat hepatocytes, by decreasing the rate of GK gene transcription. This IL-1β effect occurs without significant changes in the activation states of p42/p44 MAPK, phosphatidylinositol 3-kinase (PI 3-K)/PKB and PI 3-K/p70 S6-K signalling pathways in response to insulin, and was independent of p38 MAPK activity.

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Localisation of the cis-regulating element responsible for glucose-induced increase in the expression of the glucose-6-phosphatase gene.
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Background and Aims: Glucose is not only an energy source it is also involved in the control of fundamental cellular processes. We have shown that the gluconeogenic enzyme glucose-6-phosphatase (Glc-6-Pase) is a glucose-responsive gene. The induction of Glc-6-Pase mRNA by glucose occurs both at the transcriptional and post-transcriptional levels. We sought to determine the molecular mechanism of the glucose effects.
Materials and Methods: A reporter construct that contains the glucose-6-phosphatase promoter sequence from -751/+66 linked to the luciferase reporter gene (751/+66-Luc) was transfected into the human hepatoma cell line HepG2. Binding of nuclear proteins to the glucose-6-phosphatase gene was performed by electrophoretic mobility gel shift assays.
Results and Conclusions: We sought first to establish the responsiveness of the promoter to glucose. A 3-fold stimulation of luciferase activity was observed in the presence of 20 mM glucose compared with the luciferase activity measured in cells cultured at basal glucose concentrations (5 mM). To identify the glucose-responsive elements within the Glc-6-Pase promoter, a series of deletions in the parental promoter was created. These constructs were transiently transfected into HepG2 cells, and the response to glucose was determined either at 5 or 20 mM glucose. Deletion from –750 to -686 resulted in an increased in basal promoter activity and a 29-fold increase in response to 20 mM glucose. These suggest that the

promoter region between -751 to -686 contains a powerful inhibitory sequence repressing promoter activity. Further deletions from -686 to -135 provoke a progressive decline in promoter activity without loosing however the glucose effect. Deletion from –135 to -52 abolished the glucose effect suggesting that the region between -751 to -135 contains cis-positive elements involved in the transcriptional activation of Glc-6-Pase by glucose. The glucose-6-phosphatase promoter from -750 to + 66 was examined for sequences that might function as a carbohydrate responsive element. One site was found that matches the consensus sequence described in the literature for other glucose-responsive genes. This sequence contains a direct repeat of the E-box CANNTG separated by 5 bp. The E-box binds basic helix-loop-helix proteins like USFs (USF1 and USF2). These proteins have been suggested as major players in response to high glucose concentrations. Co-transfection experiments using USF1/USF2 transcription factors indicated these transcription factors are not involved in glucose regulation of the glucose-6-phosphatase gene. The transcription factor (s) binding the promoter and the precise mechanism responsible for the glucose effects are being investigated.

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Differential regulation of the glucose-6 phosphatase TATA box by intestine specific homeodomain proteins CDX1 and CDX2.

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Background and Aims: Glucose-6 phosphatase (Glc6Pase), the last enzyme of gluconeogenesis, is only expressed in the liver, the kidney and the small intestine and confers on these tissues only the capacity to release glucose in blood. The expression of the Glc6Pase gene exhibits marked specificities in the three tissues in various situations, particularly in insulinopenia states, but the molecular basis of the tissue specificity is not known. The presence of a consensus binding site of CDX proteins in the minimal Glc6Pase gene promoter has led us to consider the hypothesis that these intestine-specific CDX factors could be involved in the Glc6Pase expression in the small intestine
Materials and Methods: The –80/+60 bp G6Pase gene region was cloned upstream of a luciferase reporter gene. G6Pase promoter activity was assessed by transient transfections in HepG2 hepatoma cells and in intestinal CaCo-2 cells. Mutant forms of the Glc6Pase promoter constructs, as well as swapped versions of the expression plasmids encoding the murine CDX1 and CDX2 were generated by site-directed mutagenesis. DNA-protein interactions were studied using total cell extracts in bandshift assays.
Results: We first show that the Glc6Pase promoter is active in both hepatic HepG2 and intestinal CaCo2 cells by transient transfection assays. Using gel shift mobility assay, mutagenesis and competition experiments, we show that both CDX1 and CDX2 can bind the minimal promoter, but only CDX1 can transactivate it. We demonstrate that a TATAAAA sequence, located in position –31/-25 relating to the transcription start site, exhibits separable functions in the preinitiation of transcription and the transactivation by CDX1. Disruption of this site dramatically suppresses both basal transcription and the CDX1 effect. The latter may be restored by inserting a couple of CDX-binding site in opposite orientation similar to that found in the sucrase-isomaltase promoter.
Conclusion: These data strongly suggest that CDX proteins could play a crucial role in the specific expression of the Glc6Pase gene in the small intestine. They also suggest that CDX transactivation might be essential for intestine gene expression, irrespective of the presence of a functional TATA box.

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cAMP induces the transcriptionnal activity of the glucose-6-phosphatase gene in hepatocytes and enterocyte-like cells via CREB and HNF4α.

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Glucose-6-phosphatase (Glc6Pase) is a key enzyme of gluconeogenesis and is expressed in only three tissues : the liver, the kidney and the small intestine. The Glc6Pase gene expression is increased in these three tissues during fasting and diabetes, which are two situations of insulinopenia, under the control of cAMP.
We have studied the transcriptionnal regulation of the Glc6Pase gene by cAMP in human hepatoma HepG2 cells and human colonic CaCo2 cells, the latter being able to differentiate in enterocyte-like cells after several days of culture at post-confluence.
The promoter activity of the -1480/+60 bp fragment was dose-dependently induced by an adenylyl cyclase activator (forskolin : 10-8M to 10-4M). The induction was about 5 fold in HepG2 cells and 24 fold in differentiated CaCo2 cells after 6h in the presence of 10-4M forskolin. The 5' deletion fragments of the Glc6Pase promoter were induced by co-expression of the catalytic subunit of PKA in both HepG2 and CaCo2 cells. The Glc6Pase promoter exhibited two cAMP response units : a distal region of strong response upstream -500 bp (induced from 50 to 90 fold) and a proximal region of weak response downstream -500 bp (induced from 2 to 10 fold). Dominant-negative CREB and HNF4α respectively suppressed the PKA induction of the activity of the -694/+60 bp promoter fragment in both HepG2 and CaCo2 cells. Mutagenesis experiments allowed us to identify the binding sites of these two proteins. The proximal region overlaps one HNF4α binding site and two CREB binding sites. The distal region overlaps one HNF4α and one CREB binding site. A dominant-positive HNF4α synergistically enhanced the PKA induction of all promoter fragments containing CRE-sites.
These results have shown that the Glc6Pase promoter activity is inducible by cAMP, via a PKA dependant mechanism, in both hepatic and enterocyte-like cells. The transcriptional mechanism involved two distinct promoter regions and both CREB and HNF4α proteins.

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New aspects of hepatic glucokinase translocation by the glucokinase regulatory protein.

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Background and Aims: Glucokinase (GK) acts as a glucose sensor for coupling millimolar glucose concentrations to metabolism. In liver a glucokinase regulatory protein (GRP) modulates GK activity and mediates the nuclear-cytoplasmic translocation of GK in dependence of glucose and fructose metabolites. In our recent studies we investigated the molecular basis of the GK import-export mechanism and the nature of the GRP binding motif inside the GK. Through yeast two-hybrid analyses we could identify the GK amino acid residues Leu-58 and Asn-204 as most important for the GRP interaction. It was the aim of this study to characterize the molecular mechanisms of GK and GRP translocation by real time fluorescence microscopy using wild-type and mutant proteins.
Materials and Methods: COS-1 and HeLa cells were transfected with EYFP-GRP and ECFP-GK wild-type protein or proteins with a mutation of the binding motif. Protein localization and co-localization were monitored by epifluorescence and laser scanning microscopy as well as fluorescence resonance energy transfer (FRET) in cells perifused with 5.5 mM or 25 mM glucose.
Results: At low glucose concentration (5.5 mM) the ECFP-GK fusion protein was located in the nucleus together with EYFP-GRP. In contrast, cells co-transfected with GRP and the GK mutants L58R/N204Y, the ECFP-GK fusion protein remained predominantly in the cytoplasm at 5.5 mM glucose. In cells cultured at high (25 mM) glucose the ECFP-GK protein was in the cytoplasm whereas the EYFP-GRP protein showed a nuclear localization. However, after a pre-culture of the cells with low glucose the GK wild-type and GRP fusion proteins translocated together from the nucleus to the cytoplasm after perifusion with high glucose. The interaction of GK and GRP could be verified by FRET analyses. GK mutant proteins did not show any translocation from the nucleus.
Conclusion: The amino acid Asn-204 plays a pivotal role for the regulation of GK because it confers the interaction with the GRP and is localized within the substrate binding site of the enzyme protein. Importantly, our data provide evidence that GK is shuttled as a complex with GRP from the nucleus to the cytoplasm.

OP Education 1:

Long-Term Implementation of Education

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Does structured education with diabetic patients take place in Sweden?

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Background and Aims: The over all seizing goal for treatment of diabetes is to prevent acute and long term complications and maintain a good quality of life. The St Vincent Declaration and the National Guidelines of Treatment of Diabetes Mellitus describe patient education in self treatment as a pre requisit for the achievement of these goals. This survey aims to evaluate the presence of structured patient education – described as in advance planned education occation-, its organization, staff, goals and results in out patient care in Sweden.
Materials and Methods: A questionnaire consisting of 38 open and closed questions were mailed to 1250 nurses working in hospitals and primary health care in the entire country.
Results: Structured patient education was performed by 483 nurses. It was usually organized by nurses and performed in cooperation with doctors (55%), dietician (38%), chiropodist (36%), and social worker (9%). The sessions took place individually at prescheduled vistits (80%), or as group education (26%). Evident goals for the education were present at 51%, -the most common: increased general knowledge about the diabetes disease (46%), increased metabolic control (42%) and increased safety (37%). The education was evaluated by 51% of which the Hba1c-level at the next scheduled visit was the most frequently used method (44%), followed by home monitored blood glucose values (37%) and an structured evaluation form (17%). The goals had been achieved to a great or quite great extent by 67% of the responding nurses.
Conclusion: To the extent that structured patient education takes place, nurses are the usually responsible for its performance. It takes place individually as well as in groups. Many nurses lack evident goals for the education and suficient evaluation methods.

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How long does the efficacy of therapeutic education last? Four-year-follow up.

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Background and Aims: Therapeutic education has on overall beneficial impact on health and psychosocial outcomes even if is connected with the modified behaviour. Specifically, the patients who have improved knowledge and behaviour have experience of positive effects on glycemic control. But to maintain for long time the knowledge and the skill required to manage their disease, is not easy. Aim of the work is to evaluate how long the efficacy of therapeutic education lasts, by comparing effects at the end of the therapeutic programme and after 4 years.
Materials and Methods: In 1998, 268 diabetic patients (type 1 n. 122, type 2 n. 146) have been participants in therapeutic educational program for 6 months (10 hours on group and 10 hours individual). The teaching program included: diabetes clinical manifestations, prevention and management of metabolic and complication's emergency, importance of diet and physical activity. Patients had training to manage insulin therapy and blood glucose self monitoring (SMBG). Efficacy of knowledge was tested with questionnaire, before (time 0), after the therapeutic program (time 1) and after 4 years (time 2), with errors score. Efficacy on modified behaviour was evaluated (at time 0,1 and 2) by using objective variables (HbA1c, BMI) and also discretionary variables (with special score): SMBG appropriateness (how much and when), weekly time for physical activity, and how many times of uncorrected diet a week. The paired Student t-test was used.
Results: At time 1 the population have improved knowledge, score 9 vs 18, ($p < 0,0001$), SMBG appropriateness, physical activity and diet ($p < 0.0001$); also HbA1c (7.0% vs. 7.5%; $p=0.0106$) and BMI (28.8 vs. 29.1; $p=0.01$). At time 2 all variables got worse (vs time 1): knowledge, score 16 vs 9 ($p < 0.005$), SMBG appropriateness ($p = 0.013$), physical activity and diet; BMI increased 31.4 vs 28.8 ($p < 0.005$) and HbA1c 8.12 vs 7.06 ($p < 0.0001$)
Conclusion: Efficacy of therapeutic program shows better results after short time than after long. It seems that patients remember and apply only a few of the taught knowledge on their disease, but not all those they need.. Moreover some patients prefer to be supported by a stronger training relationship, based on a continuous educational program with an assistance team, to avoid discouragement at the initial failure and to be fortified to go on. Even if the economic resources in Public Health are expected to become poorer and poorer, is worthy to allocate more of them to continuous educational programs in order to strongly support the diabetic patients. The value of the therapeutic educational program in relation to the expected results has to be emphasized both with patients and physicians, and with the Health Program Decision Makers as well, also through the media.

Table 1. Indicators of program efficacy

Variables	time 0	time 1	p	time 2	p
Knowledge	18.0	9.0	0.0001	16.4	0.005
Objective variables					
HBA1c	7.51	7.06	0.0106	8.12	0.0001
BMI	29.1	28.79	0.01	31.4	0.005
Discretionary variables					
SMBG appropriateness	18.3	25.76	0.0001	22.4	0.013
Physical activity	0.66	1.77	0.0001	0.89	0.51
No diet	5.57	3.57	0.0001	5.44	0.08

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Continuous care and education for people with Type 1 diabetes: a 10 year follow-up.

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Background and Aims: The Cuban Diabetes Education Program was designed in 1980 at the National Institute of Endocrinology, on the basis of an interactive people approach. The principal aims of the program are: 1)the capability of health care providers to follow up people with diabetes; 2)therapeutic education for people with diabetes and their relatives and 3)the diabetes prevention educating people with risk factors. Different studies have been done during 20 years to determine the effectiveness of the program in its different activities and to continuously identify new needs and to reinforce the weaker aspects. On the security that therapeutic education must begin since the diagnosis and supporting the hypothesis that an interactive approach can be more efficient to develope people comprehension, skills and motivation to deal with daily self care, a special small group consultation was introduced for newly diagnosed diabetics. The aim of this paper is to show the results of a 10 years follow-up of this interactive group sessions (IGS) in 40 newly diagnosed type 1 diabetic patients comparing to 40 controls followed in one-to-one standard routine consultations (SRC).
Materials and Methods: IGS were developed on quarterly scheduled group meetings (10 patients, relatives and Health Care Providers)to check metabolic parameters and to exchange perceptions, feelings and experiences on coping with diabetes, using problem solving techniques and a patient centred approach. Individual needs were attended at the end of the meeting. SRC consisted on a quarterly routine consultations with the specialists, where direct counselling were given according to individual needs. Diabetes knowledge and feelings were evaluated before and during the 10 years follow up and validated questionnaires were applied before, 5 and 10 years later. Metabolic parameters were taken from the individual clinical record. Comparisons among groups were determined using T Student, Wilcoxon and Chi Square tests.
Results: Diabetes knowledge, skills and behaviours had significantly improved in both group but patients in IGS scored consistently higher ($p=0,000$) than those attending SRC. Feelings on treatment responsibility, self confidence and autonomy were significant higher in IGS comparing to SRC ($p=0,00002$). HBA 1c mean levels have decreased from 12,4 to 6,2% vs 11,9 to 7,6 in SRC ($p=0,001$), with less emergencies in the IGS. After 5 years follow up a significant differences was shown in the development of long term complications in favour of the IGS ($P<0,001$), situation maintained at the 10 years.
Conclusions: IGS was more efficient to develope knowledge and skills and to empower people to cope with diabetes daily care, improving the blood glucose levels, diminishing the need of emergency services and preventing the development of long-term complications.

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Long term effect of a structured outpatient education programme in Type 1 diabetic patients - a 12 year follow up.

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Background and Aims: We evaluated the long term effect of our structured outpatient diabetes teaching programme (DTTP) for intensive insulin therapy (IIT) in patients with type 1 diabetes.
Materials and Methods: 3 and 12 years after the DTTP patients, who participated between 6/1989-12/89 in the programme, were invited for a follow-up visit. Out of 69 patients 4 (2%) subjects could not or did not want to take part in the follow up. However, basic information about their status was obtained by contact of these patients or their relatives. 2 (1%) patients died and 2 (1%) were lost to follow up.
Results: 61 patients (36 female, age:[mean±SD] 54±11 years, diabetes duration: 28±11 years) completed the follow up after 12 years.

	Baseline	3 years	12 years
HbA1c (%)	8,4±1,7	7,2±1,2**	7,9±1,3 ns
Severe hypoglycemia (n/year)	0,36±0,98	0,16±0,49ns	0,10±0,48*
BMI	22,75	23,13*	24,04**

*p<0.01 and **p<0.001 vs.baseline, paired t-test
58 (98%) of the patients continued with IIT over the 12 years.

The number of daily insulin injections (2,4±0,9 vs. 4,2±1,0 after 3 years p<0.001 and 4,8±0,9 after 12 years p<0.001) and frequency of daily blood sugar monitoring (2±1,5 vs. 3,7±1,2 p<0.001 vs 4,1±1,3 p<0.001) increased significantly over 12 years, whereas daily insulin dosage (IE/kg body weight) remained unchanged (0,58±0,2 vs. 0,55±0,17 vs. 0,55±0,15). High quality of life and treatment satisfaction (DSQOLS 68,7±16,5) was demonstrated after 12 years.
Conclusion: 12 years after participation in the structured patient education programme frequency of severe hypoglycaemic episodes remained considerably reduced, whereas improvement of metabolic control could not be entirely maintained. These results clearly indicate the need of a continued patient education in patients with type 1 diabetes to maintain overall benefits.

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From recommendation to practice: implementation of the new recommendation on sugar and its impact on attitude, liking and knowledge.

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Background and Aims: In year 1988 the Finnish Diabetes Association gave a new diabetes diet recommendation in which sugar in moderate amounts was considered as a normal incredient of the daily diet. The aim of this study was to detect the changes in use of sugar and sugar containing foods, in attitude and liking of sweet taste and in knowledge.
Materials and Methods: The data was collected by a questionnaire from the patient population coming to the courses in the Diabetes Center in 1988 (N=157), 1990 (N=190), 1994 (N=168) and 1998 (N=131). The control group consisted of the personnel of two working sites in 1988 (N=89) and 1999 (N=53). Attitude, liking and use were measured with a 0 – 100 scale, other aspects were measured with 20 five point Likert scale items. The statistical analysis consisted of a chi2 test, student's t-test, correlation analysis and factor analysis.
Results: The use of sugar and sugar containing foods increased from 1988 to 1998 form 27±20 to 46±21(p<0,001) (controls 74±17 and 63±23, p<01,01) on the scale. The increase in the use of sugar was not significant before 1994. The use increased significantly also from 1994 to 1998 (p<0,01). The use of other nutritive or non-nutritive sweeteners did not change. There was a trend towards a more favourable attitude (p<0,06) and increased liking (p<0,02), almost reaching the level of controls in 1998. The use of sugar, attitude and liking, were strongly intercorrelated in the control population in 1988 and 1998. Among diabetic patients only attitude and liking correlated in 1988, but 1998 also the use of sugar correlated with attitude (p<0,001) and liking (p<0,001). Those treated with diet or 1-2 injections of insulin increased the use of sugar, but attitude or liking did not change. Those having 3 or more injections had higher use of sugar than the former group, and attitude and liking increased among them significantly through the years.
Through factor analysis two factors interpreted as "threat" and "suspicion" emerged. The use of sugar was strongly negatively correlated (p<0,001) with both factors 1988 and 1999. Among patients treated with diet or 1 insulin injection "threat" did not change but "suspicion" decreased significantly (p<0,001) from 1988 to 1998. Patients with 2 or more insulin injections felt in 1998 significantly less "threat" and "suspicion" than in year 1988 (p<0,001) and compared to the former group they felt less "threat" and "suspicion" in year 1988 but only less „suspicion" in 1998. Knowledge about the use of sugar and sugar containing foods increased over the years. In 1998 there was a significant negative correlation between knowledge and "suspicion".
Conclusion: The implementation of the new diet recommendation has taken many years. Ten years after the recommendation there was a significant increase in the use of sugar and sugar containing foods, positive changes in attitude and liking, and a trend towars reduced feeling of „threat" and „suspicion". The changes were much smaller in patients treated only with diet or 1 insulin injection, perhaps reflecting less intensive or less knowledgeable education of this group.

OP Education 2:
Psychological Impact of Diabetes

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Prevalence of psychiatric disorders in diabetic patients.
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Background and Aims: Long term outcome of diabetes mellitus is strongly related to selfmanagement abilities. Psychiatric comorbidity could be an important barrier for successful self treatment in everyday routine. Therefore this study was designed to determine the prevalence of psychiatric comorbidity in a German inpatient sample of diabetic patients.
Materials and Methods: 413 diabetic patients (age 53.4 ±14.8 years, 40% female, A1c 8.5 ±1.6%, 148 type 1 diabetic patients, 101 type 2 with oral agents, 164 type 2 with insulin) were screened by two depression questionnaires (BDI and CES-D) and an anxiety inventory (Trait version of STAI). Patients with positive screening results (1 SD above the mean of the reference population) in one of the questionnaires received a structured diagnostic interview according to research criteria of ICD-10. Prevalence rates for psychiatric disorders were standardized for gender and age, according to demographic data provided by the German Federal Statistical office.
Results: Prevalence for affective disorders based on clinical interview was 12.3% (age and gender adjusted 13.2%). Anxiety disorders had a prevalence of 5.6% (age and gender adjusted 5.7%). A prevalence of 2.2% for substance abuse was observed (age and gender adjusted 5.7%). The rate for eating disorders was 2.4% (age and gender adjusted 4.6%). Prevalence rates for eating disorders were highest in female, young type 1 diabetic patients. Overall 18.7% of the diabetic patients received a psychiatric diagnosis based on a clinical interview; 15.2% received one psychiatric diagnosis, whereas 3.5% had a psychiatric multimorbidity (2 or more diagnosis).
Conclusion: In this study an elevated prevalence of affective and eating disorders was striking. These prevalence rates where much higher than in a non-diabetic German reference sample, whereas prevalence rates of substance abuse and anxiety disorders were not elevated. In view of the facts that every fifth diabetic persons in our clinical sample had psychiatric comorbidity, a routine screening for psychiatric disorders in diabetes care seems to be sensible. Because of the elevated prevalence of affective disorders and eating disorders it can be hypothesized that there maybe a specific link between diabetes and depression respective eating disorders. On the other hand, generalization of the prevalence rates to all people with diabetes is difficult, because this clinical population may be a biased sample.

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How often do depressed diabetes patients suffer from serious levels of diabetes-specific emotional distress? A Croatian-Dutch-English survey from the European Depression in Diabetes (EDID) research consortium.
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Background and Aims: Research suggests that depression is common in people with diabetes. However, little is known yet about the co-existence of depression and diabetes-specific emotional distress. In the present paper, we set out to: 1) determine the prevalence of pervasive depression in Croatian, Dutch and English outpatient samples with diabetes, 2) determine the levels of diabetes-specific emotional distress in diabetic individuals with versus without depression.
Materials and Methods: A number of 542 outpatients with diabetes (202 Dutch, 185 Croatian and 155 English) completed the Center for Epidemiological Studies Depression (CES-D) scale and the Problem Areas in Diabetes (PAID) scale. Demographic and clinical characteristics were obtained by means of medical records (Croatia, UK) and self-reports (Dutch).
Results: Percentages of patients with CESD scores higher than 16 (indicative of depression) were 19%, 21% (Dutch men, women); 19%, 39% (English men, women); 39%, 34% (Croatian men, women). It appeared that 67% (Croatian), 39% (Dutch) and 37% (English) of the depressed patients reported to have at least four serious diabetes-specific emotional problems, compared to 18% (Croatian) and 0% (Dutch and English) of patients with a low depression score (0-1).
Conclusion: Serious diabetes-specific emotional problems are particularly prevalent in depressed diabetes patients. Health care providers who treat depression in diabetes patients should therefore have knowledge of diabetes and diabetes-specific emotional problems. Moreover, using the PAID in addition to a depression questionnaire may be useful, as this instrument could facilitate therapy by directing psychotherapeutical interventions.

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Routine assessment of psychological well-being in people with diabetes in primary care - validation of the WHO-5 Well-being Index in six countries.
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Background and Aims: The prevalence of depression in people with diabetes is around twice as high as in the general population. Depression appears to be associated with poor clinical outcomes. The WHO-5 Well-being Index (WHO-5) is a five-item generic screening instrument for well-being and depression. The purpose of this study was to evaluate the reliability and clinical validity of different language versions of the WHO-5, and to determine the feasibility of its use in people with diabetes in primary health-care .
Materials and Methods: The instrument was administered to 624 people with diabetes in Belarus, Georgia, Greece, Russia, Slovenia, and the United Kingdom attending a diabetes service as part of their routine care. The range of people completing the questionnaire included male and female adult patients of all age groups, with Type 1 or Type 2 diabetes.
Results: Using a variety of statistical techniques, the reliability and validity of the instrument were found to be high for all language versions. Thus internal consistency (α) was 0.84 to 0.90. The WHO-5 proved able to discriminate between different patient groups based on treatment modality ($p=<0.017$), metabolic control ($p=<0.000$), presence of complications ($p=<0.000$), sex ,($p=<0.000$), family status ($p=<0.001$), and country ($p=<0.000$).
Conclusion: It is concluded that the WHO-5 is a robust multi-language assessment tool useful for routine use with people with diabetes. The instrument is reliable, short and easy to use, and its ability to discriminate between patient groups suggests it may allow identification of people developing depressive symptoms in routine diabetes care.

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Perception of, and anxiety levels induced by, laser treatment in patients with sight-threatening diabetic retinopathy.
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Background: Retinopathy is a complication of diabetes that requires regular control visits and, sometimes, treatment by laser photocoagulation which is often delivered over multiple sessions.
Aims: to investigate how diabetic patients perceive Laser Treatment (LT) in terms of awareness and anxiety.
Patients and Methods: Patients waiting for LT in our Diabetic Retinopathy Centre were administered 4 questionnaires: HADS (Hospital Anxiety e Depression Scale), FA-LTE (Family Apgar-List of Threatening Experiences), STAI 1 and STAI 2 (State-Trait Anxiety Inventory). After completion of the questionnaires, all patients were asked open questions on whether: they had ever heard the word “laser”, they could describe LT and they knew why LT had been recommended for them. 48 consecutive patients (35 type 2) were interviewed, none of whom refused to participate. 47,9% had only primary education and 56,3% were retired. 35 patients had not had LT before, whereas 13 had one or more sessions within the past 12 months. Results are expressed as means ± SD. Differences between scores are shown as means and 95% CI.

Results: Patients about to receive LT displayed high levels of discomfort and anxiety, which increased among those who had to repeat treatment:

	1st laser Rx	Repeat laser Rx	Difference (95% CI)	p value
HADS	10,3±6,3	16,5±6,9	-6,2 (-10,5/-2,0)	<0.005
FA-LTE	8,0±2,2	6,3±3,0	1,7 (-0,2/3,6)	0,84
STAI 1	42,2±13,0	52,0±12,2	-9,8 (-18,2/-1,4)	<0.0023
STAI 2	41,0±13,6	53,9±9,1	-12,9 (-19,9/-6,0)	<0.001

Although 85,4% of patients had heard of LT before, most of them could neither describe LT nor explain why they were about to receive it. LT was listed among the most stressing events occurred over the past year. A woman compared it with the recent loss of her husband and another one with an accident occurred to her son. Interestingly, the patients' descriptions of LT evoked images of cutting or tearing tools and needles entering the eyes.
Conclusions: These preliminary results suggest that LT induces fear and high levels of anxiety which increase, rather than decrease, with repeating LT sessions. More data should be collected to confirm this interpretation and to guide the development of more appropriate settings to improve approach and support to patients. Lowering anxiety and promoting coping mechanisms may help to reduce fear and prevent patients' avoidance of treatment.

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Longitudinal evaluation of depression in young adults with Type 1 diabetes.
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Background and Aims: To describe the preliminary results of (3 years out of 5) of a longitudinal evaluation of depression symptoms in young adults with type I diabetes.
Materials and Methods: Two self-report questionnaires the Beck Depression Inventory II (BDI-II), and the Primary Care Evaluation of Mental Disorder (PRIME-MD) were administered to 529 young adults (mean age 27.3 ± 7.6) with type I diabetes (273 males and 256 females) attending our clinic. Four hundred ninety two patients completed the questionnaires in the second year and 402 patients completed the questionnaires in all three years.
Results: Mean BDI-II score was 9.76, 9.51, and 8.49 in the first second and third year. Females scored significantly higher than males at all time points: 11.8, 11.5, and 11.7 vs. 7.5, 7.4, and 7.5, respectively. No difference by age group was found (<24, 24-30, 30-40 and >40). Scores of 14 or higher in the BDI-II (at least mild level of depression) was found in 22.8%, 24.3%, and 18.2% of the first, second and third year. A score of 18 or higher, which was found to be the best balance between sensitivity and specificity, was found in 12.1%, 15.5%, and 12.5% of the patients in the three years of the study. Mood disorders were found in 15.4%, 13.4% and 12% of the patients in the first, second and third year of the study as compared to 16% prevalence of mood disorders found in 3000 adult patients assessed by primary care physicians (Spitzer 1999).
Conclusion: The prevalence of depression among young adults with type 1 DM treated in our center is lower than the prevalence reported by other groups. Our results corroborate with the rate of depression described in a general population of primary care clinics. Differences in social background, rate of complications, mode of therapy and the medical team approach may all attribute to our patients lower rate of depression.

OP Education 3:
How to Change Lifestyle / Behaviour

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CHOPPS: the Christchurch Obesity Prevention Programme in Schools.
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Background and Aims: The incidence and prevalence of childhood obesity is rising exponentially. The consumption of sugar-sweetened fizzy drinks has been linked to the risk of developing childhood obesity. The aim of this study was to determine if a specific school based health education programme encouraging children to consume less fizzy drinks, could reduce the risk of children becoming overweight or obese.
Materials and Methods: Six local junior schools agreed to participate in a randomised controlled trial, over 1 school year. The specific educational initiative focused on reducing the consumption of sugar-sweetened drinks. Children completed 3-day drinks diaries at baseline and completion of the trial. A 10-point multiple choice health questionnaire was also completed. Baseline and final measurements were made of weight (Seca medical scale - 770), height (portable Leicester measure) and waist circumference. Children were randomised according to their classes and comparisons were made between the control and intervention groups.
Results: Parental consent was obtained from 645 children, mean age 8.7 (± 1). 352 (55%) children completed the initial 3-day diary and 360 (56%) competed the final diary. Average consumption of fizzy drinks over 3-days decreased by 0.5 glasses; (95% confidence interval: -1.1; 0.1) in the intervention group; but increased by 0.2 glasses (95% confidence interval: -0.2, 0.5), in the control group, P= 0.03.
At baseline 19% of children in the control group were overweight or obese and 20% of children in the study group were overweight or obese. At completion of the study, 27% of children in the control group compared to 21% of children in the study group were overweight or obese, P= 0.048. (Overweight defined > 91st centile and Obesity defined >98th centile, according to the age and gender specific British BMI Chart)
414 children completed the 10-point questionnaire on healthy eating (higher value better). Average score for the intervention group was 7.6 (±0.6) compared to 6.6(± 0.7) in the control group P< 0.001 (95% confidence interval: -1.5;-0.6)
Conclusion: This specific health education initiative has been successful in reducing the consumption of fizzy drinks and increasing the level of healthy nutrition knowledge in the participating children. Decreased consumption of fizzy sweetened drinks was linked to a decrease in childhood overweight and obesity.

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The presence of distracting stimuli during meals induces increased intake in women: comparison of two distractors (television viewing versus listening to recorded story).
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Background and Aims: Diet is an important factor in the successful management of diabetes. In consequence, it is crucial to identify and possibly control the various environmental influences that affect food intake. Earlier studies suggested that the presence of certain distracting stimuli during meal eating facilitates increased intake. Television viewing is often associated with increased body adiposity, but the mechanism of this association has not been elucidated. In the present study, we measured meal size in women eating under distracted versus non-distracted conditions, and compared the effects of two distractors: television viewing and listening to a recorded story.
Materials and Methods: Healthy women (N=58; age 30 ± 1.3 years; BMI 22 ± 2) participated in four once-weekly laboratory lunches. The same menu was presented on all occasions. Subjects ate alone and ad libitum. The first and last lunches were presented without any distractor; in the other two tests, presented in random order, subjects ate while either watching television or listening to a recorded radio program. The distractors contained no food-related material. Subjects filled psychometric questionnaires (the Three Factor Eating Questionnaire and the Dutch Eating Behaviour Questionnaire) at the end of the meal series.
Results: Meal size was significantly higher in both distraction conditions than in both undistracted lunches (+11%; p<0.001). No difference in energy

intake was observed between the two distraction conditions on the one hand, or between the two undistracted conditions on the other hand. This demonstrates that the appeal of the presented food had not changed between the beginning and the end of the test series, and that television viewing induced a significant stimulation of intake, equal to, but not greater than the other distractor. In contrast to earlier reports, the stimulating effect of distraction was not related to personal characteristics, such as chronic dietary restraint.
Conclusion: Distraction during meal eating is associated with increased intake in healthy normal-weight adults. The stimulation of intake induced by different distractors is comparable. Television viewing might influence energy intake and body weight by, among other mechanisms, facilitating chronically increased intake due to mealtime distraction. The stimulating effect on meal eating induced by various distractors should be investigated in other populations, particularly in Type 2 diabetic patients with poor body weight control. A study is under way to extend the observations of the present experiment to a diabetic population.

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The role of education and weight reduction in control of Type 2 diabetes.
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Background and Aims: Unfortunately at present the majority of type 2 diabetics with high levels of blood glucose and lipids who refer to physicians, only receive more medication. In this research we have studied the effect of education and weight reduction in better control of blood sugar and lipids without increase of medication.
Materials and Methods: 94 type 2 diabetics with the history of diabetes from 1 to 27 years (average of 7 years) and age range of 26 to71 (average age of 51.4), 61 females and 33 males who referred to IDS have been studied in this research. These diabetics who mostly have high levels of blood sugar and lipids, participated in a 15-hour training course of IDS after admission. In these courses diabetics were trained about diabetes in general, self- monitoring of blood and urine glucose, proper diet, exercise, oral hypoglycemic agents and diabetes complications. We measured their weight and height, tested HbA1c and lipids, examined their eyes and feet in the first session and repeated these tests periodically till one year after the date of initial tests. The results of these findings and the number of OHA (Oral Hypoglycemic Agents) usage were studied in the beginning and at the end of the research.
Results: In this research the average of HbA1c decreased from 9.11 % to 6.2 % (by HPLC method) which has a significant difference (P=0.0000). The average weight was reduced from 70.46 Kg to 66.75 Kg (P=0.01). The average of OHA usage decreased from 3.1 to 1.8 (P=0.0000). The following results were achieved regarding the lipid profiles: The average of cholesterol and triglycerides in the beginning of the research were 234 and 276 mg/dl, respectively, which were reduced to 205 and 153 mg/dl at the end of research (P=0.0002) & (P=0.0000).
Conclusion: The statistics show a favorable metabolic control. As type 2 diabetes accounts for the majority part of diabetes, we can say that education with creation of motivation for proper diet and exercise can result in weight reduction in type 2 diabetics. This issue not only results in better control of blood sugar and lipids but also with reduces OHA usage which has an important role in reducing costs of treatment.

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Medication adherence in the Diabetes Prevention Program (DPP).
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Background and Aims: The DPP was a randomized clinical trial to compare the efficacy of an intensive lifestyle or a medication intervention to placebo with a goal of prevention or delay of type 2 diabetes among high-risk participants (ppts). Results presented here focus on the DPP medication groups, where a 31% risk reduction was shown in the metformin (MET) group (n=1,073) compared to placebo (PLC) (n=1,082).
Materials and Methods: Adherence to DPP medication was measured quarterly by pill count and recorded as 80% of prescribed dose of 850mg twice a day (or once a day, if the higher dose was not tolerated). Data on barriers to adherence and strategies to improve adherence were collected quarterly by structured interview.
Results: Overall reported adherence was 71% in the MET group vs. 76% in the PLC group (p=0.0002). Significant differences in patterns of adherence over time in study were observed by race/ethnicity (p<0.0001) and income (p<0.0001) in both groups, and by level of education (p<0.0001) in the MET group only. The most frequently reported barrier to taking DPP pills was forgetting to take the pills, with an average of 22% of ppts reporting this barrier in both treatment groups. Differences were seen in ppts reporting „no barriers," with PLC ppts more likely to report no barriers (p<0.0001). The MET group had more frequent reports of adverse reactions (usually gastrointestinal) with an average of 7% of ppts reporting this barrier, and with a significant decrease (p<0.001) over time. The most frequently reported helpful strategies to promote adherence were creating routines related to time or activities and use of reminder devices, with significant increases in use of strategies over time in both groups (p<0.0001). There were significant behavioral differences by race/ethnicity, education and income (p<0.0001), with diverse patterns of both barrier and strategy reporting over time. Medication adherence at 3 months predicted adherence at both 1 year and 3 years (p<0.0001). Persistent adherence to medication at >=80% of visits was highly predictive of lower diabetes risk in the MET but not the PLC group (p=0.008 and 0.444 respectively). Among MET ppts only, those who had persistent adherence had a 31% (CI=9%-47%) risk reduction compared with non-adherent ppts.
Conclusion: Assessing medication adherence in the DPP provided the opportunity to evaluate barriers to and strategies for improving adherence to a preventive medication in a diverse population. Adherence was fairly stable over time in the study, but significant differences were noted among racial, income and level of education groups. Barriers and strategies also differed among groups. Adherence to active metformin was associated with lower diabetes risk. These data will help inform behavioral interventions for preventing type 2 diabetes and other health problems.

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Promoting physical activity in people with Type 2 diabetes: physiological and biochemical effects.
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Background and Aims: The benefits of physical activity for management of Type 2 diabetes are well documented. Around 80 percent of people with Type 2 diabetes do not do enough physical activity to achieve these benefits. Research investigating ways to promote physical activity in this population is required. This randomised controlled trial investigates the effectiveness of exercise consultation for promoting physical activity in people with Type 2 diabetes and evaluates the resultant physiological and biochemical effects.
Materials and Methods: 70 inactive people with Type 2 diabetes were given standard exercise information and randomised to receive an exercise consultation (n=35) or not (n=35). Exercise consultation, based on the transtheoretical model, combines motivational theory and cognitive behavioural strategies into an individualised intervention to promote and maintain physical activity. Exercise consultations were delivered at baseline and 6 months with support phone calls given 1 and 3 months after each consultation. Changes from baseline at 6 and 12 months were assessed in physical activity (7-day recall & accelerometer) and physiological (BMI & BP) and biochemical variables (HbA_{1c}, fibrinogen, tPA, microalbuminuria).
Results: Between group differences were recorded in physical activity (recall & accelerometer/wk) at 6 and 12 months (p<0.01). The experimental group increased physical activity (recall & accelerometer/wk) from baseline to 6 months (p<0.05). The control group decreased accelerometer counts/wk from baseline to 12 months (p=0.03) and recorded no change on the 7-day recall. Between group differences were recorded in HbA_{1c} at 6 and 12 months (p<0.01). From baseline to 6 and 12 months the experimental group recorded a mean decrease in HbA_{1c} of 0.26% and 0.27% respectively and the control group recorded a mean increase of 0.15% and 0.34% respectively. Between group differences (p<0.05) were recorded for the change in systolic blood pressure (experimental 7.7mmHg decrease, control 5.6mmHg increase) and fibrinogen (experimental 5mg/dl decrease, control 25.8mg/dl increase) from baseline to 6 months and in total cholesterol (experimental 0.33mmol/L decrease, control 0.04mmol/L increase) from baseline to 12 months (p=0.03). No significant changes were recorded in BMI, diastolic blood pressure, tPA or microalbuminuria.
Conclusion: Exercise consultation increased physical activity and improved both glycaemic control and cardiovascular risk factors in people with Type 2 diabetes. This research provides the evidence for an innovative addition to current diabetes care.

OP Education 4:
Educational Tools

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A new pedagogical approach for both educators and diabetic patients.
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Background and Aims: Education is an important part of diabetes treatment and management. We have planned a well structured teaching program for both diabetic patients and healthcare providers. Since 2000 we have trained young and experienced nurses of teaching them some pedagogical tools to use during the education courses with patients. The goal of the study was to evaluate in both diabetic patient and educators the effect of a new pedagogical approach.
Material and Methods: Every nurse answered a questionnaire about the level of their knowledge before entering the education program. The questionnaire consisted of 55 questions separated in 7 items concerning diabetes, treatment and complications. All 6 nurses received a new complete pedagogical program with 3 60-minute courses per week during 2 months. They were videotaped twice during their patient education courses at the beginning and six months after the training program. The duration of the course, the patients speeching time and the number of open questions were evaluated. In addition, 250 diabetic patients (110 M and 140 F) received a questionnaire in order to evaluate their knowledge and skills before and after our new pedagogical approach during a 5-day hospitalization. The diabetic patients were 63.4 ± 2.2 years old, had a mean duration of diabetes of 8.7 ± 0.3 years and a mean HbA1C of 8.2 % ± 0.1 %. The self-administered questionnaire was composed by 25 questions with multiple choice responses.
Results: The results of the questionnaire for the nurses were significantly improved by 42 % after the teaching program. The mean of correct answers was 27 ± 6 before and 38.5 ± 7 after the training ($p<0.001$). The mean duration of the course was increased by 15 % (20 ± 5 min. before and 23 ± 4 min. after the training $p< 0.01$). The patients speeching time was improved by 70 % (5 ± 1 min. before and 7.2 ± 2 min. after the training $p< 0.02$). The number of open questions doubled after the training. Finally, the patients improved by 20 % their correct answers in the self-administered questionnaire ($p<0.01$).
Conclusion: This new psycho-pedagogical program is a very important part of diabetes management and treatment, not only for patients but also for educators. This approach could be of great benefit for patients in order to motivate them in the long term follow-up.

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Feasibility study of a new WHO instrument (WHO-Dia-QoL) for the measurement of well-being and treatment satisfaction in diverse health-care settings.
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Background and Aims: The Quality of Health Systems programme at WHO Regional Office for Europe, promotes a policy for quality of care which emphasises patient experience as a crucial element in the measureable long-term outcome of diabetes care. To facilitate the measurement of well-being and satisfaction in routine primary care, a feasibility study of the reliability and validity of different language versions of a brief 14-item questionnaire (WHO-Dia-QoL) was performed.
Materials and Methods: The WHO-Dia-QoL consists of the WHO-5 measure of well-being, the WHO-DTSQ measure of diabetes treatment satisfaction, a global self-rated health item, and a short section on demographic/clinical characteristics for completion by the health-care provider. It was administered to 624 people with diabetes (male and female, adults of all ages, Type 1 or Type 2 diabetes), attending diabetes services in six countries in Europe. Following data collection, participating clinicians were sent a questionnaire inviting them to comment on the feasibility of the routine use of the WHO-Dia-QoL.
Results: Participating clinicians formally reported that they were satisfied with the feasibility of the WHO-Dia-QoL in routine care (3.8 ± 0.3 (±SD) out of a maximum of 4.0 points for full agreement). All language versions of the instrument showed good psychometric reliability, with alpha ranges of 0.84-0.90 for the well-being section of the questionnaire, and 0.82-0.93 for treatment satisfaction. The questionnaire scores correlated with a number of clinical (HbA_{1c}, $r=-0.29$, $p<0.01$; presence of complications, $r=0.13$, $p<0.01$) and demographic (age, $r=-0.11$, $p<0.01$; sex, $r=-0.26$, $p<0.01$; duration of diabetes, $r=-0.15$, $p<0.01$) variables. The WHO-Dia-QoL demonstrated an ability to significantly discriminate between different patient groups including by HbA_{1C} ($p<0.001$), presence of complications ($p<0.001$), sex ($p<0.001$), and country ($p=<0.001$).
Conclusion: This feasibility study has demonstrated that a short assessment of well-being, health and treatment satisfaction, as with the WHO-Dia-QoL questionnaire, is appropriate for use as part of routine care for people with diabetes.

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Who remembers what happened in the consultation? Comparing patient and doctor recall with the video evidence.
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Background and Aims: To compare doctors and patients recall of consultations, with each other and with the video recording of the consultation. To examine whether patient distress and perceived autonomy are predictive of consultation recall.
Materials and Methods: The outpatient's consultations of 43 patients with either a consultant physician or specialist registrar were video recorded. Prior to the consultation, the patient completed the Problem Areas in Diabetes (PAID) questionnaire, measuring diabetes distress. Immediately after the consultation the patient and the doctor answered two open-ended questions about the issues discussed and the decisions made in the consultation. Patients also completed the Health Care Climate Questionnaire (HCC) measuring patient perceived autonomy in the consultation. The videos, patient and professional responses to the open ended questions were then coded into one or more of 26 categories, and coded for level of agreement.
Results: There were no significant differences between the number of topics (patient mean 3.5; dr mean 3.1) or decisions (patient mean 2.4; dr mean 2.5) recalled by the patient or doctor. However, there was a substantial discrepancy between both the patient and doctors recall of the consultation, compared with the video. The patients recall about 35% (21% shared recall with the doctor) of the issues discussed /decisions made and the doctor recalled about 28% (21% shared recall with the patient) of the issues discussed /decisions made that were identified in the videos. This left 24% of the actual consultation content not recalled by either doctor or patient. Greater autonomy in the consultations was not associated with recall. More diabetes distress was associated with less recall.
Conclusion: Patients and doctor recall of the consultation may overlap, but clear discrepancies are evident, with a quarter of the consultation not recalled by either party. If anything the patients showed better overall recall than the doctors. Training in effective consultation skills is required for both patients and professionals

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Using computer modelling to enhance structured education for people with Type 1 diabetes.
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Background: We have developed a structured patient education programme for people with type 1 diabetes. The programme utilises the DiasNet computer model to display and manipulate patient data (blood glucose, insulin dose and carbohydrate intake) as a training exercise in carbohydrate assessment and insulin dose adjustment, thus helping patient's optimise their metabolic control.
Method: Since 1999, 58 patients (28 male), age 32 (range 18 – 65) yrs, duration of diabetes 2 (0.6 – 34) yrs have completed the programme.
Results: In the 41 patients with poor control (HbA1c>8%) at entry, mean HbA1c fell from 9.6% ± 0 .15 to 8.8% ± 0.2 % ($p<0.001$ (paired t-test)) at one year and 8.3% ± 0.4 ($p<0.001$) at 2 years. Mean (95% confidence intervals) diabetes management skills, as measured by the Ipswich Questionnaire, rose from 135 (126,138) to 151 (147, 155) mean difference (95% CI, $p<0.0001$) and this was maintained to twelve months.
Conclusions: We have demonstrated that an intensive education programme for Type 1 diabetes, using the DiasNet computer model, is effective within a clinic-based population.

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The impact of an educational booklet to clarify misconceptions on diabetes mellitus in Hong Kong.

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Background and Aims: In Hong Kong, 1 in 10 people have diabetes. Due to its chronic nature, many patients take alternative or complementary medicine along with their western medications. These include herbal products or extracts, herbal formulae or other lay methods (e.g. massage, qi-kung), many of them claim to cure or improve diabetes. Hence, many diabetic patients have deep rooted misconceptions which can lead to poor compliance with conventional treatment resulting in suboptimal metabolic control and development of diabetes related complications.
Materials and Methods: Based on extensive interactions with diabetic patients, family members and medical or para-medical personnel, diabetes educators from 5 Diabetes Centres identified 28 typical diabetes-related misconceptions which can be grouped into one of the following categories: causes of diabetes, diet therapy, oral drug therapy, insulin treatment, daily living with diabetes and alternative medicine. These misconceptions were compiled in a 30-page Chinese booklet using concise messages and diagrams with an objective to rectify these common misconceptions. Before publication, the book was also reviewed by diabetologists and other nurse educators from other hospitals. The booklet has been freely distributed to many hospitals and community based clinics. The content was also available on the intranet of the Hong Kong Hospital Authority as staff teaching materials.
Results: We recently collected feedback on this educational booklet from 98 diabetic patients in 2001. The age of patients ranged from 51 to 60 years and the majority only had primary or secondary school education. They were asked to complete a questionnaire which assessed their levels of misconceptions based on the 28 items listed in the booklet. They were then given the education booklet to take home. After an average of 2-3 months, they were asked to complete the same questionnaires again. There were significant improvements in the scoring of the 28 items of misconceptions (61.2% vs. 91.1%). They were also asked to evaluate the usefulness of the booklet and more than 95% of patients found the book easy to read and informative. Patients also found it useful in clarifying common misconceptions and improving self care.
Conclusions: Our pilot project suggests that concise messages targeting at clarification of misconceptions and false beliefs improves knowledge of diabetes. Further studies will be needed to examine the effects of these educational materials on metabolic control. Last but not the least, a VCD of the booklet with animation is under production. As it's target groups are illiterate or elderly, hopefully, it can reach a wider scope of the Hong Kong population.

OP Education 5:
Psychological Insulin Resistance

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An international study of psychological resistance to insulin use among persons with diabetes.

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Background and Aims: This study examined self-reported worry about starting insulin among persons with diabetes. Our aim was to determine whether worry about insulin was associated with a variety of factors, including demographics, disease (duration, complications), regimen compliance, diabetes coping, and attitudes toward insulin.
Materials and Methods: We interviewed 2,061 adults with type 2 diabetes who were not using insulin. As part of an international study of Diabetes Attitudes, Wishes and Needs (DAWN) samples were obtained in 13 countries from 11 regions (Australia, France, Germany, India, Japan, Netherlands, Poland, Scandinavia, Spain, UK, USA). Each region contributed roughly equal numbers of respondents.
Results: All reported findings are $p<.05$ using hierarchical multivariate regression. Countries differed substantially in attitudes about starting insulin, even after controlling for respondent characteristics. The majority of respondents (57%) were worried about having to start on insulin. Worry was reported as highest in Poland, France, and Spain and lowest in Australia and India. Only a quarter of all respondents (23%) thought insulin could help them improve their own diabetes management. The perceived value of insulin for improving diabetes management was highest in Spain, Germany, Japan and India, and lowest in Australia, Netherlands, and UK. About half of respondents (48%) believed that starting insulin meant that they had not followed treatment recommendations properly. This self-blame was highest in the USA, Japan, and France, and lowest in Netherlands and Australia.
Among all countries combined, respondents were more worried about starting insulin if they were female, younger, and had more complications, but beliefs and behaviors mediated the relationships of age and complications with worry. Respondents who reported taking better care of their diabetes were less worried (but more worried patients kept more appointments). Respondents who were coping better with the burden of diabetes were less worried. Respondents who perceived starting insulin as a reflection of their failure to take care of their diabetes were more worried. Ironically, respondents who felt insulin would help them better manage their diabetes also were more worried about starting insulin.
Conclusions: This study shows that persons with diabetes report substantial worry about using insulin. This worry may be an important element in their psychological resistance to using insulin. Identifying and addressing specific sources of worry could help them begin insulin therapy without delay, when it is appropriate. More effective communication of insulin's benefits and a better understanding of the natural progression in loss of beta cell function might be helpful.

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Appraisal of insulin treatment among Type 2 diabetes Patients with and without previous experience of insulin therapy.

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Background and Aims: Recent surveys suggest that timely initiation of insulin therapy can be impaired by negative perceptions about insulin among people with type 2 diabetes. The objectives of this study were to compare perceptions about insulin therapy between insulin-naïve and insulin-treated patients with type 2 diabetes and to evaluate the psychometric properties of a newly developed assessment tool, the Insulin Treatment Appraisal Scale (ITAS).

Materials and Methods: The ITAS was developed to help clinicians assess beliefs and attitudes about insulin therapy among patients with type 2 diabetes. The ITAS contains 20 items (16 negative, 4 positive statements) scored on a 5 point Likert scale and covers a range of common beliefs and attitudes. Examples of topics included are: Feeling more sick, being more dependent, fear of hypoglycemia, pain of injections, being restricted in daily life, being protected from long-term complications and feeling better. The ITAS was administered to 146 insulin naïve type 2 diabetes patients (mean age 59.7, 46% male, 88% taking OAD, 43% working) and 136 insulin treated type 2 diabetes patients (mean age 58.4, 46% male, 56% taking OAD, 40% working) recruited from a national diabetes patient panel in the US.
Results: The insulin-naïve patients rated insulin therapy significantly more negatively than the insulin-treated patients on 15 of the 20 items ($p<0.001$, controlling for age, gender and duration of diabetes). As an example, 61% of the insulin-naïve and only 28% of the insulin-treated patients agreed that insulin therapy demanded a lot of time and energy ($p<0.0001$) and 57% of the insulin-naïve patients compared to only 6% of the insulin-treated patients were concerned with painful insulin injections. Only one item, concerning weight gain, showed an opposite trend. The ITAS showed high homogeneity (Cronbach's alpha, 0.88). The total ITAS score, calculated as the sum of the 20 items (higher score indicating a more negative appraisal) was significantly higher for insulin-naïve patients (mean, 52.0, median 52.5; SD,16.0) than for insulin treated patients (mean, 36.0, median, 36.3; SD, 13.9) ($p<0.001$).
Conclusion: The ITAS has satisfactory psychometric qualities for diagnostic and research purposes in relation to patient's perceptual barriers to insulin therapy. The ITAS can help clinicians reliably and quickly identify the patient's key concerns about insulin therapy. Patients with type 2 diabetes who have no experience with taking insulin have profound and significantly more negative beliefs about insulin therapy (psychological insulin resistance) than patients with actual experience of taking insulin. These preliminary findings highlight an important disparity between the anticipated and actual patient experience of insulin therapy, and suggest a need to provide more education about the acceptability of insulin therapy to type 2 diabetes patients.

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Compliance with recommended self-monitoring of blood glucose in patients with Type 1 diabetes mellitus.

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Background and Aims: Proper management of diabetes through appropriate self-care practices is very important to reduce/delay the complications associated with it. Regular self-monitoring of blood glucose has been found to be an important aspect of the diabetes self-care regime. However, compliance with recommended self-monitoring is found to be poor. Past research suggests that cognitive factors play a role in determining compliance with various aspects of the self-care regime. The present study used an extended Theory of Planned Behaviour (TPB; Ajzen, 1991) to predict self-monitoring in adult type 1 diabetics. According to the TPB, a person's intention or plan to perform a behaviour is the proximal determinant of behaviour. This intention is in turn predicted by the person's attitudes (positive or negative evaluations of the behaviour), subjective norm (perceived social pressure to perform the behaviour) and perceived behavioural control (the extent to which the person perceives the behaviour to be under his/her control). Past research also suggests that perceived risk of complications, perceived satisfaction with the outcomes of past monitoring, conscientiousness and past behaviour could have an impact on monitoring behaviour.
Materials and Methods: Sixty-four adult type 1 diabetics were asked to complete a questionnaire measuring the variables of the extended model. In addition information regarding demographic and diabetes-specific variables (HbA_{1c}, age at diagnosis) was also obtained. Self-report measures of compliance over a 2-week period were obtained. These were verified against objective records for 5 participants.
Results: Rates of monitoring were fairly high with over 68% of participants monitoring at least twice a day and 50% monitoring as often as or more often than recommended. The extended model was able to predict 46% of variation in intention ($F = 2.86$, $p < 0.01$) and 59% of variation in self-monitoring behaviour ($F = 3.58$, $p < 0.01$). Self-efficacy and past behaviour were the best predictors of intention to self-monitor. Past behaviour independently added 18% to the variance predicted in future monitoring behaviour ($F_{change} = 13.84$, $p < 0.001$). However, intention was still found to be a significant predictor ($p < 0.05$). The intention-behaviour correlation was found to be 0.53 ($p < 0.01$) which is comparable the average intention-behaviour correlations obtained across various TPB studies. Perceived risk, perceived satisfaction, demographic and diabetes-specific variables did not add a significant amount to the variance explained either in intention or behaviour. Significant differences were found in underlying beliefs of high and low intenders.
Conclusion: The results suggest that forming a clear plan about performing the behaviour, and building self-efficacy would help in promoting more regular self-monitoring. Self-monitoring was not seen to be very important in preventing complications associated with diabetes. Thus, educating patients about its importance could also help in improving rates of monitoring.

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Empowered patients: better diabetes control, greater freedom to eat, no weight gain!

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Background and Aims: A community based health professional-led *expert patient programme* for South Asian and White Caucasian adults with type 2 diabetes was designed based on an empowerment model and patient-centred education.
Materials and Methods: Socio-economic deprived neighbourhoods were targeted and 314 participants randomised to expert patient programme or routine treatment. Expert patient programme involved 6, 2-hour weekly sessions: what is diabetes?; weight management; glycaemic index; supermarket tour; possible complications and prevention; and goal setting. Health and psychosocial outcomes were collected at baseline and 2 months post-intervention to assess short-term impact.
Results: No significant difference between groups at baseline. At 2 months post-intervention, mean HbA1c and mean systolic blood pressure were lower in *expert patients,* 7.4% versus 7.8% (Difference 0.4%, 95% CI: 0.1% to 0.7%, P=0.02), 142 mmHg versus 147 mmHg (Difference 5 mmHg, 95% CI: 0 to 9 mmHg, P=0.06). BMI and lipid profiles remained unchanged. *Expert patients* experienced improved quality of life through freedom to eat (P<0.001), drink (P=0.005) and enjoyment of food (P=0.05). Although experiencing increased freedom to eat, mean fruit and vegetable intake was higher in the *expert patients,* 4.4 portions versus 3.4 portions per day (Difference 1 portion, 95% CI: 0.2 to 1.8 portions, P=0.01), percentage energy from fat reduced slightly, 26.4 % versus 28.8% (Difference 2.4%, 95% CI: -0.5% to 5.2%, P=0.1) and percentage energy from carbohydrate intake increased, 54.0% versus 49.9% (Difference 4.1%, 95% CI: 0.4% to 7.9%, P=0.03). Empowerment score containing 3 subscales: psychosocial adjustment (P=0.002); readiness to change (P<0.001); and goal setting (P=0.001), diabetes knowledge (P<0.001) exercise frequency (P=0.008), foot care (P<0.001), frequency of blood glucose monitoring (P=0.009) and treatment satisfaction (P<0.001), all improved in the *expert patients.*
Conclusions: Adults with type 2 diabetes trained as *expert patients* improved their diabetes metabolic control. Although they experienced greater freedom to eat, no weight gain occurred and dietary intake improved. Increased empowerment and diabetes knowledge translated to better diabetes self-management, treatment satisfaction and increased physical activity. Re-analysis of health and psychosocial outcomes at 12-months will assess the long-term impact.

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Long term effect of education program for impaired glucose tolerance in a general hospital.

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Background and Aims: Diabetes prevention program had profound effect as primary prevention of diabetes mellitus, but it requested marked manpower and cost. On the other hand, education for IGT persons was not applied in general hospital and its effectiveness is not confirmed. To clarify the long term effect of the education program specified for Japanese IGT persons in a general hospital, glucose tolerance was followed for 4 years.
Materials and Methods: The subjects were 660 glucose intolerant persons who visited yearly medical examination of health and showed mild fasting hyperglycemia of 110~139mg/dl without history of diagnosis of diabetes mellitus or oral hypoglycemic agents at initially, and were reexamined 4 years later. The education was 4 hours program specified for IGT by

multidisciplinary team (diabetologist, public health nurse, nutritionist, and health trainer). Pathophysiology and individual data of insulin secretion and insulin resistance was explained with the diabetic doll, energy balance with daily BW change curve, nutritional balance with simple figure, presentation of sugar contents of soft drinks, and training of chewing healthy lunch, etc. Glucose tolerance was judged as DM (diabetes mellitus; 2h plasma glucose >200mg/dl), BD (borderline; 2h plasma glucose 121~199mg/dl), or N (fasting plasma glucose < 110mg/dl) .
Results: Intervention of education was applied to 48 subjects, and they were compared with 612 subjects without intervention. Initial clinical characteristics were not different between 2 groups. Age was 47.9 and 49.9 years old, BMI was 24.0 and 23.7 kg/m^2 in average, respectively. 4 years later, body weight was reduced by 1.2kg (from 65.3±1.6 to 64.3±1.6) only in intervention group ($p<0.05$). Fasting plasma glucose increased significantly by 5mg/dl (from 115.1±0.4 to 120.1±1.1) in no-intervention group ($p<0.05$). Appearance rate of fasting hyperglycemia larger than 126mg/dl was 13.6% in intervention group and 18.3% in no-intervention group, reduction rate was 25.7%.Glucose tolerance was judged as N at 35.4% in intervention group compared with at 2.5% in no-intervention group, and its Ozz's ratio was 21.8($p<0.001$). Prevalence of judgement of DM and N was 60% and 20% , respectively, in intervention cases whose early insulin response (ΔIRI/ΔPG at 30min) was lower than 0.1 , but it was 15% and 37% in whom it was less than 0.4, furthermore it was 0% and 50% in whom it was 0.4 or larger than 0.4, respectively ($p<0.0001$)..
Conclusions: Our education program specified for IGT persons was cost effective and had profound power as a tool of primary prevention of diabetes mellitus. Such program should be considered in general practice, especially for IGT persons with some insulin secretary capacity . Modest body weight reduction contributes to prevention of diabetes mellitus in modestly obese Japanese IGT.

OP Education 6:
Therapeutic Education of Young Patients

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Impact of an educational video film: „The Jinn's Party" on the knowledge, practices and attitudes of school children and adolescents with Type 1 diabetes and their parents.
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Background and Aims: The WHO recognizes education as a corner stone of diabetes therapy which should be tailored to the local socio-economic and cultural circumstances. The aim of the present work included assessment of: 1) The state of knowledge, practices and attitudes of school children and adolescents with type 1 diabetes and/or their parents. 2) The impact of a short therapeutic education program including projection and discussion of a video film „The Jinn's Party" prepared to fulfill the local needs within the frame of the DESG-EASD educational guidelines. 3) The impact of this program on diabetes control namely HbA1c, frequency of absenteeism, ketoacidosis and hypoglycaemic episodes.
Materials and Methods: The study was conducted on 100 subjects (school children or adolescents) with type 1 diabetes and 56 parents randomly selected from 1600 subjects receiving health care from the Students Diabetes Center in Alexandria. Study design: Pre-test, post test quasi-experimental design. The study comprised 4 phases: 1) Initial pretest assessment, 2) Educational program including a full one day camp in a sporting club, projection of the video film & interactive discussions, 3) Immediate post-test assessment and 4) Final assessment (3 months later). Data Collection comprised: 1) Predesigned questionnaire covering knowledge, misconcepts, skills & practices, attitudes & perception. 2) Physical examination. 3) Assay of HbA1c . 4) Review of the absenteeism & hospitalization records. The Video Film describes in 60 minutes the story of a teenager with diabetes who had the visit of nice Jinnies in his dream. These Jinnies discuss with him the basic knowledge about diabetes, the local misconcepts; demonstrate the skills and practices for management and discuss his attitudes towards the disease and its management.
Results: The mean percent scores of patients knowledge was 57.20 % ± 20.10 in the pre-test, 88.79% ± 10.88 in the immediate post test and 81.60% ± 9.05 in the remote post test. Those of patients' skills were 45.35% ± 41.59; 77.47% ± 13.58 and 84.94% ± 26.3 respectively. The percent scores for attitudes were 42.53% ± 45.33; 81.33% ± 31.62 and 81.00% ± 13.64 respectively. The mean percent scores for parents knowledge were 64.85% ± 11.96; 87.32% ± 13.91 and 80.55% ± 13.55; for skills 76.26% ± 41.01; 73.00% ± 23.70 and 84.87% ± 29.03 and for attitudes 66.19% ± 39.75; 79.52% ± 33.07 and 72.50% ± 28.48 respectively. The mean HbA1c one year before the educational intervention was 9.72% ± 2.22 and 1 year after intervention 7.75% ± 1.15 ($p < 0.001$). Frequencies of absenteeism were 5.35 days/year ± 6.53 and 2.52 ± 3.10, respectively ($p < 0.001$); those of ketosis 0.90 ± 1.52 and 0.28 ± 0.62 respectively ($p < 0.001$); those of severe hypoglycaemia 0.085 ± 0.065 and 0.012 ± 0.090 respectively ($p = 0.03$) and of mild hypoglycaemia 28 ± 12 and 22 ± 15 ($p = 0.008$) respectively.
Conclusion: The present study demonstrates the positive impact of this short educational program. A result peculiarly significant, as the present intervention has been especially designed to the target population; a population with rather poor resources and special cultural background.

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Parents perceptions of diabetes learning opportunities and skills development in children and adolescents.
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Background and Aims: Children and adolescents with diabetes need to acquire skills to self manage their diabetes as adults. The process by which they acquire these skills is best accomplished by a gradual and structured process that is developmentally appropriate. Our diabetes service provides a number of educational opportunities (EOs) that focus on learning about diabetes and psychosocial wellbeing for children with diabetes and their families. These EO's include individual parent/child health professional appointments (IHPA), child education groups, camps, and parent education seminars. This study aimed to determine parent's/carers perceptions of: 1) the value of specific EOs to learning, 2) their child's diabetes self care skills and knowledge (DSCSK).
Materials and Methods: A multiple choice and short answer survey tool was developed and piloted prior to use in this study. Inclusion criteria, a

child: < 16 years, last clinic attendance < 6 months, diabetes ≥ 6 months. Following ethics approval the survey was mailed/given to all 125 eligible parents/carers. Return of survey indicated consent.
Results: 55%(69) returned the questionnaire. Respondents modal age was 36 - 40 yrs, 91% female, and 87% Australian. 62% of respondents described themselves as the main family member who helped the child with their diabetes, 33% stated that they shared the task equally with another family member and only 5% stated that they were not involved as the child could self manage. Children's mean age was 10 yrs (SD 3.4, R 2 – 16), and the mean duration diabetes 4 yrs (SD 2.6, R 1 – 11). In the past 12 months 100% of parents responded that their child had attended an IHPA, 55% a diabetes education group and 70% a diabetes camp. Parents ranked the importance of access to specific educational opportunities as: 91% IHPA, 90% parent education seminars, 84% diabetes camps, and 83% child education groups. The ways in which the parents preferred their child to learn about diabetes as: 86% IHPA, 84% discussing diabetes at home, 65% diabetes camps, 55% child education groups, 49% books and videos and 28% internet. Parent's perceptions of their child's diabetes skills and knowledge significantly correlated with increasing age of the child (r=. 9, p <.0001). Only 11% of parents perceived their child could complete all DSCK. Highest DSCSK scores were obtained in, 95% obtaining a blood sample, 93% completing a blood glucose test, 93% knowing exercise is good, 92% carbohydrate identification, and 92% knowing insulin times. Lowest DSCSK scores were: 31% knowing insulin action, 36% recognising glucose level patterns and adjusting insulin and 46% responding to hyperglycaemia.
Conclusion: Our results show that parents highly value EO's that allow them to acquire the skills to support their child's learning through discussion at home. Skills that are essential for daily diabetes management and knowledge are acquired at a younger age. Skills that require knowledge, interpretation and action are accomplished gradually by older children. Health professionals need to be aware of age appropriate expectations for diabetes education and skills attainment in their planning and teaching of other health professionals, parents/carers and children.

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Desempowerment syndrome (DS) in diabetic adolescents: integrated education and psychological care.
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Background and Aims: In diabetic adolescents, insufficient blood glucose control is frequently associated with DS. To rehabilitate such patients on a longterm scale, an 11-day education course program was developed for repeated application in a clinical camp setting at intervals of several months.
Materials and Methods: The program comprised structured seminars and metabolic visits, practical training, and individual as well as group-based cognitive behavioural therapy by a diabetes psychologist. 252 patients were followed over at least two courses (CI, CII): age and diabetes duration at entry were 13.7±SD2.4 and 4.6±3.2 years, respectively; interval between CI and CII was 13.7±2.4 months.
Results: Prevalence of DS was 30 % both in CI and CII. Practical skills (handling insulin and bred units, blood glucose self monitoring and managing logbooks, prevention and treatment of hypoglycaemia) scored on a 0-8 scale in 176 nonDS vs. 76 DS patients, was 4.1±1.8 vs. 4.0±1.9 in CI (NS), and 4.9±1.7 vs. 4.2±1.4 in CII ($p<0.01$). Multiple choice test-based decision knowledge did improve both in CI and CII but worsened during the interval between (no significant difference nonDS vs. DS). HbA1c in CI was 7.9±1.3 % in nonDS but 8.9±1.8 % in DS ($p<0.01$). Despite the average interval between CI and CII was shorter in DS, and insulin doses were identical (overall average 0.83±0.27 IU/kg/d), the increase of HbA1c between CI and CII was significantly higher in DS. The frequency per week of hypoglycaemia < 3.5 mmol/l increased significantly more in nonDS: 2.6±2.4 in CI and 4.7±3.8 in CII ($p<0.01$) vs DS: 3.1±2.5 and 4.4±3.4 ($p<0.01$).
Conclusion: After attending the program, in DS practical skills improve less, HbA1c does improve but still remains higher, and hypoglycaemia occurs less frequently than in non DS. Thus, repeated structured education in combination with diabetes-specific behavioural training may be advantageous in DS patients. Knowledge tests appear not to provide much clinically relevant information.

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Health-related quality of life in adolescents with Type 1 diabetes: the role of parental care, control and involvement.
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Background and Aims: Diabetes self-management is particularly demanding during pubertal years, and may represent barriers to functional health status, psychosocial adjustment and well being. Family life and functioning may be of critical importance for a young person with diabetes, in the transition from childhood to adult life. The aim of the study was to explore the relationships between perceived parental care, control and involvement, metabolic control and health-related quality of life in adolescents with type 1 diabetes.
Materials and Methods: In a population-based sample of adolescents with type 1 diabetes (n=130, age 11-18 yrs) participants were asked to complete the following questionnaires during a regular consultation in the out-patient clinic: Child Health Questionnaire (CHQ-CF87), Diabetes Quality of Life Questionnaire (DQOL), Parental Bonding Instrument (PBI) and Parental Involvement questionnaire. The participants (n=115, 88.5%) had mean age 14.5 (SD 1.86, range 11-18 yrs), 55 girls (47.8%), mean diabetes duration 6.7 yrs (SD 3.8, range 1-16 yrs), and mean HbA_{1c} 9.3% (SD 1.6, range 6.2-14.0%).
Results: Higher parental involvement correlated significantly with a higher level of functional health and well being (CHQ-CF87) and also with lower degree of diabetes-related impact and worry, and higher diabetes life-satisfaction (DQOL). In multiple regression analyses age and gender combined with higher degree of parental care and involvement explained 46% of the variation in mental health (CHQ-CF87). Age and gender combined with higher degree of parental care and involvement and lower perception of parental control explained 52% in diabetes life-satisfaction (DQOL). Body mass index was a significant covariate in diabetes-related worry, but other clinical variables (diabetes duration, insulin regimen, HbA1c) could not significantly explain more of the variation in neither CHQ-CF87 nor DQOL subscales.
Conclusion: The study indicates that parental care and involvement are important for psychosocial dimensions of health and well being in adolescents with diabetes, and underlines the importance of the family in diabetes health care. Early intervention involving the family is needed to facilitate adolescents` coping with everyday demands of the disease.

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Diabetes in children and adolescents from ethnic minorities - metabolic control related to family background, treatment offered and barriers to it.
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Background and Aims: To investigate whether metabolic control in children and adolescents from ethnic minorities with type 1 diabetes differed from Danish children and young people, and to obtain knowledge of factors affecting the opportunities of ethnic minorities to achieve good control - seen in the perspective of both families and professionals.
Materials and Methods: Three studies were included: Data (sex, age and diabetes duration) from The Danish Registry of childhood and adolescence diabetes including 919 Danish and 58 children and adolescents from ethnic minorities, structured interviews performed by professional interpreters of 38 families with other ethnic background than Danish, and questionnaires to the diabetes-teams in all 20 Danish paediatric departments.
Results: HbA_{1c} was significantly poorer in children and adolescents from ethnic minorities (mean 9,05 %) compared to Danish patients (mean 8,62 %) ($p = 0,018$). There was no significant difference in the prevalence of severe hypoglycaemia (unconsciousness and/or convulsions) and ketoacidosis and no significant difference in HbA_{1c} among the different ethnic groups. Compared to the Danish patients the ethnic minorities differed in mean age (ethnic 10,3 years versus Danish 11,9 years) ($p = 0,011$) and in sex ratio (ethnic 62 % girls versus Danish 45 % girls) ($p = 0,015$). The interviews of the parents revealed limited school background, lack of professional education (78 % none) and a huge need for interpreters

(64 %). This was especially prevalent in the mothers who traditionally are the main caretakers of the children. The distribution of ethnic patients over the country was very uneven with 44 patients (53 %) in a single centre, while the other centres each had 0 - 5 patients. Generally the centres provided limited specialized knowledge and offers to the ethnic minorities.
Conclusion: The investigation confirmed the hypothesis that immigrants are a vulnerable group with very different needs, and concluded that children and young people from ethnic minorities in Denmark had less chances of achieving good metabolic control compared to Danish patients. It was thus recommended that the treatment of these patients should be centralised in five or six centres, and that professional interpreters became a golden standard to all families at every visit to the centre. „Tailor-made“ offers to the individual ethnic groups, supplementary training of health care professionals as well as projects to improve methods, quality and knowledge should be encouraged.

OP Education 7:
New Models for Specific Populations

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An innovative approach to continuing professional diabetes education: evaluation of a problem-based program implemented in diabetes care in India.
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Background and Aims: India has about 31.6 millions diabetics. A review by Novo Nordisk revealed only 1200-1500 specialists in diabetes care concluding that the majority of diabetics in India are cared for by physicians with little training in this field. Didactic continuing medical education (CME) is known to be rarely successful in changing physician performance or health care outcomes. Active, learner-centred educational interventions, relevant to the learners' needs and reinforcing practice change are more successful. Hence, the aim was to design and implement a CME program in diabetes care in India to achieve these aims.
Materials and Methods: A 9 month problem-based program of 6 two-day modules was implemented in 2001-2 in 4 Indian cities. Participants were assigned to groups under an expert facilitator to discuss a series of paper-based clinical cases, with skills-based workshops, seminars and discussion forums by experts. Between modules, participants completed practice tasks. Participants identified individual and group learning issues to research between modules to bridge the learning gaps. Learning was supported by written resources. Questionnaires seeking self assessed competency information on 4 subscales- diagnosis, examination, management, and counselling, with 43 questions were administered at the start and end of the course. Internal consistency of each subscale was determined by Cronbach's alpha. The responses were compared using analysis of variance. Participant knowledge was also assessed by examination at the end of course. A questionnaire determining the longer term impact of the program on physician performance and practice outcomes was also used.
Results: In 2001–2 two cohorts, totalling 296 doctors enrolled. 93% completed all six modules. 97% of those undertook the end of course examination. 284 completed the pre-course questionnaire. 53% had no postgraduate qualifications. 22% were in family practice, 35% private practice and 41% private and/or hospital practice. Comparison of self reported competence before and after the short course revealed significant improvements on average for each of the four subscales ($p<0.001$.) 50% of participants achieved 65% or above in the examination. Follow-up data collected 4-14 months after the completion of the courses indicated that participants were providing greater patient education, closer monitoring and addressing the patient needs more effectively.
Conclusions: The provision of an interactive, learner-centred, relevant, skills based and reinforcing CME in diabetes care, resulted in a low attrition rate, significant improvement in self & objectively assessed competencies and improvements in care at the practice level.

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Taking diabetes education to the grassroots; the case of northwest province of Cameroon.
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The Northwest Province of Cameroon with a population of 2 million inhabitants is covered by 13 Health Districts, 19 Hospitals, 10 Private Clinics and many medicalized Health Centers. It is remarkable for its enclave land, multicultural ethnic groups, 204 documented vernaculars and a 60% rate of illiteracy. Before 1998, few cases of diabetes were diagnosed, and very fatal. The estimate number of deaths of diagnosed patients was almost 50%. Many came from homes of traditional healers, already with complications, some in comatose state. The challenge in the province is to educate the health care providers, the patients and their family to provide optimal care for people with diabetes. In 1998 Pan African Diabetes Educators Group trained two trainers of diabetes educators to organize, manage and train other educators to work in primary health Care. The leaders initially trained 48 diabetes educators drawn from all the Provincial Health Districts, 36 (75%) nurses, 4 (pharmacists) (8.3%) and 8 patients (16.7%). The selection of the participants took into consideration, their location, cultural background vernacular and state of literacy. The trainees in turn train others.

In 2002, there were 240 trained diabetes educators in the province. A survey questionnaire on information about diabetic educator was sent to District Health Officers, nurses patients and the Director of the Provincial Referral hospital to evaluate the activities of Diabetes Educator in the Health Care delivery system. The response was got from all the (doctors) 13 Directors of District Hospitals and the director of the Provincial hospital (100%), 24 out of 30 patients (80%) and 20 nurses all responded 100%. The survey results reveal there was a lot of controversies about diet and other method of management that both the nurses and the doctor knew. Eleven out of 13 district officers allowed diabetes educators to carry out sensitization, awareness and celebration of World Diabetes Day. These doctors did not see any reason why nurses should be trained for diabetes alone. (15.4%) 11 directors of district hospitals indicated diabetes education was very important.
All 11 directors reported diabetes educators carrying out 2 days of diabetes clinic a week where patients where taught on difference aspect of diabetes management. The provincial referral hospitals reported and increase in referral cases from diabetes educators from district hospitals to see specialist and an increase in the death tole of people with diabetes. All patients' respondent prove knowledgeable how to manage their conditions and appreciated that the learn more from their interaction with diabetes educators than with the nurses and doctors.
9 directors (69.2%) of district hospital indicated that teaching the patient to self manage their condition has reduced the number of patients coming to the hospital which might lead to more patients developing complications. 16 patient (66.7%) control their diabetes in the pharmacy because the pharmacists diabetes educator are always available and have time to tell them more about their disease and treatment.
Diabetes education in the northwest province of Cameroon has become the only one disease that has broken the barriers of ethnic culture, language and illiteracy that has always been a handicapped to the health care delivery system of the province and the Country as a whole.

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In the diabetes garden.
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Background and Aims: Lake County Tribal Health Consortium, Inc. is a health facility serving the Native American population of Lake County, California. U.S.A. It provides Medical and Dental care, Human Services, Outreach Departments and a Diabetes Program. Diabetes is the single most costly, chronic disease in the United States today with the highest rate among the Native American population. Three years ago the Tribal Health Clinic started a program called „Community Gardens For Native Americans" which provided a garden on all six reservations in our county. This project emphasizes the connection between exercise, nutrition and diabetes prevention.
Materials and Methods: Each reservation provided acreage for their garden project. Soil samples were taken for analysis to determine the saturation rate, PH levels, nutrient levels and soil values. The ground was cultivated, water systems developed and plants, fertilizer, tools and drip systems were purchased. Under the supervision of the Nutrition Education Project Developer the tribal members staked and planted their gardens together on Earth Day.
Results: This summer over 300 tribal members harvested fresh fruits and vegetables from the gardens. We started canning and food preservation classes on the reservations with many Native men participating who had helped work in the gardens. We eatablished winter gardens on the reservations and built a hothouse and greenhouse at the Tribal Health Clinic where we have started all of our seedling for the spring of 2003. We have developed interactive books for kids about nutrition and diabetes that come from stories in our gardens. This helps us to know what the children are learning about healthy eating, exercise and diabetes prevention. The Garden Project was also part of the Govenor's Panel on Health and Nutrition and we just received a California Endowment Grant to continue the project.
Conclusion: The Garden Project is rapidly growing and has been recognized as a lead project in Indian Health in the state of California. We have many visitors from other health care facilities and other states. Nutrition awareness is growing as noted by the increase in participants in the Garden Project and the Diabetes Program has seen a 2% decrease in the diabetes rate at our clinic.

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Usefulness of nursing consultation with an emphasis on psychological programs in ambulatory service in Japan - evaluation using PAID.
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Background and Aims: Generally, a very short time (5 to 15 minutes per person) is allotted to ambulatory service in Japan. Yet some people seek this service relatively frequently (6 to 24 times per year). We run two clinics, both specializing in diabetic care, to conduct clinical care of diabetic patients. To compensate for the short time allocated for patient care, we incorporated a 30-minute interview by a nurse and 15- to 60-minute nutritional consultations by a dietitian (there are very few clinical psychiatrists in Japan.) To evaluate the usefulness and results of these two types of consultation related to diabetic nutritional care, the Problem Areas in Diabetes Survey (PAID) were employed and the changes in PAID between two points in time (before and after treatment) and the shifts in illness-related emotional distress were examined.
Methods: At 2 clinics specializing in diabetic care, PAID was used to elicit information from 52 type 2 diabetic patients who were seeking care for the first time and after 105 ± 21 days (mean); and the consultative information and various clinical indices were compared. The mean duration of illness, age, and HbA1c at the initial examination and 3 months later were: 8.6 ±7.6 years, 59.3 ± 13.5 years, 8.4 ± 2.0%, and 7.2 ± 1.0%, respectively. The score 3 months after the initial examination was subtracted from the initial score and designated as ΔPAID (score).
Results: The total PAID score improved from 44.4 ± 15.8 (the initial score) to 39.3 ± 15.4 ($p < 0.0007$). The improvement was significant in those patients who were introduced to insulin therapy in comparison with those on chemo- or diet therapy ($p < 0.05$). Among diabetic complications, ΔPAID for the group of patients with diabetic nephropathy was 13.6 ± 14.7, showing significant improvement over a ΔPAID of 4.7 ± 12.0 for those without nephropathy ($p < 0.05$). The findings were similar among those with diabetic retinopathy. In a comparison of patients' age, the improvement was significantly poor for those under 40 years, but it became more prominent for those in their 50s and 60s and those over 70 ($p < 0.03$). In those patients with an initial PAID exceeding 50 and experiencing notable emotional distress, the group with an improvement in ΔPAID was found to require longer-lasting and more frequent nursing consultations ($p < 0.05$). In the nursing consultation, the patients were mainly encouraged to describe their emotional distress while no particular emphasis was placed on the diet or exercise therapy. On the whole, the emphasis was on each patient's self-determination and emotional changes. The program on nutritional consultation was not helpful in improving PAID. The improvement in HbA1c was instrumental in easing emotional distress of those whose initial PAID was 50 or higher. ($p < 0.05$). In the group of patients in whom emotional distress remained unchanged or exacerbated, a larger number of individuals were obese, or younger.
Conclusion: It was found that a program in which nurses ease the emotional distress of patients is effective in improving ΔPAID of those whose initial PAID was in excess of 50. The nutritional consultation did not contribute to this improvement.

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Health professional training - one way of coping with the epidemic of diabetes.
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Aims: To increase public & professional awareness of diabetes, and to improve the quality & availability of diabetes care & education for the people of China.
Materials and Methods: In 1998, Project HOPE began implementing a five-year nationwide Diabetes Education Program in China. The program was conceived and designed by HOPE in close partnership with the Chinese MOH and three sponsor companies - BD,Eli Lilly, and Roche Diagnostics. Collaborating institutions in China include leading medical universities & affiliated hospitals.
The program is characterized by a "train the trainer" approach, based on the HOPE principle of teaching people to teach others and helping people to help themselves. The Senior Technical Advisory Group (SENTAG),a group of renowned diabetes experts,provides guidance for program activities. The key component of the program is the training of Chinese medical professionals in diabetes care, integrating treatment and patient education. About 40 trainees from selected hospitals -(a Dr.+a nurse/ each

hospital)participate in each course. Courses consist of lectures, case studies, role-play, ward rounds, clinical demonstrations and practice and group discussions.
Results: Since 1998, the program has established 8"train the trainers" bases in Beijing, Chengdu, Xian, Shanghai, Guangzhou, Harbin, Hangzhou and Qingdao, and has conducted 24 courses in these bases. To date, 1,015 medical professionals from 494 tertiary/secondary hospitals in all provinces & regions of China except Taiwan & Hongkong, have received training. Utilizing the multiplier effect of the Train-the-Trainer methodology this has resulted in nearly 150,000 individuals trained, including:
· 118,787 patients & family members
· 29,311 health professionals
Additionally, the program has expanded to include a community-level component. Since 2000, two community education bases have been established and 553 community health care providers from 287 primary hospitals and community health centers have been trained. With the aim of providing evidence-based evaluation of our training model, HOPE initiated a study to measure the outcome of the program. A pilot study was conducted in 2002 at the two established training centers of the program . At each site, 50 patients who have received diabetes education based upon the training model (testing group-TG) and 50 patients who have not yet received diabetes education (control group-CG)were studied. The TG had an average age of 60 and had diabetes for an average of 8 yrs, whilst the CG had an average age of 51 and had diabetes for an average of 3 yrs. Both groups were tested for HbA1c analyzing with the HPLC based method (VARIANT). Statistical analysis has shown that the mean HbA1c in the 'educated' group (6.78+1.08) was significantly lower than in the 'non-educated' group (7.19+1.36).
It was observed that a significant larger percentage of the TG undertook diet control, regular exercise and medicine than the CG.
Conclusion: The pilot study appeared show that by training health professionals and establishing a national diabetes education network partnership, the program has been effective in improving public awareness and understanding of diabetes and diabetes care in China. However, a full-scale study needs to be undertaken to confirm this result and the effectiveness of this training model.

OP Education 8:
Teaching the Teachers
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Changes of perceptions and attitudes of health professionals after a 1-yr long course aiming at the implementation of diabetic patient education networks.
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Background and Aims: A one-yr long university course has been implemented in 2001 and 2002, for the training of motivated health professionals (nurses, physicians, dietitians) in diabetes patient education. It consisted of one essential module - centered on learning methods and issues, apprenticeship process, cognitive and constructive concerns, empowerment, dynamic appropriation of knowledge by patients and professionals – and 2 other modules focusing on medical, and socio-anthropological aspects. The perspective was pragmatic, theoretically based, with an integrative progressing process questioning scientific and medical knowledge, perceptions and cognitive representations of learners, and design of appropriate situations for a dynamic "Construction of Knowledge".
Materials and Methods: The health professionals were asked to produce a text at the beginning (T0), and on completion (T1) of the course. In both cases the opened question was : "Education and diabetes: give your impressions on the subject ". For each answer, we went through the analysis of the data. Results at T0 and T1 were compared. Three areas of representations were founded and analysed in the texts produced : the definition and role of the educator, the patient's part and the consideration of what he is, the necessary conditions and means to achieve the educational act. We also analysed the tone of the answers by pinpointing words used in productions.
Results: table 1
Conclusion: Health professionals switch from a representational system based on " knowledge transmission – dominant/dominated relation – techniques and structures " to a system where the following components are present : knowledge construction, participation, partnership, empowerment, action and joint representations. Health professionals and patients are integrated in a constructive dynamic : the relation is enriching, long-lasting, efficient. The patient understands, thinks, assimilates, discovers and reacts, while the health professional starts to take interest in moving from a technical prescriptive perspective to an interactive model in which the dynamic of action produces innovative situations.

Table 1, Results of qualitative investigation of HP representations

	Start of formation (T0)	**End of formation (T1)**
Education and educator's role	Relay a message. Modify behaviours. Intervene. Dedramatise. Reduction of risks	Master knowledge. Build up knowledge. Encourage to learn, act, discover. Define and adapt the patient's needs. Proceed by stages.
Position and role of the patient	Help relationship. Supervision by a professional. Adopt the correct attitudes. Respect of the guiding principles	Take into account the context, history. Start from what is known. Construct knowledge, understand. Self sufficiency, action. Improvement in life conditions
Means needed **Conditions for realisation**	Personal investment. Protocols, guidanceTechnical Input. Attitudes to adopt. Educational network. Acknowledgement of the acts	Action, construction. Adaptation, regulationActive participation. Observation, analysis. Formation, Exchange of knowledge. Creation of tools
Tonality: Verbs	To change, mourn, take care, suffer, intervene, insist, force the way, prevent, do not want, do not understand	To welcome, build, personalise, improve, act , invent, guide, increase in value, integrate, exchange, help, readjust, listen, determine, discover
Tonality: Adjectives	Insidious, fragile, chronic, everlasting, sensible, technique, unconcerned, lost, anxious, worrying, serious	Teachable, progressive, critical, pleasant, creative, conscious, thoughtful, autonomous, enriching, concrete, interactive, efficient, long-lasting, global, active

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Are healthcare professionals able to educate and support people with diabetes on carbohydrate counting?

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Background and Aims: Carbohydrates (CHO) are the main nutrient affecting blood glucose levels. It follows that, for people with diabetes, CHO-counting of a meal is the most valuable method to accurately predict glycaemia rises and consequently adjust therapy. Are Healthcare Professionals (HCPs) really able to use this method themselves and do they fully understand the patients who need to use it everyday?
Materials and Methods: During a workshop 39 HCPs in diabetes (29 doctors, 9 nurses, 1 dietician), all experts in therapeutic patient education (TPE) underwent an experiential learning session about CHO counting (4 exercises held during 2 served and 2 buffet meals). At the start of each meal the participants were given a form listing all dishes available and the ingredients contained. After deciding what to eat they filled in the form with estimates of their CHO and calorie intake. At the end of the meal a poster was displayed listing the exact values of CHO and calories per 100 g of each dish. On day 4 scales were provided to weigh the portions. At the end of the workshop the participants filled in a questionnaire listing the major difficulties encountered and the feelings experienced during the exercises, the tools they felt they needed and how this exercise would affect their strategies in educating patients about CHO counting.
Results: Thirty-five dishes were presented during the 4 sessions. CHO mean estimates per dish falling in a range of ±5 g of the exact value were considered correct. The participants erroneously estimated the CHO content in 19 (56%) dishes; underestimated foods were 5, whereas 14 were overestimated, respectively with mean estimates of –33% and +55% of the real CHO g content. These percentages were obtained from the trimmed means (α=0.05), normalised by the CHO known amount. Direct comparison of results from day 1 and day 4 (same buffet meal) shows that the dishes erroneously estimated were respectively 44% and 38%. Evaluation of the 28 final questionnaires showed the results reported in the Table 1.
Conclusion: CHO counting skills cannot be the exclusive domain of dieticians. All HCPs involved in diabetes care need to have practical knowledge of this method in order to work out personal strategies for intervention. This would allow all HCPs to be more effective and efficient in educating the patient CHO counting and in providing support for major difficulties.

Table 1: Evaluation of final questionnaires.

Which have been the greatest difficulties encountered?	Which feelings followed during the exercise? 1st day	4 th day	Which tools did you feel the need for at the start of each experiential learning session?	How do you think this experiential learning will affect your strategies to patients about CHO counting?
47% assessing weight of portions	25% funny, excitement, play	35% I improved, little easier	36% scales	44% increasing experimental learning and group work, to give them more precise instructions, be aware to recheck their knowledge
20% calculation of CHO, proteins, fat content	25% total loss, difficult	20% tiredness, frustration	16% lists of COH in ordinary food	32% I'll better understand my patients when they calculate calories
	18% resentment anger, useless	15% seriousness	16% demonstration and expert supervision	
27% lack of knowledge and/or practice	21% surprise, attentive, curiosity	15% fun	12% motivation	20% I will understand better CHO counting, learn to count COH content better
	11% I didn't realise amount of calories consumed	10% motivation to learn	12% the recipes	

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Impact of a new training programme for HCPs aimed at helping patients to develop their resiliency potential.

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Background and Aims: Empowering patients to help them develop their personal resources so as to better manage their disease and treatment is the cornerstone of **T**herapeutic **P**atient **E**ducation (**TPE**). The traditional « standard » medical interview as taught in the basic training is aimed at diagnosing the patient's pathologies and needs. The goal of our study is to evaluate the impact of a new programme that trains HCPs to adapt their questioning and listening in order to better assess patients' personal resources with the aim of helping them develop their own resiliency. Such a pilot training programme has been run and evaluated in our diabetic and/or obese patients teaching unit.
Material and Methods: HCPs were trained to improve their interviewing technique by using tools derived from the 'resiliency' concept, so as to help patients discover their personal resources during the initial interview - run by 1 resident and 1 nurse. Patients interviews (6 before and 6 after the 11-month period during which the training program took place) were performed by the same resident-nurse team. These were videotaped to allow HCPs to make an additional formative auto-evaluation under the supervision of the first author. The second author independently analysed the content of these 12 interviews to evaluate the effect of the programme.
Results: This analysis reveals that numerous significant improvements in the HCPs questioning techniques occurred after the training. Whereas the duration of the interviews (mean $\pm$ sem) before and after the training did not differ, (respectively 38 vs 41 min, ns) the better advantage HPCs took of the content of patients' replies after the training, allowed them to significantly decrease the number of questions (mean $\pm$ sem) they required with regard firstly to collecting medical information (7.9 $\pm$ 2.2 in pre- vs 3.6 $\pm$ 1.1 in post-training interviews, $p < 0.05$) and secondly, to completing the still missing medical information (16.3 $\pm$ 2.6 in pre- vs 5.8 $\pm$ 1.0 in post-training interviews, $p < 0.05$). HCPs used such « saved » time to introduce questions focusing on patients own resources (0.2 $\pm$ 0.1 in pre- vs 2.3 $\pm$ 0.4 in post-training interviews, $p < 0.05$) and also introduce comments that highlight such resources (0.4 $\pm$ 0.1 comments in pre- vs 1.6 $\pm$ 0.3 in post-training interviews, $p < 0.05$), as well as re-formulating the important messages of the interviews (1.5 reformulations $\pm$ 0.5 in pre- vs 4.8 $\pm$ 1.1 post-training interviews, $p < 0.05$).
Conclusions: Training HCPs to adapt their way of questioning will allow them to develop the resources-centred approach to the patient as required by any efficacious **TPE** programme aimed at helping patients to develop their own resiliency.

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Implementation of a structured teaching programme for social services providing outpatient care for patients with diabetes mellitus is urgently needed.

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Background: The outpatient care of elderly patients with diabetes mellitus is often ensured by social services, especially in patients with insulin therapy and diabetic foot ulceration. Further education possibilities for nursing staff of social services is still inadequate.
Aim: Assessment of problems and need for further education in outpatient nursing.
Materials and Methods: 30 social services in Thuringia/Germany, which care for nearly 450 patients with diabetes mellitus, were requested to assess the need for further vocational training and problems in outpatient routine care concerning nursing of patients at home using a semi-standardised questionaire (5 subscales: important- not important).
Results: 60% (n=18) of the social services evaluated the insulin dose adjustment by employees of outpatient nursing to be a very important subject for further vocational training. They criticised, that general practicioners are not available, when the actual blood glucose does not justice advised insulin dose and that nurses are not allowed to adjust insulin dose themselves in that case. As main subject for further education 90% (n=27) of the social services assessed the wound care of diabetic foot ulceration and 63,3% (n=19) the treatment of foot complications to be very important. Further demanded and very important evaluated subjects of outpatient nursing services are the treatment of hypoglycaemia (53,3%),

handling of insulin pens (53,3%), coma/hyperglycaemia (50%), hypertension (36,6%), possible drug therapies in diabetes mellitus (40%), behaviour of outpatient nurses in case of infectious fever diseases (36,7%), insulin pump therapy (36,7%) and nutrition of patients with (30%) and without (26,7%) insulin therapy. Furthermore the insufficient pass of information from hospitals to outpatient nursing services was criticised.
Conclusion: Adequate further vocational training possibilities for nursing staff providing outpatient care for patients with diabetes mellitus are demanded by social services, especially in insulin dose adjustment and wound care of diabetic foot ulceration. Thus, a new structured teaching programme for outpatient nursing staff will be implemented by the Working Group of the German Diabetes Association.

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Diabetes training program for dispensing pharmacists: evaluation of a French large national program.
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Background and Aims: The aim of our study was to improve diabetic patient management by dispensing pharmacists who are among the frontline providers easily accessible at prescription renewal.
Materials and Methods: The implementation of a national training program for pharmacists was set up with four national societies. Scientific Diabetes Association, Diabetic Education Study Group, Pharmacist's council and training updating for pharmacists were involved in this project. 1117 pharmacists participated in 27 trainings of half a day, each given to 40 pharmacists by diabetologists and educational nurses. Each session included a common course on new data in diabetes and then every pharmacists participated in each of 3 workshops: delivery of usual prescription; insulin injections, self monitoring of blood (SMBG) or urine glucose ; diabetic lifestyles (mainly: hypoglycaemia, foot care). The training was evaluated on a questionnaire filled in before and after the session and three to six months later.
Results: The immediate evaluation of the 27 sessions (1117 pharmacists) shows that : 88 % estimated insufficient their knowledge on diabetic devices versus 22 % after the session (943 pharmacists). The mean mark out of 20 (number of correct answers) was 13.7 at the pre-test and 15.8 at the immediate post-test and 16.8 three months later (644 pharmacists), at this point only 13 % estimated their knowledge on diabetes devices sufficient. The pre-test analysis shows that the least known topics were: allowed devices for foot care, care of chronic infected wound, behaviour in case of hypoglycaemia, use of urinary strips, SMBG for Type 2 diabetic patients, association of hypoglycaemic oral drugs. The post-test evaluation highlights the major impact of this training for pharmacists.
Conclusion: The maintenance, even the amelioration of the acquired knowledge is shown by the results of the post-test at three months. Probably there is a decreased of self confidence on own knowledge with the time requiring education supports. These results favour complementary sessions which should be set up on the whole national territory.

OP Health Care Organisation 1:
Improving the Process of Care

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The National Diabetes Register (NDR) in Sweden – improving quality in diabetes care and risk factor control during 1996-2001.
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Background and Aims: The Swedish National Diabetes Register (NDR) was initiated in 1996 by the Swedish Society for Diabetology as a response to the demands of the S:t Vincent declaration for quality assurance in diabetes care. National guidelines for diabetes care were established at the same time. Thus, NDR was started as a tool for local quality control and bench-marking against the national treatment aims.
Materials and Methods: Five samples of 27213, 41154, 36518, 41151, and 60002 diabetes patients were studied during 1996-2001, with both hospital outpatient clinics (HOC) and primary health care centres (PHC) participating. We have evaluated clinical characteristics of diabetes patients in HOC and PHC based on: age, gender, diabetes duration and treatment, HbA_{1c}, blood pressure (BP), body mass index (BMI), smoking, as well as usage of lipid- and BP- lowering drugs. This was also done separately for type 2 diabetes (DM2) patients (practical definition: diabetes onset at the age of 40 or above).
Results: During 1996-2001 the mean HbA_{1c} decreased from 7.5 (SD:1.5)% to 7.2(1.4)% in HOC patients, and from 6.8(1.5)% to 6.3(1.3)%, in PHC patients, respectively. Mean BP levels decreased from 137(20)/78(10) mmHg to 135(19)/76(10) mmHg in HOC patients, and from 151(20)/82(9) mmHg to 147(19)/79(10) mmHg in PHC patients, respectively. Only 42% of PHC patients with DM2 had BP ≤140/85 mmHg in 2001. Almost 50% of these patients were treated with blood pressure lowering drugs. Treatment with lipid lowering drugs increased from 8% to 22% of all DM2 patients treated in PHC. Increasing mean BMI values 1996-2001 in DM2 patients were related to high HbA_{1c} and BP values at follow-up (2001), independently of age, sex, diabetes duration and smoking.
Conclusion: Decreasing mean HbA_{1c} and BP values, as well as increasing use of lipid-lowering drugs during the late 1990´s among diabetes patients in Sweden could translate into clinical benefits regarding micro- and macrovascular complications. Increasing mean BMI values 1996-2001 was related to high HbA_{1c} and BP values in DM2 at follow-up (2001). Smokers showed higher HbA_{1c} and a more aggressive risk factor profile than non-smokers.

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Effect of a nationwide program of educational outreach visits to improve the processes of care for patients with Type 2 diabetes mellitus.
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Background and Aims: Despite increasing consensus regarding appropriate processes of care, variations in the quality of care exists for patients with diabetes mellitus. The objective of the study was to determine whether educational outreach visits increase the frequency with which appropriate tests for type 2 diabetes mellitus are ordered by physicians.
Materials and Methods: An interrupted time-series study with audits of practice before, during and after intervention was conducted between January 1998 and December 2000. The study was performed in physicians' offices throughout France. All physicians who diagnosed one case of type 2 diabetes mellitus during a 6-month intervention period (n =22,940) were included in the study. Educational outreach visits (office visits or phone discussions) were offered by trained medical advisors salaried by health insurance funds. During the visits, the main recommendations of national guidelines on diabetes mellitus management were discussed. The Main Outcome Measures were, the number of HbA1c measurements recorded monthly in the French national medical insurance computer database and the proportion of diabetic patients for whom one test had been reimbursed

during the previous 6 months (HbA1c, fasting blood glucose) or previous 12 months (serum cholesterol, serum creatinine, urine microalbumin, electrocardiogram, ophthalmologic examination).
Results: A total of 15,522 office visits and 9,062 telephone discussions were performed. The increase of the monthly proportion of the number of HbA1c tests to the total number of laboratory tests was higher during the intervention period than during pre-intervention period ($p < 0.0001$) and post-intervention period ($p < 0.001$). HbA1c was measured at least once in 41.2 % of patients before (n = 651,574) and in 60.5 % after the intervention (n = 911,871). The percentages were 10.6% and 15.3% for urine microalbumin, and 79.3 % and 72.0 % for fasting blood glucose, respectively. No important changes were observed for other tests.
Conclusion: Physician to physician outreach visits constitute an effective approach to improve the processes of care for diabetes mellitus. This strategy can be utilized to implement national guidelines in a national program

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Does retinopathy screening overwhelm ophthalmology resources?
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Background and Aims: Mass screening for diabetic retinopathy (DR) through fundus photography by mobile teams was introduced in 1990 in Stockholm County. Retinal specialists performing laser photocoagulation then expected their caseload to increase. Certain private practitioners however feared loss of income. We wanted to allay fears by open reporting of screening volume and yield.
Materials and Methods: From June 1990 to July 2001, 120 primary health care centres and other family doctors' offices, two hospital dpts of internal medicine and two peadiatric ones were visited and 25 000 persons with diabetes aged 9-98 years underwent dilated 45 degree Kodachrome 64 fundus photography and grading (London protocol). The first year, rollout was slow, while resources for fluorescein angiography and argon laser treatment were augmented. With a fixed annual grant, up to 5 500 examinations were performed annually. Population in the catchment area increased (Mean 1 200 000); so did the proportion (18 to 20%) annualy changing address. We used a computer registery to keep track of patients and to ensure timely re-examinations and laser treatment.
Results: Annual referral for fluorescein angiography and or photocoagulation decreased from 5% to 3% of examinees, falling to 1.5% in areas served for 10+ years. After 8 years, only three out of 100 opthalmologists still actively resisted mass screening for DR. Since then, one has retired, one changed his mind after reading a review of the literature, and the third rented a videobased fundus camera once per-patient payment became available in June, 2001. During 2002, 10 600 examinations were performed. A proxy measure for new blindness fell by 7% annually.
Conclusion: Constant exchange of information and opinion, and development of resources for further diagnosis and treatment of DR detected during screening has minimised opposition to DR screening.

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The effects of structured care by a pharmacist-diabetes specialist team versus usual clinic-based care by generalists on renal outcomes in patients with Type 2 diabetic nephropathy and mild-to-moderate renal impairment.
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Background and Aims: Type 2 diabetic nephropathy (DN) is the leading cause of renal failure worldwide. Despite evidence of benefits from aggressive control of BP, glycemia and lipid profiles, and the use of inhibitors of renin-angiotensin system, the quality of care in clinical practice remains suboptimal. We assessed the effects of a structured care model implemented by a pharmacist-diabetes specialist team in patients with Type 2 DN.
Materials and Methods: The study was a 2-year prospective, controlled study. Inclusion criteria were Type 2 diabetes, age ≤80, serum creatinine (SeCr) 150-400 μmol/L and presence of micro- or macroalbuminuria. The intervention group consisted of 80 eligible patients recruited into the DN clinic, set up in June 2000 and managed by a pharmacist-diabetes specialist team. They were followed for 2 years according to a structured care protocol: (1) diabetologist and pharmacist visits 3- to 4-monthly emphasizing patient adherence; (2) regular laboratory assessments; and (3) optimization of risk factors (BP<140/90 mmHg, HbA_{1c}<7.5% and LDL-C <2.6 mmol/L) and use of ACE inhibitors or AII antagonists unless contraindicated. Eighty age-matched patients who met the entry criteria and were attending general medical clinics in the same hospital were used as control subjects. The control group received usual care where the frequencies of doctor visits and laboratory assessments were at doctors' discretion, and structured protocol to reinforce attainment of treatment targets was not available. The primary endpoint was the composite of end-stage renal disease (ESRD, defined by SeCr >500 μmol/L or the need for dialysis or renal transplantation), or all-cause mortality. A Cox regression model was used to test the primary hypothesis and determine the treatment effect.
Results: Baseline characteristics were similar between the intervention and control groups (age, 64.5±9.7 vs 65.8±7.9 years; male, 62% vs 56%; duration of diabetes, 13.0±6.9 vs 11.4±7.5 years; SeCr, 213.5±60.4 vs 215.6±52.9 μmol/L; sBP, 151.2±26.2 vs 152.4±23.1 mmHg; dBP, 75.7±11.6 vs 74.0±14.5 mmHg; LDL-C, 3.3±1.1 vs 3.4±1.4 mmol/L; and triglycerides, 2.4±1.9 vs 2.4±1.5 mmol/L). Baseline HbA_{1c} was higher in the intervention than control group (7.8±1.3% vs 7.3±1.6%). Twenty four patients (30.0%) in the intervention group compared to 40 patients (50.0%) in the control group reached the primary endpoint (adjusted risk reduction 55.3%, p=0.005). The number treated to treat to prevent one ESRD or death in 2 years was only 5.
Conclusion: Structured care delivered by a pharmacist-diabetes specialist team reduced the incidence of the combined endpoint of ESRD or death compared to usual care in patients with Type 2 DN and mild-to-moderate renal impairment.

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International consensus on diabetic foot (ICDF) implementation in Pistoia area: is it worthwhile in terms of amputation reduction?
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Background and Aims: A comprehensive programme for prevention and treatment of diabetic foot lesions (ICDF) performed through a strict collaboration between general practitioners (GP) ,diabetologist together with the multidisciplinary foot care team has been started in 1999 in Pistoia area (157,000 inhabitants).
Materials and Methods: In this study we report the results of the strategy used in order to avoid major amputations in diabetic subjects, according to one of the major objectives of St. Vincent Declaration.In a previous work we performed a retrospective study on prevalence and incidence of diabetic foot lesions requiring hospitalization during years 1996-1999, and, among these we evaluated the ones requiring amputation (DRG Tuscany data base) (Fiuggi, DFSG Group 2000). A prospective study (2000-2002) has been performed in order to evaluate: 1) the percentage of subjects with diabetic foot lesions referred by GP to our diabetic unit (DU) never come before to our attention, 2) the possible reduction of hospitalization for leg and foot lesions in Pistoia area, 3) the final outcome of foot lesions.
Results: The preliminary data of the prospective study demonstrate a significant increase of subjects referred to DU from GP during years 2000-2002: from 40% in 2000 to 75% in 2001 up to 90% in 2002; 80% reduction of hospitalization of diabetic subjects unknown to DU; the reduction of hospitalization for major leg and foot lesions of 10% in 2000 and 35% in 2001 and 2002 , and a significant increase of access to outpatient clinic for minor grade lesions. Moreover, since 2000 it has been observed a progressive reduction of total amputations, both, in general (G) and in diabetic (D) population , compared to data related to 1999 (from 20% in 2000 to 50% in 2001 and 2002). Interestingly, since 1999 the major/minor amputation ratio has been progressively decresed from 0,83 in 1999 to 0,4 in 2002.
Conclusion: The implementation of ICDF resulting in a strict collaboration between specialist and GP could decrease diabetes-related lower extremity amputations, through a prevention programme, risk subjects follow-up and an earlier treatment of lesions. Moreover, the number and duration of hospitalization seems to be reduced by the application of ICDF, as well as the final outcome of foot lesions seems to be improved.

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Physicians' attitudes toward foot care education and foot examination and their correlation with patient knowledge and practice.
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Background and Aims: Lower limb complications are a major cause of morbidity, disability and poor quality of life in patients with diabetes. In the context of a nation-wide outcomes research program we investigated several aspects related to foot care in type 2 diabetic patients enrolled by Diabetes Outpatients Clinics (DOCs) and General Practitioners (GPs).
Materials and Methods: Overall, 3564 patients were recruited by 212 physicians practicing in 125 DOCs and 103 GPs. Patients were requested to fill in a questionnaire investigating whether they had received any information about foot care by their physician. The questionnaire also investigated how often the patients had had their feet examined in the last 12 months and how often they had been recommended to see a podiatrist. Patients were also asked how often they usually checked their feet for wounds or sores. Analyses were adjusted for patient case-mix and physician-level clustering.
Results: The prevalence of lower limb complications was 6.8%. Seventy-two percent of the patients declared they had received information on foot care by their physician, while only 49% reported they had had their feet examined. Only 9.5% of the patients were referred to a podiatrist in the previous six months, while 79.9% declared that they had never been suggested by their physician to see a podiatrist. Independent correlates of receiving foot education were longer diabetes duration, insulin treatment, and presence of eye complications. Patients with lower levels of school education and income as well as overweight individuals were less likely to receive information on foot care. Foot examination was more likely to be performed in low income patients and in those treated with insulin or with foot complications, while female patients were less likely to have their feet examined. Foot examination tended to be performed less frequently by GPs as opposed to diabetologists (OR=0.63, 95%CI 0.40-1.00). Overall, 42% of the patients declared that they never checked their feet (33%) or did it less than once a month (9%). Patients who had received foot education (OR=2.5 95%CI 2.0-3.0) and those who had had their feet examined by their physician (OR=1.7 95%CI 1.4-2.0) were significantly more likely to check their feet regularly.
Conclusion: The attention to foot complications is generally poor, and a substantial proportion of type 2 diabetic patients is not offered adequate foot care, even in the presence of major risk factors for lower limb complications. Patient knowledge and practices are strongly related to physicians' attitudes.

OP Health Care Organisation 2:
Improving Patients' Outcomes

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Michigan Diabetes Outreach Network (MDON) diabetes care improvement project.
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Background and Aims: To assure that persons with diabetes receive care according to the clinical practice recommendations of the American Diabetes Association.
Materials and Methods: The Michigan Diabetes Outreach Network (MDON) collaborates with health care providers through a systematic continuous quality improvement initiative. Consultation, education, scannable forms, data entry software, and reports are provided by the Network to partner providers.
The current MDON database includes information on over 30,000 clients (intake data) and 19,000 (follow-up data) from 165 provider agencies (physician offices, home care, state certified diabetes education programs, community health care centers, and other settings).
Changes are measured for clinical indicators such as the completion of an annual HgA1c(glycosylated hemoglobin) test, dilated eye exams, and foot exams. Behavioral and lifestyle changes are measured such as exercise, nutrition management, and smoking cessation.
MDON data reports are provided to each partner agency quarterly. Reports detail demographic trends and client outcomes serving as the basis for quality improvement activities.
Results: Trends in follow-up data from FY 1996 through FY 2001 for glycosylated hemoglobin, foot exam, and microalbuminuria (all done at least once annually) show a significant improvement in number of persons with diabetes having these tests done.
Glycosylated hemoglobin tests increased from 14 percent in FY 1996 to 78 percent in FY 2001 and foot exams done increased from 58 percent in FY 1996 to 77 percent in FY 2001. Microalbuminuria tests were added to the data system in FY 2000 and show an increase from 22 percent to 28 percent in number of persons having the test between FY 2000 and FY 2001. Individualized data analysis from the regional Diabetes Outreach Networks show a positive downward trend in the levels of glycosylated hemoglobin. Between 1995-97 there was a decrease in the glycosylated hemoglobin values of 0.69 percent (N=9,617 intakes and 4,749 follow-ups). Between 1999-01 there was a decrease value of 1.15 percent (N=10,982 and 3,158 follow-ups).
Conclusion: Results from the MDON Diabetes Care Improvement Project have helped to close the gap in Michigan's excess diabetes related mortality compared to national averages.
A regional network of collaborating agencies can improve diabetes care through use of a coordinated diabetes care improvement program and working together to problem solve with creative strategies.

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DIASTEP: improvement of management and outcomes of patient with Type 2 diabetes. A national audit undertaken by 1631 French GPs.
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Background and Aims: To improve the effectiveness of treatment in general practice for French people with type 2 diabetes by the use of audit and feed back in primary care at a national level.
Material and Methods: 202 groups of general practitioners (1631GPs) were recruited on a voluntary basis over all the regions of France. They assessed the management of respectively 15,120 and 15,106 consecutive patients in the first and the second data collection conducted 12 months apart. HbA1c were adjusted for a norm $\leq$6%.

Results: Comparison between the two phases of audit showed no difference in characteristics of patients (mean age: 66 ± 11 yrs, duration of diabetes: 9 ± 3 yrs). The monitoring by laboratory tests was improved: HDL cholesterol and microalbuminuria measurements respectively increased from 62.3% to 72.9% and from 48.3% to 59.4%. In the second data collection 4.5% of patients were treated by diet alone (-0.7%), 86.3% by oral hypoglycemic agents (OHA) (+ 0.2%), and 9.2% by insulin alone or in combination with OHA. Of patients with BMI >28 kg/m^2 and a creatinine clearance >60 ml/mn, 58.7% were treated by metformin (+ 9.3%). 37.7% performed self monitoring blood glucose (+ 4.2%). 69.6% of patients were treated by antihypertensive drugs (+ 2%) and 8.8% performed home self BP measurements (+ 2.1%). Of patients treated for hypertension, 42% took aspirin or antiplatelet agents (+10.2%). In the whole population, proportion of patients with HbA1c >8% decreased from 19.1% to 15.2% (p <0.001). In the highest and lowest quartile of baseline HbA1c, proportion of patients with HbA1c >8% decreased respectively from 28.3% to 20.4% and from 11.5% to 9.6 %. Proportion of patients with BP >140/80 mmHg decreased from 47% to 43.5% (p <0.001). Proportion of patients with LDL >1.30 g/l decreased from 44.5% to 42.3% (p <0.01). 38.6% of patients had at least one complication, including 19.1% with coronary heart disease or cardiac failure. When a coronary heart disease was present, 41.6% of patients were treated by ACE inhibitors (+ 1.5%), 45.4% by beta-blockers (+ 0,1 %), 54.6% by statins (+4.6%), 66.2% by low dose aspirin (+ 3.2%) and 7.9% by these four drugs combined (+0.4%).
Conclusion: This large French national audit improved the monitoring by laboratory tests and therapeutic practices. Blood glucose, BP and lipid controls were improved, mostly in high risk patients.

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Six-year follow-up of staged diabetes management in native Alaskan populations: improving diabetes outcomes.
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Background and Aims: Over the past 2 decades the incidence of type 2 diabetes has increased more among Alaska Natives than perhaps any other Native American population. From a prevalence of <1% of the adult population, it has now climbed to over 10%. Significant changes in diet with greater reliance on processed foods high in fat and carbohydrate combined with reduced physical activity have made diabetes the leading cause of morbidity in this population. Working with the United States Indian Health Service, the aim of this project was to improve the detection and treatment of diabetes and associated complications and to monitor the impact of our interventions over a period of 6 years in this underserved population.
Materials and Methods: Staged Diabetes Management (SDM), a systematic evidence-based approach to the detection and treatment of diabetes and metabolic syndrome, was customized and implemented in 1997 at a "model" clinic serving Alaska Natives living in both urban centers and in remote, semi-isolated villages (accessible only by airplane or boat).
Results: New SDM practice guidelines and clinical pathways were developed to reflect the needs of this unique population. Annual screening for diabetes was established starting at 30 years of age (to be initiated earlier in the presence of additional risk factors for diabetes). Diagnostic criteria were maintained at fasting plasma glucose (FPG) ≥126 mg/dL (7 mmol/L) and casual plasma glucose (CPG) ≥ 200 mg/dL (11.1 mmol/L). Treatment recommendations were modified to start all patients with FPG 200-350 mg/dL (11.1-19.3 mmol/L) on oral pharmacological therapy and to initiate insulin therapy when FPG exceeded 350 mg/dL (19.3 mmol/L). Initial treatment targets were set as: (1) <7.9 % HbA_{1C} (upper limit of normal 6.4%); and, (2) pre-meal self-monitored blood glucose 80-140 mg/dL (4.4-7.7 mmol/L). In 1996 there were 96 Alaska Natives in the clinic's type 2 diabetes registry. Prior to intervention baseline metabolic data were gathered on a sample of 81 people in active treatment. By 2002 the registry increased by 46% to 140 patients. For the purpose of analysis, clinical data were gathered on a random sample of 46 charts. Glycemic control, as measured by HbA_{1C} , showed that prior to the implementation of SDM, 19% of patients had achieved a target of <7.9% compared with 78% six years post SDM implementation (p <0.001). During the same period, the percentage of subjects with an HbA_{1C} >10% decreased from 48% to 14% (p<0.001). The improvement in glycemic control was traced to the rapid selection of effective therapies resulting in 38% treated by medical nutrition therapy, 33% oral agent monotherapy, 15% combination oral agents and 14% treated with insulin.
Conclusion: Treatment of a diverse underserved patient population living in remote villages with limited access to diabetes care present significant public health problems. Nevertheless, the SDM approach managed to result in significant long-lasting improvement in key diabetes indicators.

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Metabolic control in Type 2 diabetes patients in Chelyabinsk city: the gap between knowledge, practice and goals.
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Background and Aims: It is difficult to assess the quality of metabolic control in diabetic patients (pts) and its conformity with the standard goals of treatment in many regions of Russian Federation because of limited resources such indicator of long-term glycaemic control as HbA_{1c} is not included to the routine examinations. The aim of this study was to evaluate the level of metabolic control in type 2 diabetic pts - residents of Chelyabinsk (big industrial city with more than 1000 000 inhabitants) and its correlation with the knowledge and practice regarding diabetes.
Materials and Methods: 200 pts with type 2 diabetes (M/F 44/156; age (mean±SD) 64,5±8,2 yrs, range 41-84 yrs; duration of diabetes 9,6±7,3 yrs) were randomly selected from 1144 type 2 diabetes pts registered in one of the municipal outpatient clinics providing care to 75200 residents of Chelyabinsk city. All pts completed a questionnaire including basic demographic and social characteristics, questions regarding their knowledge about goals of treatment in diabetes and self-management issues. Data on diabetes duration, type of treatment, presence of complications and cardiovascular (CV) history were obtained from medical files. Blood pressure (BP), weight and height were measured and BMI was calculated. Fasting venous blood was drawn for HbA_{1c} and full lipid profile. The results are shown as mean±SD.
Results: 25% of pts were treated by diet alone, 58% by oral hypoglycaemic agents (OHA), 11,5% by insulin + OHA and 5,5% by insulin alone. 10 (5%) pts were current or former smokers (5/5). Mean HbA_{1c} was 7,84±1,86% (normal ≤ 6%): ≤ 6,5% in 28,5% of pts; between 6,6 and 8% in 23,5% and > 8% in 48%. Only 9 (4,6%) pts reported using self-monitoring blood glucose (BG). Mean BMI was 30,9± 5,5 kg/m^2: 36% had BMI between 25 and 30 kg/m^2 and more than a half of pts (52%) were obese (BMI ≥ 30 kg/m^2). Total cholesterol (TC) was 5,40±1,12, triglycerides (TG) 1,83±1,20, HDL-C 1,19±0,51, LDL-C 3,50±1,19 (mmol/l). Only 14 (7%, M/F 6/8) pts reached combined lipid goals: TC < 4,8, TG<1,7, LDL-C < 3,0 and HDL-C > 1,2 (mmol/l), but none of the study participants were taking lipid lowering drugs. Mean BP was 149,9±66,8 / 92,2±69,7 mm Hg. Of 169 (84%) pts with known hypertension only 49% reported regular taking recommended antihypertensive drugs. When correcting for age, sex, education, duration of diabetes, type of treatment, presence of complications, hypertension, smoking and CV events no significant correlation was found between the knowledge about BP target and regular taking antihypertensive drugs (partial correlation coefficient r=0,02; p=0,77) as well as between the knowledge about BG targets and HbA_{1c} (r=0,01; p=0,91). The level of knowledge about diet was not significantly correlated with BMI (r=0,14; p=0,07).
Conclusions: The majority of type 2 diabetes in Chelyabinsk do not achieve the standards for metabolic control presenting with high CV risk. It is a gap between patients' knowledge about diabetes, their behaviour and outcome measures. There is a need for intervention programmes to improve pts'/doctors' education and make a connection between knowledge and practice regarding diabetes.
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Non-clinical predictors of amputation among persons with diabetes mellitus in a publicly funded health care system.
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Background and Aims: Amputations represent a serious complication of diabetes (DM). Previous research suggests that rates of this adverse outcome can be reduced through appropriate interventions by patients and providers. We sought to identify non-clinical factors associated with the risk of amputation within a publicly funded health care context.
Materials and Methods: Persons with DM were identified from linked health care administrative data using a validated algorithm. Population-based annual cohorts of adults with and without DM (1995-1999) were assembled from the Ontario provincial registry (1999: n=514,755 with DM; n=8,081,557 without DM). Lower extremity amputations were identified

from a comprehensive hospital discharge abstract database. Minor and major amputations were defined as below and above the ankle respectively. Census data were used to attribute neighbourhood level socioeconomic status (SES) to individuals in the cohorts. Procedure rates were age/sex-adjusted to the 1996 Ontario population. A Cox proportional hazards model was used to identify independent risk factors for undergoing any amputation during the five-year observation period.
Results: Age/sex-adjusted odds of minor and major amputation were 24- and 14-fold greater in persons with DM than in those without and more than 100-fold greater among those under age 50 years. In a multivariate model which included age, gender and a comorbidity score, a number of non-clinical factors were significantly associated with amputation. The relative risk (RR) of amputation was 32% higher among those in the lowest income neighbourhoods compared to the highest ($p<0.001$) and there was a continuous gradient across SES quintiles. Individuals living in the northern region of the province, where communities are geographically isolated and remote from tertiary medical services, were 48% more likely to undergo amputation ($p<0.001$) than residents of Toronto. Patterns of ambulatory care were also an important independent predictor of amputation. Compared to persons with 2 or fewer primary care visits, the RR for amputation was 0.70, 0.68, 0.65 and 0.73 for 3-5, 6-8, 9-11 and 12 or more visits per year respectively (all $p<0.001$). Moreover, those who received the majority of their primary care from the same provider had a 13% lower risk of amputation ($p=0.001$).
Conclusion: Individuals with DM face a dramatically elevated risk of lower extremity amputation. In addition to known clinical risk factors, low SES and residence in remote regions were important predictors of amputation. Despite the absence of cost barriers, many patients with DM appeared to under-utilise primary care services. Infrequent or fragmented primary care was an independent predictor of amputation. Policy interventions are required to ensure adequate access to care, particularly in remote regions.

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Patterns of care delivery as a risk factor for diabetes complications: a payments register study of 4632 patients with vision-threatening retinopathy.

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Background and Aims: Studies of diabetes patients have shown that microvascular and macrovascular complications can be mitigated or delayed by improving blood glucose control. The ability to improve blood glucose control, however, is a result of a complex set of behaviours of patients and providers working within a health system. Recently, guidelines and service frameworks have been developed to facilitate a systematic approach to the prevention of diabetes complications. However, there has been no definitive evaluation of the effectiveness of this strategy in preventing complications. To address this question we investigated primary health care utilisation as a modifiable risk factor for the development of diabetes complications, specifically vision-threatening retinopathy.
Materials and Methods: The study used an extract from the Australian Medicare database. The extract comprised a national sample of patients with an existing diagnosis of diabetes in the years 1994 to 2000. Diabetes was identified using HbA1c as the index marker. The parameters tested included the major aspects of diabetes management related utilisation which were readily identifiable through specific funding items. We used a matched case-control study design. Cases were diabetes patients who received their first episode of laser photocoagulation therapy in 2000 ($n = 4632$). Controls were a random sample of diabetes patients, matched to cases on age and State of residence, who had never received this treatment ($n = 4632$). We extracted health care utilisation data for 1993 to 2000 for both groups
Results: 66% of the sample were aged over 60 with the median age being 62.5. There was a greater proportion of men in each group (cases = 53.7%, controls = 54.3%) but there was no between groups difference ($X^2 = 1.11$, 1df, $p = 0.29$). There were major differences in levels of health care utilisation. In each year, cases were less likely to attend a GP than controls, from OR = 0.21 ($p<0.0001$) in 1993 to 0.53 ($p<0.0001$) in 1999. With regard to pathology tests (markers of the process of care), cases were less likely to be tested for HbA1c, from OR = 0.24 ($p<0.0001$) in 1997 to OR = 0.38 ($p<0.0001$) in 1999, and for HDL–cholesterol, from OR = 0.017 $p<0.0001$) in 1999 to 0.04 ($p<0.0001$) in 1993. Cases were also less likely to attend specialist consultations, from OR = 0.18 ($p<0.0001$) in 1994 to OR = 0.35 ($p<0.0001$) in 1999, and consultant physicians from OR = 0.43 ($p<0.0001$) in 1993 to OR = 0.59 ($p<0.0001$) in 1999. The annual trends showed that cases were more likely to utilise health services as they approached the first episode of laser therapy: GPs, OR = 1.14 ($p <0.0001$); specialists and consultant physicians, OR = 1.12 ($p <0.0001$); and testing for HbA1c, OR = 1.14 ($p<0.0001$), although their levels of utilisation at no time reached that of controls.
Conclusions: The results suggest that low levels of diabetes management related health care utilisation may increase the risk of developing microvascular complications such as vision-threatening retinopathy. This study highlights the desirability of structuring health delivery services and interventions to increase primary care utilisation among people at risk of advanced diabetes complications.

OP Health Care Organisation 3:
Improving the Organisation of Diabetes Care

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The diabetes prevention and control program of Iran.

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Background and Aims: Diabetes mellitus (DM) has become a monumental problem and a major public health concern in the world. It is estimated that more than 1.5 million diabetics live in the Islamic Republic of Iran (I.R.Iran).
Materials and Methods: A national network for the prevention and control of diabetes began in 1996, in which four levels of health care have been designed. At the first level, behvarze (health worker) in health house, and health professional in urban health post screen the community, evaluating men and women at risk. At the second level (diabetes team), general physicians screen referred person by either FBS or 2 hr postprandial glucose and control DM according to treatment protocols. All patients would then be referred for early detection of complications to the third level, which is located in a district hospital with an internist or endocrinologist, a full-time educational nurse, and a part-time nutritionist staff (diabetes unit). Patients needing more specific facilities for diagnosis and treatment would then be referred to the fourth level, which is situated in a university (provincial) hospital.
Results: From October 1999 to October 2001, pilot areas in 18 provinces entered screening project for type 2 diabetes. Approximately 200 behvarzes in 984 health houses and 300 physicians and 700 health workers in 161 rural and 171 urban health centers participated in the screening program. 3.5 million people were screened, of which 998237 individuals were aged over 30. 59717 subjects were screened by behvarzes in the screening program. 37, 25, 16 and 22% of people studied were in age groups of 30-39, 40-49, 50-59 and >60 years, respectively. On the whole 21638 diabetics, 15091 women and 6547 men were detected. Overall 3.6% of the subjects were diabetics of which 58.3% were known and 41.7% were newly discovered. Obesity, hypertension, family history of diabetes and in female history of ≥2 abortion and newborn ≥4 kg weight were more prevalent than other risk factors.
Conclusion: A network for prevention and control of diabetes established in I.R.Iran is aimed at screening and control of type 2 DM, to counteract the rising prevalence of this disease.

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Quality of care in newly-diagnosed diabetes mellitus:"EPIDIAB" program-lessons from the first three years.

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Background and Aims: The natural history of type 2 diabetes mellitus associates macrovascular and microvascular complications. When diabetes is diagnosed, these complications can be already present. The general concept is that a complete screening and a global evaluation should be made when type 2 diabetes is diagnosed, in order to adapt the clinical management and to reduce the global risk. The objectives of this analysis were to evaluate the epidemiology of diabetes and to assess the quality of diabetes care in terms of complications screening at diagnosis.
Materials and Methods: Data have been collected through EPIDIAB Program (Epidemics of Diabetes), which has been launched in 2000. It is a prospective study having as objectives: 1). epidemiological analysis of newly-diagnosed diabetes, 2) study of quality of care; 3) strategic prediction. The study is designed to last 5 years, it includes, 14 counties, representing one third of total population.
Results: The number of newly-diagnosed persons with diabetes in the first three years was: 15.057 (7.4% type 1 DM and 92.1% type 2 DM), 16,394 (6% type 1 DM and 93.2% type 2 DM) and 15,858 (5.4% type 1 DM and 89% type 2 DM). Among persons with type 2 diabetes, overweight was present in: 48% in 2000, 19.5% in 2001 and 40% in 2002. Obesity was present in: 39.2%, 57.9% and 43%. Hypertension was present in: 45.3%, 49.9% and 48.3%. The prevalence of cardiovascular disease was 32%; 31.6% and 27.7%. The screening for complications revealed the following data:

Year	Dyslipidemia		Retinopathy		Nephropathy		Diabetic foot	
	Tested	Positive	Screened	Positive	Screened	Positive	Screened	Positive
2000	70.9	48.8	74.9	12.4	60.8	7.3	71.5	24.3
2001	65.7	51.7	59.6	15.8	50.4	5.1	53.7	22.5
2002	69	62.6	65.5	9.9	61	4.9	64.3	19.6

Data in table are %

Therapeutic education was provided for 92% patients in 2000, 90.7% in 2001 and 94.4% in 2002.
Conclusion: The data confirm the epidemic of diabetes. The high prevalence of cardiovascular risk factors and microvascular complications at diagnosis, impose a global evaluation. The screening for chronic complications and associated conditions should be improved. In order to improve the quality of diabetes care, general practitioners should be involved in the network of Romanian Diabetes Program.

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A randomised controlled trial based evaluation of delivery of diabetes care to ethnic minority groups: the United Kingdom Asian diabetes study.

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Background and Aims: The safety and effectiveness of treatments to lower blood pressure and improve glycaemic control in Type 2 diabetes has been demonstrated. However, delivery of care to ethnic minority populations may be complicated by cultural and language differences. In UK resident South Asians, Type 2 diabetes is four times more common than in Whites, and South Asians have higher risk of cardiovascular and renal complications. The aims of this project were to investigate the feasibility of using Asian link workers and a structured, nurse led and protocol driven approach to achieving risk factor targets in South Asian patients with type 2 diabetes in primary care.
Materials and Methods: Six general practices in the West Midlands were randomised to intervention or control. The intervention comprised intensive control clinics in each practice, supported by practice nurse, diabetes specialist nurse and link worker sessions, using treatment protocols and targets for glycaemic, lipid and blood pressure control. Patients were South Asians with type 2 diabetes plus at least one modifiable risk factor: blood pressure > 140/80 mmHg, total cholesterol > 5.0 mmol/l, HbA1c > 7.0%. Primary outcomes were one year follow-up changes in blood pressure, HbA1c, cholesterol and microalbuminuria, defined as an albumin:creatinine ratio >3.0.
Results: 325 (90%) patients completed follow-up at 1 year. The mean (95% confidence intervals) differences between intervention and control groups changes in risk factors were -4.6 (-8.8 to -0.3) mmHg systolic blood pressure, -3.4 (-5.7 to -1.2) mmHg diastolic, -0.4 (-0.7 to -0.1) mmol/l cholesterol and -0.03% (-0.4 to +0.3) HbA1c. Compared to controls, the intervention group had significantly reduced systolic and diastolic blood pressure, plus cholesterol. There were no significant HbA1c changes or differences between the groups. Persistent microalbuminuria, defined as albumin:creatinine ratio >3.0 on two consecutive tests, was observed for 25 (18%) intervention and 29 (20%) control patients.
Conclusions: This unique intervention facilitated significant blood pressure and cholesterol lowering in South Asian patients over a one year period and

demonstrated that primary care teams can deliver appropriate care to ethnic minority groups. HbA1c improvements may require longer follow-up or other strategies. Renoprotection may require lower blood pressure thresholds. Continuation of the intensive care clinics and follow-up measurement is essential, to allow assessment of the maintenance of risk improvements achieved.

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Can Steno-2 multifactorial intervention study and UKPDS standards be achieved in a District General Hospital in the UK?

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Background and Aims: The Steno-2 study and UKPDS showed that targeted intensified intervention on modifiable cardiovascular risk factors in patients with type 2 diabetes reduces morbidity and mortality. Can the standards in Steno-2 and UKPDS be achieved in a District General Hospital in the United Kingdom, especially in view of the historic under-resourcing of diabetes care in the UK?
Materials and Methods: The Alphabet POEM Project (**P**ractice **O**f **E**vidence-based **M**edicine) assessed the effect of systemic application of the Alphabet strategy to care of patients with type 2 diabetes, with particular focus on calculated cardiovascular risk. The Alphabet strategy is based on the mnemonic: **A**- **A**dvice, **B**- **B**lood pressure, **C**- **C**holesterol care/**C**reatinine care, **D**- **D**iabetes control (HbA1c), **E**- **E**ye examination/**E**rectile dysfunction, **F**- **F**oot examination, **G**- **G**uardian drugs (Aspirin, ACE-I, AIIA and Statins) and **H**- **H**eart disease risk. Data was collected on 400 consecutive patients with type 2 diabetes attending the outpatient department from referral to most recent follow up. Comparison was made with the Steno-2 intensive study cohort and with UKPDS. Results were analysed using the Chi-squared test.
Results: In comparison to the Steno–2 intensive cohort, Alphabet POEM fared similarly with regard to diastolic BP, HbA1c, Aspirin, ACE-I and Aingiotensin II antagonist(AIIA) use, but less well with respect to systolic BP, total cholesterol and use of statins. Alphabet POEM achieved better systolic and diastolic BP than UKPDS but glycaemic control was significantly worse.
Conclusion: The standards achieved in the Steno 2 study and UKPDS are in principle at least partially achievable in a District General Hospital in the UK, but fully achieving them in practice will probably need a radical strategy and restructuring, and greater provision of resources.

Comparison between Steno-2, UKPDS and Alphabet POEM

Variable	Intensive cohort	Alphabet POEM	Ratio (% POEM / % Steno-2 or UKPDS)	POEM: worse, similar or better ?	p value
SBP (≤130 mmHg)	Steno-2 : 45%	31%	0.69	worse	0.041
DBP (≤80 mmHg)	Steno-2 : 70%	62%	0.89	similar	0.232
Chol (≤4.5 mmol/L)	Steno-2 : 72%	33%	0.46	worse	< 0.001
HbA1c (≤6.5 %)	Steno-2 : 15%	11%	0.73	similar	0.400
Aspirin use	Steno-2 : 87%	83%	0.94	similar	0.428
Statins use	Steno-2 : 85%	54%	0.64	worse	< 0.001
ACE-I use	Steno-2 : 79%	83%	1.05	similar	0.471
AIIA use	Steno-2 : 30%	22%	0.73	imilar	0.327
SBP (≤144 mmHg)	UKPDS : 50%	57%	1.14	better	0.023
DBP (≤82 mmHg)	UKPDS : 50%	73%	1.46	better	< 0.001
HbA1c (≤7 %)	UKPDS : 50%	44%	0.88	worse	0.020

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„Meriba Zageth“ (our work) for diabetes - an indigenous model of care for diabetes in a remote Australian community.

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Background and Aims: About 10,000 people inhabit the remote islands of the Torres Strait in Far North Queensland, Australia. Diabetes was first reported in these islands in the early 1960’s. The current prevalence of Type 2 Diabetes in this population is 24% above the age of 15 years and is repotedly the highest in Australia. Obesity across all age groups is the single most important risk factor**.** In 2000, we reported the first cases of childhood Type 2 diabetes in Australia from this population. The disease is associated with excess cardiovascular mortality and morbidity. Diabetes is also the commonest cause of end stage renal failure and amputation in this population.
Materials and Methods: In 1999 a community driven diabetes programme was introduced involving a multidisciplinary team of health professionals. The programme (Meriba Zageth) based on an enhanced model of primary health care started to deliver holistic diabetes care to this population. The team made individual visits to each island community on a regular basis. A simple paper based recall system driven by health workers was set up to deliver essentials of diabetes care to patients. A computerised patient imformation recall system is now in place. The team has a full time podiatrist to deliver basic and preventative foot care to all patients. At all steps of health care, the community is involved along with a team local general practitioners. Appropriate cross referrals are made to visiting eye, renal and cardiac specialists at Thursday Island hospital. Trained midwives run the Diabetes in pregnancy programme. Suitable, locally modified and accredited training programmes have been put in place to train indigenous generalist health workers and nurses in the basic care of diabetes. In addtion, appropriate school health based screening of diabetes in children are in place.
Results: In the last three years the programme has reduced the rate of hospitalisation related to diabetes consistently by 25-30%. Major amputation rate has decreased by 50%. In an independent audit carried out in 2002 it has been demonstrated that good glycaemic control (HbA1c < 7%) has been acheived in 25% of the cohort. Blood pressure targets (BP< 130/85 mm.Hg) has been acheived in 60% of the patient population. 25% of patients are now on insulin and are self monitoring. The Diabetes in pregnancy programme has recorded significant decrease in congenital malformation and increased awarenes of diabetes in women.
Conclusions: We conclude that the current model of diabetes care in the islands of the Torres Strait can be adopted in other indigenous communities

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An evaluation of the effectiveness of a step-down clinic for diabetes in Hong Kong.

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Background: Step-down clinic for managing patients with diabetes mellitus (DM) was introduced in 1999. After attending an education program given by dietician and DM nurse specialist at DM centre, patients with stable glycemic control were channelled from a hospital based specialist clinic to a community family physician based step-down clinic. This allows a more cost-effective use of the limited resource as DM patients with different degrees of management difficulties are followed up at different levels. The objective of this study is to assess the effectiveness of the Tsan Yuk Hospital Integrated Clinic (TYHIC), a step-down clinic in Hong Kong, in managing DM patients with stable glycemic control.
Method: Two hundred patient records were randomly selected from the TYHIC. The following data were retrieved: age, sex, duration of diabetes, body height, body weight, blood pressure, lipid profiles and hemoglobin A1c (HbA1c).
Results: Demographic data of the study subjects (84 males/116 females) are as follows: age 67.3 $\pm$ 10.7 years; body weight 64.3 $\pm$ 11.9 kg; body mass index (BMI) 26.0 $\pm$ 3.8 kg/m^2 and duration of diabetes 15.1 $\pm$ 7.7 years. Eighteen percent, 75% and 7% of the subjects were on diet control alone, oral hypoglycaemic agent(s) and insulin therapy respectively. After a mean follow up of 3.1 $\pm$ 0.7 years, there was a significant improvement in BMI (26.0 $\pm$ 3.8 vs 25.5 $\pm$ 3.9 kg/m^2, p< 0.01), systolic BP (147 $\pm$ 23 vs 141 $\pm$ 19 mmHg, p< 0.01), diastolic blood pressure (76 $\pm$ 12 vs 72 $\pm$ 10 mmHg, p< 0.01) and HbA1c (8.0 $\pm$ 1.3 vs 7.6 $\pm$ 1.3%, p< 0.01). There was no significant change in lipid profiles.
Conclusion: Our study suggested that the step-down clinic is effective in managing DM patients with stable glycemic control. The risk factor profile of these patients, including BMI, blood pressure and glycemic control, showed a significant improvement after being followed up for around 3 years. With a comprehensive education and complication assessment programme co-ordinated at the specialist level, this model of shared care can further be promoted to relieve the workload in specialist clinics. The dynamic patient referral system established and supported by Diabetes Centre can further enhance the quality of care provided to the patients; enabling them to receive appropriate treatment from different levels of care according to the stage of their disease.

OP Health Care Organisation 4:
Health Care Cost

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The direct medical costs of Type 2 diabetes mellitus – a U.S. perspective.

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Background and Aims: The worldwide prevalence of diabetes is increasing. Accordingly, the demand for and cost of medical care will increase. The aim of our study was to develop a model to estimate the direct medical costs associated with type 2 diabetes.
Materials and Methods: We studied subjects with diabetes who were randomly selected from a Michigan health-maintenance organization. The response rate was 67%. There were 1,364 respondents with type 2 diabetes. Demographic characteristics, duration of diabetes, diabetes treatments, complications and comorbidities were assessed with questionnaires and medical chart reviews. Annual resource utilization and costs were assessed for each subject using health insurance claims data. The log-transformed annual direct medical costs were fit by multiple linear regression to indicator variables for demographics, treatments, complications and comorbidities. Direct medical costs associated with incident stroke, acute myocardial infarction (MI), and amputation were assessed separately.
Results: The annual direct medical costs for subjects with diet-controlled type 2 diabetes, body mass index (BMI) of 30 kg/m^2, and no microvascular, neuropathic or cardiovascular complications were $1,700 for white men and $2,100 for white women. A 10 kg/m^2 increase in BMI, treatment with oral-antidiabetic agents, proteinuria, and peripheral vascular disease were each associated with 10% to 30% increases in cost; insulin treatment, angina and MI were each associated with 60% to 90% increases in cost; and dialysis was associated with a 11-fold increase in cost. Median annual direct medical costs for diabetic patients with incident stroke, MI, and amputation were $27,000, $25,000 and $38,000, respectively.
Conclusion: Insulin treatment and major diabetes complications have a substantial impact on the annual direct medical costs of type 2 diabetes. The estimates presented in this model provide useful information for future analyses of cost-effectiveness of interventions for type 2 diabetes.

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The prevalence and cost of diabetes mellitus in metropolitan France: what trends between 1998 and 2000?

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Background and Aims: Our aim was to update available data concerning the prevalence and cost of diabetes mellitus in metropolitan France by measuring the contemporary changes recorded between 1998 and 2000.
Materials and Methods: We performed a retrospective study using patient reimbursement data from all the 128 local health offices in metropolitan France. We selected patients who received reimbursements for an oral hypoglycemic agent or insulin. Thus, 704,423 patients were studied by using data from 1998 and 1,145,603 patients were studied by using data from 2000. The expenditures studied represented the total amount reimbursed by national health insurance to diabetic patients, irrespective of the type of diabetes they had. The cost differential which could be attributed to diabetes was calculated by determining the difference between costs generated by diabetic patients to those generated by the rest of the population of the same age.
Results: Between 1998 and 2000, the prevalence of diabetes mellitus treated with oral hypoglycemic agents or insulin in the population of affiliates covered by the general scheme (France's largest health insurance fund) increased from 2.78 % to 2.96 %, corresponding to an average annual increase of 3.2 %. The total amount paid by the general scheme for care to diabetic patients (care related to diabetes or not) was 5.710 billion euros in 2000 compared to 4.862 billion euros in 1998 (2,270 euros per patient in 2000 versus 2,042 euros in 1998). The amount which can be attributed solely to diabetes can be estimated to be 2.414 billion euros in 2000 compared to 2.021 billion euros in 1998 (1,148 euros per patient in 2000 versus 1,027 in 1998). After considering the impact of the increase in the number of treated diabetics, modifications in the modalities of medical management probably account for 183 million euros of the cost increase. Medical equipment (self blood glucose monitoring devices, reagent strips, finger lancets....) accounts for 39.3 % (72 million euros) of this cost differential, medications account for 34.4 % (63 million euros), nursing care 16.9 % (31 million euros), patient transportation 6.6 % (12 million euros) and laboratory tests 2.7 % (5 million euros). There was no change in the cost of diabetes with relation to expenses for medical consultations.
Conclusion: These results confirm projected changes in epidemiology and in the cost of diabetes mellitus.

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The cost and burden of *diabetes mellitus* in Dominica's public sector.

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Background and Aims: Dominica is a developing Caribbean nation with a population of 71,532. *Diabetes Mellitus* is the fourth leading cause of morbidity on the island. The complications and hospitilization costs that result from diabetes are a great strain on the health care system. With limited available resources to address growing health needs, methods of reducing health care costs are of paramount concern.
The purpose of this study is to assess the burden and direct cost *Diabetes Mellitus* places on Dominica's public health care system using 2001 as the sample year. The results will be used to improve diabetes care by targeting those groups at risk of developing the disease and its complications, as well as maximizing the management of diabetes with existing financial resources.
Materials and Methods: The burden of *Diabetes Mellitus* is assessed by measuring all diabetes related clinic visits, hospital stays, and drugs and supplies used. The direct cost of diabetes is calculated by assigning costs to these units.
National expenditure on direct patient care is calculated using the seven district health budgets, costs of drugs and medical supplies, and all hospital expenses. From this data the proportion spent on diabetes care is calculated, along with identifying how much and to what departments funds are disbursed.
Results: Results show that *Diabetes Mellitus* has a 4.17% prevalence rate in Dominica. Of all clinic visits, 15.1% were for persons with diabetes. At district and central hospitals, patients with diabetes represented 9.0-23.9% of all admissions and on average stayed twice as long as other patients. In particular, female patients with diabetes were admitted 2-3 times as often as males with the average stay being twice as long. The average age of hospitalization for diabetes complications was 60.4 years. Ophthalmology data revealed that 8.2% of eye clinic visits and 86.2% of eye laser surgeries were performed on persons with diabetes. Due to Dominica's small population, all existing data was analyzed making sampling unnecessary, except for the Emergency Department at which a 33% sample of yearly visits was taken.
Costing calculations found the approximate costs of treating patients at the health clinic, district hospital, and central hospital levels of the public health care system. Drugs and medical supplies for persons with diabetes amounted to 15.5% of the national drug and medical supply budget. Significant to note, 10.3% of the national expenditure on direct patient care is devoted to treating persons with diabetes. All costs in this study represent minimum values.
Conclusion: The average age for hospitalization with diabetes complications is 60.4 years and females were hospitalized more often and longer than males. Based on these findings, it is imperative that preventive programs begin as early as possible to target those in high risk groups with greater priority to more effectively manage the disease. Furthermore, with the understanding that a significant portion of the national expenditure for health care is committed to treating diabetes and its related complications, it is financially prudent to invest in programs that prevent and delay the onset of complications.

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Predictors of diabetes-attributable non-blood glucose-lowering medication costs for Type 2 diabetes: the Fremantle diabetes study.

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Background and Aims: Most patients with type 2 diabetes require drug therapy in addition to that specifically for glycaemic control. This includes medications to prevent, delay or treat chronic diabetes complications. The aim of the present study was to examine longitudinally 'diabetes-attributable' medication use in a community-based patient cohort, especially the magnitude and baseline predictors of the costs of these drugs over time.
Materials and Methods: 593 patients with type 2 diabetes from the prospective observational Fremantle Diabetes Study were followed for a mean±SD 4.3±0.4 years (5 annual assessments). Costs of medications were categorised as those for blood glucose-lowering therapy (Group 1), diabetes-attributable medications (Group 2) and other treatments (Group 3). Group 2 costs were calculated by applying a range of attributable proportions, derived from the literature, for each diabetic complication for which the medication was likely to have been prescribed. Costs were the dispensed price (costs to both government and patient) calculated in year 2000 Australian dollars.
Results: Prescription medicine costs during the study comprised 25% for Group1, 13-39% for Group 2 and the remainder for Group 3 drugs. Over the study period, the median annual costs/patient of non-Group 1 medications more than doubled over four years, increasing from A$222 to A$515 (P<0.001), with median Group 2 drug costs increasing from A$15-41 to A$95-281/patient (P<0.001). The proportion of patients not requiring Group 2 drug therapy decreased from 43% to 29% whilst two-fifths of patients did not take Group 3 drugs throughout follow-up. Total costs in all groups approximately doubled between baseline and study end. Costs exhibited a skewed distribution and were square root-transformed before analysis. Using forward stepwise multiple linear regression, √Group 2 drug costs/year was positively and independently associated with baseline systolic blood pressure, coronary heart disease (CHD), total serum cholesterol, ln(serum triglycerides), ln(ACR), serum creatinine, BMI, and educational attainment, and negatively with male gender, and fasting plasma glucose (P≤0.045; R^2=28%). Age, diabetes duration, retinopathy, neuropathy, ethnicity, marital status, English fluency, smoking status, alcohol consumption, exercise, and calendar year of recruitment (1993-6) were not associated with cost.
Conclusion: The total cost of diabetes-attributable non-blood glucose-lowering medications increased two-fold over 4 years and was associated with the presence of CHD and its major risk factors, nephropathy, and obesity at baseline. Better-educated patients had increased costs which may have reflected better healthcare access. By contrast, the reduced costs associated with male gender and fasting hyperglycaemia may indicate poor self-care and/or access to services.

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Is hospital admission for diabetes inappropriate?

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Background and Aims: The expanding gap between growing costs of health care and lowering resources has recently focused attention to appropriateness of hospital admission and stay as they represent an important part of total expenditure for patient care. Admissions of diabetic patients to hospitals are often considered inappropriate as the majority of them are treatable on a out of hospital basis and Disease Related Groups for Diabetes (DRG 294 and 295) have recently been included in a list of potentially inappropriate DRG for hospital admissions in Italy, even if hyperosmolar coma and ketoacidosis patients are grouped in them. The aim of the study was to assess the appropriateness of hospital admissions and stay of patients with diabetes and its complications with a clinically oriented protocol and to test the effectiveness of an educational program for health care provider using the same appropriateness evaluation protocol.
Materials and Methods: All the files regarding admissions to the Ospedale Evangelico Valdese in Torino, Italy with a lenght of stay (LOS) longer than 2 days were analyzed according to the PRUO, a procotocol derived from the United States Appropriateness Evaluation protocol, AEP. The PRUO protocol assesses the appropriateness of presence in hospital in every single day of stay on the basis of a list of validated criteria (patients characteristics, medical or nurses procedures). The admissions to the diabetologic unit (DU) were compared to the admissions to the other surgical and medical admissions as a whole (WH). The protocol was applied to the admissions in january, march, october 2001 and march 2002. The initial (ID), median (MD) and before the last (BD) day were considered. An admission was considered completely inappropriate (CI) if all the 3 days were inappropriate. The result of the analysis was published in a letter after january 2001 while they were discussed and the files related to inappropriate admissions reviewed with the medical and nurses staff after march 2001.
Results: CI admissions for DU and WH were respectively as follows: 01/2001 62.5% and 20.7%, 03/2001 27,3% and 21,9%, 10/2001 0% and 1,3%, 03/2002 0% and 1,5%. A reduction of inappropriateness was observed for all the single day's inappropriateness both in DH and WH. The BD inappropiateness remained significantly high (DH and WH respectively): 01/2001 6,3 and 30,1%, 03/2001 0% and 68.65, 10/2001 20.1% and 54.1%, 03/2002 23.1% and 53.4%.
Conclusion: Inappropriate hospital admissions and LOS for diabetes occurs in a high proportion of cases. The direct involvement of health care providers in appropriateness analysis can enhance the appropriate use of hospitals and recover resources for out of hospital care of diabetic patients.

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Economical impact of 3 years nurse case magement. A program in 4.815 Type 2 diabetics. The financier point of view.

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Background and Aims: We designed a Diabetes Free Cost Nurse Case Management Programme to reduce medical expenses in Type 2 diabetics.
Objectives: Analysis of frequency and expenses of office and hospitalized patients reimbursement codes after 3 years implementation
Materials and Methods: We selected and trained 9 nurses and 28 diabetes monitors for associated enterprises. We selected as contents 4 Diabetes critical points: feet examination, glycaemic index, ADA treatment goals and auto control practices. 3 nurse consultations/year and one yearly educational group activity were offered to 4.815 diabetics in our screening programme. Yearly we send a summary to individual medical doctors. In nurse visit were addressed arterial pressure, feet examination, weight, nutritional & exercise patterns and adverse drugs reactions questionnaires. 3 times a year, free cost, capillary glycaemia and once a year glycosilated hemoglobin, triglicerydes and microalbuminuria were performed under nurses supervision. Nurses received phone expert support and patients calls them also for their support. With structured software, nurse teams qualified qualitatively patients diabetes trends and recommended medication, nutrition and exercise changes or medical controls. Statistical test performed; Student T and Chi2 for comparative frequency and cost analysis.
Population: 4815 diabetics were collected at voluntary screening. 66,1% were men and 33,9%, women. Two groups were analyzed: G1; in programme group, with more than 2 nurse consultation/year (569 cases) and G2; out programme group, with less than 2 consultations per year (4,246 cases). In 1.334 cases of G2 we have only initial data because they only came for screening and never came again. Mean age for G1 was 49+/-7 years in men and 42 +/- 8 years in women. G2 group mean age was 48+/-5 years in men and 43+/-6 years.
Results: G1 and G2 were not statistically different in age or sex distribution.
Post prandial glycaemic median and percentiles 25-75 were similar in both groups. G1Mean =122 and G2=137mg/dl; p25-75 for G1 was 93-154 for G2 94-155. After 3 years,G1 needed statiscally ($p<0.05$)less frequents codes for office (35% reduction) and hospitalizations (36% reduction) reimbursements. Sick days licenses were 14% reduction but not statiscally (n.s). 17% reduction was observed in total medical expenditures, but don't cover yearly programme cost (69,843,000 Chilean Pesos) mean health reimbursements expenditures for G1 were 202.608 $ Pesos and for G2 242.986 $ Chilean Pesos.
Conclusion: A nurse structured programme is a good to obtain better diabetic control and to support strategy to reduce diabetic health expenditures and office & hospital frequency uses, but more attractive benefits are needed to improve compliance and achieve the finance programme cost.

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Genetics of Type 1 Diabetes Mellitus

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Type 1 diabetes mellitus of the LEW.1AR1-*iddm* rat is linked to susceptibility loci on RNO1 and RNO20.

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Background and Aims: The LEW.1AR1-*iddm* rat is a new animal model of Type 1 diabetes mellitus (T1DM), which arose through a spontaneous mutation within the MHC-congenic inbred strain LEW.1AR1 (*RT1*^{r2}) in 1997 at Hannover Medical School. It was the aim of this study to identify diabetogenic loci within the genome of the LEW.1AR1-*iddm* rat, to obtain new insights into key pathways involved in the pathogenesis of the type 1 diabetes.
Materials and Methods: Diabetes susceptibility regions were identified by a genome-wide microsatellite scan of a (LEW.1AR1-*iddm* x BN) x LEW.1AR1-*iddm* backcross population (n=226) using 136 polymorphic microsatellite markers created from rat genome data bases.
Results: The incidence of diabetes in the diabetes-prone LEW.1AR1-*iddm* colony was 60% when both parents were diabetic. An analyses of the backcross colony (BC1) provided evidence for 3–4 predisposing genes, which were associated with the development of the diabetic syndrome. Microsatellite analyses of the BC1 revealed three susceptibility loci, two on chromosome 1 (RNO1) and one on chromosome 20 (RNO20), which were significantly linked to T1DM.
The markers, which showed the strongest association (p=0.00038) to the disease are located on RNO1q51-55 near the genes *insulin1* (*Ins1)* and the homolog of the homoeobox gene *Nkx2-3*. The second region on RNO1 is located in the centromeric region around the microsatellite *D1Rat186* (p=0.00728). The candidate region on RNO20 could be located into the MHC-region through alignment to a YAC-Contig of this chromosome. The three diabetes susceptibility loci of the LEW.1AR1-*iddm* rat show homologies to diabetes-associated genes of the human genome (IDDM1 at HSA6p21.3; IDDM5 at HSA6q24-27; IDDM8 at HSA6q25-27; IDDM17 at HSA10q25).
Conclusion: The identification of diabetes susceptibility loci of the LEW.1AR1-*iddm* rat revealed genes which play a permissive role in beta cell development and immunology. A detailed knowledge of the functional role of these genes for the pathogenesis of diabetes in the LEW.1AR1-*iddm* model may give new insights into the role of human genes in T1DM.

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Regional variation in HLA class II allele frequencies and incidence of Type 1 diabetes in Norway.

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Background and Aims: The objective was to investigate whether there are regional differences in frequencies of HLA-DR alleles and in incidence of childhood-onset type 1 diabetes, and if the two are related among the 19 counties of Norway.
Materials and Methods: Data on incidence of type 1 diabetes among children aged below 15 years in each county were taken from the National Childhood Diabetes Registry for the period 1989-1998. HLA-DR genotyping was done using PCR and sequence-specific oligonucleotide probes on 14291 samples from the Norwegian Bone Marrow Donor Registry. DR4 sub typing, and DQ typing was done on a sub sample of haplotypes with DRB1*04. Regional variation in HLA frequencies were analysed using logistic regression models, whereas regional variation in incidence of type 1 diabetes and associations between HLA frequencies and disease incidence were analysed using Poisson regression models.
Results: We found small, but significant variation in the allele frequencies of HLA-DRB1*03 by county (p=0.003), There was no significant variation in DRB1*04 (p=0.055) or DR2 (DRB1*15 or 16) (p=0.18) frequencies. There was a significant regional variation in the incidence of type 1 diabetes ($p < 0.001$), but no strong association between individual HLA-DR allele frequencies and incidence of type 1 diabetes. There was, however, an unexpected, positive association with the protective DR2 allele and a more expected positive correlation between estimated frequencies of the DRB1*0401-DQ8 and DRB1*0401-DQ8/DR3 haplotypes and incidence of type 1 diabetes. Importantly, all these associations were critically dependent on one 'outlier' observation.
Conclusions: We found evidence for some regional variation in frequencies of HLA-DR alleles known to confer risk for type 1 diabetes in individuals, however, overall this variation was small. We found only limited evidence that these minor differences could explain the regional variation in incidence of type 1 diabetes between counties of Norway. The results suggest that at least part of the regional variation in incidence may be due to influence of environmental factors or alternatively non-HLA genes.

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Genetic basis for thyroid autoimmunity in Japanese Type 1 diabetes.

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Background and Aims: Two or more autoimmune diseases of endocrine gland, such as type 1 diabetes, autoimmune thyroid disease and Addison's disease, often occur together, and this condition is termed the autoimmune polyglandular syndromes II and III (APS II/III). In particular, high prevalence of thyroid autoimmunity (TAI) in type 1 diabetes (T1D) is a common feature observed in both Caucasians and Japanese, but little has been described about the genetic basis for TAI in T1D, especially in Japanese. Therefore, in the present study, we aimed to elucidate the genetic basis for TAI in T1D.
Materials and Methods: We analyzed 101 T1D patients; mean age at onset was 39.4 years. Four HLA genes (HLA-A, DRB1, DQB1 and DPB1) and two non-HLA genes (CTLA4 G49A and IFNG intron 1 CA repeat) were genotyped by SBT (sequence-based typing), PCR-RFLP or GeneScan method.
Results:
(1) Among 101 patients with T1D, TAI was present in 42 patients (13 Graves disease patients and 29 anti-thyromicrosomal antibody positive patients). Patients with TAI were female predominant (p=0.042), developed diabetes later in life (p=0.034), required a lower insulin dose (p=0.016) and had higher prevalence of GAD-Ab (p<0.0001) than those without TAI.
(2) Patients with TAI had increased frequencies of HLA-A*02 allele (p=0.005), HLA-DQB1 0303/0303 genotype (p=0.020), CTLA4 49G allele (p=0.007), and IFNG 13 repeats allele (p=0.006), compared to patients without TAI.
(3) Logistic regression analysis including several clinical features and genetic polymorphisms revealed that, in addition to female sex and older age-at-onset, HLA-A*02 allele, CTLA4 G49A genotype and IFNG 13 repeats allele had a significant increased risk of TAI.
Conclusion: In Japanese, HLA-DQB1*0303, CTLA4 (49G allele) and IFNG (13 repeats) genes may be a background shared susceptibility genes between T1D and TAI. As for HLA-A*02, that is probably a thyroid autoimmunity-specific susceptibility gene, because it was reported that both Graves' disease and Hashimoto's thyroiditis are associated with A*02 in Japanese.

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Haplotypes of inhibitor of κB-like (IKBL) gene promoter are associated with Type 1 diabetes in Japanese.

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Background and Aims: Type 1 diabetes is a multifactorial disease in which the genes in the major histocompatibility complex (MHC) have a major role. Recently, non-MHC genes in the class III region of MHC are thought to be associated with type 1 diabetes. We analyzed the possibility of inhibitor of κB-like (IKBL, or NFKBIL1) gene as one of these candidates.
Materials and Methods: One hundred and twenty four type 1 diabetic patients and 166 control subjects were enrolled for the case-control study.

PCR-single strand conformation polymorphism (SSCP) analysis was done to detect the polymorphisms of the gene. SNP typings were carried out by SSCP, amplified fragment length polymorphism (AFLP), and sequencing. DNA typing of HLA-DRB gene was performed by the PCR-SSOP method using Dynal RELI™SSO HLA-DRB Test and sequencing. Informed consent was obtained from all the subjects. The Human Genome Committee of Oita Medical University approved the present investigation.
Results: The haplotypes of IKBL gene in the promoter region were assigned as PA, PB, and PC. The frequency of PA was significantly increased in the patients than the controls (62.9% vs. 48.8%, OR=1.78, P=0.001). The frequency of PC was significantly decreased in the patients than the controls (4.4% vs. 16.3%, OR=0.24, P=0.000015). The frequency of PB was similar between the patients and the controls (32.7% vs. 34.9%, OR=0.9, P=0.63). According to the two-locus analyses with HLA-DRB1 locus, the PA haplotype showed linkage disequilibrium with the susceptible DRB1*0405 allele, and the PC haplotype with the resistant DRB1*1502 allele. However, the PC haplotype was negatively associated with type 1 diabetes independently from the DRB1*1502 allele, and the synergistic effect was observed.
Conclusion: The present study indicated strongly the association of IKBL promoter haplotypes with type 1 diabetes in Japanese.

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Vitamin D Receptor (VDR) polymorphisms: no association among Finnish patients with Type 1 diabetes.
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Background and Aims: The effect of polymorphisms of the vitamin D receptor gene on susceptibility to type 1 diabetes has recently been studied actively. Several positive association results of gene polymorphisms have been found, and in addition, a protective effect of vitamin D supplementation has been reported in relation to diabetes risk. We therefore studied the effect of three vitamin D receptor gene polymorphisms on susceptibility to type 1 diabetes in a large sample series in the Finnish population.
Materials and Methods: A combination of case-control (944 patients, 2379 controls) and affected-family based approaches (544 nuclear families) was used. Samples were genotyped for VDRA (ApaI), VDRB (BsmI) and VDRF (FokI) single nucleotide polymorphisms using a minisequencing reaction.
Results: No significant disease association was observed in the total material for any SNPs studied. However, VDRF showed borderline significant association in the Turku cohort (controls vs. patients; 11 12,6% vs. 18,2%, 12 51,2% vs. 54,7%, 22 36,1% vs 27,0%, p=0.0063, pcorr=NS). In stratified data sets (HLA genotypes, sex, age at diagnosis) no disease association was seen in any comparisons, when correction for multiple testing was applied. Interestingly, we also observed significant geographical variation in the background allele frequencies of the studied polymorphisms in the three different regions in Finland.
Conclusion: Our data indicate that the single nucleotide polymorphisms analysed are unlikely to be associated with type 1 diabetes in the Finnish population.

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Type 1 diabetes susceptibility genes CTLA4 and INS VNTR influence initial disease presentation and residual beta-cell function during the remission period in an international cohort of young people with newly diagnosed Type 1 diabetes.
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Background and Aims:CTLA4 and INS VNTR are susceptibility genes for type 1 diabetes, with the insulin gene being identified as the *IDDM2* locus and the CTLA4 gene most likely accounting for the disease susceptibility linked to the *IDDM12* locus. Furthermore, variability within the CTLA4 gene have been associated with ketoacidosis (DKA) as initial presentation at type 1 diabetes onset, and the INS VNTR alleles have been shown to regulate insulin gene transcription *in vitro* and *in vivo*. Aim: To study the impact of CTLA4 and INS VNTR alleles on the initial presentation with or without DKA and the progressive loss of beta-cell function during the remission period in children and adolescents with newly diagnosed type 1 diabetes.
Materials and Methods: The study is an ongoing multicenter longitudinal investigation with 18 participating paediatric centres from 15 countries in Europe and Japan (84% Caucasians). Clinical information and blood samples were collected from 276 children and adolescents less than 16 years with newly diagnosed type 1 diabetes. A stimulated C-peptide was carried out at 1, 6, and 12 months after diagnosis. Genomic DNA was purified from blood samples using Qiagen DNA purification kit. PCR-RFLP analysis was performed for genotyping the population for the Thr17Ala variant in the CTLA4 gene and the INS VNTR linked – 23 HphI variant. Gene variants were investigated for their impact on the remission phase (assessed by stimulated C-peptide > 300 pmol/l) and the initial presentation of the disease with or without standard bicarbonate < 15 mmol/l using logistic regression and repeated measurement models.
Results: The genotype frequencies of CTLA4 and INS VNTR were in Hardy-Weinberg equilibrium. The homozygote Ala/Ala of the CTLA4 gene was associated with DKA at onset, (OR=2.4 C.I.=1.14-5.1). The class III/III alleles of the INS VNTR variant was associated with a higher level of C-peptide throughout the remission periode (1, 6, and 12 months) in comparison to the class I/III and class I/I alleles. This difference was, however, not significant when adjusted for age (p=0.12).
Conclusion: In an international cohort of young people with newly diagnosed type 1 diabetes we have found a significant association between the Ala/Ala variant of CTLA4 and DKA at onset, this is in accordance with a previously published study. Moreover, we found the class III/III alleles of the INS VNTR variant was associated with a higher level of C-peptide throughout the remission periode (1, 6, and 12 months), this difference was not significant when adjusted for age. The class III alleles have previously been shown to be protective against type 1 diabetes. Thus, the higher level of C-peptide among the children carrying the class III/III alleles of the INS VNTR might be due to a higher transcriptional activity of the insulin gene, which in turn leads to a higher protein translation and C-peptide level.

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Genetic analysis of putative loci located in regions 6q27 (*IDDM8*) and 11p13 in Russian families with Type 1 diabetes.
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Background and Aims: Type 1 diabetes is a complex disorder with multiple genetic loci. In the current study, we have performed the sibpair analysis to identify putative candidate genes that predispose to type 1 diabetes (T1DM) in *IDDM8* locus (6q27) and in a novel locus in chromosome region 11p13, for which we have earlier found evidence for linkage.
Materials and Methods: All families in this study (35 affected and 66 discordant sibpairs) are Caucasian Russians with nondiabetic parents. Polymorphic microsatellite markers were amplified using PCR and primer

sequences from GenBank. Data about single nucleotide polymorphisms (SNPs) were obtained from dbSNP database. Combined transmission/disequlibrium test (TDT) and sib TDT (S-TDT) were used for data analysis. Linkage disequilibrium was tested by permutation algorithm.
Results: To minimize a region of linkage with T1DM on chromosome 6q27 (*IDDM8* locus) we used a set of polymorphic microsatellites and mapped the peak LOD score marker close to telomere. Three genes were found in this area. *PSMB1* gene encodes a proteosome subunit, *TBP* gene (TATA-box binding protein) encodes a wide-range transcription factor, and *PDCD2* gene is a homolog of mouse programmed cell death activator gene. Four polymorphic markers: *T(-99)C* and (CAG)n in *TBP* gene, *G(-276)T* and *C1038T* in *PDCD2* gene were used to study an association of these genes with T1DM. Significant association with T1DM was found for marker *C1038T* (z'=2.18, Pc=0.044). All four markers were found to be in strong linkage disequilibrium (P<0.0002). Haplotype C-C-G including alleles of *T(-99)C*, *C1038T* and *G(-276)T* SNPs, correspondingly, also showed high transmission disequilibrium (z'=2.59, Pc=0.029). To minimize a region of linkage with T1DM on region 11p13 we have studied a linkage with T1DM of five polymorphic markers located nearby catalase (*CAT*) gene. Strong linkage and association evidences have been obtained for markers *C1167T* (within *CAT* gene), *D11S1392*, *D11S2008* and *D11S907*. The peak LOD score was found for *D11S907* marker (MLS=3.93, P<0.0001; z'=3.91, P<0.0001). Two genes were found in this area. *EHF* and *ELF5* genes encode the transcription factors and *D11S907* microsatellite is located within intron 2 of *EHF* gene. *T(-257)C* SNP was identified in the promoter region of *EHF* gene and strong linkage and association evidences have been obtained for this marker.
Conclusion: Chromosome regions 6q27 (*IDDM8*) and 11p13 contain the genes, which contribute to type 1 diabetes susceptibility in Russian population.

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Different contribution of class II HLA in fulminant and typical autoimmune Type 1 (Type 1A) diabetes.
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Background and Aims: Fulminant type 1 diabetes, a novel subtype of type 1 diabetes, which is characterized by 1) a markedly acute onset of diabetes, 2) a high frequency of flu-like symptoms before disease onset, and 3) an absence of islet-related autoantibodies accounts for 20% of type 1 diabetes in Japan. We aimed to clarify the contribution of genetic factor to type 1 diabetes in Japanese with special reference to the clinical phenotype.
Subjects and Methods: We investigated the class I and class II HLA (DR and DQ) in 123 patients with fulminant type 1 diabetes, 89 patients with typical autoimmune (type 1A) diabetes and 190 healthy control subjects.
Results: The frequency of HLA-DR4 in fulminant type 1 diabetes was significantly higher, while those of HLA-DR1, DR2, DR5 and DR8 were significantly lower than those in controls. In contrast, DR9 but not DR4 was frequent and DR2 was extremely rare in typical type 1A diabetes. Neither susceptible nor resistant HLA-A allele was observed both in fulminant and typical type 1A diabetes. With genotyping of DRB1 and DQB1 alleles, DRB1*0405, DRB1*1302, DQB1*0401 and DQB1*0604 were found to be susceptible, and DRB1*1401, DRB1*0803 and DQB1*0601, but not DQB1*0602, were resistant to fulminant type 1 diabetes. Furthermore, DRB1*0405-DQB1*0401 and DRB1*1302-DQB1*0604 were predisposing haplotypes in fulminant type 1 diabetes. Among HLA class II genotypes comprising of DRB1*0405-DQB1*0401, the odds ratio for homozygotes was higher than heterozygotes (12.2 vs. 4.3).
Conclusion: These results suggest that class II HLA contributes to the development of fulminant type 1 diabetes. Furthermore, susceptibile and resistant HLA subtype to type 1 diabetes are distinct between fulminant and typical type 1A diabetes.

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TNFa2 in relation to Type 1 diabetes and latent autoimmune diabetes in adults.
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Background and Aims: The genetic background and ethiopathogenesis for type 1 diabetetes is unclear. Tumor necrosis factor alpha (TNFa) is expressed by activated macrophages. An increased production of TNFa may be involved in the early stages of beta cell destruction. The TNFa locus can contatin 13 different microsatellite alleles, of which the TNFa2 allele has been associated to increased production of TNFa. The aim of this study was to find out if genotypes including TNFa2 were more prevalent among patients with type 1 diabetes or latent autoimmune diabetes in adults (LADA) compared to in the general population.
Materials and Methods: The group of type 1 patients (n=99; median age 35; range 9-89 yrs) were residents in a defined area in the southern part of Sweden. A total of 58 of 1557 (3.7%) patients (median age 51; range 21-79 yrs) were positive for at least one of ICA, GADA or IA-2A among patients clinically classified as type 2 diabetes or unclassifiable diabetes and considered as LADA. The controls were healthy blood donors (n=117; median age 35; range 19-65 yrs) and residents in the same area. TNFa microsatellite polymorphism was analysed with PCR and determination of the fragment sizes.
Results: Homozygosity for TNFa2/2 was the most frequent genotype (20/99; 20.2%) among type 1 patients and TNFa2/2 conferred a significant risk for type 1 diabetes (OR=3.4; 95%CI 1.4-8.2). Other genotypes including TNFa2 (TNFa2/x) were not significantly increased among type 1 patients (OR=0.54; 95%CI 0.31-0.94). Genotypes without TNFa2 (TNFax/x) were neutral in relation to risk for type 1 diabetes (OR=1.1 95%CI 0.62-1.8). The most frequent genotype among controls was TNFa2/11 (10/117; 8.5%). TNFa2/11 was also the most prevalent genotype among LADA-patients (11/58; 19.0%), but gave no significant risk (OR=2.5; 95%CI 1.0-6.3). Neither homozygosity for TNFa2/2 was a risk factor for LADA (OR=2.5; 95%CI 0.91-6.9) nor genotypes heterozygous for TNFa2 (TNFa2/x) (OR=1.4; 95%CI 0.76-2.7). However, when all genotypes with TNFa2 (TNFa2/2 and TNFa2/x) were combined, there was a significant risk for LADA (OR=2.3 95%CI 1.1-4.6). Accordingly, genotypes without the TNFa2-allele (TNFax/x) gave a significant protection for LADA (OR=0.44; 95%CI 0.22-0.89).
Conclusion: We concluded that TNF may contribute to the susceptibility for both type 1 diabetes and LADA since homozygosity for TNFa2 was associated to type 1 diabetes, while absence of TNFa2 conferred protection for LADA.

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Pluripotent gene expressions induced by Thyrotropin-Releasing-Hormone (TRH) in rat pancreas and β-cells: a microarray hybridization approach.
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Background and Aims: The neuropeptide TRH, originally identified as a hypothalamic hormone, is present and secreted from the pancreas. Hyperglycemia found in TRH knockout mice suggests that TRH might be a critical factor in maintaining blood glucose homeostasis. Understanding how TRH affects pancreatic gene expressions and its consequences might lead to a new approach for regulation and/or regeneration of pancreatic β cell. The aim of this study is to explore how TRH affects gene expressions in rat pancreas and β-cells by cDNA-microarray.
Materials and Methods: Total RNA (20 μg) was isolated from pancreas (treated with TRH for five days intraperitoneally given twice TRH 10 μg/kg to Male S.D. rats) as well as rat immortalized INS-1 β cells (treated with 200 nM TRH for 24 hours after serum starvation overnight) and was then hybridized by fluorescent (cy3 and cy5) labeled cDNA in chips with 1081 spots of mechanically fabricated genes (Clone Tech). Pancreas in rat and β cells were treated with vehicle served as controls. Signals were read quantitatively by Fluorescence Scanner. Standardized from nonspecific expression, genes with inconsistent data were eliminated from triple tests. TRH stirred gene expressions both pancreas and β cells were analyzed and

compared. Signal density changes greater than two folds were defined as up or down regulation while non detectable signals *vs.* control were defined as initiated or turned off genes.
Results: About 60-75% genes were detected from 1081 testing-genes and 562 genes share common expressions in pancreas and β-cells, 233 of the same genes from both responded to TRH. TRH upregulated 29 genes in pancreas and 31 genes in INS-1 cells, which included G-protein coupling receptor related genes (GPCR kinase 4 and 5, transducin-β1 subunit, Arrestin-β1, transducin-β1), Ca^{2+} channel enhancers (Ca^{2+}/calmodulin-dependent protein kinase, type I and II), Protein kinases (serine/threonine kinase-3, PKCα, PCTAIRE-3, v-mos) and proliferation or differentiation signal transduction related genes (MAPK3, growth factor receptor-bound protein 2, n-myc, GAP-43), and down-regulated pro-apoptotic Bax gene. Noticeably, TRH significantly stimulated insulin excretion genes (N-methyl-D-aspartate receptor-2A, GABA-A receptor, RAB2, Ras-related GTPase, ADP ribosylation factor 1 and 5). Conversely, there is a difference in gene expressions between the pancreas (169) and INS-1 β cells (187), which included 6 initiated and 14 turned off genes from signal transduction group plus one initiated anti-apoptotic BcLX gene from the 36 initiated and 36 turned off genes in pancreas while only 4 genes were initiated and 4 genes turned off from the 34 signal transduction genes in INS-1 β cells.
Conclusion: TRH maintaining normal insulin secretion to meet the glucose homeostasis needed might be the consequences of TRH affecting genes in both pancreas and β cells. Distinguishing gene expressions from the pancreas and β cells indicate that a different mechanism for β cell proliferation and differentiation in the pancreas. Further study is needed to determine how TRH affects genes to regulate β cell generation and insulin-secretion in the pancreas.

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Genetics and Prediction of Type 1 Diabetes Mellitus

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Suppressor of cytokine signaling-3 inhibits IL-1 beta induced NFκB signaling in beta-cells.
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Background and Aims: Type 1 Diabetes Mellitus (T1DM) is characterised by insulin deficiency due to destruction of the pancreatic β-cells. Activated cells of the immune system secrete cytokines, among others interleukin-1 beta (IL-1β) and interferon-gamma (IFNγ), which are toxic to the β-cells. IL-1β induces β-cell death through a nuclear factor kappa-B (NFκB) and mitogen activated protein kinase (MAPK) dependent expression of the gene for inducible NO synthase (iNOS), whereas IFNγ mainly induces cell death in clonal β-cells through activation of Janus activated kinases/signal transducers and activators of transcription (JAK/STAT) signalling. Cytokines induce changes of expression of more than 100 genes some of which are involved in β-cell destruction and others in β-cell defence. The latter group includes the Suppressor Of Cytokine Signalling (SOCS) - protein family. In the insulin producing cell line INS-1, we have previously shown that over-expression of SOCS-3 reduces IL-1β stimulated NO production and prevents apoptosis induced by IL-1β and IFNγ. The overall aim of the study was to investigate the mechanism(s) whereby SOCS-3 exerts its protective effect against cytokine-induced β-cell death.
Materials and Methods: We measured key signalling pathways induced by IL-1β and IFNγ in clonal INS-1 β-cells with inducible SOCS-3 expression exposed for either 6 or 24 hours +/- IL-1, +/- SOCS-3 expression. STAT-1 and NFκB DNA binding activity in response to IFNγ and IL-1β, respectively, was examined by use of Electro Mobility Shift Assay. Furthermore, selected genes, found by GeneChip microarray analysis to be IL-1β regulated and suppressed by SOCS-3 (e.g. iNOS, c-Myc, IRF-1) were investigated by Semi-Quantitative RT-PCR and Western Blotting.
Results: SOCS-3 over-expression reduced the stimulation of STAT-1 DNA binding activity in response to IFNγ by 70 %, and reduced the activation of NFκB DNA binding by IL-1β by more than 50 %. The investigated selected candidate genes were indeed found to be IL-1β regulated and suppressed by SOCS-3. This was confirmed at both mRNA- and protein-level. The IL-1β induced mRNA expression of iNOS and IRF-1 after 6 hours decreased by 40 % in the presence of SOCS-3, whereas the expression of c-Myc mRNA was completely inhibited by SOCS-3 after 24 hours of IL-1β stimulation.
Conclusion: Our data show that SOCS-3 expression significantly reduces IL-1β mediated NFκB and IFNγ mediated STAT-1 activation. Moreover, expressions of several potential pro-apoptotic IL-1β regulated genes were inhibited by SOCS-3, suggesting a mechanism for the inhibition of cytokine mediated apoptosis by SOCS-3. By elucidating how SOCS-3 protects against the β-cytotoxic effects of cytokines it is hoped that the SOCS-3 protein can be utilized therapeutically in T1DM or in β-cell-transplantation.

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Stimulation of NCAM/FGFR with the C3d peptide inhibits IL-1 mediated MAPK activation in the β-cell line INS-1E.
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Background and Aims: It is generally accepted, that Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease, where release of cytokines from active immune cells play an essential role in the β-cell destruction. In this connection several studies point at the cytokine interleukin 1 (IL-1) as an important mediator of β-cell destruction by signalling through the mitogen activated protein (MAP) kinases (ERK, JNK, and p38). In neurons, the neural cell adhesion molecule (NCAM), which activates the fibroblast growth factor receptor (FGFR) and ERK, plays an important role in neuron differentiation, neurit outgrowth, and furthermore protects against apoptosis. It is known that β-cells express NCAM, and that primary β-cells from NCAM knockout mice secrete less insulin than control cells in

response to high glucose (30 mM) challenge. Taken together these observations indicate that NCAM might have a β-cell-protective/differentiating role.
In neurons, NCAM can be activated *in vitro* with the synthetic NCAM ligand, C3d.
In this study we investigated if pre-stimulation of NCAM (and FGFR) with C3d was able to inhibit the IL-1 mediated MAPK activation, and if the potential effect was dependent on FGFR signalling.
Materials and Methods: IL-1 mediated activation of the MAPKs was investigated by an *in vitro* phosphotransferase assay and verified by Western Blotting with phosphospecific antibodies. INS-1E cells were pretreated with C3d (10 uM) for 30 min. before exposure to IL-1 (160 pg/ml) for 20 min. To investigate the potential effect of FGFR signalling a specific inhibitor of FGFR1, SU5402 (SU), (10-100 uM) was added 1 hour before addition of C3d.
Results: Pretreatment with C3d significantly inhibited (min. 40%) IL-1 mediated MAPK activation ($p<0,05$, n=5). The effect of C3d was mimicked by the SU-inhibitor (10 uM) (n=4) and the IL-1 mediated activation of especially JNK and to a minor degree ERK were decreased in a dose dependent manner with increasing SU-concentration (inhibited 74% and 43% in response to 100 uM SU, respectively). There was no significant difference between the effect of SU alone and if SU was added together with C3d, only a week tendency toward increased inhibition when both SU and C3d were added. This suggests that SU and C3d act through the same pathway, and that this pathway includes the FGFR.
Conclusion: C3d inhibits IL-1 mediated MAPK activation. We suggest that C3d in β-cells breaks NCAM dimers, leading to reduced activation of NCAM monomers contrary to findings reported in neurons, and that the effect of C3d is mediated through its indirect inhibition of the FGFR.

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Reduced primary immunization to insulin in infants who received cow milk formula with low insulin content.

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Background and Aims: Between May 1999 and May 2000 367 families with a newborn infant were recruited to the FINDIA-study to test the effect of a cow milk (CM) formula with low insulin content on the primary immunization to bovine insulin in infants at genetic risk for type 1 diabetes.
Materials and Methods: Forty-five infants carrying the HLA DQB1*0302 allele but no protective alleles were randomized into two groups and received either ordinary CM formula or a non-hydrolysed CM formula with low insulin content during the first 9 months of life as supplementary feeding. Insulin-binding antibodies were measured by EIA (IgG and IgA), by a conventional radio-binding assay for IAA using protein A immune precipitation, and by a modified radio-binding assay for IAA using biotinylated anti-human IgA-antibodies as secondary antibodies and streptavidin-IgG and protein A for immune precipitation (IgA-IAA). GADA and IA-2A were measured with conventional radioligand assays.
Results: At the age of 3 months, 14 infants were exclusively breast-fed, 14 received CM formula with low insulin content and 15 were given regular CM formula. No blood sample was available from two infants at the age of 3 months. The levels fo IgG antibodies binding to bovine insulin did not differ between the infants who received CM formula with low insulin content and those who were exclusively breast-fed. In contrast, the levels of IgG antibodies binding to bovine insulin were higher in infants who were fed the regular CM formula than in the two former groups ($p<0.05$). IgA-antibodies to bovine insulin tended to be higher in the group who received regular CM formula when compared to exclusively breast-fed children ($p=0.08$) and children who received CM formula with low insulin content ($p<0.1$), but the differences remained non-significant. Later, at the ages of 6, 9 and 12 months, the levels of IgG- or IgA-antibodies binding to bovine insulin did not differ. The levels of IgA-IAA (human insulin) were undetectable in most infants before the age of 9 months. At the age of 9 months, no differences were observed in the levels of IgA-IAA between the groups, whereas at the age of 12 months the levels of IgA-IAA were higher in the infants who received regular CM formula than in those who had received CM formula with low insulin content ($p=0.005$). The levels of IAA remained below the cut-off level for antibody positivity in all infants during the follow-up. One infant who received CM formula with low insulin content had low-titre GADA at the age of 9 months but tested negative for GADA at 12 months of age. One infant from the same group had IA-2A at 12 months of age but did not have other autoantibodies.
Conclusion: We showed here that CM formula with a low insulin content reduces the primary immunization to insulin in infants with HLA-conferred genetic risk of type 1 diabetes, and accordingly such a formula is a safe candidate to be tested in a nutritional trial aimed at primary prevention of type 1 diabetes.

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Antioxidant scavenging enzyme genes involved in the genetic susceptibility to diabetic polyneuropathy in Russian patients with Type 1 diabetes.

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Background and Aims: Clinical and experimental studies have been suggested that there is a relationship between the diabetic polyneuropathy (DPN) development and oxidative stress. Reduced efficiency of the scavenging antioxidant systems can be one of the main causes of oxidative stress development. We propose that some etiological mutations of the genes, encoding antioxidant scavenging enzymes, can be involved in the genetic susceptibility to diabetic polyneuropathy in patients with type 1 diabetes mellitus (T1DM). To examine this hypothesis we studied an association with DPN of five polymorphic markers located in four candidate genes: *Ala(–9)Val* of mitochondrial superoxide dismutase gene (*SOD2*), *Arg213Gly* of extracellular superoxide dismutase gene (*SOD3*), *C1167T* and *T(-262)C* of catalase gene (*CAT*) and *Pro197Leu* of gluthatione peroxidase gene (*GPX1*).
Materials and Methods: A case-control study was carried out in a group of 179 unrelated Russian patients with type 1 diabetes mellitus, 86 of whom had overt diabetic DPN (DPN+) and 93 had no clinical DPN (DPN–). All patients were genotyped with PCR protocols for detecting the alleles of polymorphic markers. The genotype and allele frequencies in the case-control groups were compared by exact Fisher's test. The odds ratios and 95% confidence intervals (CI) were determined to assess the strength of the relationship between the polymorphic markers and DPN.
Results: In case of polymorphic marker *Ala(–9)Val* of *SOD2* gene the carriers of *Ala* allele and *Ala/Ala* genotype had lower risk (OR = 0.57 and 0.48, respectively, $p < 0.03$), whereas the carriers of *Val* allele and *Val/Val* genotype had higher risk of DPN development (OR = 1.77 and 3.56, respectively, $p < 0.01$). In case of polymorphic marker *Arg213Gly* of *SOD3* gene the carriers of *Gly* allele had lower risk (OR = 0.61, $p = 0.02$), whereas the carriers of *Arg* allele and *Arg/Arg* genotype had higher risk of DPN development (OR = 1.64 and 3.34, respectively, $p < 0.02$). In case of polymorphic marker *T(–262)C* of *CAT* gene the carriers of *T* allele and *T/T* genotype had lower risk (OR = 0.624 and 0.46, respectively, $p < 0.027$), whereas the carriers of *C* allele had higher risk of DPN development (OR = 1.62, $p < 0.027$). No significant differences in allele and genotype frequencies were observed between DPN+ and DPN– patient groups for *C1167T* marker of *CAT* gene and *Pro197Leu* marker of *GPX1* gene.
Conclusion: The results of our study are evidence that the polymorphic markers located in *SOD2*, *SOD3* and *CAT* gene are strongly associated with diabetic polyneuropathy in Russian patients with T1DM. These data support a hypothesis concerning an involvement of etiological mutations of genes encoding antioxidant enzymes into the formation of genetic susceptibility to DPN.

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Complete mutation scanning of a β-cell protective protein, Suppressor of Cytokine Signaling 3 (*SOCS3*).
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Background and Aims: Type 1 diabetes mellitus (T1DM) is a chronic disorder caused by immune-mediated selective destruction of β-cells. It is triggered by environmental and immunological factors in genetically susceptible individuals. The pro-inflammatory cytokines interleukin-1β, interferon-γ and tumor necrosis factor-α selectively destroy β-cells in the pancreatic islets of Langerhans.
Two classes of genes are regulated in β-cells upon exposure to cytokines:
1) Those that promote β-cell dysfunction and death, and
2) Those that is protective against such effects.

Both classes are of interest as candidates for genetic susceptibility to T1DM. *SOCS3* is up-regulated in native islets and β-cell lines in response to cytokines, suggesting that it may constitute a member of the protective response. We showed SOCS3 mRNA to be up-regulated in islets and beta-cell lines when exposed to cytokines and over-expression studies proved SOCS3 to have a protective effect on the survival of the beta-cell. For these reasons we consider *SOCS3* to be candidate gene in T1DM. *SOCS3* maps to chromosome 17q25. The aim of the present study was to perform a complete mutation scanning of the exon, the 3´UTR and the promoter region of the human *SOCS3* gene.
Materials and Methods: Mutation scanning in selected panels of controls and T1DM patients was performed by single-strand conformational polymorphism (SSCP) and direct sequencing as well as by evaluation of Single Nucleotide Polymorphisms (SNP) known from databases (NCBI dbSNP). Identified polymorphisms were evaluated by means of Transmission Disequilibrium Test (TDT) in a T1DM family collection comprising 250 T1DM families.
Results: Three polymorphisms were identified in the promoter region, however none in the coding region and 3´UTR. Two of the three polymorphisms had allele frequencies below 1% and were not tested further, whereas the third one, using TDT, showed no significant linkage to T1DM in a collection comprising 250 T1DM families. In addition a NCBI dbSNP (rs10611489) in the coding region, causing an amino acid substitution, was genotyped in a panel of 101 Danish diabetics by RFLP. None carried the mutation.
Conclusion: Inherited genetic variations that changes protective mechanisms in islet β-cells are expected to contribute to the genetic basis of T1DM. A complete mutation scanning of the human *SOCS3* gene was performed, but none of the polymorphisms found, showed significant linkage to T1DM in a Danish T1DM family collection. Whether the identified mutations in the promoter region influences the level of SOCS3 expression in the β-cells when exposed to cytokines is still unknown.

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The deleted in colorectal carcinoma (DCC) gene 201 R-G polymorphism: evidence for an impact on the ability of DCC to induce caspase-3 activity but not for genetic association with autoimmune disease.
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Background and Aims: The product of the deleted in colorectal carcinoma (DCC) gene has a role in apoptosis and is a positional candidate for *IDDM6*, the putative chromosome 18q12-q23 autoimmune disease locus. We hypothesised that a non-conservative polymorphism (DCC 201 R-G; nt 601 C-G), located in an extracellular immunoglobulin-like domain of DCC, is an aetiological determinant of autoimmunity.
Materials and Methods: The hypothesis was tested by examining the impact of the substitution on DCC function in cell transfection assays and by genetically testing nt 601 C-G for association with three autoimmune phenotypes in a case-control study. There were 2 249 subjects with rheumatoid arthritis, type 1 diabetes and Graves' disease, and 2 225 control subjects, from New Zealand and the United Kingdom.
Results: Transfection of the DCC 201R and 201G variants into cultured A293 cells suggested DCC 201 R-G influences protein function; the DCC 201G variant was unable to induce caspase-3 activity, which contrasts to the 1.43-fold induction observed after transfection with the 201R variant. However, there was no evidence for genetic association of nt 601 C-G with autoimmune disease in the case control analysis (P = 0.78 in the largest data set, n = 1 209 cases).
Conclusion: Whilst the DCC 201 R-G polymorphism may impact DCC function it does not significantly influence the risk of developing the autoimmune diseases tested.

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Transforming growth factor-beta1 gene contributes to the genetic predisposition to nephropathy in Type 1 diabetes.
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Background and Aims: Diabetic nephropathy (DN) is a major long-term complication of type 1 and type 2 diabetes and is the leading cause of end stage renal disease in many parts of the world. There is strong epidemiological evidence for genetic predisposition to DN in type 1 diabetes. There is mounting evidence that transforming growth factor-beta1 (TGF-beta1), a multifunctional growth factor, plays a key role in the development of tissue fibrosis and may be involved in the pathophysiology of DN. We hypothesized that polymorphisms in the TGF-beta1 gene may affect the ability of some individuals to produce high or low levels of TGF-beta1 protein. This would subsequently predispose some individuals with type 1 diabetes to DN whilst protecting others. In this study we investigate the contributions of a T/C polymorphism in codon 10 of exon 1 of the TGF-beta1 gene to the predisposition to DN. This polymorphism is in the gene coding region of the precursor part of the TGF-beta1 protein.
Materials and Methods: Sequence specific primers were used with PCR to detect the frequency of the codon 10 genotypes in 421 Caucasian subjects with type 1 diabetes and DN (Nephs) and a control group of 411 Caucasian subjects with type 1 diabetes for at least fifty years and without DN (LTNN) and a group of 408 normal subjects with no autoimmune diseases.
Results: All groups were in Hardy Weinberg equilibrium. Frequencies of L/L, L/P and P/P genotypes were 48.7%, 39.2%, 12.1% vs 37.9%, 50.1%, 11.9% when the Neph group was compared to the LTNN group. The Neph group was significantly different to the LTNN group (ChiSq = 11.1, p = 0.004). When the Neph group was compared to the normal subject group, the TGF-beta1 codon 10 genotypes were 48.7%, 39.2%, 12.1% vs 39.9%, 49.3%, 10.5% respectively. The Neph group was also significantly different to the normal subject group (ChiSq = 9.003, p = 0.011), although the difference was less marked than with the LTNN group. A significant difference was found in the P and L allele frequencies between the Neph and LTNN groups (ChiSq = 5.1, p = 0.024) but not between the Neph and the normal subject group.
Conclusion: These results suggest that the TGFbeta1 codon 10 polymorphism is significantly associated with DN. It is likely to be one of a number of genes that form the genetic background in individuals with type 1 diabetes, upon which environmental factors will act, to give rise to DN. Further work needs to be done on the other polymorphic regions of the TGF-beta1 gene, to determine their individual and combined contributions to the genetic predisposition to nephropathy, in individuals with type 1 diabetes. Genes further up- and downstream of the TGF-beta1 gene also need to be investigated.

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Association of a functional 17β-estradiol sensitive *IL6-174G/C* promoter polymorphism with early onset Type 1 diabetes in females.
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Background and Aims: The Interleukin-6 promoter single nucleotide polymorphism (SNP) *IL6-174G/C* is a candidate gene variation in Type 1 diabetes mellitus (T1DM) and *IL6* transcription is affected by 17-β-estradiol (E_2). The aims of this study were to investigate 1) the *IL6-174G/C* SNP for linkage and association to T1DM, and 2) the impact of E_2 on the promoter activity of *IL6-174G/C* variants.

Materials and Methods: Two-hundred fifty-three Danish T1DM families were genotyped for the SNP. Linkage and association was investigated by transmission disequilibrium testing (TDT). The effect of E_2 on the SNP alleles was investigated in reporter-assays (± E_2 and ± PMA stimulation, n=5 experiments).
Results: *Linkage and association study:* Increased (56±5%) *IL6-174C* transmission was observed in the 416 T1DM offspring, P_{tdt}=0.04. However, increased *IL6-174C* transmission was found exclusively the 200 T1DM females (63±7%) and the 168 female index-cases (66±8%); P_{tdt}=0.00065 and P_{tdt}=0.00024, respectively. Random segregation was found in T1DM males, and unaffected males and females. Heterogeneity analyses excluded preferential meiotic segregation in females, P=0.0046 (affected *vs.* unaffected) and demonstrated differences in the transmission patterns between female and male T1DM offspring, P=0.005. Finally, the *IL6-174G/C* genotypes affected the age at onset of T1DM in females in an allele-dose dependent manner; CC<GC<GG; P_{trend}=0.02. *Reporter-assay study:* The PMA stimulated activity of the T1DM risk *IL6-174C* variant exceeded that of the T1DM protective *IL6-174G* variant by approximately 70% without E_2 present (P_c=0.004), but not with E_2 present. The PMA stimulated activity of the *IL6-174G* variant was repressed without E_2 present, but was derepressed by addition of E_2 (P_c=0.024), whereas the stimulated *IL6-174C* activity was unaffected by E_2.
Conclusion: The *IL6-174C* variant associates with T1DM in young females. The higher *IL6-174C* promoter activity may confer risk to T1DM in young females. This risk is negated with increasing age, possibly by increasing E_2 levels in puberty.

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Genotypic and phenotypic differences between Arabian and Scandinavian women with gestational diabetes mellitus (GDM).
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Background and Aims: GDM is a heterogeneous disorder characterized by both impaired insulin secretion and action. There are large ethnic differences in the frequency of GDM; i.e. the frequency is higher in Arabian than in Scandinavian women. The aim was to study whether differences in diabetes-associated risk genotypes explain differences in frequency and phenotypes between Arabian and Scandinavian women with GDM.
Material and Methods: 500 unrelated GDM women (100 Arabian and 400 Scandinavian) and 550 unrelated pregnant non-diabetic controls (122 Arabian and 428 Scandinavian) matched for ethnicity were screened for HLA_DQB1 genotypes, insulin gene VNTR, PPARγ2-Pro12Ala polymorphism and GAD antibodies.
Results: The frequency of HLA-DQB1*0201/0302, *02/X and *0302/X [x excludes 0602(3)] was higher in Scandinavian GDM than controls (46.2% vs. 38,7%, p =0.031) but no significant difference was seen between Arabian GDM and controls (46.7 % vs. 51.7 %, p=0.47). Scandinavian GDM women had higher frequency of GAD antibodies than controls (5.9% vs. 2.4%, p=0.043) but no difference was seen between Arabian GDM and controls (2.9% vs. 1.4%, p=0.57). There was no significant difference in genotype frequencies of the insulin gene VNTR and the Pro12Ala variant of the PPARγ gene between GDM and controls, neither Arabian nor Scandinavian. However, the Arabian women were almost twice as insulin resistant for the same BMI as the Scandinavian women (HOMA_IR; 2.8 ± 0.3 vs. 1.9 ± 0.1, p=0.018). Higher degree of insulin resistance was associated with a lower frequency of the insulin-sensitivity Ala allele of Pro12Ala in Arabian than in Scandinavian GDM (4.5% vs. 14.6%, p=0.0001).
Conclusion: Higher frequency of type-1 diabetes associated genotypes and GAD antibodies characterize Scandinavian women with GDM whereas insulin resistance and low frequency of the protective Ala 12 allele of PPARγ characterize Arabian women with GDM.

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A positive correlation between enterovirus infections and antibodies against GAD65 in siblings to newly diagnosed T1D.
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Background and Aims: Enterovirus (EV) infections have been associated with the manifestation of type 1 diabetes (T1D) in a number of reports. Recent prospective studies have suggested that EV infections initiate the autoimmune process. Variation in virulence and replication pattern between strains of a serotype has also been shown. The aim was to study if there were specific CBV strains that more often were associated with the T1D children than with controls and/or siblings and to analyse if there was any time-relationship between such infections and the appearance of antibodies against GAD65 among the siblings.
Materials and Methods: In the present study we have analysed serum from newly diagnosed T1D children, their siblings and matched controls with regard to neutralizing antibodies against different strains of CBV. Analyses for presence of antibodies against GAD65 in the same groups were also performed
Results: Newly diagnosed T1D children revealed higher titres of neutralizing antibodies against a strain of CBV-4 that has been shown to cause a persistent infection in human pancreatic islet cells. The T1D child and its sibling often encounter the same infection. Among the former 16 of 27 (59%) had a significant rise in neutralizing antibodies, the corresponding figure for the siblings were 10/13 (77%). Eight of the T1D children had such a rise against a recombinant strain, V89 4557 and only one had a titre rise against the prototype strain, JVB. In total 34/46 (72%) of the T1D revealed antibodies against GAD65, among the siblings these antibodies were detected in 5/38 (13%). All of the siblings with such antibodies also revealed a significant rise in neutralizing titre against a CBV strain, i.e a correlation between proven EV infection and the appearance of antibodies against GAD65 was found.
Conclusion: Our results demonstrate that newly diagnosed T1D have higher titres of neutralizing antibodies against certain strains of a serotype, that siblings to the T1D child often encounter the same infection, and finally, that in the latter there seems to be a correlation between the CBV infection and the appearance of antibodies against GAD65.

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Activity of antiviral enzyme 2´5´-oligoadenylate synthetase (2´5´AS) is increased in preclinical Type 1 diabetes.
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Background and Aims: Type 1 diabetes (T1D) results from autoimmune destruction of insulin-producing β-cells, a process that may be triggered by viral infection. 2´5´-oligoadenylate synthetase (2´5´AS) is a key enzyme in the antiviral immune defense system, activating RNAseL which degrades viral and cellular RNA. In previous studies, we have shown that activity of 2´5´AS is significantly higher in patients with T1D compared to control subjects (Bonnevie-Nielsen et al. 2001). These results suggest that high 2´5´AS activity could be relevant to the etiology and/or pathogenesis of T1D. For example, high enzyme activity might reflect persistent or repeated viral infection, or a dysregulated antiviral immune system. In either case, high 2´5´AS activity could induce apoptosis in some β-cells, leading to exposure of sequestered antigens and initiation of autoimmune β-cell destruction. In order to determine if this heightened 2´5´AS activity occurs before onset of diabetes, we studied children with high-risk HLA types who have been followed prospectively since birth for development of diabetes-related autoantibodies (Finnish DIPP Study).
Materials and Methods: 2´5´AS activity was determined in peripheral blood lymphocyte lysates by quantitating the enzyme products (2´5´-

oligoadenylates) in a radiometric assay using radiolabelled ATP. The subjects were 29 children seropositive for autoantibodies (very high risk of future diabetes) and 25 seronegative HLA-matched control children. All seropositive children had islet cell antibodies (ICA) and the majority (76%) had additional diabetes-related autoantibodies (GAD, IA-2, IAA).
Results: Results showed that seropositive children had significantly higher 2´5´AS activity compared to seronegative children (p=0.027).
Conclusion: This indicates that children at high risk of developing T1D (based on HLA type and the presence of autoantibodies) show elevated 2´5´AS activity, and by inference children with T1D develop their increased 2´5´AS activity prior to onset of clinical diabetes. It remains to be determined when during the preclinical period the 2´5´AS activity actually becomes elevated. Additional studies will determine the relationship of heightened 2´5´AS activity to appearance of autoantibodies and to viral infections. In summary, this study provides further support for our previous hypothesis that high 2´5´AS activity is associated with increased risk for developing Type 1 diabetes. If future studies demonstrate that high 2´5´AS activity is associated with increased risk of producing diabetes-related autoimmune antibodies, 2´5´AS activity could become a useful early predictive marker for Type 1 diabetes.

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Pleconaril inhibition of Coxsackie B virus replication in human pancreatic islets.
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Background and Aims: Coxsackie B viruses (CVB) and other enteroviruses have been implicated as environmental factors in type 1 diabetes (T1D) with evidence of infection both before and at diagnosis. CVB are able to infect human β-cells in vitro and depending on the virus strain, can cause islet degeneration or a persistent infection with possible long term consequences for the β-cells. If viruses are truly a cause of T1D, an inhibition of viral replication in T1D patients shortly after diagnosis, might be a means of saving the remaining β-cells from destruction. Our aim was to study the ability of Pleconaril, a new antiviral drug against enteroviruses, to inhibit the replication of two CVB strains in human islets in culture and to study the effects of the drug on β-cell function.
Materials and Methods: Two well-characterised CVB strains were used: one CVB4 strain, VD2921, that has been shown to establish a persistent infection in human islets and one CVB4/5 recombinant strain, V89-4557, previously shown to cause human islet degeneration. Human islets, isolated from heart-beating organ donors, were cultured 50/well in six-well plates in 3 ml RPMI1640, 5.5 mM glucose, 10% foetal bovine serum. Virus replication was studied by tissue culture infectious dose-50 (TCID-50) titrations of samples from the culture medium taken on days 0, 1, 3, 4 and 6. Pleconaril, (to a final concentration of 10 mM), was either preincubated with the virus before addition to the cells or added to the medium 30 minutes after the virus, to allow some time for virus attachment to receptors. Islets were also studied in a microscope for signs of virus-induced cytopathic effects (CPE), graded as 0-4+ where 4+ means total degradation of the islets. The function of the β-cells was studied by measuring their ability to secrete insulin into the culture medium during a two-hour stimulation with 16.5 mM glucose on day 3 post infection.
Results: Both CVB strains replicated in the human islets. Mean TCID-50 titres, day 0 and day 6 were 10^1.3 and 10^4.1 (VD2921, n=4) and 10^2.7 and 10^4.3 (V89-4557, n=10).
Preincubation with Pleconaril blocked replication of V89-4557 up to day 3 in 7/10 cases and up to day 6 in 3/10 cases. In the remaining 7/10 the replication was reduced day 3–6. When added post infection, Pleconaril inhibited V89-4557 replication up to day 3 in 10/10 experiments, but without addition of Pleconaril again on day 3 after the medium change, the virus resumed replication day 3–6 in 3/4 experiments. In 3/6 cases where Pleconaril was added again on day 3, V89-4557 replication day 3–6 was inhibited completely. VD2921 replication was completely inhibited in 4/4 experiments where virus was preincubated with Pleconaril and 4/4 experiments where Pleconaril was added post infection. CPE did not seem different between groups and insulin release in response to high glucose (n=3-6) did not differ significantly between any of the groups.
Conclusion: There seem to be virus strain differences in the sensitivity to Pleconaril treatment. Pleconaril clearly inhibited the replication of the CVB4 strain VD2921, whereas the replication of CVB4/5 strain V89-4557 was in most experiments lowered but not completely inhibited.

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Investigation of risk factors associated with different HLA genotypes of susceptibility to Type 1 diabetes (The DIABFIN Project).
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Background and Aims: The DIABFIN project is the first Italian study aimed at predicting type 1 diabetes (T1D) risk in the general population by HLA typing at birth. The present study aims to correlate the different class II HLA genotypes, categorized by their effect on T1D susceptibility, with a number of associated risk factors occurring during pregnancy and the neonatal period.
Materials and Methods: In this study, cord blood from 4869 babies born in Milan, Genoa and Rome was collected with the intent of HLA-typing for the identification of subjects at high (DRB1*03/DRB1*04-DQB1*0302), moderate and low genetic risk for type 1 diabetes. Of the 4855 newborns who were HLA-typed, 0.91% were at high risk, 13.8 % at moderate risk, and 85.3 % at low risk. Data was collected for 41 risk factors regarding mother's pregnancy, type of delivery, characteristics at birth and family of origin of the newborns with special emphasis on the history for autoimmune diseases.
Results: There was equal division between genders in the moderate and low risk groups, with male excess in the high risk group (61.9% vs. 38.1%). Risk factors were analyzed for correlation with respect to the HLA risk genotypes. When adjusted for sex, mother's age, drug usage during pregnancy and number of siblings, a significant association between HLA risk categories and length of gestation was observed (high risk = 271.24 days; moderate risk = 273.27 days; low risk = 274.60 days; $p<0.03$). This association was significant only considering the lowest quartile of gestation (days) of each HLA risk category and was not observed for higher quartiles.
Conclusion: In the Italian population, the length of gestation is associated with HLA risk categories for T1D. The shortest length of gestation recorded in the high risk HLA category might have identified those neonates at the highest risk of developing T1D.

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Differentiation of risk for Type 1 diabetes in probands with single autoantibodies from the general population.
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Background and Aims: As already shown for autoantibody (AAb) positive first degree relatives of patients, the risk to develop type 1 diabetes (T1D) is also increased in probands from the general population when multiple AAbs against different beta cells antigens occur. More than 80% of them have the T1D associated HLA-DRB1 and -DQB1 risk markers, while the protective haplotypes are absent. The study was aimed to estimate the risk in probands with single AAbs from the general population by differentiating them regarding to AAb specificity, titer and occurrence of distinct HLA markers.
Materials and Methods: By combined AAb testing a general population of 6,337 healthy schoolchildren without T1D heredity, 147 probands with single AAb positivity were identified. AAbs against GAD65 (GADA), protein tyrosine phosphatase (IA-2A), insulin (IAA) were detected by 125I-antigen binding assays ≥99. percentile, and AAbs against islet cell antigens (ICA) immunohistochemically ≥20 JDF units. HLA-DRB1 and -DQB1 specificities were defined of the 147 AAb positive children and compared to those of 339 AAb negative controls as well as of 274 patients with T1D.
Results: In AAb positive children, GADA occur significantly more frequent (44.2%; $p<0.01$) compared to IA-2A (23.1%), IAA (8.8%) and

ICA (23.8%). Furthermore, the T1D associated DQB1 alleles *0302 and/or *02 occur most frequently, although also 18.5% (12/65) of children with GADA and 28.6% (10/35) with ICA bore the protective allele DQB1*0602. In contrast, only one proband with IA-2A and one child with IAA has the protective allele. If AAb positive children were stratified regarding to AAb titer (≥99.0 and < 99.9 percentile (n=138), their genetic markers did not differ from those of AAb negative controls. However, probands with single AAbs at high titers ≥99.9 percentile (n=9) reflect the same positive and negative genetic association as the patients with T1D. In this group the risk was enhanced for homozygosity for DQB1*0302 and DRB1*03 (odds ratios; OR=14.0) as well as for heterozygosity for DQB1*0302/*02 and DRB1*04 homozygosity (OR=10.48). None of the probands with high titer single AAbs has the protective haplotype DRB1*15 or DQB1*0602.
Conclusion: Single AAbs below the 99.9 percentile, especially GADA and ICA, are not associated with T1D. Only probands with single AAbs at high titers recruited from the general population have the same genetic predisposition as patients with T1D and are therefore at increased risk for the disease.

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Induction of diabetes-related autoantibodies below cut-off for positivity in young non-diabetic children.
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Background and Aims: Information about the natural fluctuation of beta-cell autoantibodies in healthy children is scarce. The aim was to study the natural course of diabetes related auto-antibodies at low concentrations, below the cut off for positivity used for prediction of type 1 diabetes, in a non-diabetic population followed from infancy.
Materials and Methods: Blood samples were taken from 205 children at 6 weeks, 6 and 18 months and at 5 years of age. Autoantibodies against GAD65 (GADA), tyrosine phosphatase (IA-2A) were determined by radioligand binding assays.
Results: None of the 205 children had IA-2A above the cut-off for positivity used for prediction of type 1 diabetes. One had GADA above the cut-off for positivity at 6 weeks of age, four at 6 months of age, two at 18 months of age and seven at 5 years of age. All children had detectable levels of GADA and about half had IA-2A during the follow-up period. Many children developed IA2-A already at 6 months of age, similar concentrations were seen at 18 months, and then a decrease in the levels of IA-2A occurred until 5 years of age ($p<0.001$). GADA were less often induced at 6 months of age, increased up to 18 months ($p<0.001$) and fluctuated at similar levels up to 5 years of age.
Conclusion: We conclude that there is a natural induction of humoral immune response to beta-cell autoantigens early in life. Our results suggest that the mechanisms of B-cell tolerance to GAD and IA-2 differ in healthy children, so that after induction of autoantibodies the levels of IA-2A decreased whereas the levels of GADA increased during the first 5 years of life.

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Evaluation of a new method for quantification of insulin autoantibodies.
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Background and Aims: Among predictive autoantibody markers associated with type 1 diabetes (T1D), insulin autoantibodies (IAA) are usually the first markers to appear and they are present in the vast majority of young children predestinated to develop T1D. Most laboratories measuring IAA use various modifications of liquid-phase radio-binding assays (RBA). These methods are relatively expensive and labour intensive, using reagents that are unstable and environmentally inappropriate. The objective of this study was to evaluate a new, time-resolved fluorescence (TRF) immunoassay for the measurement of IAA.
Materials and Methods: The evaluated DELFIA IAA assay uses a two-phase approach: (i) in the first phase, the sample is incubated with europium (Eu) labelled insulin at +4ºC for 16-20 hours, after which (ii) the IgG molecules are captured using Protein A Sepharose beads (+4ºC for $^1/_2$ hour). Finally, the plate is washed repeatedly in vacuum and the captured immunocomplexes are measured using TRF- technology. Results are derived from a standard curve constructed from serial dilutions of a rabbit polyclonal anti-human Insulin IgG antibody.
IAA status was assessed in 232 subjects of whom 75 were subjects with newly diagnosed T1D (mean age 17.9 years, range 1.6 – 61.9 years), 78 were prediabetic subjects (periodical samples, the last sample on the day of clinical diagnosis of T1D; mean age 8.5 years, range 3.0 – 18.3 years) and 75 IAA-negative control subjects (mean age 11.3 years, range 9.2 – 14.4 years). Also, the IAA status of 100 subjects (50 diabetics and 50 non-diabetic siblings of children with T1D; mean age 8.7 years, range 0.7 – 20.5 years) were assessed in a double-blind manner. The results were compared to the results obtained with the *in-house* RBA.
Results: Performance characteristics of the DELFIA IAA assay; The calibration curve was linear over the whole standard range (0 – 50 µg/mL, $r>0.9991$, n=8). The average intra-assay coefficient of variation (CV) was 15.2 % at concentrations between 0-5µg/mL, but at the higher concentrations (10-50µg/mL) the precision improved to 6.8%. The analytical sensitivity of the assay was generally better than 1.3 µg/mL (mean + 2SD). The preliminary cut-off value 5.2 (90% confidence interval: 3.4-6.0) µg/mL (n=164, 97.5th percentile) was used in the comparison studies.
The DELFIA IAA assay gives good agreement in terms of clinical sensitivity and specificity with the RBA: among T1D subjects, DELFIA IAA found 31 IAA-positive samples (RBA-positive n=25); among prediabetics, DELFIA IAA found 20 IAA-positive samples (RBA-positive n=28); and in relation to the double-blinded samples, DELFIA IAA detected 17 IAA-positive samples among subjects with T1D (RBA-positive n=13) and 15 IAA-positives among the siblings of affected children (RBA-positive n=0). Some conspicuously high IAA-values were observed in a few control samples (tested negative with RBA).
Conclusion: The DELFIA IAA assay offers a reliable, relatively rapid, non-isotopic alternative to radio-IAA methods. The assay requires comparatively low sample volume and is simple enough to allow screening of large numbers of samples.

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Does genetic screening for Type 1 diabetes in newborns affect mothers and fathers in the same way?
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Background and Aims: Screening for type 1 diabetes (T1DM) risk in newborns has shown little negative emotional impact on most mothers, but father's reactions have not been studied. To support vulnerable parents or oppose negative reactions mothers' as well as fathers' opinions and reactions should be identified. This study aims to investigate differences in mothers' and fathers' reactions to participation.
Materials and Methods: All parents with a newborn child in the county of Skåne, Sweden are invited to a prospective screening study of risk for development of T1DM in the child (The DiPiS project - Diabetes Prediction in Skåne). During pregnancy parents are informed about DiPiS. At delivery blood are obtained from the mother and cord blood from the child. The cord blood is analyzed for HLA and autoantibodies (GAD, IA2, IAA). Two months after delivery parents give written consent to participation, fill out a psychosocial and a hereditary questionnaire on diabetes. Parents are not informed about their child's risk status.
Results: During the first year of study 10 854 mother/child blood samples were collected and 6909 (63.6%) of the parents agreed to participate. The invitation was not answered by 2959 parents (27.3%), 828 (7.6%) denied participation and 158 (1.5%) were excluded. The psychosocial questionnaire was filled out by 6773 (62.4%) mothers. Seven questions in the psychosocial questionnaire were addressed to and answered by 6178 (92%) fathers. A vast majority of parents were satisfied with the information about DiPiS, but significantly more fathers than mothers (12.5 vs 6.5%; $p<0.001$) were dissatisfied. Most parents knew about diabetes before joining DiPiS, although more fathers than mothers (8.9 vs 5.3%; $p<0.001$) reported they didn't. Fathers more often estimated (41.8 vs 34.3%; $p<0.001$) that their child had a risk of getting diabetes. A majority of parents (70.2% mothers, 69.5% fathers) were not at all affected by participation in DiPiS. Sixtyeight of 6628 mothers (1%) and 73/6069 fathers (1.2%) stated they felt anxious by participating, but 1909 (28.8%) mothers and 1778 (29.2%) fathers felt reassured. Mothers and fathers often agreed in their reactions with 73.7% answering in the same way. Anxious mothers and fathers were often born abroad. When „thinking of the possibility that the child could get a chronic disease in the future" the same proportion of mothers and fathers (42% and 41.6%) answered they were not affected by such a thought, but more mothers than fathers (29.8% vs 23.4%;

p< 0.001) felt worried, particularly single mothers and mothers expressing lack of support.
Conclusions: The enrollment of newborns in a screening study for T1DM risk did not affect most parents. A quarter of both mothers and fathers felt reassured. Conclusive differences between mothers and fathers were not observed, but information and attention must be directed to parents born abroad. Results must be interpreted with great caution as this part of DiPiS interfered little with the family's life, contained no invasive procedure on the child and did not give information of possible risk status - factors that could affect anxiety in the parents.

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T-cell function in latent autoimmune diabetes in adults.
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Background and Aims: We have recently reported that in patients with anti-glutamic acid decarboxylase (GAD) 65+ diabetes with residual beta-cell function (=latent autoimmune diabetes in adults; LADA), most with a „high-titer" (over 10 U/ml) required insulin within 5 years, whereas most with a „low-titer" (1.3-9.9 U/ml) did not need insulin for over 15-20 years after the onset. We therefore examined T-cell function in LADA to evaluate the difference between the „high-titer" and „low-titer" groups.
Materials and Methods: Blood samples were obtained from the enrolled subjects with informed consent, and cytokine production upon polyclonal activation, the serum level of interferon-inducible protein-10 (IP-10), and numbers of GAD65-reactive CD4+ cells in the periphery were examined.
Results: Interleukin (IL)-10 production upon polyclonal activation was significantly lower in the „high-titer" group than in the "low-titer" group. The serum level of IP-10 was higher in the „high-titer" than the "low-titer" group. Although GAD65-reactive CD4+ cells in the periphery were detected in both groups, a significant positive correlation between serum IP-10 level and the number of GAD65-reactive CD4+ cells was observed only in the „high-titer" group. Therefore, it has been speculated that the "co-existence" of GAD65-reactive IFN-gamma-producing CD4+ cells and a high serum IP-10 level may be important for rapid disease progression as seen in the "high-titer" group.
Conclusion: Based upon these results, T-cell function is considered to be different between the „high-titer" and „low-titer" groups in LADA, supporting our previous findings regarding the clinical outcome of insulin-dependence in the two groups.

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Multi-center prevention trial of slowly progressive Type 1 diabetes with small dose of insulin (The Tokyo study)-Sixth report.
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Background and Aims: A recent preliminary study demonstrated a preventive effect of a small dose of insulin on progressive beta cell dysfunction in islet cell antibody (ICA)-positive patients initially diagnosed as having type 2 diabetes (LADA or slowly progressive type 1 diabetes). We have designed a randomized multi-center study (the Tokyo Study) with larger patient population in non-insulin-requiring stage of slowly progressive type 1 diabetes. In this congress, we present the results of up to 60 month follow-up of the Tokyo study.
Subjects and Methods: Patients were selected as previously described (Kobayashi T et al. Ann. N. Y. Acad. Sci., vol 958). Fifty-eight GADA-positive patients were randomly divided into 2 groups: one group received insulin (Ins G, n=28), and the other received sulfonylurea (SU G, n=30). All patients underwent a 75g oral glucose test (O-GTT) every 6-12 months. The insulin-dependent stage was defined based on an integrated value of serum C-peptide levels on O-GTT (sigma CPR ; sum of CPR at 0, 30, 60, 90, 120 min) falling below 4.0 ng/mL.
Results: Sigma CPR value in SU group decreased progressively from 21.9±10.5 to 14.9±9.8 ng/ml after follow-up period($p < 0.05$ vs baseline). The sigma CPR value in insulin group remained unchanged(20.4±15.4 vs 17.8±16.2). Among SU group, 30% (9/30) of subjects progressed to IDDM stage, while 10.7% (3/28) of subjects in insulin group progressed to IDDM stage ($p = 0.07$). With regard to the subjects who had preserved C-peptide response at recuruitment (sigma CPR > 10ng/ml), proportion of SU group progressed to IDDM stage was significantly higher than that of insulin group (7/28, 25% vs 0/25, 0%, $p<0.01$). All patients who progressed to IDDM stage showed higher GADA titer (over 10 U/ml).
Conclusion: It was suggested that small dose of insulin treatment is effective to prevent beta cell failure in slowly progressive type 1 diabetes, especially in the patients who initially have preserved insulin responce. We recommend avoiding SU treatment and instead administering insulin to LADA patients with higher GADA titer.

PS 4

Type 1 Diabetes Mellitus and Mortality

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Left ventricular hypertrophy in Type 1 diabetes: prevalence and relation to coronary heart disease and cardiovascular risk factors. The EURODIAB IDDM Complications Study Group.

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Background and Aims: An excess mortality has been reported in subjects with left ventricular hypertrophy (LVH). LVH prevalence and association with cardiovascular events have been described in the general population, whereas there is a lack of such information in type 1 diabetes, with the exception of patients selected for the presence of albuminuria. We evaluated the prevalence of LVH in the EURODIAB cohort of type 1 diabetic patients and the relation of LVH to coronary heart disease (CHD), cardiovascular risk factors and chronic diabetic complications.
Materials and Methods: The EURODIAB population consisted of 3250 type 1 diabetic patients attending 31 centres in 16 European countries. LVH was defined by ECG Cornell voltage-duration product (RaVL + SV3 x QRS duration) >2623 mm.ms in men and >1558.7 mm.ms in women.
Results: Prevalence of LVH in the whole population was 3.4%, higher in females (4.6%) than in males (2.3%) (p=0.001). Subjects with LVH had higher systolic (p=0.001) and diastolic (p=0.02) blood pressure, triglycerides (p=0.01) and QT interval duration (QTc) (p=0.0001) than subjects without LVH. Prevalence of LVH was significantly higher in subjects with CHD (p=0.001), hypertension (p=0.011), diabetic nephropathy (p=0.027), QTc>0.44 s (p=0.001) and distal symmetrical polineuropathy (p=0.044). Standardised regression showed a significant relation between Cornell voltage-duration product and age (p=0.0001), diabetes duration (p=0.0001), body mass index (BMI) (p=0.0001), waist to hip ratio (WHR) (p=0.0001), insulin dose (p=0.0001), systolic and diastolic blood pressure (p=0.0001), total- (p=0.007), LDL- (p=0.046) and HDL-cholesterol (p=0.0002), triglycerides (p=0.0016) and albumin excretion rate (AER) (p=0.0001); no relation was observed with HbA1c, QTc and QTd. Cornell voltage-duration product was positively associated with physical activity (p=0.0001). In multivariate analysis by sex, Cornell voltage-duration product persisted significantly associated with age (p=0.04), BMI (p=0.0001), WHR (p=0.007), HDL-cholesterol (p=0.02) and CHD (p=0.0001) in men, and with age (p=0.03), systolic blood pressure (p=0.0006), moderate (p=0.02) and vigorous (p=0.05) physical activity, BMI (p=0.0001), AER (p=0.03), QTc>0.44 s (p=0.002) and CHD (p=0.004) in women.
Conclusions: Prevalence of LVH in type 1 diabetic subjects was 3.4% and was higher in females than in males. LVH was associated with CHD and cardiovascular risk factors and could contribute to the increased cardiac morbidity and mortality in type 1 diabetic patients. Prospective studies are needed to assess the predictive value of LVH with respect to mortality and cardiovascular events in type 1 diabetic patients.

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The association between birth weight and survival in persons with diabetes.

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Background and Aims: Several studies reveal that low birth weight (BW) babies are at increased risk of both adult morbidity, including obesity and type 2 diabetes mellitus (DM2), and mortality. Studies from the Pima Indians and ours from Rochester, MN, also suggest high BW is predictive of DM2; in both populations, higher body mass index (BMI) in adults with DM2 is predictive of mortality. The association between BW and mortality in persons with DM2 is unknown.
Materials and Methods: Using the longitudinal, population-based resources of the Rochester Epidemiology Project, we previously identified all individuals born locally who, based on retrospective record review, first met National Diabetes Data Group criteria on or after age 20 years as a Rochester resident from 1945-1995. Birth weights were available for 220 term singleton births. Thirty-eight likely type 1 DM cases (BMI <30 as of the date they first met NDDG criteria and on insulin within 1 year of that date and at last follow-up) and 8 cases with incomplete information were excluded from the analysis. The remaining 174 cases were followed through 12/31/2000 for vital status and date of death. Cox proportional hazards, adjusted for age and sex, was used to estimate the contribution of BW to survival. BW was entered both as a continuous variable and as a catgorical variable, with 6.5-8.4 lbs (2.9-3.8 kg) as the reference value.
Results: The 174 cases [53% male; mean BW = 7.4 ± 1.1 lbs (3.4 ± 0.5 kg); mean age as of the date NDDG criteria were first met = 44 ± 11 years] were followed for a median of 10.5 person-years, during which time there were 30 deaths, double the 15 expected (p<0.001) based on rates for the White North Central population of similar age, sex, and year of birth. When BW was entered as a continuous variable, there was a significant inverse association between BW and mortality; the relative hazard (RH) was 0.69 (95% CI = 0.49-0.97, p = 0.03). When entered as a categorical variable, the RH for low compared to normal BW subjects was 2.39 (95% CI = 1.01-5.66, p = 0.047); the RH for high compared to normal BW subjects was 1.59 (95% CI = 0.62-4.05, p = 0.33).
Conclusion: Low BW is not only associated with increased risk of DM2, it is also associated with a marked increased risk of death among persons with DM2. There was a slight but not statistically significant increased risk of death among high BW subjects with DM2 that merits further investigation.

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Mortality in Type 1 diabetes patients with onset before age 40 years.

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Background and Aims: 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 before 40 yrs.
Materials and Methods: We designed a retrospective study of T1DM patients with onset before 40 yrs., registered at Bucharest Diabetes Center and deceased between 1942 and 1995. We analyzed 789 cases, 447 (56.65%) males and 342 (43.35%) females. For each patient the age at diabetes onset, disease duration, cause of death and sex were recorded. Statistical analysis was made using Student's t test and ANOVA.
Results: The mean age at onset was 31.31±8.43 yrs, at death 51.44±15.17 yrs. and the mean survival period was 20.13±11.58 yrs. Comparing 1942-1950 with 1990-1995 period, we found a statistical significant increase of survival period from 2.97±2.07 yrs to 30.04±11.59 yrs (p<0.0001) and of life expectancy from 31.14±9.24 yrs to 60.35±14.63 yrs (p<0.0001). There is no statistical significant difference for the age at onset (28.16±8.53 yrs vs. 30.3±8.51 yrs, p=0.181). Comparing the two sexes, we found a statistical significant difference for survival period i.e. 20.96±11.59 yrs (M) vs. 19.04±11.51 yrs (F), p=0.02 and for life expectancy i.e. 52.7±14.9 yrs (M) vs. 49.81±15.39 yrs (F), p=0.008. There is no statistically significant difference for age at onset (31.73±8.03 yrs (M) vs. 30.76±8.9 yrs (F), p=0.11). The major causes of death during the 1990-1995 period were: cardiovascular diseases (37.33%), cerebral vascular diseases (17.33%), chronic renal failure (10.67%), malignancies (10.67%), gastrointestinal and hepatic diseases (6.67%), infectious diseases (4%), acute diabetic complications (2.67%) and others (10.66%). The percentage of cardiovascular and cerebral vascular diseases related deaths rose 5.3 times, chronic renal failure related deaths rose 5 times and gastrointestinal and hepatic diseases rose 3 times from 1942 to 1995, while infectious diseases decreased 7.3 times during the same period.
Conclusions: Comparing 1942-1950 with 1990-1995 period, we found a statistical significant increase of survival period and of life expectancy, while the age at onset remained the same. Females have a significantly lower age at death then males explained by a lower survival period with diabetes, the age at onset being the same for both sexes. The percentage of cardiovascular and cerebral vascular diseases related deaths rose 5.3 times, chronic renal failure related deaths rose 5 times and gastrointestinal and hepatic diseases rose 3 times from 1942 to 1995, while infectious diseases decreased 7.3 times during the same period.

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Higher relative mortality in females with type 1 diabetes mellitus compared to men – a cohort study of persons with childhood onset Type 1 diabetes.

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Background and Aims: The higher premature mortality in persons with type 1 diabetes is known. A previous study in Norway found a doubled relative mortality compared to the background population, but no significant sex difference. The aims of the study were to study the mortality trend in the same cohort during a 12year period and compare the results to studies from Norway and other countries.
Materials and Methods: The cohort includes all individuals' diagnoses 1973-1982 in Norway and age at onset below 15 years, a total of 1914 subjects, 1039 males and 875 females. Average observation time was 23 years. Their mortality status was determined as of 31.12.2000 by linking a file from the National Diabetes Registry to census data in Statitics, Norway and cause of death was also recorded. Comparable mortality data for the background population were obtained from Statistic Norway, by calculating the observed mortality rate in 9 year old persons during 23 year observation period in Norway.
Results: Out of the 1914 persons included in the cohort, 86 (4,5 %) were deceased during the observation period, 50 males (4,9 %) and 36 females (4,3%). No significant difference in mortality was found by gender or by age at onset.
However, the relative mortality was significant higher in females (RR=6,4; CI 4,5 – 8,4) compared to males (RR=2,7; CI 2,0 – 3,4).
Conclusions: In a national cohort of subjects with child-onset type 1 diabetes, approx. 4,5 % deceased during a 23 years observation period. The lower female mortality in the background population is outweighed by type 1 diabetes and the relative mortality is doubled in females compared to men after 23 years mean observation time. However, the life expectancy for subjects with type 1 diabetes diagnosed in the persons seems to be more favourable compared to previous studies.

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Temporal trends in the cumulative incidence of late Type 1 diabetes complications: Pittsburgh Epidemiology of Diabetes Complications Study 1950-2000.

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Background and Aims: To determine whether recent (post 1980) improvements in diabetes management may have led to reduced complication rates, three cohorts of childhood onset type 1 diabetic subjects diagnosed in the 1950's, 1960's, and 1970's were studied.
Materials and Methods: All (n =658) were participants in the Pittsburgh Epidemiology of Diabetes Complication Study and have been followed since 1986-1988 with biennial exams (up to 1996-1998) and annual surveys. Those dying before 1986 (n = 145), have also been included in the mortality data, and where indicated with the complication data. Follow up was censored at 2000 with durations of 20 and 25 years studied for all cohorts.
Results: Mortality at both 20 and 25 years declined significantly among all cohorts (diabetes duration 20 years: 21%-12%-1% and 25 years 35%, 22%, 5%, 1950's to 1970's respectively, p<0.0001). A similar pattern was observed for End Stage Renal Disease (ESRD): 20 years: 14%-9%-1% and 25 years 24%-13%-6%, 1950's-1970's respectively, p<0.0001). Interestingly, for Coronary Artery Disease (CAD death or non-fatal MI only), there was a trend at 20 years for lower rates by diagnosis cohort (1.5%, 3.6%, 6.4%, 1950's-1970's p=0.051), however this trend was no longer observed at 25 years (8.7%-14.5%-12.4%, 1950's-1970's p=0.26).
Conclusions: These results suggest (1) more recently diagnosed with type 1 diabetes subjects had a larger duration before dying or developing ESRD and (2) rates have not decreased by diagnosis cohort for CAD cumulative incidence, which merits further exploration.

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Epidemiology of Type 1 Diabetes Mellitus

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Incidence of Type 1 diabetes mellitus in La Palma Island (1993-2002).

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Background and Aim: The aim of this study was to ascertain the incidence of Type 1 diabetes mellitus in the 0-29 yr-old group in La Palma island (the most northwest of Canary Islands, Spain: 730 Km^2, upper 81000 hab and subtropical climate).
Subjects and Methods: All subjects younger than 30 yr with Type 1 diabetes (according WHO 1985 and/or ADA 1997 criteria) diagnosed between january 1993 and december 2002 (prospectively 1995-2002) were included. All subjects were resident in La Palma island at least 6 months prior to diagnosis of Type 1 diabetes. All the reported cases were on insulin treatment. The population at risk (0-29 yr) fluctued between 36419 hab -1991 General Census- (15711 hab in the 0-14 age group) and 32271 hab -2000 General Census- (12752 hab in the 0-14 age group). Using the capture-recapture method (primary source was hospital records, while secondary sources was membership files of La Palma Diabetic Association and Primary Care Physicians), the ascertainment was 100 %. The incidence rates were expressed as number of cases per 10^5 hab per year. The 95% Confidence Intervals were estimated assuming the Poisson distribution of the cases. The age adjustment for the rates was done using the direct method with a World and European Standard Population.
Results: Sixty eigth subjects younger than 30 yr had presented Type 1 diabetes at the last 10 yr (43 male, 25 female; sex ratio 1.7; medium age: 12.9±7.6 yr (95% CI: 12-13.8). In 8 cases (11.8%) Type 1 diabetes was present in another family member (brothers, but not twins). The annual incidence fluctued between 5.5 and 27.9/10^5, resulting the overall annual incidence 19.9/10^5 (95% CI: 15.1-24.5), being 32.2/10^5 (95% CI: 22.7-41.7) in the 0-14 age group, and 11.7/10^5 (95% CI: 7-16.3) in the 15-29 age group. The incidence in males, 24.4/10^5 (95% CI: 17.1-31.7), was higher than in females, 15/10^5 (95% CI: 9.1-20.9). The age-adjusted incidence to World Standard Population was 16.2/10^5 (95% CI: 12.7-19.7) [26.1/10^5 (95% CI: 19.9-32.2) in the 0-14 age group and 9.3/10^5 (95% CI: 5.5-13.2) in the 15-29 age group]. The age-adjusted incidence to European Standard Population was 16.3/10^5 (95% CI: 12.5-20.1)[26.4/10^5 (95% CI: 19.6-33.1) in the 0-14 age group and 9.5/10^5 (95% CI: 5.4-13.7) in the 15-29 age group].
Conclusions: The incidence of Type 1 diabetes in La Palma island is the most higher reported up to date in a spanish community, and in the 0-14 yr group is close to the highest of the world. It was inconsistent with the hypothesis of a north-south gradient in diabetes risk. The knowledge of the incidence rates in La Palma island can contribute to study the role that genetics and environemental factors may play in these differences.

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Increasing incidence of Type 1 diabetes during the second year of life in Italian children.

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Background and Aims: Recent studies report an increasing incidence of type 1 diabetes mellitus (T1DM) in children aged less than 5 years. Environmental agents operating early in life have been suggested to play a major role triggering the disease's process. We analysed the temporal pattern of incidence of childhood-onset T1DM in Italian children aged 0-4 years according to age.
Materials and Methods: Registry for type I Diabetes mellitus in Italy (RIDI), was established in 1997 aiming at co-ordinating the pre-existing local registries and promoting new registries. This report is based on 857

cases aged 0-4 years, prospectively registered during 1990-1999 upon 9 registries, covering about 35% of the Italian population. Data from registries belonging to Peninsular Italy were considered as a whole, while data from Sardegna region were considered separately. The change in incidence during the 10-year study period was analysed by fitting the Poisson regression models to the number of cases with resident population as the normalising constant.
Results: Incidence rates (per 100 000 p-years) are shown in the table.

		Peninsular Italy			Sardegna		
Age (years)		Cases (no.)	Age-specific Incidence Rates	95% Confidence Intervals	Cases (no.)	Age-specific Incidence	95% Confidence Intervals
Males	0	13	1.4	0.7-2.4	7	8.8	3.5-18.0
	1	76	8.1	6.4-10.2	32	39.6	27.1-56.0
	2	4	6.8	5.2-8.7	37	43.9	30.9-60.4
	3	95	10.0	8.1-12.3	37	42.7	30.0-58.8
	4	86	8.9	7.2-11.1	30	33.7	22.7-48.2
Females	0	16	1.8	1.0-2.9	8	10.7	4.6-21.0
	1	45	5.1	3.7-6.8	14	18.1	9.9-30.4
	2	77	8.6	6.8-10.8	18	22.9	13.6-36.2
	3	66	7.3	5.7-9.4	28	34.3	22.8-49.7
	4	83	9.1	7.3-11.3	25	29.9	19.3-44.2

The incidence of T1DM showed an average increase, statistically significant, in male aged 1 year either in mainland Italy (13.7%, 95% confidence interval: 6.6%-21.3%, p<0.001) and in Sardegna region (13.3%, 95% confidence interval: 1.5%-26.4%, p=0.026). No significant incidence trend was observed for the other years of age .
Conclusions: Our study confirms the striking difference in incidence rates of T1DM between mainland Italy and Sardegna region for each year of age among children aged under 5. The differences for incidence and temporal trends between children aged <1 year and children aged 1-4 years suggest to consider the former group separately. The increasing incidence trend during the second year of life in males strongly suggest the need of further studies in younger children for a better understanding of environmental factors.
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Familial risk of Type 1 diabetes mellitus in preschool age.
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Background and Aims: Both genetic and environmental factors are thought to play an important role in the aetiology of Type 1 diabetes. To quantify in detail the familial risk to Type 1 diabetes in children under 5 years of age a nationwide population-based case-control study was performed in Germany during 1992-95.
Materials and Methods: Data from 760 incident cases (71% of eligible) and 1871 population controls (43% of eligible), individually matched for age, sex, and place of residence, were analysed. Information on family history of type 1 and type 2 diabetes in parents, siblings and grandparents as well as on putative environmental risk factors were collected using a mailed parent-administered questionaire. Data were analysed by multivariate conditional logistic regression, in particular adjusting for relevant confounders: family' socio-economic status, duration of overall breastfeeding, maternal age at delivery, number of children in the family.
Results: A family history of type 1 diabets was reported in 9.9% (75) of cases and 1.1% (20) of controls. The respective numbers regarding first-degree relatives were 7.5% (57) and 0.7% (13). Among cases 3.8% (29), 2.0% (15), 1.7% (13), 2.8% (21) had a type 1 diabetic father, mother, sibling or grandparent. The respective estimates among controls were 0.2% (4), 0.4% (7), 0.1% (2) and 0.4% (7). A family history of type 1 diabetes was significantly associated with an increased risk for type 1 diabetes (OR (95%-CI): 12.8 (7.2-22.9)). Regarding first-degree relatives the OR was 14.5 (7.3-28.6). Further, the risk of type 1 diabetes was also increased by an isolated history of type 1 diabetes in fathers, mothers, siblings or grandparents. The respective ORs were 24.6 (7.4-82.4), 6.9 (2.6-18.5), 23.1 (5.0-108.0) and 8.0 (3.0-21.6). A family history of type 2 diabetes did not significantly affect the type 1 diabetes risk. When adjusting for relevant environmental confounders OR estimates were only slightly altered.
Conclusion: This large nationwide population-based case-control study provides detailed valid estimates of the familial risk of type 1 diabetes in preschool age relevant for patients' advice. It is confirmed that a history of type 1 diabetes in fathers or siblings confers a three times higher risk than a type 1 diabetes history in mothers or grandparents.

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Epidemiology of islet cell autoimmunity in the United States: racial and ethnic differences among US adults.
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Background and Aims: In the United States the number of adults being diagnosed with diabetes is increasing each year and is approaching an epidemic level. Ten to fifteen percent of recently-diagnosed adult onset non-insulin requiring diabetics have immune and genetic features of Type 1 diabetes (DM) and tend to progress to insulin therapy. Hence, the identification of autoimmune DM is of importance for diabetes prevention and treatment. Despite the high prevalence of DM in racial and ethnic minority adult populations in the US, little is known concerning the prevalence of islet cell autoimmunity in these groups.
Materials and Methods: We evaluated diabetic (n=1064; diabetes by history and ADA fasting criteria) and non-diabetic (n=1036) participants 40-90 years old (mean SD; 63 12) from the Third National Health and Nutrition Examination Survey (NHANES III). We estimated the prevalence of GAD65AA in Non-Hispanic Whites (NHW; n=920), Non-Hispanic Blacks (NHB; n=534) and Mexican Americans (MA; n=646). GAD65 were detected in triplicate by immunoprecipitation of serum samples with the *in vitro* transcribed/translated ^{35}S-[Met]-labeled recombinant human glutamic acid decarboxylase (GAD65, 65 kDa isoform). In house laboratory thresholds for GAD65AA positivity gave excellent performance in multiple international workshops.
Results: The prevalence of GAD65AA was higher in diabetic individuals as compared to non-diabetic individuals in both NHW (6.3% versus 2.0%, p=0.001; diabetes versus no diabetes) and NHB (3.7% versus 1.3%, p=0.08), and it was associated with insulin requirement. This difference in GAD65AA prevalence was not evident in MA (1.2% versus 2.6%, p=0.18). A similar racial/ethnic pattern of GAD65AA prevalence was also evident when excluding diabetic patients diagnosed with diabetes before the age of 40 and currently treated with insulin. The lower prevalence of islet cell autoimmunity among diabetic MA suggests that MA have a lower frequency of autoimmune diabetes.
Conclusion: Based on the prevalence of DM in the US reported by NHANES III and the US population (US Census 2000), we estimate that there are 720,000 NHW, 67,000 NHB and 13,000 MA adults 40-74 years old in the US with autoimmune diabetes, a prevalence, as high, if not higher than that of Type 1 diabetes.

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The association between self-reported and directly measured Type 1 diabetes (T1D) complications across the globe.
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Background and Aims: Little is known about the global distribution of complications in T1D. Recent data from the WHO DIAMond COMPlications (DiaComp) population-based study has demonstrated substantial geographic variation in complication prevalences. However, this variation has not been explained and may be confounded by differences in the assessment of complications and risk factors, e.g. self-report of physician diagnosis (SR) as opposed to direct measurement (DM) by clinical examination.
Methods: Physician's exam diagnoses were correlated (Spearman) to survey responses of a prior physician diagnosis using data from the DiaComp study, which consisted of 15 centers in 12 countries (n=986). Centers were located in Argentina, Finland, Israel, Italy, Japan, Lithuania, Puerto Rico, Romania, Slovakia, Sweden, UK, and US. Individuals had been diagnosed with T1D at less than 15 yrs of age and were within 5 to 25 yrs duration. Center effects on complication prevalence were modeled using logistic regression, while also controlling for demographic variables (DV): duration and sex, demonstrated risk factors (RF): smoking (ever), HbA1c and hypertension (≥140/90 or on medication), and the health care practice

variables (HC), e.g., number of physician visits in the previous year, daily self-monitoring of blood glucose (SMBG), and intensive insulin therapy (>2 shots/day) (IIT).
Results: Center rates of SR and DM neuropathy (Michigan Neuropathy Screening Instrument exam plus monofilament) were correlated(r=.50, p=.05), while for renal disease (RD), SR was not correlated (r=.01, p=.9) to DM (20mg/l using Micral II strips) . The highest rates of SR neuropathy were seen in Lithuania (24.2%), Israel (17.3%), and Romania (13.9%), and DM neuropathy in Puerto Rico (59%), Israel (39.2%), Pittsburgh (24.4%). For RD, high prevalence of SR was seen in Israel (19.2%), Argentina (14.3%), and Sweden (11.8%), and high DM RD prevalence in Ancona (100%), Argentina (42.4%) and Puerto Rico (28.3%). Moreover, no major disparities (being in the top tertile for one measurement, SR or DM, and the bottom for the other) were seen for neuropathy, while RD did show major disparities for 2 centers. SR and DM neuropathy both showed substantial reduction in center effects after controlling for DV and RF but neither showed any further reduced effect after controlling for HC. Likewise, SR RD did not show substantial reduction in center effect after controlling for HC, but DM RD did show such a reduction after controlling for HC in Romania (OR reduced from 2.7 (95% C.I. = 1.1 - 6.3) to 1.5 (95% C.I. = .56 - 4.3).
Conclusions: Both SR and DM show moderately consistent geographic variation in neuropathy, while these are less consistent for RD, which may reflect different screening and patient informing practices for microalbuminuria. Importantly, health care variables did not appear to explain much of the geographic variation.

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Prevalence of overweight and obesity in Type 1 diabetes.
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Background and Aims: The increase of obesity worldwide and the excessive weight gain with intensive insulin therapy could result in a new situation in type 1 diabetic patients where overweight is also becoming an important problem. The aim of our study was to evaluate the prevalence of overweight and obesity in type 1 diabetic patients followed at our hospital during a period of 6 months and to correlate anthropometric measurements with demographic and clinical factors.
Materials and Methods: We studied 170 (89 females) type 1 diabetic patients (age of onset of diabetes < 30 yrs), being 14 children (age <10 yrs), 51 adolescents (10-19 yrs) and 105 adults (age >19 yrs), mean age 24,4± 11,9 yrs. Adults were classified according body mass index (BMI) and children and adolescents as risk for overweight (≥ 85th and < 95th percentile) and overweight (≥ 95th percentile). We also calculated the BMI z score. The whole group was classified as normal blood pressure (NBP), high-normal BP (HNBP) and hypertension (HBP). Waist circumference was measured in adults and measurements ≥ 80cm(F) and ≥ 94cm(M) were considered indicators of visceral adiposity.
Results: The prevalence of risk of overweight, overweight and/or obesity was 21,2% (n=36), without difference between sex, duration of diabetes, insulin dose or age group. BMI in adults was 23,2± 3,4kg/m^2, 22 (21%) with overweight and 3 (2,9%) with obesity. Among children and adolescents 9 (13,8%) subjects were at risk of overweight and 2 (3%) had overweight. BMI z score and BMI percentile were highly correlated (r=0,97). Female children and adolescents had greater BMI (20,9± 0,7 vs. 19,1± 0,4 kg/m^2; p=0,04) and z score (0,53± 0,74 vs. -0,04± 1,07; p=0,02) than males. There were 23 (36,5%) women with waist circumference ≥ 80 cm and 4 (9,5%) men with waist circumference ≥ 94cm (p=0,006). There was difference in systolic blood pressure (SBP) (p=0,004) and in diastolic blood pressure (DBP) (p=0,0007) between subjects with normal BMI and high BMI. A trend for increase waist circumference was observed in the groups of NBP, HNBP and HBP, respectively: 73,2± 8,6, 81,5± 11,6 and 82,9± 11cm (p=0,0000). By multivariate analysis BMI was dependent of age (p=0,008, OR: 1,04 95% CI=1,01-1,07). Using stepwise analysis SBP was dependent of waist circumference (r=0,57) and of age (r=0,63) and DBP was dependent of waist circumference (r=0,53).
Conclusion: The prevalence of overweight and obesity in type 1 diabetic patients reflects the global tendency of weight excess and their clinical outcomes. Among the anthropometric measures waist circumference seems to be related to a increase of risk for cardiovascular complications. Awareness of overweight in type 1 diabetes needs to be intensified.

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Secular trend of glucose intolerance in obese Caucasian children in Italy.
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Background and Aims: We examined the change in the prevalence of glucose intolerance in obese Caucasian children referred to the Istituto Auxologico Italiano, a specialized centre for the study of obesity, over a period of 27 years.
Materials and Methods: Three cohorts of Italian obese children were studied in the period 1976-83 (period I, n=437, % male: 48), 1984-95 (period II, n=381, % male: 44), 1996-2002 (period III, n=441, % male: 49). Age ranged between 6-18 yr in all three cohorts with mean age 12 vs 14 vs 14 yrs respectively. All subjects underwent an oral glucose tolerance test (1.75 g/kg glucose in 250 ml of water). Insulin resistance was determined by $HOMA_{IR}$ and insulin secretion by ΔI30/ΔG30.
Results: BMI and waist circumference increased with time (period I vs II vs III, BMI: 29.3±4.9, 34.1±5.4 and 35.3±6.5 kg/m^2, p< 0.0001 for trend; waist 78.4±7.5, 75.4±20.7 and 107.3±17.3 cm). The frequency of impaired glucose tolerance increased from period I to period III (3.1%, 4.2% and 6.1% respectively, p<0.05 for trend). Consistently, 2-h post load glycaemia was higher in the more recent cohorts after adjustment for differences in age and BMI (mean ± ES: 4.8±0.06, 5.8±0.07, 5.9±0.06, p<0.001 for trend). The prevalence of type 2 diabetes was similar (0.2%, 0.5% and 0.2% respectively). Insulin sensitivity was reduced, but did not change over the three successive 9-yrs periods; by contrast insulin response declined with time (ΔI30/ΔG30: 406±34, 335±37, 255±32, pmol/mmol, p<0.01 for trend). Two-hr glycaemia was independently and positively related to $HOMA_{IR}$ within each of the three 9-yrs period (β 0.322, p<0.0001 vs β 0.163, p<0.0005 vs β 0.141, p<0.001) and negatively to insulin secretion in period II and III (β -0.210, p<0.0001 vs β -0.161, p<0.01).
Conclusion: In the last 27 years, there has been a deterioration of glucose tolerance in obese children which appears to be, at least partly, related to a progressive impairment of insulin secretion. The increasing degree of obesity may contribute to this trend but its true impact requires assessment in a population based study.

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The estimation of DRB1, DQA1, DQB11 HLA gene alleles in gestational diabetes mellitus.

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Background and Aims: Gestational diabetes mellitus (GDM) is associated with an increased risk of maternal and fetal complications and further development of diabetes after delivery. There is an increasing evidence that glucose level disturbances observed during pregnancy have heterogeneous etiologies and that genetic factors could play a key role in the predisposition to different "subtypes" of gestational diabetes.The aim of the study was the estimation of the frequency of diabetes type1-associated HLA alleles and haplotypes of DRB1, DQA1 and DQB1 genes in women with gestational diabetes in comparison to subjects without glucose intolerance during the pregnancy. The association between the studied alleles and the risk of diabetes development in the next 5 years after delivery or the insulin therapy were also analyzed.
Materials and Methods: The study was performed in 168 subjects with gestational diabetes and 144 healthy age and BMI matched women with normal glucose tolerance during the pregnancy. Alleles of DRB1, DQA1, DQB1 genes were genotyped using SSP-PCR method.
Results: The frequency of DRB1*03 or DQB1*0302 alleles were significantly higher and the frequency of DQB1*0602 or *0603 alleles were lower in subjects with gestational diabetes treated with insulin in comparison to the group treated with low carbohydrate diet only or controls. Moreover higher frequency of DRB1*03 and/or *04 and DQB1*02 allels were observed in GDM women, who developed diabetes in the next 5 years after delivery in comparison to the group with normal postpartum glucose tolerance (44.4% vs. 19.1% and 55.6% vs.38.5%). After delivery none of the women with DQB1*0602 or *0603 alleles and previous GDM have clinical indications for the insulin treatment during the next 5 years after delivery.
Conclusion: The genetic profile (diabetes type 1-associated HLA alleles) could be useful for the diagnosis of different "subtypes" of gestational diabetes mellitus and could serve as a predictor of insulin-dependency during the next 5 years after delivery.
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Lymphocyte subsets and cytokines in women with gestational diabetes and in their newborn.

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Background and Aims: Gestational diabetes mellitus(GDM)is considered a condition related to further diabetes development, mainly type 2 ,but in 2-4 % of cases type 1.Aim of our study was to identify some possible immunological markers that could predict type 1 diabetes development in GDM patients, and the possible immunological impairment in their newborn.
Materials and Methods: 60 GDM women ,diagnosed by Carpenter e Coustan criteria, 27 women with normal glucose tolerance (NGT) and their babies were evaluated. As for immunological parameters total lymphocytes, T lymphocyte subsets:CD3,CD4 and CD8 expressing T cell receptor(TCR)αβ or γδ,CD16 and CD19, pancreatic auto antibodies (ICA,IA2, antiGAD), citokine IL-2,Il-5 and soluble receptor Il-2 were evaluated in all patients at 3rd trimester of pregnancy. At delivery a blood sample from babies umbilical cord was taken for measurement of lymphocyte subpopulations and cytokines.
Results: As for pregnancy outcome, a higher rate of caesarean section($p<0.001$) and of babies large for gestational age ($p<0.05$)was observed in GDM vs NGT group.The analysis of the immunological parameters showed an increase in CD4 and CD8expessing TCRγδ (CD4γδ:2.29±1.44 %vs 1.42±1.13% ,$p<0.009$; CD8γδ:3.15±1.92% vs 1.73±1.48% ,$p<0.002$), a reduction of CD3 expressing TCRαβ(66.3±8.7 % vs70±6.4%,$p<0.04$) in GDM mothers compared to NGT ones. A reduction of CD4, an increase of CD8 ,a significantly reduced CD4/CD8 ratio and an increase of IL5 was found in GDM mother insulin-treated with respect to GDM diet treated and controls.We found 6 women with positive pancreatic auto antibodies (2 GDM and 4 NGT) ,these patients presented a reduction of CD4($p<0.01$) and an increase of CD8($p<0.03$) with a reduction of CD4/CD8 ratio($p<0.05$)with respect to GDM and controls with negative auto antibodies.An increase of CD3αβ(64±8.6% vs. 57±15%,$p<0.02$) ,of CD4(50.4±10.2% vs. 43.3±12.3%, $p<0.04$), and of CD8γδ(1.47±1.39% vs 0.93±1.32 %,$p<0.02$) and a reduction of CD 16(10.9%±vs 18.1±10.5%,$p<0.01$)was found in GDM newborn with respect NGT ones. About cytokines evaluation a decrease of IL 5 and TNFα was found in GDM babies with respect to NGT ones.
Conclusion: In conclusions our data show that GDM women and their newborn present impaired lymphocyte subsets ,these modifications seem more relevant in patients with positive auto antibodies and/or insulin treatment. A follow up study is necessary to evaluate in the mothers the possible relationship of lymphocyte subpopulations modification and the out set of auto antibodies positivity and/or type 1 diabetes mellitus development and in the newborn the time course of impairment.

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Autoimmune gestational diabetes is not too rare.

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Background and Aims: In this study we investigated the frequency of autoimmune diabetes in a group of patients with gestational diabetes (GDM).
Material and Methods: The study included 287 GDM patients and 72 healthy pregnant women. All cases were followed until delivery, and they were seen at postpartum 1 year. The predictors of insulin requirement during pregnancy and denominators of type 1 diabetes as well were defined.
Results: Among GDM patients all four autoantibodies (ICA, Anti-GAD, IAA, and Anti-TPO) were tended to be higher in frequency than in control group. However, only ICA (12.9%) and Anti-GAD (15%) were found significantly more common. At least one antibody was detected positive in 22.6% of our GDM group. Of these 58.4% had isolated, 36.9% had two and 4.6% had three, but none had four antibodies. Compared to control group, in the GDM group CD3, CD8, CD16 and CD95 lymphocytes were increased, whereas CD25 lymphocytes and CD23/CD19 and CD4/CD8 ratios were decreased significantly. Based on each antibody status, GDM group have been evaluated for phenotypic, biochemical and immunologic variables. Besides that CD23/CD19 ratio and infant body weight were higher in the ICA (+) subgroup, we did not found any significant difference in terms of ICA. Similarly, there was no difference between Anti-GAD (+) and Anti-GAD (-) subgroups except that infant body weight was higher in the Anti-GAD (+) subgroup. Again we did not found any difference between IAA (+) and IAA (-) subgroups except that CD8 and CD23 lymphocytes were increased significantly in the IAA (+) subgroup. Moreover, antibody positive and negative subgroups in terms of Anti-TPO were similar. 27.5% of GDM patients required insulin during pregnancy, of these 11.3% had one, 8.8% had two and 2.5% had three antibody positive. No correlation was found between antibody status and lymphocyte subgroups. Four patients (1.4%) maintained insulin requirement after delivery, and diagnosed with type 1 diabetes as they showed ≥1 antibody and low C-peptide levels. Fasting glucose (OR 6.4, 95%CI: 2.1-19.6) and the CD19 lymphocytes (OR 10.3, 95%CI: 1.4-73.2) were only variables that were found to be associated with insulin requirement. At postpartum 1 year, standard OGTT revealed that 34.6% of preGDM women had abnormal glucose intolerance. 10 patients had diabetes. Slowly progressive type 1 diabetes was diagnosed in 3 (2.8%) of them due to ≥1 antibody, and low-normal C-peptide levels. Additionally, preclinical type 1 diabetes might be present in 4 (3.8%) cases with impaired glucose metabolism as they had ≥1 antibody but normal C-peptide.
Conclusions: Finally, the prevalence of autoimmune GDM in our study was 8% in total. We conclude that autoimmune GDM patients is a high risk group for future type 1 diabetes, and GDM patients need to be screened for islet autoantibodies during pregnancy.

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Fasting or post-challenge blood glucose values should be used for classification of glucose intolerance in subjects at risk for metabolic syndrome?

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Background and Aims: The normal-pathological threshold of fasting blood glucose values was modified by the new WHO diagnostic criteria (1999) and, in addition, impaired fasting glucose (IFG) was introduced as a new clinical entity. Nevertheless, the 2-h post-glucose challenge criteria and the concept of the impaired glucose tolerance (IGT) remained unchanged. There is no unequivocal agreement whether new fasting or unchanged post-challenge blood glucose criteria should be used for classification of glucose intolerance. The aim of this study was to assess the reliability of the fasting blood glucose values for classifying different categories of glucose intolerance in subjects screened for metabolic syndrome.
Materials and Methods: In order to detect subjects with metabolic syndrome, a mass-screening was performed in a certain part of north-west Hungary. At inclusion, subjects of both sexes aged 20 to 65 years exhibited at least one of the following clinical characteristics: hypertension (treated or newly diagnosed), obesity (BMI >30.0 kg/m^2) or elevated waist-hip ratio (>0.85 in women, >0.90 in men). An oral glucose tolerance test (OGTT) with 75 g glucose was performed. Subjects known to have diabetes were not involved.
Results: In the total cohort (n=944; women/men: 545/399; age: 46.1±7.3 years; BMI 32.2±5.4 kg/m^2; waist-hip ratio 0.90±0.09; x±SD) newly diagnosed diabetes mellitus (based on the 120 min post-challenge glucose values) was found in 87 subjects (9.2 %), IGT was detected in 136 cases (14.4 %) while normal glucose tolerance was documented in 721 subjects (76.4 %). Using fasting blood glucose values for classification, diabetes mellitus was detected in 79 subjects (8.4 %), IFG was found in 124 cases (13.1 %) while 741 subjects (78.5 %) had normal glucose tolerance. Impaired glucoregulation (IGT + IFG) was found in 223 subjects (IGT alone 99 cases [44.4 %], IFG alone 87 cases [39.0 %], IGT and IFG in combination 37 cases [16.6 %]). The sensitivity and specificity of fasting blood glucose criteria for detecting diabetes were 63.2 % and 97.1 %, respectively, while those for detecting glucose intolerance (IFG and diabetes as well as IGT and diabetes) were 52.9 % and 88.2 %, respectively. Clinical characteristics of subjects with abnormal post-challenge but normal fasting blood glucose values (n=105) did not differ significantly from those of subjects with normal post-challenge but abnormal fasting blood glucose values (n=85) (age: 46.7±6.9 years vs 46.7±6.1 years; BMI: 33.1±5.4 kg/m^2 vs 32.3±4.5 kg/m^2; waist-hip ratio: 0.91±0.09 vs 0.92±0.07; p>0.05).
Conclusions: OGTT and 2-h post-glucose challenge criteria should be used for the diagnosis of different categories of glucose intolerance in screening for metabolic syndrome.

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Adiponectin concentrations relate to increased plasma lipids and intramyocellular lipid content and further decrease in insulin resistant women with prior GDM.

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Background and Aims: Adiponectin (AC), expressed in white adipose tissue only, has been shown to have insulinsensitizing and antiinflammatory properties. Decreased concentrations of AC and increased intramyocellular lipid content (IMCL) have been shown to precede type 2 Diabetes (DM2). We have shown that AC is decreased in women with prior gestational diabetes (pGDM) compared to women with normal glucose tolerance (NGT) 3 months post partum (pp). Aim of this study was to restudy AC in pGDM 1 year after delivery.
Materials and Methods: 44 pGDM underwent oral glucose tolerance tests (OGTT) both 3 months and 1 year pp, plasma AC concentration, plasma lipids and body fat mass (bfm, by bioimpedance analyzer) were measured at the same time; according to the Insulin Sensitivity Index (S_I, FSIGT: 3 months pp), pGDM were devided into a resistant (S_I<2.8, pGDM-R, n=15) and sensitive (S_I>2.8,pGDM-S,n=24) subgroup; in addition IMCL (by localized ^{1}H NMRS in M. tibialis anterior) was assessed 3 months pp.
Results: At baseline, AC (7.0±0.4 µg/ml) was 29% lower in pGDM compared to NGT (9.8±0.6 µg/ml, p<0.0001), whereas there was no difference between pGDM-R and pGDM-S. Comparing baseline results with the follow-up examination, pGDM (age: 33.7±0.7 yrs, BMI: 27.4±0.8 kg/m^2, waist: 91.3±1.8 cm, TG: 101.7±9.2 mg/dl, chol: 197.5±5.2 mg/dl, HDL: 55.6±2.3 mg/dl, bfm: 26.4±1.7 kg, basal glucose: 91.8±2.2mg/dl, 2h-glucose: 120.6±5.3 mg/dl) had lower plasma concentrations of HDL (49.1±1.9, p<0.0002) as well as higher TG (120.2±11.3, p<0.007) after 1 year. There was no difference in plasma concentrations of AC. In pGDM-R, however, plasma concentrations of AC further decreased (6.6±0.5, p<0.05), although glucose tolerance, plasma lipids and bfm did not change. At baseline, AC correlated with bfm (r=-0.3, p<0.05), IMCLTA (r= - 0.3, p<0.004), 2h-glucose (r= -0.3, p<0.03) as well as with HDL (r=0.5, p<0.001). At the follow-up examination, AC correlated with bfm (26.6±1.8, r=-0.4, p<0.008), waist (88.6±2.0, r=-0.4, p<0.009), TG (r=-0.4, p<0.01), basal glucose (96.3±4.1, r=-0.4, p<0.02), 2h-glucose (124.6±8.2, r=-0.5, p<0.004) and with HDL (r=0.6, p<0.0001).
Conclusion: Decreased plasma concentrations of AC relate to increased plasma lipids and IMCL as well as glucose tolerance 3 months and 1 year after delivery. In the resistant subgroup plasma concentrations of AC further decrease at the follow-up investigation. Our results suggest that AC plays a substantial role in the pathogenesis of DM2 and might serve as a new indicator of risk in addition to the established risk factors like obesity and insulin resistance.

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B-Cell function is highly heritable in Mexican-American families of probands with gestational diabetes.

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Background and Aims: We have shown that Mexican-American (MA) women who develop gestational diabetes (GDM) have a B-cell phenotype characterized by failure in the presence of chronic insulin resistance. This failure is predictive of type 2 diabetes.
Materials and Methods: We are recruiting MA families consisting of a GDM proband, her non-diabetic siblings, 1st-cousins, and available parents to assess genetic determinants of this B-cell phenotype. Phenotyping includes oral (OGTT) and tolbutamide-modified intravenous glucose tolerance tests (IVGTT), and body composition by DEXA. IVGTTs are analyzed by Minimal Model to derive insulin sensitivity (S_I). B-cell function is assessed as incremental 30-minute insulin (30'dINS) from the OGTT and acute insulin response (AIR) from the IVGTT. Disposition index (DI), a measure of B-cell compensation for insulin resistance, is computed as AIR×S_I. Narrow sense heritability (h^2) adjusted for age, gender, and BMI is estimated by variance components using SOLAR.
Results: To date, we have analyzed the data of 88 males and 177 females in 48 families; 10 families include 1st-cousins. Average number of sibs is 3.6 (range 1-9) and of 1st-cousins is 4.8 (range 2-9). Subjects were non-pregnant with fasting plasma glucose <126 mg/dl (7 mM) at time of testing. Mean (±SD) age was 35±9 years and BMI was 29±6 kg/m^2. We observed strong h^2 for AIR (72%±16; p<0.0001), DI (34%±16; p=0.004), 30'dINS (44%±13; p<0.0001), and S_I (55%±18; p<0.0001). BMI (71%±15; p<0.0001) and total body fat (63%±14; p<0.0001) also exhibited strong familiality. Removing BMI as a covariate did not qualitatively alter h^2 for AIR or 30'dINS, but increased h^2 for S_I to 67±17% and DI to 53±16%.
Conclusion: Significant h^2 for two independent measures of insulin secretion and a measure of B-cell compensation suggest there are genetic determinants of the B-cell defect that predicts diabetes in these individuals. The impact of BMI on our h^2 estimates for S_I and DI also suggests an important intereaction that may have a genetic basis. Our findings support a genetic approach to identify the fundamental cause(s) of this B-cell defect, which underlies the development of type 2 diabetes.

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Lack of excess maternal transmission of Type 2 diabetes in a Korean population.

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Background and Aims: The purpose of this study was to assess the familial clustering of type 2 diabetes and to investigate the presence of excess maternal transmission of type 2 diabetes in Korea.
Materials and methods: The medical records of 56,492 subjects (31,680 men, 24,812 women), who attended the Health Promotion Center in Samsung Medical Center between 1998 and 2001, were examined for this analysis. The subjects were questioned about their parents' (whether living or deceased) diabetes status. All study subjects were classified into the three groups (normal fasting glucose, impaired fasting glucose, and diabetes) according to their fasting plasma glucose value and previous history of diabetes. In addition to the parental diabetes status and the degree of glucose tolerance, several clinical and biochemical parameters such as age, gender, body mass index, systolic blood pressure, diastolic blood pressure, serum cholesterol, serum triglyceride, serum HDL-cholesterol and serum LDL-cholesterol were also investigated.
Results: A total of 48,810 subjects (86.4 %) presented with a normal fasting glucose, 3,587 subjects (6.3 %) with impaired fasting glucose, and 4,095 subjects (7.2 %) with diabetes. Offspring with paternal diabetes were at increased risk for diabetes (odds ratio 2.54, 95% CI 2.22-2.91, $p < 0.001$) and for impaired fasting glucose (odds ratio 1.61, 95% CI 1.39-1.88, $p < 0.001$) when compared to the normal group and adjusted for other clinical and biochemical variables. Furthermore, offspring with maternal diabetes were also at increased risk for diabetes (odds ratio 3.10, 95% CI 2.76-3.49, $p < 0.001$) and for impaired fasting glucose (odds ratio 1.59, 95% CI 1.37-1.84, $p < 0.001$) when compared to the normal groups and adjusted for putative risk factors. Offspring with bilineal parental diabetes were at a greater risk for diabetes (odds ratio 6.09, 95% CI 4.55-8.16, $p < 0.001$) and for impaired fasting glucose (odds ratio 3.99, 95% CI 2.89 - 5.50, $p < 0.001$) when compared to the normal groups and adjusted for putative risk factors. In both genders, offspring with maternal diabetes showed no increased risk for diabetes (odds ratio 1.22, 95% CI 0.92 - 1.37, $p = 0.266$ in men; odds ratio 1.31, 95% CI 0.95 - 1.81, $p = 0.104$ in women) when compared with those with paternal diabetes.
Conclusion: The data suggested that parental type 2 diabetes was an independent risk factor for offspring type 2 diabetes in this Korean population. Excess maternal transmission of type 2 diabetes was not observed.

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Thiazolidinedione therapy in the prevention/delay of Type 2 diabetes in patients with impaired glucose tolerance and insulin resistance.

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Background and Aims: This prospective analysis examined the effects of early thiazolidinedione (TZD) treatment on the prevention or delay of type 2 diabetes mellitus (T2DM) in a multi-ethnic population with impaired glucose tolerance (IGT) or insulin resistance (IR).
Materials and Methods: The analysis included 172 patients (aged 29-86 years) with IGT and IR (normal or borderline glycosylated hemoglobin [HbA_{1c}], C-peptide levels >2 mg/ml, fasting blood glucose 100-125 mg/dl). Patients in the treatment group (n=101) had received troglitazone for an average of 10 months before being randomly switched to rosiglitazone (4 mg/d) or pioglitazone (30 mg/d). Patients were switched when troglitazone was withdrawn from the U.S. market because of liver toxicity concerns. Seventy-one patients who received no antidiabetic medication served as a control group. HbA_{1c} and C-peptide levels were measured at baseline, 2 years, and study end point (3 years.
Results: Mean HbA_{1c} and C-peptide levels decreased significantly for patients receiving TZD therapy. At the end of the study period, 3 patients in the treatment group had progressed to T2DM, compared with 19 patients in the control group. The estimated cumulative incidence rate of diabetes at 3 years was lower for the TZD group (2.97%) than for the control group (26.8%). The crude incidence rate was lower for the TZD group (1.4 cases per 100 person-years) than for the control group (9.4 cases per 100 person-years) ($p<.001$ vs control). The incidence of diabetes (risk reduction) was 88.9% lower in the TZD group than in the control group ($p<.001$ vs control).
Conclusions: TZD treatment was efficacious in reducing HbA_{1c} and C-peptide levels in patients with IGT/IR. Progression of IGT/IR to T2DM appears to be significantly delayed or prevented with early TZD treatment.

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Predictive properties of IFG and IGT for diabetes.

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Background and Aims: To prospectively evaluate progression to diabetes in individuals with glucose intolerance (IFG, IGT or both IFG and IGT) and the predictive properties of associated CVD risk factors for diabetes.
Materials and Methods: After 5 years follow-up, in 486 glucose intolerant (172 IFG, 245 IGT and 69 both IFG and IGT) Caucasians, 75g-OGTT was repeated. Study subjects were categorized according to 1999 WHO criteria as having DM, IFG, IGT or normal glucose tolerance (NGT). Total plasma cholesterol, triglycerides, systolic and diastolic blood pressure (BP) were measured at baseline and at follow-up.

Table 1-*Study subjects baseline characteristics*

	IFG	*IGT*	*IFG+IGT*
Number	172	245	69
Age (years)*	54.72 ± 7.13	52.14 ± 6.28	53.72 ± 5.18
Sex - M [n(%)]	82 (47.67)	114 (46.53)	31 (44.93)
- F [n(%)]	90 (52.33)	131 (53.47)	38 (55.07)
BP - Systolic (mmHg)*	132.72 ± 21.14	138.14 ± 23.31	141.14 ± 24.82
- Diastolic(mmHg)*	84.34 ± 16.14	89.72 ± 14.32	90.02 ± 12.14
Total cholesterol (mg/dl)*	202.13 ± 24.13	214.82 ± 32.73	229.14 ± 32.44
Triglycerides (mg/dl)*	212.72 ± 36.82	235.19 ± 47.62	249.72 ± 48.16

*Data are means ± SD.

Results:

Table 2-*Progression to diabetes and other categories of glucose tolerance, by baseline status, at 5 years follow-up*

At baseline		*At 5 years follow- up* DM	IFG	IGT
IFG	172	51 (29.65)	53 (30.81)	33 (19.19)
IGT	245	92 (37.55)	21 (8.57)	98 (40)
IFG+ IGT	69	41 (59.42)	7 (10.14)	17 (24.64)

Data are n (%)

Subjects who developed diabetes had higher systolic and diastolic blood pressure values and higher total cholesterol and triglycerides plasma levels than those who did not progress to diabetes.
Conclusion: Both IFG and IGT are associated with increased risk of diabetes. Risks are higher when IFG and IGT coexist. Associated CVD risk factors have predictive value for the development of diabetes.

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Lower progression of carotid intima media thickness under acarbose: the STOP-NIDDM study.

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Background and Aims: There is evidence that the atherosclerotic complications in type 2 diabetes start at an early phase and even in prediabetes, whereby postprandial hyperglycemia plays an important role. The STOP-NIDDM study showed that under acarbose the conversion rate to diabetes in IGT subjects could be reduced significantly. The aim of the present analysis was to examine whether the progression of the intima-media thickness (IMT) of the common carotid artery (CCA) could be also reduced under acarbose.
Materials and Methods: A total of 117 subjects were examined in one of the sites of the international STOP-NIDDM study, a trial on primary prevention of type 2 diabetes in IGT using acarbose. Both study groups –

under acarbose (n=57) and placebo (n=60) did not differ significantly for age (65 and 64 years respectively) and gender. At the beginning and at the end of the study IMT of the CCA was determined by B-mode ultrasound using Acuson 128XP. IMTmean was determined as the average of double measurements bilaterally in the distal 10 mm of the ACC and IMTmax was visually determined by the observer independent of the localization**.**
Results: The subjects under placebo showed a highly significant progression of the IMTmean (from 0.92 to 0.97 mm; p<0.001) and IMTmax (from 1.05 to 1.08 mm (p=0.004) during the 4-years follow-up period. In the acarbose group a significant rise was observed of the IMTmean (from 0.91 to 0.93 mm (p=0.01), but not of the IMTmax (from 1.03 to 1.05 mm). The progression of the IMTmean was significantly lower (p=0.035) under acarbose (0.02 mm) than under placebo (0.05 mm), and the progression of the IMTmax under acarbose (0.02 mm) was slightly lower (NS) than under placebo (0.04 mm).
Conclusion: The analysis shows that in the primary prevention of type 2 diabetes in IGT using acarbose a significantly lower progression of the carotid IMT is observed, which could point to the protective vascular effect of the preventive measures.

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Epidemiology of Diabetes and Social Sciences

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Ethnic profile of young people aged 0-29 years with Type 1 and Type 2 diabetes in west Yorkshire, UK.
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Background and Aims: The epidemiology of diabetes is changing as more young people are being diagnosed with type 2 diabetes. Such cases are associated with ethnic minority groups. The northern English county of West Yorkshire contains a population of 850,000 with a high proportion of south Asians. We aimed to describe the distributions of type 1 and type 2 diabetes by ethnic group, age and sex.
Materials and Methods: Information was extracted from a population based Register on patients diagnosed aged 0-29 years with any form of diabetes between 1991-2002 in the study area of West Yorkshire. Diagnosis was based on information contained in hospital records and classified according to WHO criteria. Any cases described as "uncertain" had their clinical and biochemical profile reviewed by a panel to classify diagnosis. Ethnic group was assigned as being south Asian (Indian, Pakistani or Bangladeshi) or not by a computer algorithm based on name recognition and designed for the population of West Yorkshire. The proportion of subjects identified as south Asian was compared across 5-year age groups by sex and also with the background population from the 1991 national UK Census.
Results: 1800 cases of diabetes were identified, of which 1670 (93%) were classified as Type 1, and 86 (5%) as Type 2. We excluded 39 unclassified patients, 3 with MODY and 2 with gestational diabetes. Ascertainment was 99% and 97% complete for the 0-14 and 15-29 age groups respectively. 10% of males and 11% of females were classified as Asian. The relative proportion of Asians with type 1 diabetes decreased as age increased (12% of those aged 0-4, 8% aged 25-29, test for trend P=0.04). However, this was similar to the distribution of ethnic groups seen in the background population apart from a slight excess for south Asians aged 25-29 (8% vs. 5%, P=0.02). Females did not follow any clear pattern with age although exhibited a similar excess of Asians aged 25-29 (11%). For type 2 diabetes, south Asians comprised 43% of patients aged 10-29 in comparison to 9% of the background population (P=0.01)
Conclusions: The proportion of south Asian patients with type 1 diabetes largely mirrored the background population of West Yorkshire. However, for type 2 diabetes there was a striking excess of Asian teenagers and young adults confirming previous observations in other areas of the UK. Future work will closely examine the relationship between ethnicity, deprivation and obesity.

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Prevalence of diabetes in the Goulburn Valley, Australia.
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Background and Aims: The Goulburn Valley (GV) (population 120,000) has one of the highest patient:general practitioner (GP) ratios in Australia. We hypothesized that the associated reduced access to primary care would be associated with a greater proportion of undiagnosed diabetes than in other parts of Australia. We have compared the prevalence of diabetes including undiagnosed diabetes in the major towns in the GV with randomly selected towns of 25,000 or more across Australia in the AusDiab study (AD) reported in 2002.
Materials and Methods: Houses were randomly selected from local government residential lists in the regional centre (RC, population 43,000) and the capitals of the 6 surrounding shires (SC, populations 3,000-13,000) 50-120km away. The sampling was stratified such that 50% of subjects were from the RC and 1/12 from each of the SC. All residents aged 25 years and over were invited to attend a 75g oral glucose tolerance test (GTT) using World Health Organisation diagnostic criteria. Subjects also completed a standard questionnaire including demographic data. Those with known diabetes completed an additional more detailed questionnaire and their diabetes was confirmed using historical data. The survey used

identical questionnaires, sampling methods and laboratories as the AD. Members of the AD team were present at each GTT session to ensure consistency. AD included 11,247 participants.
Results: The survey is due to be completed in March 2003, with 1413 GTTs undertaken from 2378 invited to date. As the study is incomplete, statistical testing has not yet been conducted. The prevalence of diabetes was similar in the RC and SC and these have been pooled for comparison with AD. The overall prevalence was similar in the GV and AD (7.2% vs 7.4%). However, the prevalence of undiagnosed diabetes was 2.0% in GV and 3.7% in AD. The ratio of diagnosed:undiagnosed overall, adjusted for age, was 2.56:1 in GV but 1.00:1 in AD. This ratio was similar between RC and SC (2.36 vs 2.76). The combined prevalence of impaired glucose tolerance and impaired fasting glucose (IGT/IFG) was also lower in the GV vs AD (10.4% vs 16.4%).
Conclusion: These preliminary data suggest that the prevalence of diabetes is similar in this study population to that in the wider Australian population found in AD. However, there are fewer people with undiagnosed diabetes in both the regional centre and the Shire capitals. The reason for this difference warrants further study and may reflect a different approach to screening in areas with shortages of GPs to those where GPs are more plentiful. Such analyses may lead to enhanced approaches to diabetes screening. The reasons for the lesser proportion with IGT/IFG remain unclear.

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Prevalence of diabetes and nutritional status in patients with cystic fibrosis: results of a multicenter diabetes screening program.
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Background and Aims: Cystic fibrosis related diabetes mellitus (CFRD) has been reported to be the second most prevalent form of diabetes in children and the third most common type in adults. It is also associated with increased morbidity and mortality. Prevalence rates range from 3% to 25% depending on age and diagnostic criteria all derived from relatively small cohorts.
Materials and Methods: As part of a prospective randomised multicenter study based on an initiative of the German Cystic Fibrosis Foundation, an annual oral glucose tolerance test (OGTT) following standard WHO recommendations was conducted as a screening test in order to identify patients (≥ 10 years) with CFRD. As an age-independent parameter of nutritional status we used body-mass-index(BMI)-Z-scores based on the recent German reference data, using LMS transformation. The Kruskal-Wallis-Test was performed to compare the groups with different OGTT-results.
Results: Up to now 618 patients (54 % males, 46 % females aged 10-64 years, median 16.6 years) were screened by an OGTT. A prevalence of 71% with a normal (n), 6.3% with an impaired fasting glucose (IFG), 14.1% with an impaired -(IGT) and 8.6% with a diabetic glucose tolerance was calculated. Out of the 53 patients with a diabetic response 56% were without fasting hyperglycaemia. There was no significant difference among the groups concerning the BMI-Z-scores (mean ± SD; n:-0.65± 1.15, IFG:-0.56± 1.28, IGT:-0.95± 1.30, CFRD:-0.83± 1.21).
Conclusion: In this large cohort CFRD has a prevalence of 8.6 %. With this screening approach we could not see any age independent difference regarding nutritional status between the early diagnosed patients with CFRD and other CF patients.

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Socio-economic factors and prevalence of diabetes and hypertension-the Chennai Urban Rural Epidemiology Study (CURES).
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Background and Aims: The aim of this study was to assess the association of socio-economic status with diabetes and hypertension in large representative sample of Chennai (formerly Madras) city in South India.
Materials and Methods: As part of the urban component of the Chennai Urban Rural Epidemiology Study (CURES), a systematic sampling method was used to obtain a representative population of Chennai (aged >= 20 years-26001 individuals). A structured questionnaire was used to obtain details on demography, occupation, family income, medical history, depression, stress scale, physical activity and dietary pattern. Anthropometric measurements included height, weight, waist and hip measurements. Blood pressure estimations were obtained using electronic Omron apparatus (Omron Corporation, Tokyo, Japan). Fasting capillary blood sugar estimations were performed on all subjects using One Touch Basic plus (Lifescan Johnson & Johnson, California, USA). Diabetes was diagnosed based on previous medical history and ADA fasting criteria. Hypertension was diagnosed in individuals who reported to be known hypertensives and based on JNC VI criteria. Monthly income was graded as grade1:Rs <2000 (~$40), grade 2: Rs 2001- Rs.5000 (~$41-100), grade 3:Rs.5001 - Rs 10,000 (~$101 -200), grade 4: Rs10,001-Rs20,000 (~$201-400) and grade 5: > Rs 20,000 (> ~$ 401).
Results: The study population comprised of 49.2% males. The overall prevalence of diabetes was 18.3% (95% confidence interval {CI} 17.8 - 18.8) and hypertension was 23.2% (95% CI - 22.7 -23.7). The first analysis was carried out using occupation as a marker for socio-economic status. The prevalence of diabetes was 20.1% among businessman and executives, 19.7% among clerical workers, 18.7% among housewives and retired individuals and 16.5% among skilled labourers (trend chi square - 17.2, $p < 0.0001$). The prevalence of hypertension was 25.1 % among businessman and executives, 24.4% among clerical workers, 23.5% among housewives and retired individuals and 20.9% among skilled labourers (trend chi square - 19.1, $p < 0.001$). A significant increase was observed in the trend of prevalence of diabetes (trend chi square - 13.2, $p < 0.001$) and hypertension (trend chi square - 3.64, $p = 0.05$) with income grades. Body mass index was significantly lower in subjects with monthly income <Rs.2000/- compared to other grades ($p<0.001$).
Conclusion: Higher socio-economic status is associated with the prevalence of diabetes and hypertension in this urban south Indian population which is in contrast to the situation in developed countries.

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Does glucose tolerance status affect quality of life?
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Background and Aims: The aim of this study was to examine the association of quality of life with glucose tolerance status in the Australian population.
Materials and Methods: The Australian Diabetes, Obesity and Lifestyle study (AusDiab) was a population-based study of 11,247 people from randomly selected areas of Australia. As part of the study, participants underwent an oral glucose tolerance test and completed the SF 36 quality of life questionnaire. Dimensions of the SF 36 scale were assessed using logistic regression modeling (the lowest quartile of each scale dimension of the NGT group was used to define those with a poor quality of life).
Results: Table 1 shows the dimensions of the SF 36 - quality of life scale by glucose tolerance status. Previously diagnosed diabetes was associated with a significantly greater risk of being in the lowest quartile of each dimension of the SF 36 scale and this association was only partially attenuated by adjustment for age and sex. Among those with newly diagnosed diabetes and impaired glucose tolerance there was also evidence of reduced quality of life on some dimensions of the scale. The associations were still evident after further adjustment for body mass index.
Conclusion: These findings show that diabetes is associated with a reduced quality of life and that this is evident in the early stages of the disease. Table 1 Odds ratios of being in the lowest quartile of each dimension of the SF 36 by glucose tolerance status (adjusted for age and sex)

	Physical functioning	Physical role	Bodily pain	General health	Vitality functioning	Social role	Emotional health	Mental
NGT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IFG	1.03 (0.9-1.2)	1.04 (0.9-1.2)	0.97 (0.8-1.2)	1.25 (1.1-1.5)*	0.93 (0.8-1.1)	1.17 (0.9-1.5)	1.19 (0.9-1.4)	0.93 (0.8-1.12)
IGT	1.15 (1.0-1.3)*	1.15 (1.0-1.3)*	1.23 (1.1-1.4)*	1.33 (1.2-1.5)*	1.20 (1.1-1.4)*	1.37 (1.2-1.6)*	1.16 (1.0-1.3)*	1.01 (0.9-1.2)
NDM	1.36 (1.1-1.7)*	1.36 (1.1-1.7)*	1.16 (0.9-1.4)	1.68 (1.4-2.1)*	1.24 (1.0-1.5)*	1.11 (0.9-1.4)	1.23 (0.9-1.5)	1.14 (0.9-1.4)
KDM	1.88 (1.5-2.3)*	1.88 (1.5-2.3)*	1.56 (1.3-1.9)*	2.99 (2.4-3.7)*	2.38 (1.9-2.9)*	2.13 (1.7-2.7)*	1.64 (1.3-2.0)*	1.41 (1.1-1.7)*

NGT – normal glucose tolerance, IFG – impaired glucose tolerance, IGT – impaired glucose tolerance, NDM – newly diagnosed diabetes mellitus & KDM – known diabetes mellitus. * - significant p<0.05

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Initiative for Quality Promotion and Epidemiology for Diabetes (IQPED): excellent tool to advise the government on diabetes.

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Background and Aims: In Belgium a system of quality promotion was set up for centres with multidisciplinary diabetes teams treating diabetic patients on ≥ 2 daily insulin injections.
Participation was a condition to receive recognition from the government and financial support to provide education and material for self-monitoring of blood glucose. Data were collected and analysed by the IPH. The aims were:
1. provide the centres with a benchmarking of their data, allowing them to compare their results with other (anonymous) centres.
2. obtain data to advise the government on diabetes care (only pooled data were transmitted).
Materials and Methods: The centres were asked to provide data on a random sample (based on an alphabetical patient list) of 10% of diabetic patients (at least 50 patients for small centres) on ≥ 2 daily insulin injections. The basic information sheet of the DiabCare Quality Network (http://www.diabcare.de) was used for data collection. Information on patient characteristics, blood glucose control, cardiovascular risk status, diabetes complications, self-monitoring, and drug treatment were retrieved.
Results: All 134 Belgian diabetes centres participated. Data form 7599 patients were retrieved (13.7% of patients under study). 36.3% had type 1 (DM1), 59.4% type 2 (DM2) and 4.3% had other types of diabetes. The average age, duration of diabetes and male/female ratio were resp. 46 y, 17 y and 1.2/1.0 for DM1; and 67 y, 13 y and 0.8/1.0 for DM2. The median HbA1c was 7.7% in DM1 and 7.5% in DM2. Cardiovascular risk factors were rather well controlled, except for blood lipids. A LDL-cholesterol ≥ 115 mg/dl was present in 49% of DM1 and 56% of DM2 patients. There was a high prevalence of diabetes complications. Nephropathy was present in 12.3% of DM1 and 23.0% of DM2. Advanced eye disease with maculopathy, proliferative retinopathy and/or blindness was present in resp. 9.2, 19.6, and 1.7% of DM1 and 14.2, 19.3 and 1.5% of DM2. Risk factors for diabetic foot were highly prevalent with loss of protective sensation in resp. 23.6 and 36.3% of DM1 and DM2, peripheral arterial disease in 10.2 and 21.0%, and a history of minor or major amputation in 1.9 and 3.0%. A history of acute myocardial infarction was present in resp. 7.2 and 15.9% of DM1 and DM2, and of stroke in 4.8 and 9.3%. A report written for the government proposed several measures to optimise diabetes care in Belgium (e.g. the lipid results were used to urge the government in lowering the lipid levels for reimbursement of hypolipidemic drugs; the high prevalence of complications in DM2 to advise on taking measures to improve care of DM2 by general practitioners).
Conclusion: The results show that it is possible to collect a large number of relevant data from a representative sample of diabetic patients followed in diabetes centres. Benchmarking with individual feedback and the assurance of confidentiality are stimuli to obtain reliable data. The data can be used for local quality improvement and for counselling the government. This data collection will be repeated annually.

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World Health Organisation two-step strategy for the screening of the high-risk populations, the missing cases.

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Aims: The new diagnostic criteria recommended by the ADA will detect only the diabetic patients with fasting hyperglycemia, and leave the isolated post-challenge hyperglycemia (IPCH) and impaired glucose tolerance (IGT) unidentified. The WHO recommended that all those with IFG have an OGTT to exclude the diagnosis of diabetes (two-step strategy). This two-step strategy will leave the subjects with normal fasting glucose (≤ 109 mg/dl). The aims of this study is to compare the WHO two-step strategy and the standard OGTT for all subjects.
Subjects and Methods: We reanalyzed the results of 1061 high-risk population which has been screened for diabetes mellitus and IGT. All subjects were screened with OGTT 75 gram glucose load after fasting for 10 hours. The results were divided in three categories: ADA criteria, two-step strategy, and the OGTT.
Results: Using the ADA criteria, these 1061 subjects can be classified to normal fasting glucose (FPG ≤ 109 mg/dl) 851 subjects (80.2%), IFG (FPG 110 – 125 mg/dl) 116 subjects (10.9%), and diabetes mellitus (FPG ≥ 126 mg/dl) 94 subjects (8.8%). The WHO two-step strategy performed in 116 IFG subjects identify 36 diabetic patients (FPG < 109 mg/dl and 2 h post load ≥ 200 mg/dl = IPCH) or 31.0%, and 52 IGT or 44.8% from all subjects. If the OGTT is performed to the 851 normal fasting glucose, it can identify another 47 diabetic patients or 8.0% and 217 IGT or 36.9% of all subjects. This means that without OGTT to all subjects, 36 diabetic patients or 20.3% from all diabetic patients and 36 IGT or 19.3% from all IGT subjects will left unidentified.
Conclusions: Applying the WHO two-step strategy in subjects with IFG will fail to detect 20.3% of diabetic patients and 19.3% IGT subjects. It is recommended that in high-risk populations the old strategy of screening – the gold standard OGTT – should be used instead of the two-step strategy.

PS 8
Genetics of Type 2 Diabetes and Animal Models

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Fetal malnutrition causes the changes in mitochondrial DNA content and muscle fiber type profile in adult life.

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Background and Aims: Poor intrauterine nutrition is associated with impaired pancreatic β-cell function and development, major changes in glucose metabolism in animal studies, but the mechanism is not yet clear. Qualitative or quantitative changes of mitochondrial DNA(mtDNA) may impair the production of ATP which plays a central role in insulin release. Since insulin has a major role in fetal growth, changes of mtDNA content could be a possible mechanism explaining the association between fetal malnutrition and type 2 diabetes in adult life. Muscle fiber type profiles are affected by exercise training, innervation, hormones, and aging. However, the effect of fetal malnutrition on muscle fiber type is unknown. So we aimed to investigate the effect of protein malnutrition in fetus and early life on mtDNA content and muscle fiber type in adult life.
Materials and Methods: We have developed a rat model of fetal malnutrition. Male offspring of dams fed a low-protein(8% casein) diet during pregnancy and lactation were weaned onto either control(18% casein) diet (recuperated group, R) or a low-protein diet (low-protein, LP), and they were compared with control group (C). We quantitated mtDNA content by competitive PCR with internal standard and examined muscle fiber compositions.
Results: MtDNA content in liver was lower in R and LP than C(by 53%, 60%; $p<0.001$) at 5 wk of age, but higher in R and LP than C(by 78%, 65%; $p<0.05$) at 15 wk of age. MtDNA content in skeletal muscle was significantly lower in R and LP than C (by 50%, 28%; $p<0.05$) and mtDNA content in pancreas also lower in R and LP than C(by 53%, 24%; $p<0.05$) at 25 wk of age. In soleus muscles of fetal-malnourished rats, a significant decrease in type II fibers(R 10.4%, L 7.8%) concomitant with an increase in type I fibers was observed, compared with control group(C 31.8%; $p<0.001$). On the contrary, the fiber type profile of EDL or gastrocnemius muscles showed no significant differences. Values of areas under the curve for insulin during intravenous glucose tolerance test (IVGTT) were lower in R and LP than C(by 29%, 21%; $p<0.05$). However, rats with fetal malnutrition showed no major impairment in glucose tolerance during IVGTT, or glucose utilization in euglycemic hyperinsulinemic clamp studies. Both pancreas weights and the relative β-cell mass were significantly lower in LP than C.
Conclusion: Rats with fetal protein malnutrition showed the changes in mtDNA content and muscle fiber type profile, and persistently impaired pancreatic β-cell function and development which may contribute to the development of type 2 diabetes in adult life.

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Low mRNA antioxidant enzymes levels associated with death and neural tube defects in embryos of the Cohen diabetic sensitive rat.

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Background and Aims: We investigated the role of the antioxidant defense mechanism, genetic predisposition and environmental factors in embryopathy of the Cohen diabetic sensitive (CDs) rat under diabetic conditions. We have previously shown that genetic susceptibility plays an important role in the reduction of superoxide dismutase (SOD) activity under diabetic conditions, inducing underdevelopment and neural tube defects (NTD) in embryos of the hyperglycemic CDs rats. SOD was elevated in embryos of the contrasting resistant strain (CDr) under the same conditions, thus protecting the embryos of the teratogenic effect of this diet.
Methods: Studies were performed on 11.5 day old live embryos of the hyperglycemic CDs rat [fed a diabetogenic high sucrose low copper diet (HSD), n=37] and from normoglycemic CDs rats [fed regular diet (RD) n=162], and on embryos CDr rats fed RD (n=225) or HSD (n=39). Embryos were monitored for growth and congenital anomalies. Levels of mRNA of Cu/Zn SOD, glutathione peroxidase (GSHpx) and catalase (CAT) were assessed by RT-PCR in embryonic homogenates.
Results: Embryos of CDs fed RD had a significantly lower score (underdeveloped) and were smaller when compared with embryos of CDr fed RD (a score of 36.7 ± 0.3 Vs 41.6 ± 0.13 and length of 4.2 ± 0.05 mm Vs 5.2 ± 0.05, $P < 0.01$ respectively) and with embryos of the CDs fed HSD (score of 36.7 ± 0.3 Vs 31.0 ± 1.4 and length of 4.2 ± 0.05 mm Vs 3.6 ± 0.1, $P<0.01$ respectively). When fed HSD, more than 50% of the CDs embryos were dead and 44% of the live embryos exhibited NTD. There was a similarly high survival rate and low incidence of NTD in embryos of CDs and CDr fed RD or CDr fed HSD. The CuZn-SOD mRNA basic levels were significantly lower in embryos of CDs fed RD when compared with that of embryos of CDr fed RD. Basic mRNA levels of GSHpx and CAT were not different in embryos of CDs and CDr when fed RD. When fed HSD, mRNA levels of CuZn-SOD increased in both CDs and CDr embryos; GSHpx mRNA levels increased in embryos of CDr but decreased in embryos of CDs; CAT mRNA levels were increased in embryos of CDr and unchanged in embryos of CDs. β-actin mRNA levels were not different between groups.
Conclusions: This study demonstrates a genetically determined reduction in the basic expression of CuZn-SOD in the embryos of the CDs rats, and a decreased expression of GSHpx in response to HSD diet pointing to a reduced antioxidant defense mechanism. This may suggest an important role for genetic susceptibility in determining the capacity of the embryo to cope with diabetes-induced oxidative stress.

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Genetic dissection of a diabetes QTL in congenic lines of the GK rat and synteny conservation with diabetes susceptibility loci in human 1q.

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Background and Aims: Genetic studies of the spontaneously diabetic (Type 2) Goto Kakisaki (GK) rat have demonstrated the existence of a quantitative trait locus (QTL) on rat chromosome 2 (*Nidd/gk2*) affecting diabetes phenotypes. The aims of the study are to test the existence of *Nidd/gk2* in congenic lines derived from GK and non diabetic Brown-Norway (BN) rats and identify the homologous regions of the refined rat QTL in the human genome.
Materials and Methods: Congenic lines were designed to fix GK alleles in overlapping regions of the locus *Nidd/gk2* on the genetic background of the BN rat through repeated backcross breedings. Using a genetic marker assisted protocol, the following congenics were produced: 2a (34cM; D2Wox26-D2Mit16), 2c (51cM; D2Mit6-D2Got149), 2e (23cM; D2Wox17-D2Rat63) and 2k (11cM; D2Wox17-D2Rat157). Intravenous glucose tolerance and *in vivo* insulin secretion tests were performed in 3 months old congenic and BN rats. Both radiation hybrid chromosomal mapping and computational analyses of rat, mouse and genomic sequence data were carried out for comparative genome analyses of the rat QTL.
Results: Overall glucose tolerance expressed as the cumulative glycaemia during the test was not significantly different between the lines, but the peak of glucose response was significantly higher in 2e and 2k rats (270±4mg/dl and 270±5mg/dl, respectively) than in BN (250±3mg/dl) and 2a (248±3mg/dl) rats ($p<0.015$ to $p<0.002$). Cumulative insulinaemia, which reflects the overall insulin response to glucose *in vivo*, was significantly higher in 2k rats (9.8±1.1µmol/l) than BN (5.8±0.4µmol/l; $p<0.002$), 2a (5.6±0.5µmol/l; $p<0.005$) and 2e (5.2±0.5µmol/l; $p<0.003$) rats. Rats of the line 2c also showed a higher insulin response (7.3±0.9µmol/l) than BN, 2a and 2e rats, but differences were not statistically significant. Rat gene mapping and genomic sequence data analyses allowed us to confirm evidence of synteny conservation between the GK QTL interval introgressed in the congenic line 2k and a region of human 1q21-24 linked to diabetes in several independent studies.
Conclusion: These results confirm the existence of gene(s) at the QTL *Nidd/gk2* involved in the control of glucose homeostasis and insulin secretion *in vivo*. Although the 11cM chromosomal region targeted in the congenic line 2k is likely to contain gene(s) responsible for both enhanced glucose induced insulin secretion and impaired glucose tolerance, other genes lying outside this region appear to modulate the phenotypes. The congenic line 2k could provide a new original model to test diabetes candidate genes localised in the genomic region conserved between human 1q21-24 and the GK QTL interval introgressed in this congenic.

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QTL dissection using a GK.BN F2 cohort derived from a congenic line.
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Background and Aims: Genetic investigations in the spontaneously diabetic (type 2) Goto Kakizaki (GK) rat have consistently demonstrated the importance of diabetes quantitative trait loci (QTLs) in rat chromosome 1 (*Nidd/gk1* and *Niddm1*). Recent studies in congenic lines derived for these QTLs have implicated non-overlapping chromosomal regions controlling diabetes related phenotypes and highlighted the complex structure of QTLs in inbred models. Here, we describe a novel strategy that allows fine QTL mapping of multiple diabetes related traits.
Materials and Methods: Using a genetic marker assisted protocol, we initially produced a congenic line specifically designed to carry GK alleles in a 105cM region (between markers D1Wox18 and D1Got 154) covering both loci *Nidd/gk1* and *Niddm1*, and the genetic background of the non diabetic BN rat. This congenic line was backcrossed to BN rats and progenies were intercrossed to produce a cohort of 221 hybrids, in which GK alleles segregate only at chromosome 1 loci. Genotype screening was therefore limited to chromosome 1 markers. Intravenous glucose tolerance and insulin secretion tests were performed in all hybrids at 3 and 6 months. Interval mapping, analysis of variance and permutation tests were used to test for evidence of genetic linkage between markers and genotypes.
Results: A total of 55 chromosome 1 markers were typed in the cross, leading to an average spacing of 2cM between adjacent loci. In 3 and 6 months old rats, glucose tolerance, expressed as the cumulative glycaemia following glucose injection, was significantly linked to a 13cM region between markers D1Rat164 and D1Mgh21. Strongest evidence of genetic linkage was observed at marker D1Got172 (LOD=5.42 at 3 months and LOD=3.7 at 6 months). Markers did not show evidence of linkage to insulin variables in 3 months old hybrids. At 6 months however, plasma insulin levels 15 and 20 minutes after glucose injection were linked to a 25cM region between markers D1Rat115 and D1Rat83 (Maximum $LOD^{Ins15mins}$=3.8 at marker D1Uia13; Maximum $LOD^{Ins20mins}$=3.9 at marker D1Got237). This QTL, which possibly contains two peaks of linkage, maps >13cM away from the region linked to glucose in tolerance.
Conclusions: Our strategy allowed us to create an epistasis-free model applied to the fine mapping of diabetes QTLs in the GK rat. Our results demonstrate the possibility to dissect several susceptibility loci affecting distinct but closely related diabetes variables that can be further investigated in congenic lines and sublines.

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One of the hyperglycemia QTL in OLETF rat, *Nidd3/of*, showed dominant inheritance evidenced by characterization of its congenic rat.
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Background and Aims: Numerous genome scan analyses identified QTL affecting diabetic trait in various animal models. Subsequently established congenic strains provided more direct evidence of those QTL. However, the confirmation using congenic animals so far verified only recessive loci. Our previous genetic analyses on OLETF (Otsuka Long-Evans Tokushima Fatty) rat identified 14 QTL and predicted that some of them were dominant mutation. Here we characterized one of the loci to test the mode of inheritance using the congenic rat.
Materials and Methods: Heterozygote males were obtained by mating F.O-*Nidd3/of* males with F344 females. Males of F.O-*Nidd3/of*, F1[F.O-*Nidd3/of* X F344] and control non-diabetic F344 rat were raised on standard laboratory diet till 30 weeks of age and phenotyped by OGTT (oral glucose tolerance test) assay.
Results: Plasma glucose levels of both F.O-*Nidd3/of* and F1[F.O-*Nidd3/of* X F344] rats were higher than that of the F344 rat throughout the course of OGTT analysis. Sixty-minute postprandial blood glucose levels for F.O-*Nidd3/of* (128.9±2.5 mg/dl, p<0.0001) and F1[F.O-*Nidd3/of* X F344](125.2±6.5 mg/dl, p<0.05) were significantly higher than that of F344 (104.0±3.3 mg/dl). Similarly, 90-min. postprandial blood glucose levels for F.O-*Nidd3/of* (124.5±2.0 mg/dl, p<0.0001) and F1[F.O-*Nidd3/of* X F344](126.5±5.3 mg/dl, p<0.01) were clearly elevated than that of F344 (104.0±3.3 mg/dl). Body weight for F.O-*Nidd3/of* (409.9±5.3 g, p<0.01) and F1[F.O-*Nidd3/of* X F344](419.0±4.9 g, p<0.0001) were also higher than that of F344 (378.2.0±5.3 g), suggesting that the *Nidd3/of* locus may have dominant effect on both phenotypes.
Conclusion: These results indicate that the mode of inheritance of *Nidd3/of* locus on diabetic trait is clearly dominant. In our knowledge, this is the first demonstration of dominant QTL affecting diabetic phenotype found in animal model. Because the locus influences on body weight, its effect on glucose regulation may be mediated by body weight control.

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Effect of high caloric diet for congenic strains carrying OLETF diabetes *Nidd2/of* and *Nidd10/of* locus.
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Background and Aims: Previously we identified 14 quantitative trait loci (QTLs) responsible for hyperglycemia using genome wide scan in the Otuka Long-Evans Tokushima Fatty (OLETF) rat. Subsequently generated congenic strains confirmed the successful isolation of the OLETF-derived diabetic QTL for all the loci. To further characterize these loci, we examined the genetic interactions between *Nidd2/of* and *Nidd10/of* locus by using double-congenic strain, F.O-*Nidd2&10/of*. Furthermore, we also tested how the high caloric diet influences on this gene-to-gene interaction.
Materials and Methods: Males of the F344 rat, a control strain, F.O-*Nidd2/of*, F.O-*Nidd10/of* and F.O-*Nidd2&10/of* congenic strains were raised either on standard laboratory diet(C) or on high caloric diet with 10% additional sunflower oil (H). At 20 weeks of age, they were examined for body weight and plasma glucose levels in OGTT (Oral glucose tolerance test).
Results: On standard diet, the body weight of only congenic strains containing *Nidd10/of* locus was significantly higher than that of the F344 rat, suggesting that *Nidd10/of*, not *Nidd2/of*, has an effect on the body weight. On the other hand, only congenic strains carrying *Nidd2/of* locus responded to high caloric diet and showed increased body weight, when the same dietary treatment had no effect on the F.O-*Nidd10/of* or the F344 rat. On high caloric diet, the total increment in double congenic strain can be explained by the additive effect of the two loci. Similar but slightly different propensity was found for blood glucose levels at 60 min. in OGTT. On standard diet, the glucose levels were higher for all congenic strains compared with the control strain. High caloric diet elevated glucose levels of F.O-*Nidd2&10/of* only such that the total increase in double congenic strains was equal to the addition of increase in F.O-*Nidd2/of* and F.O-*Nidd10/of* strain.
Conclusion: These data further confirmed that both *Nidd2/of* and *Nidd10/of* loci are involved in body weight as well as blood glucose regulation. For both phenotypes, we found that there is an interaction between these loci and the interaction was clearly different depending on the dietary condition. Therefore, we propose that this is an excellent model for studying genetic interaction as well as gene-to-environment interaction in the expression of type2 diabetes.

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NN414, a potent potassium channel opener (K_{ATP}CO) selective for SUR1/Kir6.2 prevents development of diabetes in *Psammomys obesus*.
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Background and Aims: We have previously shown that treatment with NN414, a SUR1/Kir 6.2 selective K_{ATP} channel opener, ameliorates postprandial glycemia and reduces deterioration of glucose intolerance in Zucker obese rats. *Psammomys obesus* is a desert gerbil that spontaneously develops diabetes when fed a high energy (HE) diet. This nutritionally induced diabetes can be reversed by return to low energy (LE) diet. The aim of this study was to investigate whether treatment with NN414 prevents the progression to overt diabetes in *Psammomys obesus* fed a HE diet.
Materials and Methods: To test for the ability of the animals to become diabetic, 40 female *Psammomys obesus* were transferred to HE diet for up to 2 weeks (W) followed by a reversal period on LE diet. The animals (N=22) that developed hyperglycemia on the HE diet were randomised into two groups receiving NN414 (15mg/kg) or vehicle once daily for 4W and the remaining animals (N=18) were discarded from the study. Blood glucose and body-weight (BW) was measured once daily, food intake was measured over 6 days after 2W of dosing and plasma insulin, HbA_{1C} and pancreas histology at the end of the study.
Results: After 4W of treatment, the NN414 treated remained normoglycemic (5.7±1.0 mM) whilst the vehicle treated animals had

developed significant hyperglycemia (14.2±1.7 mM, p<0.01). HbA_{1C} levels at end of study were significantly lower in the NN414 (6.9±0.4%, p<0.01), as compared to vehicle (10.7±1.6%) treated animals. There was no significant difference in BW or food intake between the groups. Sections of pancreas were immunostained for beta and non-beta islet cells and quantitated. Preliminary results indicate no difference between the beta cell mass of the NN414 and the vehicle treated animals as groups, due to high variability, but at corresponding beta cell masses the NN414 treated animals had lower HbA_{1C} values, suggesting superior functioning beta-cells in the NN414 treated animals.
Conclusion: This study demonstrates that administration of NN414 to *Psammomys obesus* during HE feeding, prevents nutritionally induced development of diabetes.

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Novel G protein coupled receptor kinase modulation utilized for treating Type 2 diabetes and for induction of melanogenesis.
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Background and Aims: Diabetes is diagnosed at an alarming rate around the world. More than 90% of the estimated 200 million persons affected with diabetes worldwide, have type 2 diabetes, which is a challenge for applying new treatment modalities. According to the thrifty gene hypothesis, the code of β3-adrenergic receptor is considered as one of the potential thrifty genes. A novel approach to enhance GPCR activity by GRK2/3 induced negative modulation is presented.
Material and methods:A GRK2/3 negative modulator was developed by our KinAce technology to create a series of overlapping peptides covering the entire sequence of GRK2/3 (amino acids 383-404, hereby HJ loop). Myristoyl-glycine or leuryl-glycine was attached to the N terminus of the peptides to facilitate intracellular entry and their C-terminus was amidated.
Results: The peptides were tested for their ability to induce melanogenesis (intracellular process that is initiated by activation of specific GPCR – melanocortin receptor) in B16 cells. Two peptides KRX-683107, and KRX-683124, derived from the C-terminus end of the HJ loop of GRK2/3 efficiently enhanced melanogenesis of B16 cells without stimulation by the melanocortin peptides (αMSH). In these peptides the 387th glycine of the native sequence was substituted by d-arginine to enhance the metabolic and peptide conformation stability. The common effect of differential concentrations of αMSH and increased concentrations KRX-683 was examined. When increasing concentrations of KRX-683 peptides, a typical dose dependent and saturable induction of melanogenesis was observed. The αMSH enhanced the melanogenesis induced by both peptides at their non-effective concentrations but did not affect the melanogenesis at the saturated concentrations of KRX-683 peptide. These results suggest that KRX-683 peptide and αMSH use a similar pathway to induce melanogenesis, supporting our basic hypothesis. The KRX-683 compounds have been found to be effective also as antidiabetic agents in an animal model of Type 2 diabetes – the gerbil *Psammomys obesus*. Treatment of *Psammomys*, rendered hyperglycemic by high energy diet, with KRX-683124 restored the blood glucose levels to normal in 90% of animals after 5 weeks of once a week administration of 12.5 mg/kg of the peptide (n=18). KRX-683107 peptide in a similar dose also lowered the blood glucose level of hyperglycemic *Psammomys* to normal in 65% of the animals after 4 weeks of treatment vs. no change in the vehicle treated controls. Moreover, a marked decrease (50%) in the adipose tissue weight and a significant improvement in glucose tolerance, after a 1g/kg i.p. glucose load, were observed in all peptide treated animals.
Conclusion: The efficacy of KRX-683, low molecular weight peptides in reversing the nutritionally elicited diabetes process suggests that this drug may be a useful candidate for treatment of type 2 diabetes.

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Polymorphism Pro12Ala of the PPARγ2 gene is associated with lower insulinemia and HOMA-R in Czech Type 2 diabetes mellitus patients.
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Background and Aims: The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor subfamily of transcription factors. PPARγ2 plays a key role in regulation of adipocyte differentiation and energy balance. Numerous recent studies provide the evidence that the Pro12Ala polymorphism is linked to insulin sensitivity and type 2 diabetes mellitus but the results are controversial and depend on the ethnicity. The aim of this study was to determine allele frequencies and to study the influence of the polymorphism on biochemical and anthropometric parameters in a group of DM2 patients (n=246; age 58.81±6.98; BMI=30.68±5.55kg/m^2), in a group of healthy offsprings of diabetics (n=93; age 39.54±10.45; BMI=25.85±4.14kg/m^2), and in healthy Czech adult population without family history of DM2 (n=123; age 32.23±10.64; BMI=23.21±3.67kg/m^2).
Materials and Methods: The Pro12Ala substitution was detected by PCR-RFLP method (HgaI). For statistical analyses, the NCSS 2000 program was used.
Results: χ^2 test did not reveal significant differences in 12Ala allele distribution between the group of DM2 patients and controls (hetero/homozygotes: 28.57%/1.22%; vs. 25.00%/0.86%, χ^2=0.79; p=0.67). The distribution in the group of offsprings was similar (hetero/homozygotes: 26.44%/2.30%). The 12Ala allele frequencies in diabetics, offsprings, and controls were 0.15, 0.15, and 0.13, respectively. The Mann-Whitney test revealed significant differences in a group of non-insulin treated diabetics: the fasting insulin levels and HOMA-R index were significantly lower in Pro12Ala compared to Pro12Pro genotype (insulin: mean±SD 13.62±9.27 vs. 16.36±10.58mIU/l, median=11.0 vs. 14.6mIU/l, p=0.028; HOMA-R: mean±SD 5.54±7.05 vs. 7.18±6.63mIU*mmol/l, median=3.98 vs. 5.10mIU*mmol/l, p=0.007). No significant differences were observed in C-peptide, proinsulin, and glucose levels. Lipid parameters (total cholesterol, HDLcholesterol, LDLcholesterol) did not differ between the particular genotypes in any group. Analysis of genotype in relation to body composition (BMI, WHR, %fat, %muscles) revealed no significant differences.
Conclusion: The frequency of the Pro12Ala substitution in the PPARγ2 gene did not differ significantly between DM2 patients and controls. In non-insulin treated diabetics, the fasting insulin levels and HOMA-R index were significantly lower in the Pro12Ala genotype. This observation supports the evidence that the 12Ala allele is associated with better insulin sensitivity.
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The association between leptin gene variants Codn25 (CAA/CAG), G-2548A and obese subjects in Chinese.
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Background and Aims: Variant of leptin gene or functional variant of linkage disequilibria may contribute to the pathogenesis of obesity, but differences may exist between populations with different genetic background. The present study was to observe the association between leptin gene variants [codn25(CAA/CAG), G-2548A] and obesity or it's phenotype in Han population in Chinese.
Materials and Methods: There were 271 unrelated subjects, including 153 I degree obesity patients (BMI 25.0~29.9 kg/m2, age: 51.5±14.9 years), 52 II degree obesity patients (BMI ≥30 kg/m2, age: 48.1±15.7 years) and 66 normal control (BMI 18.5~22.9 kg/m2, age: 49.1±11.1 years). We recruited the subjects with diagnostic criteria for obesity, which was recommended in 2000 in Asia-pacific area. The leptin gene variants were detected by PCR-RFLP.
Results: (1) Both genotype and allele frequencies of codn25 CAA/CAG, CAG were significantly different between II degree obesity and normal group in women (p=0.032, 0.047). The CAA, CAG allele frequencies in the two groups were 81.9%, 18.1% and 92%, 8%. But this significant difference was not observed between I degree obesity and normal group in

women (p=0.673, 0.685), and this difference was not observed between obesity and control in men yet (p>0.05). (2) There were significant differences in waist circumferences among different G-2548A genotype groups (F=6.259, p=0.015) in obese patients (BMI≥25 kg/m2). The waist circumferences in the group carried with A/A homozygous was remarkably lower than that in the group carried with A/G and G/G genotypes without reference to sex. (3) In different obese groups the synergic effect of Codn25 CAA/CAG, G-2548 variants on obese phenotype such as serum leptin, lipid profile, waist circumference, waist to hip ratio, body fat mass were not found.
Conclusion: (1) There is a close relationship between leptin gene variant of codn25 (CAA/CAG) in II degree obese of China Han women. This association indicates that the effect of this variant on obesity susceptibility is different in different obese degree and sex. (2) The G-2548A variant in leptin gene contributes to the regional body fat distribution in China Han obese, especially the adipose depots in abdomen. There isn't relationship between this action and sex. (3) There is no obvious synergic effect of these two leptin gene variants on obese phenotype.

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Identification and clustering of pathology-associated genes in the liver of Type 2 diabetic patients.
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Background and Aims: Type 2 diabetes is characterized by excessive hepatic glucose production by the liver, and is often associated with non-alcoholic fatty liver disease (NAFLD). The histological spectrum of NAFLD includes fatty liver alone to non-alcoholic steatohepatitis (NASH) with inflammation and fibrosis. We have found that fatty liver is closely associated with insulin resistance and the components of metabolic syndrome leading to increased risk for cardiovascular diseases. To deepen our understanding of type 2 diabetes and related liver disease, we used complementary DNA (cDNA) microarray technology to analyze relationship between gene expression profiles, and the livers pathology of patients with type 2 diabetes.
Materials and Methods: Liver biopsy samples were obtained from 12 patients with type 2 diabetes (11 males, age 46±12 yr, BMI 28±4 kg/m^2, FPG 121±30 mg/dl, HbA_{1c} 7.1±1.5%, ALT 55±38 IU/L, Mean±SD) and 9 non-diabetic patients (5 males, age 55±11 yr, ALT 29±14 IU/L). The severity of steatosis, inflammation and fibrosis was graded histologically from score 0 to 4. Mean scores for steatosis, inflammation and fibrosis were 2.0±1.0, 1.0±0.7 and 1.2±0.7, respectively in diabetic patients. All of the non-diabetic liver samples were histologically normal. We made cDNA microarrays consisting of 1083 human cDNAs. To determine the relative expression ratios of individual genes, Cy3-labelled cDNA from the specimens obtained from the diabetic patients were compared with Cy5-labelled cDNA from reference RNA from the liver of a non-diabetic patient.
Results: Hierarchical clustering of the gene expression of all 21 patients was assessed as for similarities between the differentially expressed genes. One cluster was obtained for the 12 diabetic patients and a separate cluster was obtained for the 9 non-diabetic patients. In the diabetic patients, the genes also clustered into 2 groups. Of these clustered genes, 266 genes were significantly up-regulated, and 94 genes were significantly down-regulated in the diabetic patients (P<0.05). To clarify a molecular link between fatty liver and NASH, we identified differentially expressed genes between 0-1 and 2-4 of steatosis scores, between 0-1 and 2-4 of inflammation and fibrosis scores in the diabetic liver. Twenty eight, 31 and 103 genes were significantly up- or down-regulated in severe group of steatosis, inflammation and fibrosis, respectively. These gene sets clustered diabetic patients according to the severity of each histology. Steatosis shared 15 genes with inflammation, and 3 genes with fibrosis. Although histological scores of inflammation and fibrosis correlated to each other (p=0.017), inflammation and fibrosis shared only 2 genes.
Conclusion: Expressed genes profiles of the liver of diabetic patients are different from those of non-diabetic patients. In view of gene expression profiles in the liver of type 2 diabetic patients, hepatic steatosis is closely associated with inflammation, whereas fibrosis seems to be an independent event.

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The role of sialidase gene on insulin resistance; sialidase transgenic mice and human sialidase gene polymorphisms.
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Background and Aims: It has been previously reported that gangliosides and sialic acids play an important role in both type1 and type2 diabetes, obesity and hypertension. Sialidase is a key enzyme removing sialic acids from gangliosides. We generated transgenic mice (TG) expressing plasma membrane-associated sialidase specific for gangliosides. After an intraperitoneal glucose tolerance test, they had hyperglycemia, and moreover, plasma insulin levels were increased 30-fold in TG compared with control mice. Insulin tolerance test showed insulin resistance in TG. Insulin-stimulated phosphorylations of insulin receptor and IRS-1 in skeletal muscle of TG. Glycogen synthase activity in skeletal muscle of TG were much higher than in those of control. Hematoxylin and eosin stain showed hyperplasia of pancreatic islets, and immunohistochemistry showed diffused stain with anti-insulin antibody. These results provide evidence that overexpression of plasma membrane-associated sialidase can lead to insulin resistance. To examine the association between human sialidase gene and diabetes mellitus, we screened polymorphisms in the gene in Japanese diabetic patients.
Materials and Methods: Using genomic DNA extracted from 250 diabetic patients and 213 normal control subjects, we performed PCR-direct sequencing with the specific primers for all exons of the gene to screen polymorphisms and mutations with ABI-SNaPshot. And then the data were statistically analyzed.
Results: We determined SNP (T/C) in exon3 codon 46. The allele frequencies of the SNP were different between diabetic group and control group (diabetics: C 0.596, T 0.404; control: C 0.751, T 0.249)(p<0.001). The values of body mass index (BMI) of subjects with normal glucose tolerance were higher in T/T genotype than in C/C genotype. The values of plasma insulin both at fasting state and 2 hours after 75 g glucose loading were also higher in T/T genotype than in C/T genotype. HOMA (R) and HOMA (beta) were also higher in T/T genotype. And moreover, we determined SNP (-505 C/T) in promoter region of the gene. But unlike SNP (T/C) in exon3, the allele frequencies of the SNP (-505 C/T)Å@were not significantly different between diabetic group and control group (diabetics: C 0.960, T 0.040; control: C 0.975, T 0.025). And the values of clinical data of subjects with normal glucose tolerance were not significantly different between in three genotype (C/C, C/T and T/T) of SNP (-505 C/T). In addition to two polymorphisms, we determined mutation (A to G) converting lysine to arginine in exon 3. This mutation was not found both in the all other diabetics and controls. The male patient aged 39 years with the mutation suffered from type1 diabetes at the age of 35, and had poor control of diabetes (HbA1c 12.8%).
Conclusion: From these results, it may be concluded that the polymorphism of sialidase gene, SNP (T/C) in exon 3, may play an important role in diabetes mellitus and insulin resistance.

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Haplotype construction of the FRDA gene and evaluation of its role in Type 2 diabetes mellitus.
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Background and Aims: Friedreich's ataxia (FRDA), caused by an expansion of an intronic trinucleotide repeat (GAA) in the *X25*-gene (9q13) is one of the most common hereditary neurodegenerative diseases. Almost 20% of the FRDA patients develop type 2 diabetes mellitus (T2DM) and some case control studies have shown association between T2DM and an intermediate (>10<66) GAA expansion. In addition, we and others have shown suggestive linkage between the 9q13-q21 locus and T2DM. We aim to study whether certain haplotypes and/or SNPs in the *X25*-gene is associated with T2DM.
Materials and Methods: 220 parent offspring trios with IFG, IGT or T2DM were genotyped for the GAA repeat and six additional SNPs in the *X25*-gene using the single base extension-technique (SNaPshot™, PE Biosystems) on an ABI3100. Association was tested with TDT and

haplotype blocks were created based on the TDT data. Also a meta-analysis comparing data from all published studies was performed.
Results: Transmission from heterozygous parents to affected offspring did not significantly differ from the expected for any allele (transmission of the rarer alleles for SNP's 1-6: 51%, 49%, 47%, 47%, 51% and 47%). Haplotype blocks did not provide evidence for excessive transmission to T2DM patients. Finally, the meta analysis did not support an association between intermediate GAA repeats and diabetes (OR=1,04 p=0,66).
Conclusion: Our data challenge a significant role of genetic variation in the *X25*-gene in the pathogenesis of T2DM.

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The mutation of the signal peptide of the insulin gene.
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Background and Aims: The mutations of insulin gene rarely cause diabetes mellitus. To date, seven missense mutations have been reported in insulin/proinsulin gene and all cases accompanied hyperinsulinemia or hyperproinsulinemia, and all presented mild hyperglycemia. Recently, a missense mutation (A7, Cys>Thr) was found in the insulin II protein in the Akita mouse. This mutation appears to function as a dominant negative manner and was associated with hypoinsulinemia and severe diabetes in this mouse model. This observation suggests that insulin mutation may result in hypo- or hyperinsulinemia depending on the locus of mutation. From the screening of insulin gene among Japanese subjects with type 2 diabetes, we found a family with a point mutation of Ala(GCA)>Thr(ACA) in the signal peptide region (Metabolism 50(6), 2001). However we could not confirm the relation of this mutation with diabetes. In the present study, we aimed at disclosing the relation of this mutation with diabetes, in vitro.
Materials and Methods: The wild and mutant form of the insulin cDNA was subcloned into the mammalian expression vector pTARGET and then trnsfected into CHO and MIN6 cells, (transiently). We carried out immunostaining using anti-C-peptide antibody. In addition, both anti-C-peptide and anti-endoplasmic reticulum antibodies were used for double immunostaining.
Results: In the mutant gene-transfected CHO cells, C-peptide localized to the circumference of the nucleus, and such localization was not shown in the wild type gene-transfected cells. We failed to detect the same localization of C-peptide in the mutant gene-transfected MIN6 cells, probably because of the presence of intrinsic mouse insulin and C-peptide.
Conclusion: Our previously reported point mutation of Ala(GCA)>Thr(ACA) in the signal sequence of insulin gene may cause disturbance of insulin secretion through the variation of synthetic process.

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The human G-protein beta-3 subunit gene 825 C/T dimorphism is associated with Type 2 diabetes.
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Background and Aims: The T allele of the 825C/T dimorphism of the gene encoding the human G-protein beta3 subunit (GNB3) has been associated with hypertension, obesity, carotid atherosclerosis and reduced insulin sensitivity. The present study was performed to evaluate the potential association of this genetic marker with prevalent diabetes.
Materials and Methods: The 825C/T dimorphism was determined in a population-based cohort of 702 healthy Caucasian subjects of middle-European (Austrian) origin without a previous diagnosis of diabetes. Fasting plasma glucose levels were used to classify subjects according to ADA criteria as normal (≤ 110 mg/dl), impaired fasting glucose (IFG, >110 and ≤ 126 mg/dl) or diabetes mellitus (> 126 mg/dl). An oral glucose tolerance test (OGTT) was performed to classify subjects according to WHO criteria as normal (2h-post-prandial glucose < 140 mg/dl), impaired glucose tolerance (≥ 140 and <200 mg/dl) or diabetic (≥ 200 mg/dl).
Results: The cohort consisted of 399 (56.8%) men and 303 (43.2%) women. The mean age was 56 ± 11 years (range 20 to 87 years). GNB3 genotype was successfully determined in 688 (98.0%) subjects. Fiftyone subjects were classified as diabetic by WHO or ADA criteria, 522 subjects were classified as normal by both ADA and WHO criteria. Carriers of a 825T allele were found more frequent among diabetics (68.7%) than among normal subjects (49.4%; $p < 0.001$), the resulting odds ratio was 2.2 (95% confidence interval 1.2 – 4.1). In contrast, fasting or post-prandial plasma glucose levels were not significantly associated with GNB3 genotype, neither in the whole cohort nor in any subgroup stratified for ADA or WHO criteria or sex.
Conclusion: In summary, our results demonstrate a strong linkage of the GNB3 825T allele with prevalent diabetes. The mechanism for this association is currently unclear and seems to be independent of glucose metabolism.

Group, n	CC, n (%)	CT + TT, n (%)	P (compared to normal)	Odds ratio of T-allele carriers
Normal, 522	264 (50.6)	258 (49.4)	-	1 (reference)
IGT or IFG, 106	50 (47.2)	58 (52.8)	0.76	1.1 (0.8 – 1.7)
Diabetes, 51	16 (31.4)	35 (68.7)	0.001	2.2 (1.2 - 4.1)

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A case of Type 2 diabetes with Thr149Met mutation in glucagon-like peptide-1 receptor gene.
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Background and Aims: Glucagon-like peptide-1 (GLP-1), an incretin hormone, is released from the intestinal L-cells. GLP-1 binds to the specific receptor (GLP-1 receptor; GLP-1R), a member of G-protein-coupled receptor, on the pancreatic β-cell and stimulates glucose-dependent insulin secretion and also growth and proliferation of β-cells. To address the possibility that the partial disruption of GLP-1 signaling could cause diabetes, we tried to detect the mutation in GLP-1R gene in the population with type 2 diabetes and found a rare case with mutated GLP-1R in this study.
Materials and Methods: (1) Genomic DNA was extracted from the peripheral blood from 36 unrelated Japanese type 2 diabetic. Polymerase chain reaction (PCR) was carried out to amplify the coding region of GLP-1R gene. PCR products were directly sequenced to determine the polymorphism.(2) Study subjects included 410 patients with type 2 diabetes, and 320 control subjects. The GLP-1R Thr149Met variant results in another *NLaIII* site and, therefore, subjects were screened for this substitution by PCR-restricted fragment length polymorphism.(3) 2-compartment model of C-peptide kinetics and minimal model approach in the intravenous glucose tolerance test were carried out for the patient with Thr149Met mutation in GLP-1R gene. The first phase C-peptide secretion rate (CS1) and minimal model parameters, insulin sensitivity index (Si) and glucose effectiveness (Sg) were obtained.
Results: (1) Five missense mutations were detected: Pro7Leu (CCG>CTG), Arg44His (CGC>CAC), Arg131Gln (CGA>CAA), Thr149Met (ACG>ATG), Lue260Phe (TTA>TTC). (2) Only the proband had Thr149Met mutation in GLP-1R gene.(3) The proband was 44-year-old man. Since 35 years old, he had been treated with sodium valproate because of epilepsy. His mother was diabetic. He developed diabetes at age about 20. He was administered with glibenclamide but could not get the glycemic control (HbA1c, 10.9 %). So he was admitted to the hospital. Height and body weight were 159 cm and 52 kg. The blood pressure was 132/90 mmHg. Physical examination showed no abnormalities except for short forth metatarsal bones, orthostatic hypotension and decreased Achilles tendon reflex. Antibody to GAD was negative. No mitchondrial DNA mutation at positions 3243, 1555, 11778, 3460, 14484, 9101, 9804 and 14498 was detected in peripheral white blood cells. Ophtalmological study showed proliferative retinopathy. In spite of intensive insulin treatment, his glycemic control was brittle. Fasting and postprandial CPR were 0.2 and 0.4 ng/ml, respectively. C-peptide secretion rate and minimal model analysis revealed that CS1 and Sg were extremely low and Si was relatively low: CS1, 0.122 ng/ml/5min (normal range: 6.8-18.5); Si, 1.91×10^{-4}/min/(μU/ml) (normal range: 2.6-7.6); Sg 0.774×10^{-2}/min (normal range: 1.15-4.1).
Conclusion: Thr149 is located in the first transmembrane domain of GLP-1R and thus this mutation could impair its function, though function analysis remains to be examined. The proband exhibited the severe impairment of both insulin secretion and glucose effectiveness. This diabetic phenotype in this case could be partially explained by Thr149Met mutation in GLP-1R .

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The Pro12Ala polymorphism of PPARγ gene and susceptibility to Type 2 diabetes mellitus in a Polish population.

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Background: Evidence has recently been shown that the polymorphisms of some genes might influence genetic susceptibility to complex, multifactorial forms of type 2 diabetes mellitus (T2DM). One of those genes is PPARγ (peroxisome proliferator activated receptor γ). The PPARγ gene product is a nuclear hormone receptor that regulates adipogenesis and is a target for thiazolidinediones, medications enhancing sensitivity to insulin. The sequence difference of this gene that was associated with T2DM in several populations was amino acid variant Pro12Ala.
Aims: 1) To determine the allele and genotype frequency of Pro12Ala PPARγ amino acid variant in a Polish population; 2) To search for the association of Pro12Ala polymorphism with T2DM and related phenotypes in the examined population.
Materials and Methods: We included 644 individuals (366 T2DM patients with the age of diagnosis above 35 years and 278 non-diabetic controls) into the case-control analysis. Oral glucose tolerance test (75 g) with plasma glucose and insulin measurement, homeostasis model assessment (HOMA) were accessed in 169 normoglycaemic individuals. The fragment of the PPARγ gene which contains the examined amino acid variant was amplified by polymerase chain reaction (PCR). Alleles and genotypes were determined based on electrophoresis of the DNA digestion products by specific restriction enzyme BshI. Differences in distribution between the groups were examined by χ^2 test. A general linear model was used to test variables for differences between genotype groups.
Results: The frequency of Pro/Ala alleles was similar in T2DM patients and in the controls (83.5%/16.5% vs. 84.5%/15.5%, respectively, p=0.607). Similarly, there was no difference between the groups when we analysed the genotype distribution. In addition, we performed some stratification analyses based on age of diagnosis, body mass index (BMI), and family history of T2DM. We found that the Pro/Ala and Ala/Ala genotypes were more frequent in the group of T2DM cases with the age of diagnosis above 50 than in the controls (36.1% vs. 27.3%, p=0.046). This difference was not significant after the Bonferroni correction for multiple comparisons. The other stratification analyses were not able to show any differences between the groups. Similarly, no difference was observed in respect to prediabetic traits between the carriers of various genotypes.
Conclusion: The frequency of the Pro12Ala PPARγ polymorphism in the studied Polish population is similar to other Caucasians. We were not able to confirm earlier reports that Pro allele is associated with T2DM or diabetes-related traits. Moreover, the results of the stratified analysis in case-control analysis suggest an opposite trend in late onset T2DM.

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Cosegregation of MIDD and MODY: functional and clinical consequences.

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Background and Aims: Several forms of diabetes are associated with monogenic defects in beta-cell function. MODY3 is explained by mutations in the gene HNF1α, whereas MIDD is associated with a mutation at position 3243 in the mitochondrial DNA. The aim of this study was to explore the pathophysiology of diabetes in a family with both a mutation in HNF1α (M626K) and mtDNA3243 mutation.
Materials and Methods: We studied a Finnish family including six carriers of mtDNA 3243 mutation and six carriers of both 3243 and M626K mutation. The degree of 3243-heteroplasmy was determined by PCR in DNA extracted from peripheral blood. The M626K-mutation was generated in HNF1α cDNA by in vitro mutagenesis, and transcriptional activity, DNA-binding capacity and intracellular localisation of the mutated HNF1α protein were investigated by Dual Luciferase assay, electrophoretic mobility shift assay and immunolocalisation studies.
Results: Carriers of the 3243 mutation showed marked variation in the amount of mutated DNA, i.e. heteroplasmy which was also reflected by large variation in insulin sensitivity and b-cell function. The M626K mutation in HNF1α decreased transcriptional activity by 46 % in a non-insulin producing cell line (HeLa). The intracellular localisation was normal, whereas the DNA binding ability was increased.
Conclusion: Co-segregation of the two mutations was not associated with a more severe form of diabetes than the individual mutations, suggesting that they are not additive.

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Genetics of Type 2 Diabetes Mellitus and MODY

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HNF-1α transactivates the L-PK proximal promoter differentially in a HNF-1α producing cell-type dependent manner.

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Background and Aims: The liver-type pyruvate kinase (L-PK) gene is expressed in a few organs such as the pancreas and the liver. It encodes a major glycolytic enzyme and shows polymorphisms, associated with type 2 diabetes. Very recently decreased mRNA level of the liver-type pyruvate kinase (L-PK) gene was observed in the pancreas of *Hepatocyte nuclear factor–1α* (*Hnf-1α*)$^{-/-}$ mice, but not in their liver. Here we examined whether HNF-1α is directly involved in HNF-1α producing cell-type dependent transcription of the gene.
Materials and Methods: Transient transfection assays were carried out using HNF-1α-expressing cells, namely, hepatocyte-derived HepG2, and Huh7 and pancreatic β cell-derived HIT cells and the L-PK proximal promoter harboring its TATA box and a binding site (LF-B1BS) for HNF-1α. Electrophoretic mobility shift assays (EMSA) were performed using nuclear extracts derived from those cells and LF-B1BS as a radio-labeled probe.
Results: Transfection assays show that HNF-1a transactivates efficiently the L-PK proximal promoter in HIT cells, but hardly in HepG2 and Huh7 cells. Deletion/point mutations of its LF-B1BS cause a dramatic decrease in its transactivation by HNF-1α in HIT cells. EMSA shows that the HIT nuclear extract-derived protein(s) binding to the LF-B1BS is recognized specifically by antibody to HNF-1α. Finally quantitation analyses show that HNF-1α DNA binding activity is detected comparablely in those HNF-1α-expressing cells.
Conclusion: Taken together, these results strongly indicate that HNF-1α transactivates differentially the L-PK proximal promoter depending on the cell type of HNF-1 α producing cells and mutations in the regulatory cis elements, especially LF-B1BS, required for expression of the L-PK gene, may be linked to diabetes mellitus.

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Novel mutations and promoter sequence variants of the HNF4A/MODY1 gene in the Norwegian MODY registry.

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Background and Aims: Recent evidence suggests an important role of the gene HNF4A causing MODY1 in the transcriptional network regulating pancreatic insulin secretion. We have investigated the prevalence of MODY1 sequence variants in the Norwegian MODY Registry.
Material and Methods: Patients in the MODY registry were recruited form primary care and hospitals and subjected to expert diagnostic review. DNA from MODY2- and MODY3-negative patients was sequenced directly.
Results: Two novel sequence variants were found: GGA326AGA (giving the amino acid substitution G326R) and ACC339ATC (giving the amino acid substitution T339I) in exon 8, and the formerly reported sequence variant GTG255ATG(giving the amino acid substitution V255M) in exon 7, all in the transactivating domain of the protein. Resequencing exon 8 with a second primer set showed a single nucleotide substitution in the original primer binding site giving rise to an allelic dropout. Two novel intron variants, -50G/C and -192C/G, in the P2 promoter region were also observed. These were not found in 53 unrelated MODY probands nor in 50 healthy blood donors. The localization of the two novel coding sequence variants in the transactivation domain predicts a reduction of transactivation activity in accordance with previous functional studies of mutations in this region.
Conclusions: Four novel sequence variants and the formerly reported GTG255ATG (V255M) were found in Norwegian MODY2/3-negative patients, three causing amino acids substitutions and two being single nucleotide substitutions in the P2 promoter region. We observed a sequence variant in an established primer binding site for exon 8. Hence, re-

sequencing using a new primer set should be considered in HNF4A negative MODY patients.

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WITHDRAWN

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***HFE* gene mutations and diabetes in an urban Australian community: the Fremantle Diabetes Study.**

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Background and Aims: Although the relationship between iron overload and glucose intolerance is well-recognised, a clear association between mutations in the *HFE* gene and diabetes is yet to be established. This may be because most previous studies have included small samples of selected patients. The aim of the present study was to determine the frequency of common *HFE* mutations in a large, population-based sample of diabetic patients, and to assess the frequency of biochemical and clinical expression of iron overload in subjects homozygous, heterozygous and wild type for these mutations.
Materials and Methods: We studied 1355 (95%) of the 1426 patients recruited to the Fremantle Diabetes Study (FDS), a prospective study in a postcode-based catchment area of approximately 120,000 people. C282Y mutations in the *HFE* gene were determined from buffy coat DNA by PCR and restriction enzyme cleavage. H63D mutations were determined only in C282Y heterozygotes. Serum iron, transferrin and ferritin concentrations were also measured on all homozygotes and heterozygotes, and 300 randomly-selected wild types
Results: 1198 patients were wild type (W/W; Group 1), 133 patients were C282Y/W (Group 2), 15 were C282Y/H63D (Group 3) and 9 patients were homozygous for C282Y (Group 4). The breakdown of these genotypes in relation to previously-published data from the Busselton Study, an Australian community study of diabetic and non-diabetic subjects, is shown in the table. There were expected increases in ferritin (geometric mean [SD range]; 110 [41-292] vs 186 [26-1,317] mg/L in Group 1 vs Group 4) and transferrin saturation (mean ± SD; 23 ± 8 vs 59 ± 32 % in Group 1 vs Group 4) in patients with mutations. Homeostasis model assessment (HOMA)-derived measures of beta cell function and insulin sensitivity were similar across the four genotype groups.
Conclusion: The frequency of common *HFE* gene mutations in a community-based sample of diabetic patients similar to that observed in the general population. Mild to moderate degrees of iron overload did not alter glucose tolerance. These data imply that diabetes mellitus is a late, and therefore rare, manifestation of iron overload.

Genotype	Busselton(n=3011)	FDS type 1(n=117)	FDS type 2(n=1224)
W/W	85.4%	94.9%	87.9%
C282Y/W	11.9%	4.3% 10.3%	
C282Y/H63D	2.2%	0.9%	1.1%
C282Y/C282Y	0.5%	-	0.7%

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Maternally inherited diabetes and deafness (MIDD): a study of a diabetic population in Denmark.

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Background and Aims: Mutations in the mitochondriel genome (mtDNA) can cause diabetes mellitus (DM). The most common mutation is the A3243G pointmutation at the tRNA (leu,UUR) gene. This mutation can cause a variety of clinical phenotypes e.g: Mitochondriel myopathy, Encephalopathy, Lacto-Acidosis and Stroke-like Episodes (MELAS) and Maternally Inherited Diabetes and Deafness (MIDD). MIDD is associated with maternal inheritance, early onset of DM (often < 40 years) caused by a progressively insulin secretion defect and development of a perceptive hearing impairment (HI). The patients are lean with normal/low body mass-index. The titers of islet cell-antibodies are most often negative and the diabetes may initially be treated with oral antidiabetic drugs. Studies from a number of countries estimate the prevalence of MIDD in diabetic popultions to 0.5-2.8%. At the present time there are no Danish prevalence estimate or clinical studies of MIDD.
The aims of the study are:
1.Estimation of the prevalence of MIDD in Ribe County (225.000) persons.
2.Description of the relationship between the genotype and pheno-type with focus on the importance of the amount of mutant mtDNA (degree of heteroplasmicity).
3.Sceening of the maternal relatives to the MIDD patients for the A3243G mutation.
Materials and Methods: 1. Qualitative and quantitative analyses of the A3243G mutation. 2. Insulinsecretionanalyses; Oral Glucose Tolerance Test (OGTT), Insulin-Modified frequently sampled Intra-Venous Glucose Tolerance Test (IM-IVGTT) Controls: (2:1) matched by gender, age and bodymass index. 3. Audition test. 4. Exercise test. 5. Ophthalmologic examination. 6. Muscle biopsi. Criteria of inclusion: Patients with diabetes and hearing impairment and/or maternal predisposition to diabetes and /or hearing impairment.
Results: At present time (February 2003) we have pre-screened all the diabetic patients in our outpatient clinics (1700) and tested 45 patients with possible MIDD. We have identified two families with a total number of 18 persons with the mutation. Phenotypes presenting so far: Four patients with MIDD, three with HI, one with HI and impaired OGTT, one with MELAS as well as 9 asymptomatic carriers. Several patients in family 1 have pronounced symptoms of myopathy. The diagnosis has been confirmed by excercise- and handgrip test. In the other family none of the patients suffer from myopathy. The degree of heteroplasmicity in blood vary from: 40 to 61% and in muscle from: 40 to 90%. Prevalence of MIDD in the diabetic out-patient clinics in Ribe County, Denmark: 0,29% with 95% confidence intervals [0,67-6,0/1000]. Prevalence of A3243G positive persons in Ribe County, Denmark: 0,008% with 95% confindence intervals [4,3-11,7/100.000].
Conclusion: The prevalence of MIDD in a diabetic population in Denmark seems to be equal to the prevalences found in other studies.The clinical presentations are very heterogenous.

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Metabolic effects of the Gly1057Asp polymorphism in *IRS-2* and interactions with obesity.

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Background and Aims: Insulin receptor substrate (IRS)-2 plays an important role in insulin signaling and its disruption results in diabetes in mice. In an Italian population, the IRS-2 Gly1057Asp substitution was associated with lower risk of type 2 diabetes in lean, but with a higher risk in obese individuals.
Materials and Methods: To clarify the role of this mutation in Pima Indians, its effect on type 2 diabetes was tested in a large cohort (n=998). A subgroup of non-diabetic individuals (n=233), had measurements of body composition, insulin action (M), endogenous glucose production (EGP), acute insulin response (AIR), and subcutaneous abdominal adipocyte size (SAAS). 132 subjects had these measurements on more than one occasion in a longitudinal study.
Results: The frequency of the Asp1057 allele was 0.6. Subjects homozygous for this allele (Asp/Asp) had a higher prevalence of type 2 diabetes than both of the other genotypes combined [X/Gly, Odds ratio 1.5 (95% CI 1.02-2.29)]. Subjects with Asp/Asp had higher percent body fat, BMI and waist circumference (all $p<0.05$), but there was no difference in metabolic characteristics. However, the relationship between percent body fat and fasting glucose, basal EGP, EGP during the clamp, AIR and SAAS was different in the Asp/Asp group (p for interaction=0.02, 0.06, 0.0007, 0.08, 0.006) compared to the X/Gly group, suggesting a more detrimental effect of Asp-homozygosity on these traits with increasing percent body fat. These associations were mostly confirmed in longitudinal analyses.
Conclusion: Our findings suggest that the association of homozygosity for the Asp1057 allele in IRS-2 with type 2 diabetes in Pima Indians, may be mediated by interaction of the polymorphism with obesity on several diabetes-related traits.

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Distinct LMNA mutations result in insulin resistance.

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Background and Aims: Mutations within the LMNA gene encoding the nuclear envelope proteins lamin A and lamin C were recently described to cause the autosomal diseases familial partial lipodystrophy, Emery-Dreifuss muscular dystrophy, Limb-Girdle muscular dystrophy, dilated cardiomyopathy, and atrioventricular conduction system disease. Features of familial partial lipodystrophy include muscle disease, regional loss of adipocytes, and insulin resistant diabetes mellitus. The role of lamin A and C in this context has not been elucidated so far. Therefore, mutation analysis of LMNA is currently of great interest to gain more insights into the functional aspects of lamin A/C.
Materials and Methods: Patients with suspected laminopathy were analyzed for mutations by direct sequencing of all exons, exon/intron boundaries, and the promoter region of LMNA. This was performed by cycle sequencing using fluorescent dye terminators and the ABI Prism 310 automatic sequencer (Applied Biosystems, Darmstadt, Germany).
Results: We identified the disease causing mutations in 15 families so far. Within these 15 families we detected 11 different mutations within LMNA: S22L, T27I, R190W, Q355X, R482W, R482Q, W498C, R582H, R644C, 1397delA, and 425-426ins21nt. The mutations S22L, R190W, Q355X, R644C, 1397delA, and 425-426ins21nt resulted in the phenotype of dilated cardiomyopathy with or without atrioventricular conduction system disease and variable symptoms of peripheral neuropathy, but no hyperinsulinemia. The mutations T27I and W489C resulted in variable forms of muscle dystrophies. In contrast the mutations R482W, R482Q, and R582H resulted in the phenotype of familial partial lipodystrophy.
Conclusion: Extended family analysis revealed so far a total of 44 gene carriers. The corresponding epitopes of these variants are located in the conserved regions of exon 8 and 11, which have been predicted to appear in close localization using crystallization analysis. This in turn suggests, the identical mechanism of these identified mutations in altering functional aspects of lamin A/C. Most interestingly, SREBP1 has been shown to interact with lamin A/C in this region and therefore, may serve as a potential ligand with altered affinity due to these distinct mutations. This may initiate complex pathways, which finally result in the pathogenesis of insulin resistance. In conclusion, LMNA associated familial lipodystrophy serves as an excellent monogenetic model to study the pathogenesis of insulin resistance. Most interestingly, patients show clearly a phenotype reflecting altered regulation of myocytes and adipocytes. This presented genotype/phenotype relation demonstrates that both the kind of mutation and its localization within the LMNA gene plays an important role in the pathogenesis of the phenotype.

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RAGE polymorphisms and the heritability of insulin resistance: the Leeds Family study.

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Background and Aims: Activation of the receptor for advanced glycation end products (RAGE) leads to a cascade of proinflammatory and procoagulant responses which have an important role in the pathogenesis of the vascular complications of diabetes mellitus. Additionally, there is evidence that proinflammatory mechanisms underpin the development of type 2 diabetes which implicates RAGE in the pathogenesis of insulin resistance. To investigate the relationship between RAGE allelic variation and insulin resistance, the Gly82Ser variant and three promoter variants, (-429, -374 and 63bp deletion) were studied in 482 subjects of known family pedigrees characterised for insulin resistance (using HOMA) and for atherothrombotic risk.
Materials and Methods: Genotyping was performed as previously described and statistical analysis was undertaken using SPSS and SOLAR software packages.
Results: Carriage of the –429 C allele was associated with increased insulin resistance (p=0.020). Pedigree analysis was performed using SOLAR software and the relationship remained significant when family structure was considered (p=0.023). Insulin resistance was estimated to have a heritability of 23.8% before the addition of covariates. Analysis of the relationship between RAGE and insulin resistance indicated that –429 C allele reduced the residual heritability of insulin resistance after adjusting for covariates (age, sex, BMI) from 20.8% to 20.0% and contributed approximately 2% to the total heritability of insulin resistance
Conclusion: The results indicate that the RAGE gene either directly affects the development of insulin resistance or is in linkage disequilibrium with a locus involved in this process.

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UCP-2 promoter polymorphism (-866G/A) affects its expression in beta cells and modulates clinical profiles of Japanese Type 2 diabetic patients.

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Background and Aims: UCP-2 promoter polymorphism (-866G/A) is reported to be associated with its expression in adipose tissue and obesity in Caucasians; G allele is associated with lower promoter activity and risk of obesity. However, our previous study showed no differences of allele frequency between obese and lean Japanese type 2 diabetic patients. On the other hand, the frequency of insulin therapy is significantly higher in patients with A allele. Therefore we have investigated the promoter activity of each allele in beta cells and the relationship between this polymorphism and clinical profiles of Japanese type 2 diabetic patients.
Materials and Methods: DNA fragment of UCP-2 promoter region with –866G or A is inserted into pGL3-Basic vector, transfected to INS-1 cells and promoter activity is analyzed by dual-luciferase system. Four hundred and thirteen type 2 diabetic patients and 68 non-diabetic subjects were analyzed by PCR-RFLP method using Mlu I. To minimize the mixing of type 1 diabetes or MODY, the following patients were excluded; patients diagnosed before 25 years of age or patients receiving insulin therapy within 3 year from onset. Non-diabetic subjects were defined as over 60 years of age, HbA1c less than 5.6% and no positive family history of diabetes.
Results: The promoter activity of A allele was significantly higher than that of G allele (57.8+/-7.3 vs 49.4+/-6.8, shown as the ratio to that of pGL3-Basic: p=0.032) in glucose concentration of 11.1mmol/L. Similar results were obtained under glucose concentration of 5.6 or 22.2 mmol/L. There are no significant differences of allele frequency between type 2 diabetic patients and non-diabetic subjects. Between type 2 diabetic patients with A allele and those without A allele, there are no significant differences of age, gender, max BMI, duration of the disease, HbA1c level, incidences of diabetic microangiopathy, hypertension, and hyperlipidemia, and average intimal-medial thickness of carotid artery estimated by ultrasonography. However, the patients with A allele showed significantly earlier onset of the disease (46.5+/-10.3 vs 49.2+/-9.0 years of age: p=0.020) and higher frequency of insulin therapy (48.5% vs 34.7%: p=0.017). Both logistic regression analysis (p=0.0153) and Kaplan-Meier analysis (p=0.0365) confirmed the independent effect of UCP-2 genotype on the insulin requirement.
Conclusion: These results indicate that UCP-2 (-866G/A) polymorphism does affect its transcription in beta cells and modulate the clinical profile such as age of onset or insulin requirement in Japanese type 2 diabetic patients.

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Relationship between cholesteryl ester transfer protein and nephropathy in Type 2 diabetes.

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Background and Aims: Genetic susceptibility is implicated in the genesis of microangiopathy. Among the different genes implicated in the development of diabetic nephropathy (DN),very few studies have assessed the potential role of cholesteryl ester transfer protein (CETP) polymorphism.
Materials and Methods: In a group of 404 type 2 diabetic patients (mean (SD) age 59.5(10.7), sex ratio 0.76 (female/male), diabetes duration 11.8 (7.7)years, BMI 28.9(5.3), HbA1c 8.2(1.9)%, cholesterol(CT)5.8(1.2), triglycerides (TG)2.2(1.8), HDL 1.3(0.4), LDL 3.5(1,0) mmol/l we studied prospectively during 8 years the expression of DN (creatinine > 90micromol/l and/or microalbuminuria > 30microg/mn)according to TaqIB CETP polymorphism. Genetic variants were determined by PCR , identifying 2 alleles (B1: absence and B2: presence of the restriction site).
Results: TaqIB allele frequency were the following :B1B1 (31%), B1B2(49.5%),B2B2(19.5%), respecting Hardy-Weinberg equilibrium. TG levels were significantly higher in B1B1 subjects (2.3(1.7)mmol/l) and HDL levels lower in B1B1 male patients (1.2(0.6)mmol/l). At entry , 226 diabetic patients (56%) presented a nephropathy,its prevalence was significantly higher in B1B1 subjects (80/124=64.5%) than B1B2 and B2B2 (146/279=52%)($p<0.05$). After 8 years of following the incidence of DN was significantly higher in B1B1 patients (34%) than B1B2 and B2B2 (12.6%)($p<0.01$). In multivariate analysis, CETP seems to influence the expression of nephropathy particularly in hypertensive patients.
Conclusion: These results suggest that TaqIb CETP polymorphism may be implicated in the development of DN. The role of lipids modifications may be a link that needs further studies .

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Adiponectin: T45G polymorphism and plasma concentration are independently associated with onset of hyperglycemia during a 3-year period in DESIR study.

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Background and Aims: The plasma concentration of the adipocyte derived peptide, adiponectin, is decreased in patients with obesity, type 2 diabetes and coronary artery disease. The adiponectin gene is located on chromosome 3q27 where a diabetes susceptibility locus has been mapped. A silent polymorphism in exon 2 (T45G) has been associated with BMI, insulin sensitivity and type 2 diabetes in some cross-sectional studies. Our aim was to assess the contribution of this polymorphism in the development of features of the insulin resistance syndrome in a 3-year prospective study in 4448 French Caucasian subjects from the D.E.S.I.R cohort.
Materials and Methods: The study population consisted of men and women, aged 30 to 64 years, who participated in D.E.S.I.R., a 9-year follow-up study that aims to clarify the development of the insulin resistance syndrome. Participants were volunteers from Health Examination Centers in the western central part of France. Genotyping was performed using polymerase chain reaction followed by hybridization with allele-specific molecular beacon fluorescent probes. The association of genotypes with continuous variables was tested by ANOVA or ANCOVA. Adjusted odds-ratios were calculated by a multivariate logistic regression.
Results: After 3 years, GG subjects had a greater increase in BMI (body weight gain in kg: 1.04, 1.03, 2.01 for TT, TG and GG genotypes respectively; $P = 0.009$) and in waist-hip ratio (WHR) (increase: 0.007, 0.010 and 0.023 for TT, TG and GG genotypes respectively ; $P = 0.007$). For subjects normoglycemic at baseline (n = 3948), the 3-year risk of becoming hyperglycemic (type 2 diabetic or impaired fasting glucose) was increased in GG carriers: odds-ratio adjusted for sex (vs TT) = 2.71 (95%CI 1.31-5.60) ($p = 0.007$). When adjusted for multiple factors (sex, age, BMI, WHR, insulinemia, glycemia, and also body weight gain and WHR increase), the risk associated with GG remained unchanged: OR = 2.88 (95%CI 1.29 - 6.46), $p = 0.010$. Baseline plasma adiponectin was lower in subjects who later became hyperglycemic (24,8 ± 11,1 µg/ml) than in subjects still normoglycemic (27,1 ± 12,9 µg/ml), matched for sex, age and BMI ($p = 0,019$). Multiple logistic regression showed that T45G polymorphism and baseline adiponectin level were independently associated with onset of hyperglycemia.
Conclusion: T45G adiponectin polymorphism affects body weight gain, with android distribution, and onset of hyperglycemia in a 3-year period. Reduced concentration of plasma adiponectin is also predictive of hyperglycemia, which is in favor of its causal role in the insulin resistance syndrome. Nevertheless, since the genetic variation and plasma concentration act independently from each other, the mechanism of this relationship needs clarification.

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AGT203, a new Type 2 diabetes target.

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Background and Aims: After extensive critical review of published and in-house genome-wide linkage scans for type 2 diabetes, we focused on chromosome 3q27 as a region likely to contain a diabetes susceptibility gene. The aim of this study was to identify genes in this region that contribute to the development of type 2 diabetes.

Materials and Methods: We examined all genes in the 95% confidence interval of this linkage peak taking into account factors including predicted structure and expression, proximity to the linkage peak and similarity to known drug target families.
Results: This screen highlighted AGT203, whose structure suggests it may be a membrane-associated enzyme. Resequencing of AGT203 in 50 individuals identified 21 novel polymorphic variants, which were genotyped in 1100 U.S. Caucasian subjects. 56 haplotypes were observed (9 with frequencies >0.01). 2 SNPs were independently associated with fasting plasma insulin (I1Y193 p=0.0457, I6S-153 p=0.0239), and another was associated with the insulin:glucose ratio (I1Y-35e p=0.0451). All 3 of these SNPs were non-coding. In Psammomys obesus, an animal model of obesity and type 2 diabetes, AGT203 was expressed predominantly in skeletal muscle. AGT203 gene expression was reduced by ~50% in red gastrocnemius muscle of obese, diabetic compared with lean, nGT P. obesus ($p<0.05$). Furthermore, AGT203 expression was negatively correlated with plasma insulin ($r=-0.51$, $p<0.01$) and blood glucose concentrations ($r=-0.48$, $p<0.05$). In vitro studies in C2C12 myotubes showed that both glucose and insulin suppressed AGT203 gene expression ($p<0.01$).
Conclusion: These preliminary data suggest a role for AGT203 in the pathophysiology of type 2 diabetes, and further studies are underway to determine the precise role of AGT203 in the disease process.

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A novel missense mutation (Val1481Ile) in the fatty acid synthase gene (*FAS*) is associated with percentage of body fat and lipid oxidation rates in Pima Indians.

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Background and Aims: Recent studies show that inhibition of fatty acid synthase (FAS) induces a rapid decline in fat stores in mice, suggesting its role in energy homeostasis. The human fatty acid synthase gene (*FAS*) maps to chromosome 17q25, a region showing suggestive linkage with adiposity in a genome wide scan for genetic determinants of type 2 diabetes mellitus (T2DM) and obesity in Pima Indians. Therefore, we investigated the role of *FAS* in the pathophysiology of obesity in Pima Indians.
Materials and Methods: We screened *FAS* for genetic variation by sequencing the coding region, and the 5' and 3' untranslated regions (UTRs) in DNA from 32 full-blooded non-first degree related Pima Indians. Selected variants were genotyped in a cohort of full-blooded Pima Indians (N = 216) and analyzed for associations with adiposity and related metabolic phenotypes.
Results: Eleven single nucleotide polymorphisms (SNPs) were identified: two SNPs in the 5'UTR, 4 SNPs in exons (two silent mutations in exon 4 - Asn189 and exon 20 - Ala1089; two missense mutations in exon 8 - Arg419His and exon 25 - Val1481Ile), 2 SNPs in introns and 3 SNPs in the 3'UTR. Six of these SNPs were further genotyped in a group of non-diabetic Pima Indians (N = 216) with measurements of body composition (DEXA), and energy expenditure and macronutrient oxidation rates under resting and hyperinsulinemic conditions (indirect calorimetry). The Val1481Ile polymorphism (G to A; allele frequency of A = 0.10) was associated with percentage of body fat and lipid oxidation rates. Compared with homozygotes for the Val variant, subjects with Ile/x had a lower mean percentage of body fat (30% ± 1 vs. 33% ± 1; p = 0.002; adjusted for age, sex, family membership) and a higher mean resting (0.76 ± 0.04 vs. 0.69 ± 0.02 mg/kgEMBS/min, p = 0.04) and insulin suppressed lipid oxidation rates (0.05 ± 0.06 vs. 0.01 ± 0.03 mg/kgEMBS/min, p = 0.04; both adjusted for age, sex, family membership, percentage of body fat).
Conclusion: Our findings suggest that the Val1481Ile mutation of *FAS* protects Pima Indians against the development of obesity, an effect possibly explained by the role of this gene in the regulation of lipid metabolism.

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A QTL for the disposition index (DI) maps to human chromosome 11: the IRAS Family Study.

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Background and Aims: The IRAS Family Study is a multicenter project designed to study the genetic epidemiology of insulin resistance and adiposity. Insulin resistance is a risk factor for a variety of chronic diseases, including type 2 diabetes and cardiovascular disease. Type 2 diabetes can be delayed or possibly prevented by identifying subjects with a predisposition to insulin resistance and reversal of the predisposing risk factor profile with lifestyle changes and/or pharmacologic agents (insulin sensitizers). Reversal of insulin resistance will be aided by the detection of quantitative trait loci (QTLs) controlling variation in insulin resistance and insulin sensitivity phenotypes.
Materials and Methods: The IRAS Family Study is a multicenter family study focused on large Hispanic and African-American pedigrees. We have completed a genome scan for measures related to glucose homeostasis in the first set of IRAS Family Study DNA, using the insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT). Assessed phenotypes included insulin sensitivity (S_I), glucose effectiveness, (S_G), first-phase insulin response ($AIR_{glucose}$) and disposition index (DI, the ability of the pancreatic β-cells to compensate for insulin resistance, where $DI = S_I \times AIR_{glucose}$). All analyses were conducted using a genetic variance component approach as programmed in the SOLAR software. A total of 66 extended families (21 African-American and 45 Hispanic) were used in the current analysis (over 1200 with DNA).
Results: The strongest evidence for linkage was for DI, occurring at two locations on chromosome 11. In African-Americans, there was significant evidence for linkage at D11S2371 (lod = 3.21). In the combined sample, there was strong evidence at GATA117D01 (lod = 2.21). Other sites of linkage include: S_I: chromosome 15 in Hispanics (lod = 2.28, D15S822). S_G: chromosome 18 in African-Americans (lod = 1.45, ATA82B02). $AIR_{glucose}$: African-Americans on chromosomes 4 (D4S1625, lod = 2.73), 12 (PAH, lod = 2.56) and 14 (GATA198A07, lod = 1.61).
Conclusions: These results suggest that several candidate regions exist that may contain genes that account for variation in glucose homeostasis. Strongest evidence exists for β-cell function in African-Americans as reflected in DI. Identifying genes in the identified regions should facilitate the development of more effective therapies for prevention of diabetes and other syndromes associated with metabolic disease.

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A quantitative trait locus on 7q31 for the changes in plasma insulin in response to exercise training: the Heritage Family Study.

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Background and Aims: Several genome-wide linkage scans have been carried out to identify quantitative trait loci for type 2 diabetes and related metabolic phenotypes. However, no previous linkage scans have focused on the response to exercise training of relevant metabolic traits.
Materials and Methods: We performed a genome-wide linkage scan for baseline fasting plasma glucose, insulin and C-peptide and their responses to a 20-week exercise training program in non-diabetic White and Black men and women from the HERITAGE Family Study. Baseline data were available for 507 Whites and 283 Blacks and exercise training data for 459 Whites and 211 Blacks. A sib-pair linkage procedure implemented in the SIBPAL program of S.A.G.E and 509 markers with an average spacing of 6.0 Mb were used. The maximum number of sibpairs available was 344 in Whites and 93 in Blacks.
Results: The strongest evidence of linkage was found for the changes in fasting plasma insulin in response to exercise training with a marker in the leptin gene on chromosome 7q31 (P=0.0004) in Whites. In Blacks, the strongest evidence of linkage was observed for baseline fasting plasma glucose on chromosome 12q13-q14 (P=0.0006).
Conclusions: The present study in non-diabetic individuals provides the first evidence that a genomic region close to the leptin locus contributes to the fasting plasma insulin response to exercise training. This region harbors several potential candidate genes.These findings may be important in the ongoing effort to identify individuals at increased risk of developing type 2 diabetes and who are most likely to benefit from a physically active lifestyle.

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Novel candidate genes in Asian Indians with Type 2 diabetes mellitus.

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Background and Aims: Asian Indians ranking high in ethnic susceptibility to type 2 diabetes could be explained by specific patterns of gene expression. Identifying gene markers for diabetes will facilitate preventing or delaying type 2 diabetes.
Materials and Methods: Microarray gene profiling of 13,474 sequence-verified, non redundant human cDNAs was employed to compare leucocyte gene expression in Asian Indians with type 2 diabetes (DM: n=3) and matched non-diabetic controls (n=3).
Results: Significant differential expression (and fold change <0.3 or >3) was noted for 897 genes in DM vs. controls in following function groups (%) : enzyme (31), nucleic acid binding (23), ligand binding or carrier (10), signal transducer (9), transporter (6), structural protein (6), cell adhesion (4), tumor suppressor (3), transcription factor binding (2), enzyme inhibitor (2), chaperone (2), cell cycle regulator (1) and defense/immunity protein (1). Some of them were also associated with other phenotypes (fold change) : congenital afibrinogenemia, dysfibrinogenemic thrombophilia (16.8), Canavan disease (10.0), Laron dwarfism, idiopathic short stature (8.3), familial chylomicronemia syndrome, lipoprotein lipase deficiency (8.0), involutional osteoporosis, vitamin D-resistant rickets (7.9), susceptibility to cerebral malaria (7.2), elliptocytosis-2, recessive spherocytosis (7.1), infantile form Refsum disease, Zellweger syndrome-3 (6.0), Bardet-Biedl syndrome 2 (5.9), , autosomal recessive 12 deafness, , type 1D Usher syndrome (5.9), hypertriglyceridemia (5.4), acatalasemia (5.4), argininosuccinicaciduria (5.2), Griscelli syndrome-type pigmentary dilution with mental retardation (4.9), renal amyloidosis (4.2), Charcot-Marie-Tooth neuropathy-4A, autosomal recessive axonal neuropathy with vocal cord paresis (4.0), cerebellar, somatic hemangioblastoma, von Hippel-Lindau syndrome (3.9), breast cancer-1, papillary serous carcinoma of the peritoneum (3.7), autosomal recessive inclusion body myopathy, sialuria (3.5), argininemia (3.3), 3 or more types amyloidosis, hypoalphalipoproteinemia (3.3), Opitz G syndrome, type I (3.3). Other Genes (fold change) with potential roles in type 2 diabetes were : dihydrofolate reductase (7.1), ferredoxin 1 (5.9), IGF-II mRNA-binding protein 3 (4.2), deiodinase, iodothyronine, type II (3.6), zona pellucida binding protein (3.1), Human glucocorticoid receptor alpha mRNA, variant 3' UTR (3.1), Glucosamine-6-phosphate isomerase (0.2); nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (0.3), glucokinase (hexokinase 4) regulatory protein (0.3), glucocorticoid receptor DNA binding factor 1 (0.3); major histocompatibility complex, class II, DO alpha (0.3), high density lipoprotein binding protein (vigilin) (0.3), cholecystokinin (0.3).
Conclusions: Microarray gene profiling has revealed candidate genes, some of them novel, which may account for high ethnic susceptibility of Asian Indians to type 2 diabetes mellitus.

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Permanent neonatal diabetes mellitus due to glucokinase deficiency - an inborn error of the glucose-insulin signalling pathway.

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Background and Aims: Neonatal diabetes can be either permanent or transient. Recently, we have shown that permanent neonatal diabetes mellitus (PNDM) can result from complete deficiency of glucokinase (GK) activity (Njølstad et al., NEJM, 2001). The aim of this study was to investigate if there were more cases in order to verify the syndrome.
Material and Methods: We screened ten probands with neonatal diabetes for mutations in GK. Probands from three families had mutations. These probands had intrauterine growth retardation (IUGR; birth weights mean 1770 g) and insulin-treated diabetes mellitus from birth (diagnosis at mean 4 $^1/_2$ days). There was consanguinity in family 1 and 2 while family 3 was related to family 2.
Results: The homozygous mutations A378V and IVS8+2 nt T>G in GK were identified in the probands of family 1 and family 2, respectively. The proband of family 3 was compound heterozygous for IVS8+2 nt T>G and G264S. Five parents were available for this study and all had hyperglycemia and were heterozygous. GK A378V had an enzyme activity of 0.06 percent that of wild-type, indicating complete enzyme deficiency in the homozygous proband. The nature of the splice site mutation IVS8+2 nt T>G suggested enzyme inactivity. Recombinant GK G264S activity was near normal.
Conclusion: We present three new patients with GK-deficient PNDM, in addition to our previous two cases. The following clinical picture is emerging: Severe IUGR, permanent insulin-requiring diabetes from first week of life, recessive inheritance and hyperglycemia in both parents. The pattern of inheritance and the enzyme deficiency fulfill the criteria of an an inborn error of metabolism.

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The SNP-43 polymorphism of the Calpain-10 gene is associated with insulin secretion and action in children of patients with Type 2 diabetes.

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Background and Aims: The SNP-43 polymorphism of the Calpain-10 (CAPN10) gene has been associated with insulin resistance and type 2 diabetes (T2DM). However, the mechanism behind this association has remained unclear. We investigated the effect of this polymorphism on

insulin secretion, insulin action and the risk of T2DM in the Finnish population.
Material and Methods: The effect of the SNP-43 polymorphism on insulin secretion and action was studied using the intravenous glucose tolerance test (IVGTT) followed by the hyperinsulinemic euglycemic clamp study in 129 nondiabetic children [age 34.0±6.2 (mean±SD) years, body mass index (BMI) 27.11±5.03 kg/m^2] of patients with T2DM. The effect on the risk of T2DM was evaluated in 490 participants [age 55.3±7.1 years, BMI 31.2±4.6 kg/m^2, all with impaired glucose tolerance (IGT)] of the Finnish Diabetes Prevention Study.
Results: The G allele of the SNP-43 polymorphism associated with low insulin area under the curve (AUC) during the first 10 minutes of the IVGTT (2118±953 pmol/L*min in 85 subjects with the GG genotype, 3093±2238 in 39 subjects with the GA genotype and 5079±3398 in 5 subjects with the AA genotype, p<0.001) and with high whole body glucose uptake (WBGU) during the hyperinsulinemic clamp [58.73±17.25 vs. 49.71±16.28 vs. 44.42±17.23 µmol/min/lean body mass (kg), p=0.026]. The correlation between insulin AUC during the IVGTT and WBGU during the clamp was weaker in subjects with the GG genotype (R^2=0.077, p=0.013) than in subjects with the A allele (R^2=0.343, p<0.001). However, no effect of the polymorphism on the risk of T2DM during the 3 year follow up in subjects with IGT was observed (p=0.766).
Conclusions: The SNP43 polymorphism of the CAPN10 gene modifies insulin secretion and action in nondiabetic children of patients with type 2 diabetes. This may contribute to the risk of T2DM although no effect of this polymorphism on the risk of T2DM was seen in the Finnish Diabetes Prevention Study.

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Haplotype combination 11.21 in the promoter of the adiponectin gene (APM1) is associated with increased diabetes risk in Caucasian population.

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Background and Aims: Adiponectin, a novel adipocyte-derived collagen-like protein, encoded by the APM1 gene that has been considered to modulate insulin sensitivity and glucose homeostasis. This protein protects obese mice from diabetes and have anti-atherogenic effects. Furthermore plasma adiponectin levels are decreased in both type 2 diabetes (T2DM) and obesity. The aim of our study was to analyse genetic variation in adiponectin with respect to risk of getting type 2 diabetes.
Material and Methods: We screened 365 German subjects with type 2 diabetes mellitus (mean age: 60,5 ± 11,2 years, BMI 28.7 ± 4.8 kg/m2, age of disease onset 52.7 ± 13,8) and 323 random controls (mean age: 59,2 ± 15,2 years, BMI 24,7 ± 4,1kg/m2). 3 common SNPs, one in the coding region of exon 2 +45T>G, and 2 promoter variants SNPs -11391G>A and -11377C>G were analysed. Polymerase chain reaction (PCR) was performed using LightCycler™ Technology (Roche Diagnostics, Mannheim, Germany).
Results: The allelic distributions of the SNPs -11377C>G and +45T>G (G15G) revealed none or only a mild effect on diabetes risk in our sample but the SNP -11391G>A variant allele in the minimal promoter showed a significant higher frequency in the diabetic patients group (p=0,003). Carrying the heterozygote genotype at SNP -11391G>A was associated with a significant 1,85 fold (95% CI 1,21 - 2,82) increased risk of type 2 diabetes. Carrying the variant allele at SNP -11391G>A and the C allele at SNPs -11377C>G on one haplotype (21) was associated with an 1.50fold (95% CI 1.02-2.21), p=0.03 increase in diabetes risk. The haplotype combination 11.21 was significantly more frequent in the diabetes group (p=0,004) and was associated with an significant elevated diabetes risk (OR=2,82 (95% CI 1,35-5.91), p=0.006) after correction for BMI and age.
Conclusions: Our observations suggest that genetic variants in the adiponectin gene promoter region defining a risk haplotype combination (11.21) which is associated with significant increased risk of type 2 diabetes. This genetic variant may be part of the genetic determinants of type 2 diabetes in the German Caucasian population.

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Adiponectin gene is associated with obesity and obesity correlated traits in childhood.

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Background and Aims: The search for candidate genes for obesity has been active. Little is know so far about the role of the newly identified fat secreted hormone, the 30kDa adipocyte complement related protein (ACRP30/adiponectin), only expressed in differentiated adipocytes, and encoded by the APM1 gene. Association studies between several APM1 SNPs and obesity and correlated traits were conducted to evaluate the contribution of APM1 genetic variants to obesity and obesity correlated traits.
Material and Methods: The study was conducted on 130 consecutive cases of Italian children recruited from the obesity's center of the Pediatric Department at University "La Sapienza" (Rome) (centiles of BMI by age, mean 106.8 ± Std.E. 1.4, were calculated by the LMS method of Cole (1990), age mean 10.4 ± Std.E. 0.25; male 70 female 60). Any subjects with previous diagnosis of endocrine diseases, hypertension, were excluded from the study.
The following traits were analyzed in subjects: anthropometric variables (BMI), and laboratory data (blood fasting glucose, total cholesterol, HDL, LDL triglycerids,) and blood pressure (PA). Genomic DNA was extracted from peripheral venus blood specimens by a conventional salting-out.
Three different SNPs of the APM1 gene were evaluated: -11391 G>A; +45 T>G; and +276 G>T. The typing of the SNPs was performed using the fluorogenic 5' nuclease assay designed by our group and detected with ABI PRISM® 7900HT Sequence Detection System (USA, CA). Statistical analysis: the effect of the polymorphisms on quantitative variables was tested (age and sex adjusted) by a multivariate analysis performed using the SPSS 11 software program (SPSS, Illinois, USA).
Results: Children carrying the A allele at position –11391 have a significantly higher blood fasting glucose levels (89 ± 1 vs. 84± 1, p=0.02) and significantly higher centimes of BMI (112 ± 2 vs. 104±2 p=0.018) compared with non carriers. In addition we observed significantly lower levels of HDL (53± 2 vs. 45.8± 2 p=0.024) in subjects carrying the A allele at position –11391 compared with those negative for the A allele. There were no significant differences in each variable analyzed between subjects with and without the G allele at position +45 and between subjects with and without the T allele at position +276.
Conclusions: the present study suggests that adiponectin is a novel susceptibility gene for obesity and obesity correlated traits in the Italian population.

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Interethnic diversity of SNP and haplotypes of IRS-2 gene explains variability in insulin resistance associated with PCO syndrome.

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Background and Aims: Discovery of SNP (single nucleotide polymorphisms) and their unequal distribution through human genome opened large perspectives for linkage disequilibrium mapping of disease-related genes in complex diseases. To understand the role of IRS-2 (insulin receptor substrate-2) gene (Chr 13q34) in insulin resistance, and particularly of the allelic variant Gly1057Asp we have studied gene-based complex haplotypes in Caucasian populations (n = 156) with polycystic ovary syndrome (PCOS) from Eastern Europe or South of France.
Materials and Methods: Insulin resistance was estimated by HOMA index while glucose intolerance by the OGTT. Gene SNP-ing was performed by fluorescence-based sequencing of unphased DNA (ABI-373A) covering the coding region of IRS-2 and haplotypes were reconstructed by computing procedures (PHASE) taking into account variations at sites 816 (T/C), 829 (C/T), 879 (G/A), 1031 (G/A), 1033 (A/G) et 1057 (G/A).
Results: In Romanian and French PCOS populations, obesity (48.2 vs 44.7%), insulin resistance (57 vs 46%) and Acanthosis Nigricans (27 vs 23.3%, respectively) were equally represented. SNP of IRS-2 were organized as 8 and 13 haplotypes in two populations, the mutation Gly1057Asp being located in 2 haplotypes (H2 and H4) and 4 haplotypes (H2, H4, H10 and H11) respectively. Although the prevalence of the allelic variant 1057 (44.8 vs 66.3 %) can distinguish Romanian and French populations at low statistical level ($P < 0.003$), four distinct haplotypes were strikingly different between populations: 7 vs 30%, 42 vs 18%, 3.5 vs 50% and 21.4 vs 7.4% for H1, H2, H4 and H8 respectively. In logistic regression significant differences were obtained for H8 ($P < 0.002$, OR 3.9 95% CI 1.65-9.6) and H1 ($P < 0.002$, OR 0.16 (0.05-0.52) for French PCOS, but only for one haplotypes containing the 1057 mutation : H4 ($P < 0.0001$, OR 0.04 (0.01-0.19) for Romanian population. Although insulin resistance may be explained by shared haplotypes such as H2, better correlation was found with haplotypes with ethnic specificity such as H10 in French population ($P < 0.0001$) responsible for increase in HOMA index from 2.2 ± 0.13 to 14.1 ± 6.9. Interestingly impaired glucose tolerance was explained in French population by the mutation Gly1057Asp ($P < 0.007$, OR 8.1, CI 1.7-37.3) or by H4 haplotype which was poorly represented in Romanian population.
Conclusion: These data suggest that case control studies may identify pathogenic haplotypes od IRS-2 but the interethnic diversity explain better the variability in the role of IRS-2 in insulin resistance and glucose intolerance and therefore can lead easier to identification of causative SNP in candidate genes. In conclusion ethnic diversity in haplotype structure in various populations may considerably contribute to understanding disease related genes in complex disorders such as PCOS, obesity and type 2 diabetes.

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The G972R variant of the insulin receptor substrate-1 (IRS-1) gene is associated with insulin resistance evaluated by clamp in obese subjects without metabolic complications.

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Background and Aims: Insulin-resistance plays a central role in the pathogenesis of type 2 diabetes, hypertension and atherosclerosis. Several association studies have shown that the IRS-1 gene G972R variant is a genetic risk factor for insulin-resistance, suggesting a possible interaction between obesity and the IRS-1 gene in the development of impaired insulin action. Other studies have suggested a possible effect of the G972R variant on insulin secretion.
Aim of this study was to evaluate the possible role of the IRS-1 gene G972R variant in obese subjects without clinical and metabolic alterations (diabetes, hypertension, dyslipidemia, CAD). All subjects have been studied by the euglycaemic hyperinsulinaemic clamp associated with indirect calorimetry, and the presence of the G972R variant was related to indexes of insulin sensitivity. Furthermore, the possible role in insulin secretion of this variant was studied by means of indexes of insulin release derived from the OGTT (1st phase, 2nd phase, Insulinogenic Index, $HOMA_{ins}$).
Materials and Methods: 51 obese subjects (BMI=38.9±6.2) without clinical and metabolic alterations underwent the euglycaemic hyperinsulinaemic clamp and the IRS-1 genotype was detected in real-time with LightCycler hybridization probes, using fluorescently-labelled nucleotides. 25.5% (n.=13) of the obese subjects resulted carriers of the G972R variant, a prevalence significantly higher compared to a control population previously studied (6-9%).
Results: Comparison of clinical parameters (BMI, waist, FFM, FAT, etc.) did not show significant differences between carriers and non-carriers of the variant. Comparison of indexes of insulin sensitivity showed a significant difference between G972 carriers compared to wild-type carriers in the M index, ($p<0.05$), in the non-oxidative glucose ($p<0.02$), in the insulin clearance ($p<0.03$) and in the Insulin Sensitivity Index ISI ($p<0.005$). Finally, the analysis of indexes of insulin release did not show significant differences.
Conclusion: In conclusion, our results confirm the association of the G972R variant of the IRS-1 gene with insulin-resistance, but from our data it appears that this variant does not play a role in insulin secretion in these subjects.

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An association between Interleukin-1 Beta (IL-1 beta*2) and Interleukin-1 Receptor Antagonist (IL-1RN*2) alleles and diabetic microvascular complications.

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Background and Aims: Diabetic microvascular complications affect only a subset of diabetic patients. It is believed that the occurrence and progression of the microvascular complications may be influenced by genetic, hemodynamic and metabolic factors. Interleukin 1 and similar pro-inflammatory cytokines have been implicated in the pathogenesis of diabetic complications. The aim of this work has been to test for possible genetic association with the polymorphic IL-1 receptor antagonist (IL-1RN) locus and IL-1 beta locus.
Materials and Methods: IL-1 beta and IL-1 RN polymorphism has been analysed by PCR amplification method in a cross section of 76 patients with diabetes mellitus (20 type 1 and 56 type 2) and 28 age- & sex-matched healthy controls. Diabetic microvascular complications were documented by clinical and laboratory evaluation.
Results: There has been no evidence to suggest that genotype at IL-1 beta (-511) had any effect on nephropathy status. However, genotype 2,2 at IL-1 RN (VNTR) was reduced in the nephropathy cases compared to those DM cases without nephropathy (empirical $p=0.05$). Within the diabetic patients no evidence was found to suggest that genotype at either locus had any effect on retinopathy status. There was no evidence to suggest that genotype at either locus had any effect on type 1 DM. There was also no evidence to suggest that genotype at IL-1 RN (VNTR) had any effect on type 2 DM. On the other hand, there was some evidence to suggest that genotype at IL-1 beta (-511) had an effect on type 2 diabetes status (OR=5.3 [0.6-44.4], $p=0.05$). But this effect was shown to be a result of the hypertensive patients within the DM type 2 group and not an indicator for susceptibility to DM type 2 itself. There was no evidence to suggest that genotype at IL-1 RN (VNTR) had any effect on hypertensive status in diabetic patients, while genotype 2,2 at IL-1 beta (-511) was elevated in hypertensive diabetic patients (OR=6.5 [1.8-23.4], $p=0.002$). Type 2 DM without hypertension compared to controls showed no difference in genotype at IL-1 beta (-511). Whereas, type 2 DM with hypertension compared to controls showed significant evidence of different genotype distributions (p-value = 0.006).
Conclusion: Our results reveal that genotype 2,2 at IL-1RN (VNTR) was reduced in the nephropathy cases, and that the elevated levels of genotype 2,2 at IL-1 beta (-511) seen in type 2 DM cases was due to the hypertensive status of those cases and not due to their DM status.

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Heritability of plasma leptin and associations with the metabolic syndrome and haemostasis.

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Background and Aims: Leptin is synthesized and secreted mainly from adipocytes and is correlated with obesity and features of the insulin resistance syndrome. Insulin resistance underlies the development of both type 2 diabetes and cardiovascular disease and leptin levels are elevated in both diseases. The relationship between leptin and haemostatic risk factors has not been determined. The aim of this study was to investigate the contribution of heritability and haemostatic and metabolic risk factors to circulating leptin levels in a healthy Caucasian family study.
Materials and Methods: Leptin levels were determined by ELISA in 531 individuals from 89 families. Statistical analyses were carried out using the SPSS and SOLAR software packages.
Results: In keeping with previous studies plasma leptin levels were significantly higher in women (13.2 [11.8-14.7] ng/ml compared with men (3.3 [2.9-3.8] ng/ml, p<0.0001). Age and sex-adjusted leptin concentrations were significantly associated with haemostatic cardiovascular risk factors: fibrinogen (r=0.20, p<0.0001), factor VII (r=0.23, p<0.0001), PAI-1 (r=0.43, p<0.0001), and tPA (r=0.30, p<0.0001). In addition, in keeping with previous reports leptin was significantly associated with classical cardiovascular risk factors and features of the insulin resistance syndrome: systolic BP (r=0.22, p<0.0001) and diastolic BP (r=0.18, p<0.0001), calculated insulin resistance (HOMA, r=0.40, p<0.0001), BMI (r=0.70, p<0.0001), fasting plasma glucose (r=0.21, p<0.0001), fasting plasma insulin (r=0.41, p<0.0001), fasting plasma cholesterol (r=0.19, p<0.0001), triglyceride (r=0.28, p<0.0001), LDL (r=0.15, p=0.001), HDL (r= - 0.18, p<0.0001), and WHR (r=0.37, p<0.0001). Finally, leptin was significantly lower in current smokers (5.1 [4.1-6.3] ng/ml) compared with non-smokers (8.4 [7.5-9.5] ng/ml, p=0.01). In quantitative genetic analyses, additive genetic components explained only 10.3% of the variance in plasma leptin whilst BMI, sex, smoking, PAI-1, WHR and fibrinogen together explained a further 53% of the variance in plasma leptin.
Conclusion: Our results suggest that the association of plasma leptin with haemostatic cardiovascular risk factors may underpin the increased cardiovascular risk associated with the metabolic syndrome and points to a central role of the adipocyte.

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Effect of endothelial nitric oxide synthase polymorphism (Glu298Asp) on the progression of glycaemia in Chinese subjects with impaired glucose tolerance.

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Background and Aims: Subjects with impaired glucose tolerance (IGT) have increased risk of progressing to type 2 diabetes mellitus. Such progression may be delayed or reversed with the use of peroxisome proliferator-activated receptor gamma (PPAR gamma) ligands, such as troglitazone. PPAR gamma ligands enhance the release of nitric oxide from endothelial cells and in mice, endothelial nitric oxide regulates insulin sensitivity. In this study, we determined whether endothelial nitric oxide synthase (eNOS) exon 7 polymorphism was associated with the progression or regression of glucose intolerance in Chinese subjects with IGT over a period of 5 years.
Materials and Methods: 258 subjects with IGT at baseline from the Hong Kong Cardiovascular Risk Factors Prevalence Study underwent oral glucose tolerance tests at 2 years and 5 years for re-assessment of glycaemic status. The genotype of each patient for Glu298Asp (G298T) variant in exon 7 of eNOS gene was determined with PCR-RFLP.
Results: The alleleic frequency was 88.9% and 11.1% for G and T alleles respectively, consistent with a control Chinese population of 300 screened previously (87.3% and 12.3% for G and T alleles respectively). At 5 years, 40.0% of the subjects had reverted to normal glucose tolerance (NGT), 39.5% had remained in IGT and 20.5% had progressed to diabetes. Subjects heterozygous (18.4%) or homozygous (2.0%) for T were grouped together for analysis. A significant effect of genotype on the glycaemic status of the subjects at 5 years was observed (p=0.012). Significantly fewer subjects with at least one T allele reverted back to NGT compared to G/G (22.6% vs 44.4%, T/T or G/T vs G/G, p=0.004). A similar trend was seen at 2 years, but the difference did not reach significance (36.5% vs 46.3%, T/T or G/T vs G/G, P=NS). In multiple logistic regression analysis including also age, sex and body mass index (BMI), the eNOS polymorphism remained a significant independent determinant of regression to normoglycaemia at 5 years (p=0.009), together with BMI and sex.
Conclusion: In this group of Chinese IGT subjects, the eNOS Glu298Asp polymorphism appeared to be predictive of their glycaemic status at 5 years. The progression of glycaemia in subjects with IGT is likely to be multifactorial and the eNOS gene may be a significant contributing factor in Chinese.
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Phenotypic analysis of childhood obesity linked to 16q22.1-q23 chromosomal region.

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Background and Aims: We previously evidenced the linkage of childhood obesity and quantitative associated traits with the chromosomal region 16q22.1-22.3. Here, we compare the phenotypic characteristics of 92 children whose obesity is linked to 16q with 133 children whose obesity is not linked to this region.
Materials and Methods: Comparisons between groups were performed using Chi2 and T-tests.
Results: The penetrance of obesity is significantly higher in 16q-linked families than in 16q-non linked ones (mean percentage of obese children per family : 91,1 % vs 72.7 %, p = 0.001). Comparison of anthropometric data (BMI Z score, fat mass percentage from DEXA, waist circumference SDS) in the two groups shows a predominance of moderate to severe obesity in 16q-linked obesity, while extreme forms of obesity were only found in 16q unlinked families. Waist circumference (expressed in SDS according to sex and age) is lower in 16q-linked obesity for every range of its values. Comparison of means of data discloses a gender effect : birth weight and size are higher (p = 0.02 and 0.01) in obese males but not in obese females linked to 16q while diastolic blood pressure and insulin plasma levels are lower (p = 0.02 and 0.03) and HOMAs insulin sensitivity index higher (p = 0.03) in obese females but not in obese males, than in their non-16q-linked counterparts.
The correlation of adiponectin (an insulin-sensitizing hormone secreted by adipocytes) plasma levels with insulin sensitivity index HOMAs is strengthened by the linkage to 16q (p value shifting from 0.05 to 0.001). Familial segregation analysis of microsatellite markers shows an excess of transmission of alleles linked to obesity by fathers to their children in 16q22-q23, which suggests a paternal imprinting of obesity.
Conclusion: (1)16q-linked childhood onset obesity is less severe than obesity unlinked to this locus; (2) a gender effect on insulin resistance associated phenotypes which is independent of puberty, is noted in 16q-linked obesity; (3) adiponectin is more closely related to insulin sensitivity in 16q-linked obesity; (4) a paternal imprinting may contribute to16q-linked obesity; and (5) CTCF (CCCTC-Binding Factor), a transcription factor regulating paternal imprinting of IGF-2 (a growth factor involved both in adipocyte differentiation and in insulin sensitivity) is a candidate gene of chromosome 16q childhood obesity susceptibility locus.

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Is ACE gene insertion/deletion polimorphism one of the common determinants of the carbohydrate metabolism and hypertension in patients with type 2 diabetes mellitus?

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Background and Aims: We hypothesized that the ACE gene polymorphism influences the effectiveness of the treatment of carbohydrate metabolism and hypertension in type 2 diabetes.

Materials and Methods: Our patients (n=145, type 2) were characterized by 64 (29-82) years of age (median and range); HbA1c, 7.0 (5.2-16.3) %; fructosamine, 319 (180-640) µmol/l. Diabetes was treated by oral hypoglycemic agents (n=39) or by insulin therapy (n=106). Non-parametric Mann-Whitney U, Kruskal-Wallis, median and chi-square tests, non-parametric Kendall's tau b and Spearman's rho correlations were used for statistical analysis (SPSS v. 10.1, Chicago, Ill., USA).
Results: There were no significant differences in HbA1c, plasma glucose, systolic, diastolic BP and albuminuria between groups of patients carrying genotypes II (n=27), ID (n=68) or DD (n=50). Serum level of fructosamine was in the II group 294 (197-460), in the ID group 310 (180-640) and in the DD group 331 (218-581) µmol/l (p=0.052). Using median test there were 8 II, 35 ID, and 31 DD patients whose fructosamine-level was higher than the median, and 20 II, 36 ID, and 20 DD patients whose level was lower than the median (p=0.023). Dividing the patients into two groups (II and ID+DD) fructosamine-level was 294 (197-460, II) and 328 (180-640, ID+DD) µmol/l (p=0.007). In this grouping the number of antihypertensive drugs was 4 (0-7) in subgroup ID+DD, which was significantly higher than that in subgroup II, 3 (0-7) (p=0.015). ACE gene polymorphism correlated with fructosamine level (tau b: r=0.153, p=0.017, rho: r=0.199, p=0.014). Association test between ACE gene polymorphism (II vs ID+DD) and type of treatment of diabetes (oral vs. insulin; chi-square=3.234, p=0.072, OR=2.210, 95% CI: 0.919-5.312) and treatment of hypertension (number of combination; chi-square=3.075, p=0.079, OR=1.866, 95% CI: 0.926-3.762) seems to suggest that treatment with insulin and higher antihypertensive combination may be more prevalent in patients carrying allele D.
Conclusion: Summarizing, carriers of D allele need more antihypertensive drugs in type 2 diabetes, and have higher fructosamine level. Concluding, on the one hand enhanced insulin resistance due to allele D may induce more insulin secretion and this way these patients can be more prone for exhaustion of beta-cells leading to the prevalent need of insulin therapy and, on the other hand, patient with more severe insulin resistance state due to allele D have drug-resistant hypertension.

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The association of aldose reductase gene (AR2) polymorphisms with diabetic neuropathy in adolescents.
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Background and Aims: Variants in the AR2 gene have been implicated in the development of diabetic retinopathy and nephropathy, with the most convincing data identifying a CA repeat microsatellite allele (Z-2), which has a functional role in gene expression. Aldose reductase inhibitors have been used in the management of diabetic neuropathy with varying effectiveness, which could be due to variable genotypic expression. The pupillary response to light may be the most sensitive and reproducible of the currently available tests for subclinical neuropathy and thus was used as the major outcome in this study. The aim was to investigate potential associations of AR2 polymorphisms (including the CA microsatellite repeat) with autonomic and somatosensory nerve test abnormalities
Materials and Methods: 374 adolescents (less than 20 years) underwent nerve testing and genotyping of the AR2 gene. The microsatellite (CA repeat) and two single nucleotide polymorphisms (-12C>G, -107C>T) were investigated by RFLP. Infrared pupillometry measured the resting dark-adapted pupil diameter and the phasic light response (maximum constriction velocity and reflex amplitude), and the thermal threshold for heat discrimination was measured in the foot. The median duration was 7.0 yrs [4.9-10.3] and HbA1c was 8.5% [7.7-9.4].
Results: 69% had pupillary abnormalities (30% with two, 15% with three abnormalities). The Z-2/Z-2 genotype increased the risk nearly threefold for pupillary abnormalities (OR 2.81, CI 1.14-6.95) after allowing for diabetes duration, HbA_{1c} and blood pressure percentiles. 85% of adolescents with this genotype had at least one abnormality compared with 67% occurring in those with the other genotypes (p=0.02). 78% had two or three pupillary abnormalities compared with 53% of those with other genotypes (p=0.014). In combination with the CC genotype (-12C>G), 88% had at least one (p=0.038) and 81% had 2-3 abnormalities (p=0.0036). However, this did not improve the logistic regression model.
21% had an abnormal threshold for heat discrimination. The microsatellite was not associated with this abnormality. The CC (-107C>T) genotype was associated with a twofold increased risk of abnormal hot thermal threshold (OR 2.36, CI: 1.34-4.16). 30% of adolescents with this genotype had the abnormality compared with 17% occurring in those with the other genotypes (p=0.0055).
Conclusions: Polymorphisms in the AR2 gene are associated with the development of subclinical diabetic neuropathy in adolescents with type 1 diabetes.

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Differences in paraoxonase phenotype and genotype between diabetics and non-diabetics (the CODAM Study).
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Background and Aims: Oxidation has been implicated in the etiology of type 2 diabetes. The paraoxonase Q192R genotype (PON1) has been associated with higher risk of coronary heart disease and fasting glucose levels in subjects with type 2 diabetes. The ratio of paraoxonase to diazoxonase activities has been shown to largely depend on the PON1 Q192R genotype. Our aim was to compare activities of paraoxonase and diazoxonase, the derived PON1 Q192R genotype and IMT in subjects with different stages of glucose intolerance.
Materials and Methods: We included the 566 members of the Cohortstudy Diabetes and Atherosclerosis Maastricht (CoDAM study), 303 with NGT, 121 with IGT, 80 with newly diagnoses diabetes and 62 with treated type 2 diabetes. Paraoxonase and diazoxonase activities were measured in serum using paraoxon and diazoxon as substrates. Genotype was determined from the ratio of diazoxonase to paraoxonase, which showed clear cut-off points in its distribution at 6.6 and 17.6.
Results: The study population included 351 men and 215 women, mean age 59.1 yr (range 42-72yr). Subjects with newly diagnosed diabetes had similar paraoxonase activities. However, diazoxonase activities were significantly lower than subjects with NGT, respectively 5185 and 5959 u/l. Based on the ratio the PON1 Q192R genotype was assessed. The observed frequency of the PON1 192R allele was 0.369 among newly diagnosed diabetes and 0.272 among NGT subjects and the observed PON1 genotype distributions were in Hardy-Weinberg equilibrium. The RR-genotype was significantly more present in newly diagnosed diabetics (p=0.02) compared to NGT subjects.
Adjusted for age, gender, BMI, smoking, physical activity and triglycerides the odds ratio and 95% confidence interval for newly diagnosed diabetes was 3.29 (1.24-8.70). For treated diabetes it was 3.43 (1.13-10.39) and for IGT it was 1.96 (0.82-4.69) compared to NGT. Among subjects with diabetics there was a tendency for high IMT in those with the RR-genotype (p=0.16). No association was observed in NGT or IGT.
Conclusion: The PON1 192R variant is more frequent in subjects with newly diagnosed diabetes compared to NGT subjects. This variant is associated with higher levels of paraoxonase (lower activity) and lower levels of diazoxonase (higher activity). This suggests less protection to oxidation of lipoproteins in diabetic. It also suggests that it plays a role in their increased risk of atherosclerosis. In addition, as the PON1 192R variant is especially high in newly diagnosed diabetes our results suggest that oxidative stress also play a role in the development of diabetes.

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Adiponectin in a native Canadian population experiencing rapid epidemiological transition.

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Background and Aims: Adiponectin is emerging as an important protein in the etiology of obesity and related metabolic disorders. The objectives of this study were to determine cross-sectional and prospective associations of adiponectin concentration with adiposity, type 2 diabetes mellitus (DM) and cardiovascular disease (CVD) risk factors in a population-based study of Native Canadians, a group experiencing dramatic increases in DM and CVD.
Materials and Methods: During the 1993-1995 baseline survey, samples for glucose, insulin, adiponectin, and lipids were drawn after an overnight fast. Waist circumference and % body fat were measured, and a 75g oral glucose tolerance test was administered (n=505 NGT, 74 IGT, 149 DM). In 1998, 95 high-risk subjects, defined as those who at baseline had IGT or NGT with an elevated 2-hour glucose concentration (≥ 7.0 mmol/l), participated in a follow-up examination using the protocol employed at baseline.
Results: After adjustment for covariates including % body fat and HOMA insulin resistance (IR), adiponectin concentrations were significantly lower among males vs. females (10.8 vs. 15.0 µg/ml, p<0.0001) and among DM vs. NGT subjects (11.1 vs. 13.1 µg/ml, p<0.05). Adiponectin was inversely correlated with % body fat (r=-0.43, r=-0.39, for males and females, respectively), waist circumference (r=-0.42, -0.38), HOMA IR (r=-0.30, -0.26) and triglyceride (r=-0.43, -0.27) and positively correlated with HDL (r=0.43, 0.37) (all p<0.0001). In multivariate linear regression analysis in non-diabetic subjects, HDL and % body fat were significantly related to adiponectin variation among both males and females (R^2 = 23-28%). In the prospective study of high-risk subjects, higher adiponectin at baseline was significantly associated with increases in HDL (r=0.24, p=0.03) and decreases in HOMA IR (r=-0.29, p=0.009) after adjustment for covariates including age, adiposity, and diabetes status at baseline and follow-up. Baseline adiponectin was not associated with risk of progression to diabetes (adjusted OR=1.01, p=0.98 per SD change).
Conclusion: These population-based findings support the hypothesis that low circulating levels of adiponectin are an important determinant of CVD risk.

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Visceral adiposity is an independent predictor of incident hypertension in Japanese Americans.

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Background and Aims: Visceral adiposity is generally considered to play a key role in the insulin resistance syndrome, including hypertension. The aim of this study is to investigate whether visceral adiposity increases the risk of hypertension independent of other adipose depots and fasting plasma insulin
Materials and Methods: We studied 300 Japanese Americans with systolic BP <140 mmHg, diastolic BP <90 mmHg, not taking antihypertensive medications, and not taking oral hypoglycemic medication or insulin at entry. Variables included plasma glucose and insulin measured after an overnight fast and during an OGTT; and abdominal, thoracic, and thigh fat areas by computed tomography. Visceral adiposity was measured as intra-abdominal fat area (IAFA) at the umbilicus level. Total fat area (TFA) was calculated as the sum of these fat areas. Total subcutaneous fat area was defined as TFA minus IAFA. Hypertension was defined as a systolic BP ≥140 mmHg, a diastolic BP ≥90 mmHg, or taking antihypertensive medications.
Results: During the 10-11 years follow-up period, there were 92 cases of incident hypertension. IAFA was associated with an increased risk of hypertension. Multiple-adjusted odds ratio of hypertension for IAFA was 4.58 (95% CI, 1.63-12.85) for Quartile 3 and 4 compared with Quartile 1 after adjusting for age, sex, fasting plasma insulin, 2-hour plasma glucose, and BMI. IAFA remained a significant risk factor of hypertension even after adjustment for TFA, total subcutaneous fat area, or abdominal subcutaneous fat area. No measure of regional or total adiposity was associated with the risk of hypertension in models that contained IAFA.
Conclusion: Greater visceral adiposity increases the risk of hypertension in Japanese Americans independent of other adipose depots and fasting plasma insulin.

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Comparison of WHO and NCEP definition for metabolic syndrome in prediction of mortality in Type 2 diabetes in Chinese population.

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Background and Aims: To examine the prevalence of metabolic syndrome (MES) in Chinese type 2 diabetic patients and predictive values of the World Health Organisation (WHO) and National Cholesterol Education Program (NCEP) definitions and the individual components of MES on all cause mortality.
Materials and Methods: A prospective analysis of a consecutive cohort of 5202 Chinese type 2 diabetic patients recruited between July 1994 and April 2001. All patients underwent comprehensive assessment with ascertainment of survival status in May 2001. Definition of obesity was modified using the Asian criterion. Metabolic syndrome was defined as presence of 2 or more of the components in the respective definitions.
Results: Mean age was 59.1±13.5 years and 43.8% were male. Duration of diabetes was 7.4±6.6 years and follow up period was 2.2±1.8 years. Overall, 49.2-61.8% of patients had MES depending on the use of definitions. Using modified criteria with Asian definition of obesity, the prevalence increased by 3.7-12.1%. Patients with MES according to WHO criterion were older, more likely to be men, had longer duration of diabetes, higher HDL-C and urinary albumin excretion rate when compared with NCEP criterion after adjustment for age, gender and diabetes duration. Using the NCEP criterion survival rate was similar between patients with or without MES (3.51% vs 3.85%) whereas with the WHO criterion, patients with MES had higher mortality (4.17% vs 2.76%, p=0.02) than those without. Using Cox regression analysis, only age, male gender and duration of diabetes were independent factors for death. When individual components of the MES were analyzed, for the NCEP definition, hypertension (RR (95% CI): 2.32 (1.62,3.31) p<0.001) and obesity [0.66 (0.49-0.89, p=0.006) were significant predictors for death. For the WHO criterion, apart from hypertension (RR (95% CI): 1.5 (1.11-2.02) p=0.008) and obesity [0.53 (0.39-0.72, p<0.001), microalbuminuria was the strongest predictor [3.08 (2.23-4.23) p<0.001] for death.
Conclusion: In Chinese type 2 diabetic patients, WHO criterion appeared to have a better sensitivity than the NCEP criterion for predicting death, probably due to the inclusion of albuminuria as one of the components. Age, duration of diabetes and male sex remained the most important predictors for death. When individual components of the MES were analysed, hypertension and albuminuria were strong predictors. In this relatively lean Chinese population, a low index of obesity also had predictive value for death.

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The metabolic syndrome and total and cardiovascular mortality in non-diabetic European men and women.

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Background and Aims: Few studies have evaluated the associations between the metabolic syndrome with any definition and mortality. This study is to estimate association of the metabolic syndrome with total and cardiovascular mortality for men and women.

Materials and Methods: Existing baseline data on glucose at fasting and two hours after a 75g oral glucose tolerance test were available for 11 prospective European cohort studies comprising 6156 men and 5356 women without diabetes aged 30-89 years, with a median follow-up of 8.5 years. A modification of the WHO definition of the metabolic syndrome was used. The subjects were considered to have the metabolic syndrome, if they had hyperinsulinemia (fasting plasma insulin in the highest cohort- and sex-specific quartile of the non-diabetic background population), and two or more of the following components: 1) obesity (body mass index ≥ 30 kg/m^2); 2) hypertension, systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or using of antihypertensive drugs; 3) dyslipidemia, raised plasma triglycerides (≥1.7 mmol/l) and/or low HDL-cholesterol (<0.9 mmol/l in men, <1.0 mmol/l in women); and 4) impaired glucose regulation. Hazard ratio for all-cause or cardiovascular mortality was estimated with Cox mode in each cohort. Meta-analyses were performed to assess the overall association of the metabolic syndrome with the risk of all-cause and cardiovascular mortality.
Results: The age-standardized prevalence of the metabolic syndrome was higher in men (15.7%) than in women (14.2%). During follow-up, 1049 deaths were recorded, of which 404 deaths were ascribed to cardiovascular disease. The overall hazard ratio for all-cause mortality in people with the metabolic syndrome versus those without was 1.41 (95%CI 1.10-1.82) in men and 1.42 (1.03-1.95) in women after adjustment for age, cholesterol, and smoking. The overall hazard ratio for cardiovascular mortality in people with the metabolic syndrome versus those without was 2.03 (1.39-2.98) in men and 2.48 (1.36-4.52) in women after adjustment for confounding factors.
Conclusion: Non-diabetic subjects with the metabolic syndrome showed an increased risk for death from all-cause and cardiovascular diseases.

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Increased mortality associated with the metabolic syndrome in post-menopausal women: the study of osteoporotic fractures.
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Background and Aims: Despite the marked increase interest in identifying persons with the metabolic syndrome, as defined by the World Health Organization (WHO) and National Cholesterol Education Panel (NCEP), few prospective data are available on the effects of the syndrome on mortality. This study examined total and cardiovascular (CVD) mortality associated with the metabolic syndrome in elderly women.
Materials and Methods: The study sample comprised 9,704 ambulatory white women aged 65 or older who were enrolled from 1986 to 1988 in the Study of Osteoporotic Fractures (SOF). The sample was population-based, and women were not selected based on osteoporosis. We assessed the longitudinal associations of the metabolic syndrome components that were measured at baseline with total mortality, as well as with coronary heart disease (CHD) and CVD mortality. Diabetes, blood pressure, waist and hip circumference, and body mass index—were measured at the baseline exam; 7% (682) of participants reported physician-diagnosed diabetes. Lipids were not measured on all participants at the baseline exam and are therefore not included in this analysis. Participants or family members were contacted by mail or telephone every four months after baseline, and more than 98% of these follow-up contacts were completed; follow-up for vital status was 100% complete. Cause of mortality was adjudicated by a study physician from death certificates and medical records, based on the International Classification of Diseases, 9th edition (ICD-9). Age-adjusted Cox proportional hazards models were used to estimate the hazard ratios (HR) for mortality, with 95% confidence intervals (CI) associated with the metabolic syndrome (by the WHO and the NCEP definitions).
Results: At the time of enrollment, 317 (3.3%) women met the WHO criteria, and 261 (2.7%) met the NCEP criteria for the metabolic syndrome. During a mean (±SD) of 12.2 (±3.9) years of follow-up, 3,427 women died (507 of CHD, 1,264 of CVD). Overall mortality was 2 to 3 fold greater in those with the metabolic syndrome by the WHO (HR 2.7; 95% CI 2.3 to 3.1) and NCEP definitions (HR 2.4; 95% CI 2.1 to 2.8). The associations were even stronger for CHD mortality (HR, 4.0; 95% CI 2.8 to 5.6 by WHO criteria; and HR, 4.4; 95% CI 3.3 to 5.9 by NCEP criteria). Similarly, deaths from CVD were associated with the syndrome (HR, 3.6; 95% CI 2.9 to 4.5 by WHO criteria; and HR, 3.2; 95% CI 2.6 to 4.0 by NCEP criteria). Results were similar after further adjustment for smoking. Additional analyses were performed to evaluate the relative contribution of the components of the metabolic syndrome to mortality. None of the individual components, however, was as strongly associated with any of the mortality outcomes as was the metabolic syndrome.
Conclusions: The metabolic syndrome, as defined by both the WHO and the NCEP, strongly predicts long-term total mortality, as well as cause-specific CHD and CVD mortality, in elderly women.

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Cardiovascular risk associated with impaired fasting glucose in urban north India.
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Background and Aims: The burden of diabetes and cardiovascular disease (CVD) is rapidly increasing in India, especially in urban areas. Data regarding the cardiovascular risk of persons having impaired fasting glucose (IFG), which has a high rate of progression to diabetes, are scarce in India. We carried out a cross-sectional community based study in Delhi to estimate the prevalence of CVD and its risk factors in indviduals detected to have impaired fasting glucose..
Materials and Methods: 3050 urban adults (52% women), aged 35-64, selected using random multistage startified sampling, underwent comprehensive assessment for cardiovascular risk. ADA criteria were used to define diabetes and imapired fasing glucose. Metabolic syndrome was defined using NCEP ATP III criteria. Diabetics (415 in number) were excluded from this analysis
Results: The mean age of those with or without IFG was same, 47 years. Overall prevalence of IFG was 11.7%. The prevalence of IFG increased with age and was more in men (13.2%) as compared to women (10.4%). After adjusting for age and sex, abdominal obesity (as defined by NCEP ATP III) and overweight had significantly higher odds of having IFG (OR 1.5, p<0.01). Mean blood pressure, serum cholesterol, serum triglycerides, waist circumference, body-mass index and serum insulin levels were significantly higher in those with IFG as compared to those without, all p<0.001 (see table). Serum HDL levels were lower in those with IFG but not significantly (40 vs 41 mg/dl). The prevalence of risk factors in individuals with IFG were as follows: hypertension 33.2%, abdominal obesity as per NCEP criteria 25%, abdominal obesity as per WHO-EGIR 70%, overweight 51% , hypercholestrolemia 44%, and hypertriglyceridemia 50%. The corresponding prevalence of these in individuals with normoglycaemia were 23%, 14%, 60%, 37%, 33% and 35%. The prevalence of these was significantly higher in those with IFG (all p<0.001). Metabolic syndrome, as per NCEP ATP III guidelines, was present in 61% in those with IFG as compared to 16% in those with normoglycaemia (p<0.001)
Conclusion: The study demonstrates the clustering of cardiovascular risk factors with IFG in urban Indians. This is likely to increase greatly the absolute cardiovascular risk for these individuals. Such persons should be routinely screened for other CVD risk factors . Furthermore urgent preventative measures for promotion of a healthier lifestyle are required to control the epidemic of diabetes in India.

Mean values in IFG and normoglycaemia

Variable	Normoglycaemia	IFG
Systolic blood pressure(mmHg)	119	127
Cholesterol HDL ratio	4.4	4.9
Triglyceride (mg/dl)	148	166
Body mass index	23	25
Waist circumference (cm)	82	88

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Predictive properties of isolated impaired glucose tolerance for incident coronary heart disease and cardiovascular mortality are not explained by the development of diabetes during the follow-up.

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Aims: To evaluate whether the relation between impaired glucose tolerance (IGT) at baseline and cardiovascular morbidity and mortality at follow-up was confounded by the subsequent development of diabetes.
Research Design and Methods: A screening survey for diabetes was made in 1987 using 2-hour 75 g oral glucose tolerance test. 1234 men and 1386 women aged 45-64 years who were free of diabetes at baseline were followed up for 11 years. Multivariate hazard ratio (HR) was estimated using Cox regression analysis for incidence of coronary heart disease (CHD) and for mortality from all cardiovascular disease (CVD) and from all-causes.
Results: In subjects who had isolated IGT (2-hour blood glucose 6.7-9.9 mmol/l and fasting glucose <5.6 mmol/l) at baseline and who did not progress to diabetes at follow-up, the multivariate adjusted HRs (95% CI) were 1.70 (1.12-2.58) for CHD incidence, 2.28 (1.37-3.79) for CVD mortality and 1.65 (1.12-2.41) for all-cause mortality.
Conclusion: Baseline IGT was an independent risk predictor for cardiovascular morbidity and mortality and for all-cause mortality, which was not confounded by the subsequent development of overt diabetes.

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Prediction of Insulin Resistance and Diabetes Development

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Relation of smoking with factors associated with insulin resistance syndrome in Japanese male Type 2 diabetic patients.

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Background and Aims: It is known that smoking is one of risk factors for atherosclerosis. To clarify the relation of smoking with factors associated with insulin resistance syndrome in Japanese type 2 diabetes, we compared the clinical characteristics of smokers and non-smokers with type 2 diabetes.
Material and Methods: As the hospital-based Shikoku Diabetic Study, 2940 type 2 diabetic out-patients were registered since April 2000 until May 2001. These patients were divided into smoker and non-smoker groups. The smoker was defined as a current smoker, and the non-smoker was defined as a person without any smoking history. Smokers were found more frequently in male patients than female patients (male 687/1720 (39.9%) vs. female 96/1218 (7.9%)). In these male patients, the mean age of smokers was significantly older than that of non-smokers. Thus, we analyzed only male type 2 diabetic patients with an age less than 65 years for meaningful comparison. Under this condition, the prevalence of smokers was quite high (547/1013, 54.0%). Mean age and body mass index were not significantly different between these 547 smokers and 466 non-smokers (age; smokers 53.2 ± 7.9 vs. non-smokers 54.1 ± 8.6 yrs , body mass index; smokers 24 ± 3.3 vs. non-smokers 24.4 ± 3.8 kg/m2).
Results: The mean levels of HbA1c and systolic blood pressure of smokers at the initial visit were significantly higher than those of non-smokers (HbA1c; smokers 7.0 ± 1.4 vs. non-smokers 6.8 ± 1.4 %, p=0.024, systolic blood pressure; smokers 133.7 ± 16.8 vs. non-smokers 131.7 ± 16.6, mmHg, p=0.014). The mean plasma glucose level and diastolic blood pressure were not significantly different between these two groups. The mean serum triglycerides level of smokers was significantly higher than that of non-smokers (187.0±138.2 vs. 166.3 ± 122.7 mg/dl, p=0.014), and the mean serum HDL-cholesterol level in smokers was significantly lower than that of non-smokers (50.3 ± 14.8 vs. 52.8 ± 15.1mg/dl, p=0.000). Total serum cholesterol and LDL-cholesterol levels were not different between these two groups.
Conclusions: In Japanese male type 2 diabetic patients, smokers have a worse blood glucose control, and are more likely to have multiple factors associated with insulin resistance syndrome than non-smokers. Since the prevalence of smokers was quite high in these patients, the cessation of smoking should be postulated to prevent atherosclerotic complications.

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Differences in the prevalence of the metabolic syndrome between ethnic groups in recently diagnosed Type 2 diabetes in the North American cohort of the ADOPT Study.

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Background and Aims: Previous studies have reported ethnic differences in the prevalence of the Metabolic Syndrome (NHANES III), but few data exist in diabetes. This analysis set out to assess ethnic differences in the prevalence of the Metabolic Syndrome in a cohort of patients from the ADOPT study (a global, randomised, controlled clinical trial).
Materials and Methods: Subjects had recently diagnosed (≤ 3 years) type 2 diabetes and fasting glucose < 10 mmol/l at study entry. The prevalence of the Metabolic Syndrome using NCEP ATP III (National Cholesterol Education Program Adult Treatment Panel III) criteria was assessed in different ethnic groups from the North American cohort of the study [Caucasians (n = 1756), African Americans (n = 164), Asian Americans (n = 74) and Others (principally Hispanic; n = 215)].
Results: The highest rate of the Metabolic Syndrome was in Caucasians. The pattern of abnormalities differed, with prevalence of all 5 criteria 3-fold higher in Caucasians than in Asian Americans. C-reactive protein (CRP) levels were lowest in Asian Americans, possibly reflecting differences in adiposity, or insulin resistance.

	Caucasians	African Americans	Asian Americans	Others	*P*
BMI (kg/m^2)	33.0 ± 6.0	34.5 ± 6.5	28.2 ± 4.7	33.1 ± 6.3	< 0.0001*
HOMA IR (μU/ml.mmol/l)	7.2 (7.0, 7.5)	7.1 (6.4, 7.8)	5.5 (4.8, 6.4)	7.4 (6.7, 8.0)	< 0.0001*
CRP (mg/dl)	0.38 (0.35, 0.41)	0.49 (0.39,0.61)	0.16 (0.11, 0.20)	0.40 (0.33,0.48)	0.0003*
Metabolic Syndrome +ve (%)	83.4	75.0	60.8	76.7	< 0.001**
Diabetes (%)	100	100	100	100	
HTN (%)	73.1	78.0	58.1	63.3	< 0.001**
Low HDL (%)	52.7	39.6	41.9	48.8	< 0.01**
High TG (%)	63.4	32.9	48.6	54.0	< 0.001**
Waist Circ (%)	72.9	75.0	43.2	65.6	< 0.001**
All 5 criteria (%)	25.3	12.8	8.1	14.0	< 0.001**

* Ethnic difference by ANOVA adjusted for age and gender;
**Chi-square test of association between criterion satisfaction and ethnicity.
†Geometric mean (95% CI)

Conclusions: There are marked differences in the prevalence of the Metabolic Syndrome, in addition to insulin resistance and subclinical inflammation among the different ethnic groups studied. This may translate into differences in the prevalence of cardiovascular disease.

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Does the association of BMI with components of the metabolic syndrome differ between populations?

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Background and Aims: The metabolic syndrome has been associated with an increased risk of type 2 diabetes and cardiovascular disease. This study examined whether the associations of body mass index (BMI) with components of the metabolic syndrome differed between a French and an Australian population.
Materials and Methods: Participants from the Australian Diabetes, Obesity and Lifestyle Study (AusDiab) and the French Epidemiological Study on the Insulin Resistance Syndrome (DESIR) were studied. Subjects analysed were 33-68 years and had complete data (AusDiab n=8201, DESIR n=4334). The metabolic syndrome was defined using the National Cholesterol Education Program (NCEP) criteria (≥3 abnormalities, including those on treatment for an abnormality).
Results: In both populations the frequency of the metabolic syndrome was higher among men than women, and higher among the Australian population (24.0%) compared to the French population (14.6%). For both men and women, the regression lines relating BMI to each of triglycerides, fasting plasma glucose (FPG), HDL-cholesterol, systolic BP in women and diastolic BP in men had the same slopes in both populations (those on treatment were excluded). However for any given BMI in both men and women, these concentrations were significantly worse in the Australian population for triglycerides, HDL-cholesterol and FPG (in women). In contrast, systolic and diastolic BP were significantly worse (higher) in the French population for any given BMI.
Conclusion: The frequency of the metabolic syndrome in the Australian population was almost double that of the French population and at each level of BMI the Australian population had a worse glucose and lipid profile, but lower BP.

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Low levels of testosterone and sex hormone-binding globulin predict development of the metabolic syndrome in middle-aged men.

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Background and Aims: Mild hypoandrogenism is associated with factors related to insulin resistance, but little is known about its association with the development of the metabolic syndrome itself. We assessed the association of low levels of testosterone and sex hormone-binding globulin with development of the metabolic syndrome in a population-based cohort of 617 non-diabetic middle-aged men who did not have the metabolic syndrome at baseline.
Methods and Results: After 11 years of follow-up, 130 men had developed the metabolic syndrome (National Cholesterol Education Program definition). Baseline serum total and calculated free testosterone and sex hormone-binding globulin levels were lower in men who developed the metabolic syndrome. Men with total testosterone, calculated free testosterone and sex-hormone-binding globulin levels in the lower third had a 2.9 (95% CI 1.8 – 4.8), 2.0 (95% CI 1.3 – 3.2) and 4.5 (95% CI 2.7 – 7.7) -fold increased risk of developing the metabolic syndrome after adjustment for age (P<0.01 to < 0.001). Further adjustment for potentially confounding factors such as cardiovascular disease, smoking, alcohol intake and socioeconomic status did not alter the associations. Adjustment for components of the metabolic syndrome attenuated the associations, but they remained statistically significant. The association of low testosterone and sex hormone-binding globulin levels with the metabolic syndrome as defined by the World Health Organization was similar, but the association was no longer significant after adjustment for baseline components of the metabolic syndrome.
Conclusion: Hypoandrogenism predicts development of the metabolic syndrome in middle-aged men, possibly independently of other factors related to insulin resistance.

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Low prevalence of tumor necrosis factor-α gene polymorphisms in the 5' promoter region (-238G/A and -308G/A) in subjects of Papua New Guinea.

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Background and Aims: Tumor necrosis factor-α (TNFα) is a cytokine, which is expressed primarily in adipocytes. TNFα expression in adipose tissue is increased in the obese, and is closely correlated with level of insulin resistance. Recently, two polymorphisms were identified in the 5' promoter region of the TNFα gene. The first polymorphism was G to A substitution at position -308 (-308G/A), which was reported to be associated with obesity and increased insulin resistance. The second was G to A substitution at position -238 (-238G/A), which was speculated to be associated with reduced insulin resistance. The aim of our study was to investigate whether -238G/A and -308G/A polymorphisms were involved in obesity of Papua New Guinea (PNG), where remarkable increase in the prevalence of type 2 diabetes (T2DM) had been reported in urban areas.

Materials and Methods: This study was conducted in 12 Balopa villages of PNG in 1994 with approval of the PNG Medical Advisory. The blood samples were collected from 750 residents of the villages. Polymerase chain reaction (PCR)-restriction fragment length polymorphism method was used to analyze these polymorphisms. To detect the polymorphisms the PCR product was digested with NcoI for -308G/A, or with MspI for -238 G/A. To examine whether these polymorphisms were associated with obesity, the polymorphisms of the subjects belonging to the non-obese group (20≤ BMI<23) and the obese group (30≤ BMI) were analyzed. The subjects who had obvious family history of obesity were omitted from the non-obese group.
Results: The -308G/A polymorphism was analyzed in a total of 253 subjects; 133 in the non-obese, and 120 in the obese. Analysis of this polymorphism revealed that the -308G/A was not present in each group. We then examined the -238 G/A polymorphism. Although a total of 253 subjects were analyzed, there were not any subjects carrying the -238G/A substitution. Our data showed that frequencies of two polymorphisms in the 5' promoter region of TNF-α gene were very low, indicating these polymorphisms were not associated with obesity in PNG.
Conclusion: We could not detect -238G/A or -308G/A substitutions in the 5' promoter region of TNFα gene in subjects of PNG. The -308G/A polymorphism was reported to be associated with obesity and/or insulin resistance, mostly in Caucasian. The allele frequency of -308A was 24% in Australian, 18% in African American, 17% in Caucasian American, 1.5% in Japanese, and 0% in PNG. The prevalence of -308G/A in PNG was one of lowest in the world. Although subjects carrying -308G/A were thought to have higher insulin resistant level, it was not likely that this substitution was a gene polymorphism that explained the recent increase in T2DM of PNG. The association of -238G/A with insulin resistance is controversial, and its importance in the pathogenesis for T2DM remains unknown. It was suggested, however, that -238G/A polymorphism was not involved in development of T2DM in PNG population.

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Is insulin resistance the main cause of impaired fasting glycemia (IFG)?

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Purpose: It has been reported that IFG is caused by insulin resistance and IGT by reduction of insulin secretion. The beta cell function of the pancreas is weaker in the Japanese than that of the people of Europe and America. This study was conducted to clarify the difference between IGT and IFG in the Japanese.
Methods: Subjects of this study were 12,357 OGTT examinees composed of 5,841 males and 6,516 females who were registered in 1982 - 2002. Cases with DM under treatment, gastrectomy and liver dysfunction were excluded from the study. The mean age at registration was 62.5 years. The subjects were divided into four groups based on WHO criteria, i.e. normal, IFG, and IGT which was divided into two groups, isolated IGT (FPG <110 and 2 h PG 140-199 mg/dl) and IGT (FPG 110 -125 and 2 h PG 140-199 mg/dl). Plasma glucose levels were measured by glucose oxidase method and IRI by double antibody method. HOMA-IR was calculated by Matthews' equation.
Results: 1) As for prevalence of IFG, we used cases that received OGTT within one month after general health examination at fasting. Of the 2,310 cases with FPG < 110 mg/dl, the rate of IFG was 3.1%, IGT 35.1%, and DM 9.6%. Of the 1,274 cases with FPG ≧126 mg/dl, the rate of IFG was 5.0%, IGT 14.1%, and DM 70.5%, and of the cases with FPG 110-125 mg/dl, the rate of IFG was 11.3%, IGT 36.0%, and DM 29.0%. In examining the FPG distribution of the 9,718 examinees, the rate of cases with FPG<110 mg/dl was 83.7%, FPG≧126 mg/dl 6.3%, and FPG 110-125 mg/dl 10.0%. The prevalence of IFG in the examinees was estimated to be 4.1% and that of glucose intolerance was 10.7%.
2) In comparing mean ± S.D of IRI at fasting, 1/2 h-IRI, and 2 h-IRI by OGTT results, fasting IRI were 6.4±3.6, 1/2 h-IRI 39.6±27.1, and 2 h-IRI 37.1±25.4μU/ml in normal, 8.3±4.5, 38.4±24.9, 44.8±33 in IFG, 7.6±4.3, 38.9±26.7, 64.8±44.3μU/ml in isolated IGT, 9.2±5.2, 36.7±24.9, 66.0±48.4μU/ml in IFG and IGT, respectively. F-IRI has elevated in the order of normal, isolated IGT, IFG, and IFG and IGT. As for ΔIRI/ΔPG(1/2 h), it has decreased gradually in order of normal being 0.67±3.53, IFG 0.50±0.63, isolated IGT 0.49±0.60, and IFG and IGT 0.31±1.45. Though decrease of insulin secretion in early phase was observed in IFG and IGT, there was hardly any difference between IFG and isolated IGT.
3) HOMA-IR was 1.5±0.9 in normal, 2.4±1.3 in IFG, 1.9±1.1 in isolated IGT, and 2.6±1.5 in IFG and IGT. FPG was 94.7±7.5, IFG 115.0±4.2, isolated IGT 98.0±6.9, and IFG and IGT 115.7±4.1mg/dl. On the other hand, HOMA-IR was calculated using FPG and F-IRI; the ratio of FPG between IFG and isolated IGT is 1.2:1.0 and ratio of HOMA is 1.3:1.0. No remarkable difference was observed between two groups. Therefore, it cannot be suggested that IFG is caused by insulin resistance and isolated IGT is caused by reduction of insulin secretion.
Conclusion: In the Japanese the frequency of IFG only is low with glucose intolerance of 10.7%. According to data of IRI value, ΔIRI/ΔPG (1/2 h), and HOMA-IR, it cannot be concluded that IFG depends on insulin resistance and isolated IGT depends on reduction of the insulin secretion. It is assumed that the gradual reduction of insulin secretion together with glucose intolerance will lead to onset of diabetes mellitus of IFG and IGT.

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Definitions of the metabolic syndrome are useful for predicting Type 2 diabetes.

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Background and Aims: Screening for diabetes has become particularly important because of the recent results on the prevention of type 2 diabetes. We have compared IGT, a commonly used screening test for diabetes, with proposed definitions of the metabolic syndrome by the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII) and World Health Organization in 1999 (WHO-1999). WHO-1999 criteria require an OGTT.
Materials and Methods: Using the 1999 WHO definition of type 2 diabetes, we ascertained a 7-8 year incident diabetes in non-diabetic subjects of the San Antonio Heart Study (n = 2452). Predictive discrimination was determined by receiver-operating characteristic (ROC) curves.
Results: Baseline prevalence of IGT was 12.9%, NCEP-ATPIII definition 17.3%, WHO-1999 definition 16.9%, and IGT combined with the NCEP-ATPIII definition 25.2%. The area under the ROC curve for the 2-h glucose value was 0.797. In comparisons with this area, the area for the NCEP-ATPIII criteria was 0.770 (p = 0.229) and for the WHO-1999 criteria 0.831 (p = 0.050). However, the area for the 2-h glucose value combined with the NCEP-ATPIII criteria was 0.841, which was greater than the area of the 2-h glucose value (p <0.0001) but similar to the area of the WHO-1999 criteria (p = 0.207).
Conclusion: The WHO-1999 definition may be a better predictor of diabetes than IGT and the NCEP-ATPIII definition. However, the NCEP-ATPIII definition requires no OGTT, and has a predictive discrimination that is complementary to the predictive discrimination of IGT.

Table: Sensitivity, specificity, and predictive values of IGT, and the NCEP-ATPIII and WHO-99 definitions

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
NCEP-ATPIII	50.8%	86.4%	31.8%	93.4%
WHO-1999 *	53.5%	86.6%	33.2%	93.7%
IGT	51.9%	91.5%	43.2%	93.8%
IGT + NCEP-ATPIII	70.8%	80.1%	30.8%	95.7%

* microalbuminuria is lacking in the SAHS, and the glucose uptake requirement has been changed with the homeostasis model of assessment of insulin resistance

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Comparison of metabolic syndrome definitions in the prediction of diabetes over 5 years in Mauritius.

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Background and Aims: The metabolic syndrome has been shown to be associated with an increased risk of developing cardiovascular disease and Type 2 diabetes. Two definitions of the metabolic syndrome have been proposed. The World Health Organisation (WHO) and an expert panel from the (American) National Cholesterol Education Program (NCEP) have recently developed independent working definitions of the syndrome. Little research has been undertaken into the ability of these definitions to predict Type 2 diabetes.

Materials and Methods: A total of 3,771 Mauritians participated in a population-based survey in 1987 with follow-up in 1992. All those with previously or newly diagnosed diabetes in 1987 (n=489), together with all those without complete data for each component of the metabolic syndrome (n=111) were excluded. Incident diabetes was defined by glucose tolerance test and self-report. Obesity was defined using waist circumference (WC) and waist-hip ratio (WHR) cut-points for Asians. The metabolic syndrome was defined by the NCEP as 3 or more of obesity by WC, elevated triglycerides, reduced HDL, hypertension and fasting plasma glucose ≥6.1mmol/L. The WHO definition included impaired fasting glucose, impaired glucose tolerance or in the top quartile of fasting insulin PLUS 2 of hypertension, dyslipidaemia (elevated triglycerides or reduced HDL) or obesity defined by BMI and/or WHR.
Results: The prevalence of the metabolic syndrome at baseline, as defined by the WHO and the NCEP, was 19.1% and 13.8% respectively. A total of 291 individuals (9.2%) developed diabetes between the 1987 and 1992 surveys. The WHO definition of the metabolic syndrome had a higher sensitivity for predicting Type 2 diabetes than did the NCEP definition (0.48 [SE=0.03] vs. 0.34 [0.03], p<0.001). Specificity (0.84 [0.007] vs. 0.88[0.006], p<0.0001) was significantly greater for the NCEP definition, while positive predictive value (0.23[0.02] vs. 0.22[0.02]) and negative predictive value (0.94[0.005] vs. 0.93[0.005]) were similar for both definitions. When analysed separately by gender, there was no significant difference in specificity, sensitivity, positive predictive value or negative predictive value between the two definitions among women, however among men, sensitivity was significantly higher for the WHO definition (0.52 vs. 0.33, p<0.001) and specificity was higher for the NCEP definition (0.83 vs. 0.91, p<0.0001).
Conclusion: The WHO definition of the metabolic syndrome appears to be a better predictor of diabetes among Mauritian men based on its considerably higher sensitivity, with a comparably smaller (although still significant) reduction in specificity. Both WHO and NCEP definitions performed similarly in Mauritian women.

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The metabolic syndrome in recently diagnosed Type 2 diabetic patients.

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Background and Aims: Type 2 diabetes mellitus (DM2) is a common feature of the metabolic syndrome (MS). However, the prevalence of the MS in DM2 is not well known. The aims of the study were: a) to quantify the prevalence of the MS as defined by according to WHO (1999) and NCEP ATP III (2001) criteria; b) to evaluate the concordance between the two diagnostic criteria; c) to define the clinical and metabolic characteristics associated with the presence of an increasing number of the MS components in recently diagnosed type 2 diabetic outpatients.
Material and Methods: 247 outpatients with recently diagnosed DM2 (160 males and 87 females, mean age 52±8 years, range 26-65), consecutively recruited, in good glycemic control, without retinopathy, neuropathy nor other chronic disease, were studied. Besides the evaluation of the common clinical and metabolic parameters, the basal and post-glucagon insulin and C-peptide serum levels, the urinary albumin excretion rate (UAER, the average of two 24-hours urine collections) and the insulin sensitivity, by means of the Short Insulin Tolerance Test (K_{ITT}) ed $HOMA_{IR}$, were measured.
Results: The prevalence of the MS was 82.2% and 74.9% according to WHO e NCEP criteria, respectively. The diagnosis was concordant for 79.7% of the patients (68.4% with and 11.3% without the MS, k 0.40, p < 0.0001). Moreover, the prevalence of the MS did not differ among decades of age (from 26-35 to 56-65 years) (p for trend = NS). Only 3 patients (1.2%) did not have any other component of the MS by both criteria, apart from DM2. The population was subdivided in five groups, on the basis of the number of the MS components actually present. By the WHO criteria, no difference was found among the five groups for age, sex, fasting glycemia, glycated haemoglobin, creatinine, LDL-cholesterol and heart rate (ANOVA or χ^2, p = NS). On the other hand, a linear, statistically significant, increase of insulin resistance (both K_{ITT} and $HOMA_{IR}$), fasting insulinemia, basal and post-glucagon C-peptide, non HDL-cholesterol, uric acid, leukocytes, smokers ($p \leq 0.001$), pulse pressure ($p = 0.002$), total cholesterol ($p = 0.013$) and ischemic heart disease ($p = 0.01$) was observed from the group without any component of the MS to the group with all the four features of the MS. The same analysis, conducted using the NCEP criteria, showed similar results, with the exception of the non significant association between the presence of an increasing number of the MS components and leukocytes, UAER, total and non-HDL cholesterol, whereas female sex was significantly more represented in the groups with the higher number of the MS features.
Conclusions: In recently diagnosed type 2 diabetic outpatients, the prevalence of the MS according to the WHO and NCEP ATP III criteria is high and not influenced by age. The two criteria show a good, even if not excellent, concordance. The more numerous the components of the MS, the higher are the insulin resistance, serum insulin, C-peptide, total and non HDL-cholesterol, uric acid, leukocytes, pulse pressure, prevalence of smokers and ischemic heart disease. DM2 without any feature of the MS is rare.

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Prediction of Type 2 Diabetes Mellitus

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Impaired fasting glycaemia, HbA(1C) and BMI are significant predictors for the development of Type 2 diabetes in middle-aged subjects. The Fredericia Study, second generation.

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Background and Aims: Both impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT) are known as strong risk markers for the development of Type 2 diabetes. The aim of this study was to evaluate IGT, IFG and other possible predictors for the development of Type 2 diabetes in a middle-aged population.
Materials and Methods: In 1997-98, we examined 282 offspring of Type 2 diabetic patients and 275 offspring of non-diabetic subjects. Diabetes was diagnosed according to the WHO criteria (1999). The 557 subjects had already been examined in 1991-92 , where anthropomorphic measurements and fasting levels of level of blood glucose, cholesterol, HbA(1C) and insulin were measured. Urinary albumin excretion rate (UAER) was measured on 3 overnight samples. Subjects with fasting blood glucose ≥ 4,4 mmol/l had been asked to come back for an oral glucose tolerance test (OGTT). Fasting glucose was measured on whole blood. Using a logistic regression model we evaluated possible predictors of Type 2 diabetes including BMI, parental Type 2 diabetes, IFG , IGT, UAER, HbA(1C) and fasting insulin.
Results: The subjects were aged 26-64 years(mean:47.5) at the first examination in 1991. 31 subjects (5.6 %) of the total 557 subjects developed Type 2 diabetes between 1991 and 1998. 40 % (6/15) of the subjects with IFG and 37,5 % (3/8) of the subjects with IGT developed type 2 diabetes. IFG, HbA(1C) and BMI were found to be significant predictors for the development of type 2 diabetes. Odds ratio were: IFG = 4.7 ($p<0.01$); HbA(1C)(cut-off value: 4.8) = 4.4 ($p<0.001$) and BMI (cut-off value: 30 kg/m^2) = 2.4 ($p<0.05$). IGT, UAER, parental Type 2 and fasting insulin were eliminated from the model using forward elimination procedure.
Conclusion: IFG, HbA(1C) and BMI were found to be significant predictors for the development of Type 2 diabetes over 7 years in a middle-aged Danish population. The use of OGTT on subjects with fasting whole blood glucose ≥ 4,4 mmol/l or information about parental Type 2 diabetes did not add further information, when the aim was to predict Type 2 diabetes in this population.

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Different patterns in iron parameters and inflammatory parameters between newly diagnosed diabetics, IGT and NGT (the CODAM Study).

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Background and Aims: In diabetes patients a 2-3 fold higher oxidative stress levels are usually observed. The iron metabolism plays a crucial role in this process because its components are the initiators of reactive oxygen species. Parameters of interest are ferritin and the so-called non-tranferrin-bound-iron (NTBI). In this study we measured these parameters in newly diagnosed diabetes type 2, in subjects with impaired glucose tolerance (IGT) and in subjects with normal glucose tolerance (NGT) of the CODAM Study. A comparison was made with other inflammation parameters, such as C-reactive protein (CRP) and complement-3 (C3). Our aim is to determine whether an increased iron status may be one of the factors in the development of diabetes type 2.
Materials and Methods: We included the 504 members of the CODAM Study, 303 with NGT, 121 with IGT and 80 with newly diagnosed diabetes. NTBI was measured in serum with a homogenous assay using a fluorescein-apotranferrin conjugate. The other parameters were determined routinely with a Hitachi 912 autoanalyzer.
Results: Subjects with newly diagnosed diabetes have higher NTBI, ferritin, CRP and C3 values than subjects with NGT. NTBI values were not significant different between both groups. After adjustment for age, gender BMI, smoking and physical activity the CRP values were borderline significantly different, probably due to the contribution of adipocytes (BMI adjustment). Both ferritin and C3 values were significantly different after adjustment. The IGT group showed a remarkable difference between the relative levels of the iron parameters NTBI and ferritin on one hand and CRP and C3 on the other hand. NTBI and ferritin levels were more closely to those of the NGT group whereas the CRP and C3 levels where close to those of the newly diagnosed diabetes group. Mean values were for NTBI: 1.92 1.34 and 1.52 µmol/l; for ferritin: 290, 205 and 181 µg/l; for CRP: 4.95, 4.72 and 3.86 mg/l; for C3: 1.97, 1.93 and 1.72 g/l for newly diagnosed diabetes, IGT and NGT group, respectively.
Conclusions: In the IGT group the values of the inflammation parameters CRP and C3 are closely to those of the diabetes group, reflecting a 'diabetes-like' inflammatory state of the IGT group. The values of the iron parameters ferritin and NTBI of the IGT group are more closely to those of the NGT group indicating a 'non-diabetes' state of the iron status in the IGT group. From these data we might conclude that a high iron status is probably not a risk factor for the development of diabetes type 2. Why patients with type 2 diabetes have increased levels of iron parameters remains to be elucidated. Information on oxidative stress parameters will possible give more information in this complicated matter.

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Higher coffee consumption is associated with lower risk of Type 2 diabetes and impaired glucose tolerance in Swedish men and women.

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Background and Aims: It has recently been reported that coffee consumption is associated with a substantially lower risk of clinical type 2 diabetes. It has been speculated on mechanisms that could explain these associations. Among coffee constituents, chlorogenic acid may decrease hepatic glucose production.
We investigated the association between coffee consumption and type 2 diabetes and impaired glucose tolerance (IGT) in middle-aged Swedish men and women. In addition, we examined possible mechanisms of expected associations by analysing insulin sensitivity and β-cell function.
Materials and Methods: This population-based cross-sectional study comprised 3128 Swedish men and 4821 women aged 35-56 years living in five municipalities on the outskirts of Stockholm. An oral glucose tolerance test identified 55 men and 52 women with previously undiagnosed type 2 diabetes and 172 men and 167 women with IGT. Odds ratios (OR) accompanied by 95% confidence interval (CI) were calculated in multiple logistic regression analysis. Insulin sensitivity and β-cell function were estimated according to the homeostasis model assessment (HOMA). We have tested for potential confounders such as age, family history of diabetes, body mass index, smoking, physical inactivity and socio-economic position.
Results: We found that coffee consumption i.e. ≥5 cups/day compared to ≤2 cups/day had an inverse association with type 2 diabetes in both men and women, OR=0.45 (CI: 0.22–0.91) and OR=0.28 (CI: 0.12-0.69), respectively. There was also an inverse association with IGT and coffee consumption; OR for men 0.63 (CI: 0.41-0.96), and for women OR=0.49 (CI: 0.30-0.78). HOMA was analysed in subjects with normal glucose tolerance. High coffee consumption was positively associated to insulin sensitivity in women, OR=1.38 (CI: 1.12-1.71), but not in men, while no relation was found to β-cell function.
Conclusion: Our data suggest that higher coffee consumption is associated with a decreased risk of type 2 diabetes, as well as its pre-clinical IGT stadium, and more pronounced in women than in men. A possible mechanism could be through increased insulin sensitivity.

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Prevalence and clinical significance of Glutamic Acid Decarboxylase (GAD) antibodies in recently diagnosed Type 2 diabetes in the ADOPT study cohort.

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Background and Aims: A number of patients with clinical type 2 diabetes mellitus (T2DM) are glutamic acid decarboxylase antibody positive (GAD+). The aim of this study was to assess the GAD status of patients enrolled in ADOPT.
Materials and Methods: ADOPT (A Diabetes Outcome Progression Trial) is a randomised, double-blind, comparative drug trial in 4,293 drug-naive, recently diagnosed T2DM patients. The GAD status of these patients was evaluated at baseline in the context of anthropometric and biochemical characteristics.
Results: Although BMI and age were similar, the 159 (3.7%) GAD+ patients tended to have a lower waist circumference, higher HbA_{1c}, and lower fasting insulin accompanied by decreased measurements of β-cell function (pro-insulin/C-peptide, Δ I30/Δ G30) during an OGTT. However, when β-cell function is corrected for insulin-resistance [Δ I 30/Δ G 30]/insulin, GAD+ and GAD- patients were similar.

Parameter	GAD-positive*	GAD-negative*	*P*-value
Age (yrs)	59.0 (51.0, 65.0)	57.0 (50.0, 64.0)	$P = 0.14$
BMI (kg/m^2)	29.9 (27.3, 35.7)	31.1 (27.8, 35.3)	$P = 0.26$
Waist Circumference (cm)	103.0 (94.0, 113.0)	104.1 (96.0, 113.0)	$P = 0.09$
Fasting Glucose (mmol/l)	8.2 (7.6, 9.4)	8.2 (7.5, 9.1)	$P = 0.37$
HbA_{1c} (%)	7.5 (6.8, 8.0)	7.3 (6.7, 7.9)	$P = 0.06$
Fasting Insulin (pmol/l)	102.0 (64.6,150.0)	122.0 (86.1,186.6)	$P = 0.03$
Pro-insulin/C-peptide ([pmol/l]/[nmol/l])	43.8 (28.8, 62.1)	39.3 (27.1, 57.1)	$P = 0.07$
Δ I30/Δ G30 ([pmol/l]/[mmol/l])	26.4 (14.0, 51.9)	33.2 (18.7, 58.7)	$P = 0.01$
[Δ I30/Δ G30]/insulin ([[pmol/l]/[mmol/l]]/[pmol/l])	0.26 (0.16, 0.41)	0.27 (0.17, 0.43)	$P = 0.49$

*median (IQR)

Consistent with increased fasting insulin as a surrogate for insulin resistance, GAD- patients had lower HDL (median [IQR] 1.20 [1.01, 1.42] vs. 1.26 [1.06, 1.48] mmol/l; $P < 0.05$]) and higher triglycerides (1.80 [1.29, 2.61] vs. 1.33 [1.12, 2.49] mmol/l; $P < 0.05$).
Conclusion: Newly diagnosed patients with T2DM who are GAD+ appear otherwise phenotypically similar to GAD- patients. Although measures of β-cell function appear to be poorer in GAD+ patients, when ambient insulin resistance is corrected for, β-cell function is similar. Nonetheless, the natural history and progressive nature of T2DM may be different in these two groups over time.

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Prospective study of gamma glutamyl transferase in relation to the development of Type 2 diabetes in the D.E.S.I.R. study.

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Background: It has been shown that γ-glutamyl transferase (GGT) predicts the development of type 2 diabetes in men.
Methods: We examined the association between GGT levels and risk of diabetes in a 3 years study of 2080 men and 2137 women from the D.E.S.I.R. cohort, aged from 30 to 65 years and coming from the centre west of France.
Cases were ascertained by yearly self-questionnaire and after 3 years of follow-up by a physician consultation with a fasting plasma glucose measurement (FPG) to detect undiagnosed diabetes if FPG equal or greater than 1,26g/l.
Results: 89 subjects developed diabetes during follow-up (1.4% men, 2.8% women).
At baseline, GGT correlated significantly with alcohol consumption (r=0,36) and all the main risk markers of diabetes: glucose (r=0,31), BMI (r=0,37), age (r=0,15).
In a univariate logistic regression analysis, GGT were associated with the 3 years occurrence of diabetes in men (p<0,001) and in women (p<0,02). Compared with quartile 1, men with GGT in the highest quartile had an increased odds ratio of developing diabetes 7,3 (95% CI: 2,5-20,9) and women by 4,0 (95% CI:1,1-14,3).
After adjustment on confunding factors: age, alcohol consumption, smoking habits, physical activity and BMI, the association was still significant in men (OR = 4,5 (1,5-13,3)) in men but not in women (OR=1,9(0,5-7,4)). In men, this association was weaker after adjustment on FPG (OR =3,1 (1,1-9,8)).
Conclusion: These finding suggest that a raised GGT level is a risk marker for diabetes in both sexes. The GGT level is a more independent marker in men than in women. To clarify this relation and to confirm GGT as a possible marker of hepatic fat or hepatic insulin resistance, it will be necessary to have a longer follow-up.

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High prevalence of glucose abnormalities in patients with hepatitis C virus infection. A large cohort study considering the liver damage.

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Background and Aims: There is growing evidence to suggest an association between hepatitis C virus (HCV) infection and diabetes mellitus, two common disorders which cause devastating long-term complications in a significant number of patients. To further explore the link between HCV infection and diabetes, we have compared the prevalence not only of diabetes mellitus but also the impaired fasting glucose (IFG) -an early predictor of diabetes mellitus- between HCV infected patients and patients with other HCV-negative liver diseases. In addition, in the analysis of the results both the degree of liver damage and HCV genotypes were considered.
Materials and Methods: A total of 642 consecutive patients attending the outpatients Liver Unit of a University Hospital (498 anti-HCV positive and 144 anti-HCV negative) were prospectively recruited. Patients were classified as having chronic hepatitis (n=498) or cirrhosis (n=144) by means of the result of either a liver biopsy or by typical clinical features. Serological testing for anti-HCV was done using a second-generation commercial enzyme-immunoassay. The HCV genotype was determined by RT-PCR on a segment from the core region and by hybridization of this fragment with oligonucleotide specific probes.
Results: In patients with chronic hepatitis, both diabetes and IFG were significantly more prevalent among anti-HCV positive patients than in those anti-HCV negative patients (17% vs. 7% and 15% vs. 5% respectively; p<0.05). In addition, when the prevalence of glucose abnormalities were evaluated in patients with normal transaminases (n=187) the differences remained statistically significant (anti-HCV positive: 24% vs. anti-HCV negative: 5%; p=0,003). In contrast, among patients with cirrhosis, although both diabetes mellitus and IFG were more prevalent in anti-HCV positive patients than in anti-VHC negative patients, the differences were not statistically significant (diabetes: 40% vs. 36% and IFG: 12% vs. 7%). Differences in the prevalence of glucose abnormalities among genotypes were not observed.
Conclusion: The higher prevalence of glucose abnormalities observed in HCV infected patients in comparison with other liver diseases is mainly due to patients with chronic hepatitis and is unrelated to HCV genotypes.

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Upper normal 2-h plasma glucose values derived from a standard OGTT are associated with decreases in insulin sensitivity and secretion that may predispose to Type 2 diabetes.

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Background and Aims: Little information is available on differences in glucose metabolism measures, independant of adiposity differences, in subjects with apparently normal glucose levels. Therefore, we evaluated differences in measures of glucose metabolism and cardiovascular disease (CVD) risk factors among subjects with normal 2-h plasma glucose (2hPG).
Materials and Methods: We compared insulin secretion and sensitivity by using several indices derived from an oral glucose tolerance test (OGTT) in

681 subjects (aged from 18 to 65 y) from the Quebec Family Study who had varying degrees of 2hPG. Subjects were categorized by using 2hPG following a 75-g OGTT, as having a low normal 2hPG (LN2hPG) (2hPG<5.6mM), high normal 2hPG (HN2hPG) (2hPG between 5.6 and 7.8mM), impaired glucose tolerance (IGT) (2hPG between 7.8 and 11.1mM) and type 2 diabetes (2hPG≥ 11.1mM).
Results: There was a progressive decline in beta-cell function and in insulin sensitivity when moving from LN2hPG to type 2 diabetes. Compared with subjects with LN2hPG, subjects with HN2hPG were more insulin resistant (as evaluated with Cederholm, Matsuda, Metabolic Clearance Rate (MCR) of glucose, Insulin Sensitivity Index (ISI) and OGTT-Belfiore indices) ($p< 0.05$) and had reduced insulin secretion (adjusted for insulin sensitivity) as estimated with the homeostasis model assessment (HOMA) beta-cell index ($HOMA_{BC}$), Stumvoll indexes, insulin to glucose ratio, C-peptide to glucose ratio and with the insulinogenic index during the first 30 min and the 120 min OGTT ($p< 0.001$). They also had higher plasma triglyceride (TG) concentrations ($p< 0.01$) and higher cholesterol to HDL-cholesterol (chol/HDL-chol) ratio ($p< 0.05$). These differences remained even after adjustment for age, sex and adiposity (body mass index (BMI) and waist circumference). Significant decreased in insulin sensitivity and insulin secretion were observed in the IGT group compared with subjects with LN2hPG and subjects with HN2hPG ($p< 0.001$).
Conclusion: These results suggest that decreases in insulin sensitivity and secretion, independant of age, sex and adiposity, are already present in subjects with 2hPG concentrations in the upper normal range. Since these changes may predispose to type 2 diabetes, the clinical significance of these glucose values might need to be adressed.

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Excess risk of Type 2 diabetes in lower socioeconomic groups is explained differently in men and women by psycho-social and other risk factors.

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Background and Aims: In Western societies, people in socioeconomic deprivation are more likely to develop type 2 diabetes than those less deprived. Established risk factors such as physical inactivity, obesity and smoking are suggested to account for this difference. In addition, the influence of psycho-social factors has been discussed. With regard to this background, we aimed at investigating to what extent the excess risk of type 2 diabetes in lower socioeconomic groups was due to established risk factors (physical inactivity, overweight, smoking and diabetes heredity) and psycho-social factors (work stress and low sense of coherence).
Materials and Methods: This cross-sectional study comprised 3,128 Swedish men and 4,821 women, aged 35 to 56 years, living in five municipalities in the Stockholm area. An oral glucose tolerance test identified 55 men and 52 women with previously undiagnosed type 2 diabetes. Information on lifestyle factors was collected in a questionnaire. Socioeconomic position was classified according to a Swedish socioeconomic index and divided into three categories (high, middle and low). Odds Ratios (OR) with 95% confidence intervals (CI) were estimated in a logistic multiple regression analysis. The relative contribution of different factors to the socioeconomic differences in diabetes risk was assessed by comparing analysis with adjustment for different sets of risk factors.
Results: The age-adjusted OR for type 2 diabetes in middle and low socioeconomic groups in men were 2.4 (CI: 1.0-5.3) and 2.9 (CI: 1.5-5.7), and in women 3.1 (CI: 1.5-6.6) and 2.7 (CI :1.3-5.9), respectively. In men, the OR in these groups changed to 2.0 (CI:0.9-4.5) and 2.2 (CI:1.1-4.3) after adjustment for established risk factors; no change was found when we included psycho-social factors. In women, the OR changed to 2.7 (CI:1.3-6.0) and 2.0 (CI:0.9-4.5) after adjustment for established risk factors and when we included both established and psycho-social factors the OR was 1.9 (CI:0.8-4.5) and 1.3 (CI:0.5-3.2), respectively.
Conclusion: In men, established risk factors but not psycho-social factors explained some of the excess risk of type 2 diabetes in middle and low socioeconomic groups (29% and 37%, respectively). In women with middle or low socioeconomic position a new and important finding was the influence of psycho-social factors which together with established risk factors explained 57% and 82%, respectively of the excess risk of type 2 diabetes.

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Analysis of gene polymorphisms in „thrifty genes“ in subjects of Papua New Guinea.

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Background and Aims: Type 2 diabetes (T2DM) is thought to be a multifactorial disorder. Although reduced energy expenditure and excess calorie intake are involved in the development of T2DM, recent gene analysis has revealed that so-called „thrifty genes“ have crucial roles in the pathogenesis of T2DM. The aim of this study was to elucidate whether polymorphisms in „thrifty genes“ were involved in the explosion of T2DM in the South Pacific region, especially in Papua New Guinea (PNG).
Materials and Methods: In this study we analyzed PPARγ 2 Pro12Ala polymorphism, β 3-adrenergic receptor (AR) Trp64Arg polymorphism, the substitutions in the 5'untranslated region (-112A/ C) and Met229Leu in uncoupling protein (UCP)-1, and the substitution in the 5'flanking region (-55C/ T) of UCP-3. Blood samples were collected from Austronesian-speaking Balopa Islanders of PNG. This study was conducted in 12 Balopa villages in 1994 with approval of the PNG Medical Advisory. Polymerase chain reaction (PCR)-restriction fragment length polymorphism method was used to analyze gene polymorphisms. To elucidate whether these polymorphisms were associated with obesity, the subjects were enrolled into three groups: non-obese group (20 ≤BMI<23), overweight group (25 ≤BMI<30), and obese group (30 ≤BMI). The subjects who had obvious family history of obesity were omitted from the non-obese group.
Results: Although more than 250 samples were analyzed, PPARγ 2 Pro12Ala was not found in any groups of PNG subjects. The allele frequencies of the Arg64 of β3AR in the overweight and the obese were significantly higher than in the non-obese group, which indicated this polymorphism was associated with high BMI in PNG. Frequencies of -112A/ C and Met229Leu in UCP-1, which were associated with susceptibility to T2DM in Japanese, were rare. The frequency of -55C/ T in UCP-3, which is though to be associated with obesity in some populations, was significantly lower in the obese group than in the non-obese and the overweight.
Conclusion: As PPARγ 2 Pro12Ala, which is thought to have protective effect against obesity and T2DM, was not found in subjects of PNG, people in PNG were thought to have susceptibility to obesity and T2DM. In addition, the frequency of Trp64Arg polymorphism in the β3-AR in PNG subjects was significantly higher in the overweight and the obese subjects than in the non-obese subjects. Thus, the Trp64Arg polymorphism in the β3-AR seems to be one of major genetic factors related to obesity in subjects of PNG. No association of polymorphisms in UCP-1 and UCP-3 genes with obesity in PNG was found. Our data clarified some genetic backgrounds that explain the recent increase in T2DM in urban areas of PNG.

Frequencies of Gene Polymorphisms

Allele frequency (%)	Non-obese	Overweight	Obese
PPAR γ 2 Pro12Ala	0	0	0
β 3AR Trp64Arg	9.4	21.4	18.8
UCP-1 -112A/C	1.5	0	1.4
UCP-1 Met229Leu	0	0	0
UCP-3 -55C/T	34.1	36.1	24.3

PS 16

Prediction of Type 2 Diabetes and Screening Methods

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Effect of metformin on hepatic steatosis and leptin in Type 2 diabetic patients.

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Background and Aims: Hepaic steatosis is prevalently associated with obesity and diabetes, and plays an important role in insulin resistance. On the one hand, obesity is associated with elevated leptin. Leptin is expressed primarily by adipocytes, which secrete this hormone into blood. Plasma leptin levels correlate with percent body fat, suggesting that leptin is an important signal of fat score. On the other hand, metformin reduce insulin resistance in type 2 diabetic patients. The present investigation was undertaken to clarify whether metformin affect fatty liver and leptin in type 2 diabetic patients.
Materials and Methods: Eighteen type 2 diabetic patients with fatty liver were selected from our department for this study. There was not documented any evidence of liver disease or drug abuse including alchol. Of 18 diabetic patients, 10 patients (metformin group) were treated with metformin(750mg/day) orally for 3 months. 8 patient (glybenclamide group) were treated with glybenclamide (2.5mg/day). They were matched for clinical characteristics such as age, BMI, duration of diabetes mellitus, blood pressure, fasting plasma glucose(FPG) and HbA1c. After a 3-month pretreatment period, clinical variables were assessed at 3rd month. HbA1c, IRI, FPG, leptin, waist hip ratio and liver spleen ratio of computed tomography (CT ratio) were compared between two groups. During the study period, we did not change antihypertensive drugs.
Results: In the metformin group, CT ratio increased from 0.76 ±0.06 at baseline to 0.92 ±0.06($p<0.005$) after 3 months. In the glybenclamide group, CT ratio remained virtually stable from 0.85 ±0.08 at baseline to 0.91 ±0.06 after 3 months. In the metformin group, IRI decreased from 14.1 ±2.8 U/ml at baseline to 9.1 ±0.9U/ml ($p<0.05$)after 3 months. In the glybenclamide group, IRI remained unchanged 14.2 ±3.8 U/ml at baseline to 12.1 ±2.5U/ml after 3 months. Leptin-changing rate in the metformin-treated patients was significantly smaller ($p<0.05$) than that in the glybenclamide group(–6.4 ±0.1% v.s.22.3 ±12.9%). No significant changes were seen in BMI, blood pressure, FPG, HbA1c, lactic acid and waist hip ratio after 3 months between two groups.
Conclusion: These results suggest that metformin ameliorate hepatic steatosis and leptin in type 2 diabetic patients and that decreased liver fat may be associated with reduced insuin resistance.

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Characteristics	glybenclamide	metformin
Sex(M/F)	6/2	8/2
Age(years)	55.4± 4.9	57.7± 3.8
Known duration of diabetes(years)	5.4± 1.8	5.3± 1.8
BMI(kg/ m^2)	29.4± 1.8	29.1± 1.2
Systolic blood pressure(mmHg)	134±4	134±4
Diastolic blood pressure(mmHg)	80±2	84±4
Waist hip ratio	0.90±0.03	0.93±0.03
Antihypertensive drugs(patients)	5	7
Liver spleen ratio	0.759±0.07	0.846±0.06
Baseline clinical characteristics	mean±SEM	

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	glybenclamide	metformin
HbA1c(%)	7.7±0.5	7.8±0.4
IRI(U/ml)	14.2±3.5	14.1±2.8
Total cholesterol(mg/dl)	209.3±7.8	201.7±11.1
Triglyceride(mg/dl)	199.5±27.2	172.5±21.1
High density lipoprotein cholesterol(mg/dl)	47.3±2.9	47.5±3.8
Lactic acid(mg/dl)	14.7±2.2	15.6±1.1
leptin(ng/ml)	8.2±1.4	7.4±1.1
Fasting plasma glucose(mg/dl)	164.8±10.9	172.2±15.5
Baseline clinical data	mean±SEM	

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Inflammation increases the risk of the metabolic syndrome and diabetes in middle-aged men.

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Background and Aims: Inflammation has been suggested to play a role in the pathogenesis of the metabolic syndrome and type 2 diabetes mellitus, but there is a paucity of prospective data from population-based studies. We assessed the association of high CRP levels with development of the metabolic syndrome in a population-based cohort of 615 non-diabetic middle-aged men who did not have the metabolic syndrome at baseline, and with incident diabetes mellitus in 777 men who did not have diabetes at baseline.
Methods and Results: After 11 years of follow-up, 114 men had developed the metabolic syndrome (World Health Organization definition). Baseline serum high sensitivity C-reactive protein (CRP) levels were higher in men who developed the metabolic syndrome. Men with CRP levels in the upper third had a 2.9 (95% CI 1.7 – 4.9) -fold increased risk of developing the metabolic syndrome after adjustment for age. Further adjustment for potentially confounding factors such as cardiovascular disease, smoking, alcohol intake and socioeconomic status had no effect, but adjustment for body mass index (BMI) decreased the association (OR 1.9 [95% CI 1.1 – 3.4]). Adjustment for other components of the metabolic syndrome attenuated the associations such that they were no longer statistically significant. Of 777 non-diabetic men, 79 men developed diabetes during the 11-year follow up. Men with CRP levels in the upper third were 2.2 (95% CI 1.2 – 4.2) times more likely to develop diabetes, but not after adjustment for BMI or other components associated with insulin resistance.
Conclusion: High CRP levels increase the risk of the metabolic syndrome and diabetes 2 – 3 -fold in middle-aged men, but not independently of factors related to insulin resistance. Inflammation may be a mechanism by which obesity and insulin resistance promote the development of the metabolic syndrome and diabetes.

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C-reactive protein predicts the deterioration of glycaemia in Chinese subjects with impaired glucose tolerance.

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Background and Aims: It has recently been shown that C-reactive protein (CRP) predicts future risk for diabetes in healthy Caucasian. We determined whether plasma CRP level was elevated in Chinese subjects with impaired glucose tolerance (IGT) and whether CRP level could be used to predict progression to type 2 diabetes or reversion to normal glucose tolerance (NGT) in these high risk individuals.
Materials and Methods: 228 subjects with IGT at baseline from the Hong Kong Cardiovascular Risk Factor Prevalence Study underwent a repeat oral glucose tolerance test after 2 years. CRP level was measured in their stored baseline plasma samples and from 228 subjects with NGT matched for age and BMI by a high sensitivity immunoturbidimetric assay. Subjects with plasma CRP levels >15 mg/l (6 from the IGT group and 4 from the NGT group) indicating clinically relevant inflammatory conditions were excluded from the subsequent analysis.
Results: Subjects with IGT at baseline had higher plasma CRP levels than subjects with NGT [1.18 mg/l (0.52 - 2.52) vs 0.87 (0.37 - 1.84), median (interquartile range), $p = 0.01$]. At 2-year, 117 subjects with IGT reverted to NGT, 84 remained in IGT and 21 progressed to diabetes. Individuals who progressed to diabetes had the highest plasma CRP levels at baseline ($p < 0.0001$). Those with baseline CRP levels in the third and top quartile had a relative risk of remaining in IGT or progressing to diabetes of 2.94 ($p = 0.03$) and 2.65 ($p = 0.04$) respectively after adjusting for age, sex, BMI, smoking and fasting glucose.

Conclusion: CRP independently predicts the risk for remaining in IGT or progressing to diabetes in Chinese subjects with IGT. CRP might provide an adjunctive measure for identifying those subjects with the highest risk of progression to diabetes, who would derive the greatest benefits from preventive interventions.
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Insulin sensitivity and secretion in the first two years of Type 2 diabetes.
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Background and Aims: To increase understanding of early progression of type 2 diabetes (T2D) in Caucasians, we measured insulin sensitivity and secretion over a 2 year period after diagnosis and investigated the relationship with glucose control.
Materials and Methods: In yrs 0, 1, 2 after diagnosis of T2D, 54 subjects (M/F 43/11; age 56±1; BMI 30.0±0.6kg/m^2) underwent insulin-modified IVGTT (0.3g/kg glucose; 0.05U/kg insulin at 20min) and meal tolerance test (MTT; 500kcal). Subjects were treated conventionally by diet (D; 23 in yr1, 16 in yr 2), sulphonylureas (S; 6, 7), metformin (M; 16, 14), or combination (M+S; 9, 17). Minmod analysis of IVGTT gave insulin sensitivity (S_I), glucose effectiveness (S_G), first-phase insulin secretion (AIR_G), and disposition index ($D_I=S_I x AIR_G$). Insulin secretion model of MTT gave fasting (M_0) and postprandial (M_I) β–cell responsiveness.
Results: After reductions in yr1, HbA_{1C} but not FPG increased in yr2. Insulin sensitivity and postprandial β–cell responsiveness remained unchanged. First-phase insulin secretion but not fasting or postprandial β–cell responsiveness increased continuously over 2 yrs.

Type 2 diabetes progression (N = 54; adjusted for BMI)

	Year 0	Year 1	Year 2	P-Value
FPG (mM)	10.6	8.4	8.5	<0.001
FPI (pM)	57.6	63.9	65.6	0.042
HbA_{1C} (%)	7.6	6.1	6.8	<0.001
$S_I x10^{-5}$ (/min per pM)	1.12	1.30	1.17	NS
$S_G x10^{-2}$ (/min)	1.51	1.49	1.54	NS
AIR_G (pM per 6min)	325	409	470	<0.001
$D_I x10^{-5}$ (/min per 6 min)	364	532	549	<0.001
$M_0 x10^{-9}$ (/min)	4.8	6.3	5.2	0.020
$M_I x10^{-9}$ (/min)	16.4	19.3	15.8	NS

The improvement in glucose control, ΔHbA_{1C}, in yr1 and yr2 was negatively associated with ΔM_0 (r_s=-0.49, P <0.001) and ΔM_I (r_s=-0.36, P=0.001) but not with ΔS_I or Δ of other metabolic indices.
Conclusions: At presentation of T2D in Caucasians, insulin sensitivity and insulin secretion are maximally reduced. On the population level, insulin sensitivity and postprandial β–cell responsiveness fail to improve over 2yrs with conventional treatment. Individual amelioration/deterioration of glucose control is associated with fasting and to a lesser degree postprandial β–cell responsiveness but not insulin sensitivity or disposition index suggesting the importance of targeting fasting and postprandial β–cell responsiveness as the first line naïve treatment of T2D.

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Screening methods for diabetes in United Kingdom primary care.
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Background and Aims: Although there is no formal national screening programme for diabetes in the UK, primary care undertakes new patient health-checks (NPHC) that can detect asymptomatic disease using dipstick urinalysis and the UK government has recently introduced a National Service Framework (NSF) for ischaemic heart disease (IHD) which includes annual (non-fasting) blood glucose testing (BGT). This study compares the detection rate between the different methods and population groups screened in a large primary care practice (PCP) of over 17,500 patients.
Materials and Methods: Clinical data obtained by either the practice nurse or doctor during the course of the screening consultation was entered onto the patient's computerised records. Computer programme enquiry reports were written to generate patient demographic details and test results from the PCP database for further statistical analysis using Epi Info 2002.
Results: In a 12 month period 642 newly registered non-diabetic adult patients (mean age 40.03 years, SD +/- 17.9) of a PCP were screened during a NPHC. One (0.2%) was found with previously undiagnosed diabetes. In the pre-registered PCP population, 342 non-diabetics (mean age 54.6 years, SD +/- 18.7) who were transferred onto a new general practitioner's list were entitled to a NPHC and 9 (2.6%) new cases were found. BGT detected 27 (4.3%) new cases of diabetes in 628 patients with IHD (mean age 68.2 years, SD +/- 8.52). In the IHD population 15.8% (173/1096) have pre-existing diabetes.
Conclusion: BGT had the best detection but was in the group at most risk of developing diabetes due to age and IHD. There were significant differences between the mean ages of all groups. Although BGT may be appropriate in high-risk groups who are having blood testing for other reasons, this would be less acceptable to the general population. NPHC using dipstick testing for glycosuria would detect a majority of undiagnosed diabetes with a better sensitivity rate if restricted to an older population.
The UK government is proposing to change the contract of primary health care service provision that may end NPHC's. These are an inexpensive and useful resource in detecting diabetes in populations before macrovascular complications have developed. The UK National Screening Committee (NSC) has been asked to examine the effectiveness of diabetes screening and to submit any proposals by 2005 that could be incorporated into the NSF for diabetes. Screening has the potential to save lives or improve quality of life through early diagnosis so enabling preventative therapy and the treatment of complications. These results suggest that is imperative for the NSC to make urgent recommendations to continue some form of screening programme for populations most at risk in the intervening years, when opportunistic/case-finding screening would be the least preferred and unfortunately the only available option.

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Increased levels of triglycerides, body mass index and blood pressure and low physical activity increase the risk for diabetes in Swedish women. A prospective 18-year follow-up study.
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Background and Aims: To investigate risk factors for the development of diabetes in middle-aged women.
Materials and Methods: A random population sample of 1351 women without prior diabetes or cardiovascular disease, aged 39-65 years, took part in a screening study in 1979 to 1981 with questionnaires, physical examination and blood sampling. Development of diabetes until 1998 was identified at a second examination in 1997-1998.
Results: 73 women (5,4%) were diagnosed with diabetes during follow-up. As expected, obesity carried a steeply increasing age adjusted risk with HR 3.2 (95%CI 1.3-8.1) already at body mass index (BMI) 24-27, and 8.3 (3.5-19.7) at BMI ≥ 27, compared to BMI < 22 kg/m2. Increasing systolic blood pressure (SBP) 130-144, 145-159 and ≥ 160 mmHg respectively, escalated the HR of diabetes to 1.6 (0.8-3.3), 3.6 (1.7-7.4) and 5.6 (2.7-11.4) compared to SBP < 130 mmHg. Also, low physical activity predicted diabetes, HR 2.1 (1.3-3.3) for sedentary compared to non-sedentary activity. The strongest predictor, however, was s-triglycerides with HR 4.0 (2.1-7.6), 7.1 (3.6-14.0) and 9.3 (4.3-20.2) in women with s-triglycerides (TG) 1.0-1.4, 1.5-1.9 and ≥ 2.0 mmol/l respectively, compared to women with s-TG < 1,0 mmol/l at first examination. Smoking was not associated with increased risk of diabetes. After adjustment for BMI, SBP and physical activity, increasing TG level remained a strong and significant risk factor for diabetes [HR 3.0 (1.6-5.7), 3.7 (1.8-7.7) and 4.5 (2.0-10.0), p<0,001].
Conclusion: : Even slightly elevated s-TG result in a considerably enhanced risk of diabetes in middle-aged Swedish women, independently of age, BMI, blood pressure and physical activity.

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A model to predict abnormal glucose tolerance.

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Background and Aims: The progression of IGT to Type 2 diabetes mellitus can be prevented. The aim of the study was to outline a cheap, non-invasive and easy method to identify the subjects at high risk for abnormal glucose tolerance (AGT) and who should be scheduled for oral glucose tolerance test (OGTT).
Materials and Methods: 1097 out of the 1508 born in 1933-1956 and living in the Finnish municipality of Savitaipale participated in a clinical cross-sectional population-based survey. Self-administered questionnaire (smoking habits, long-term medication, and family history of DM), anthropometric measurements (BMI, WHR, BP) and laboratory tests, including 75-g oral glucose tolerance test and lipids, were made.
Age, systolic(sBP) and diastolic(dBP) blood pressure, BMI, WHR, waist circumference (waist), triglycerides (TGL) and HDL cholesterol (HDL) were divided into quintiles. All associations between the known risk factors for diabetes and AGT were analysed by cross-tabulations. The data were compiled into four models using single and multiple logistic regression analyses with AGT as the outcome of interest. Model 1 included age, waist, sBP and antihypertensive medication(HTM). Model 2 further included smoking habits and model 3 the family history of diabetes. HDL, TGL and antilipid medication were included in model 4. The logistic equations relating the predictors of AGT were used to calculate the probability that a subject would have AGT. Receiver operating characteristic (ROC) curves were drawn for sensitivity against 1-specificity. The areas under the ROC curves (AUC) were compared by deLong's non-parametric test.
Results: The statistically significant crude ORs to predict AGT in men were age ≥ 45.5 years, BMI ≥ 29.1, waist ≥ 103 cm, WHR ≥ 0.991, sBP ≥ 146.5 mmHg and the use of HTM. In women, the corresponding values were age ≥ 54.9 years, BMI ≥ 22.4, waist ≥ 76 cm, WHR ≥ 0.811, sBP ≥ 134.5 mmHg, dBP ≥ 83.5 mmHg, use of HTM and TGL ≥ 1.189 mmol/l. The AUC of model 1 compared to model 4 was 0.695 vs 0.728 (p=0.036) in men and 0.769 vs 0.782 (p=0.126) in women. The AUCs of model 2 in men was 0.705 (p=0.098; vs model 4) . The quintiles were scored from the lowest to highest by using coefficient B in model 1(table) and combined. The AUC for this model was 0.692 in men (p=0.36 vs model 1, p=0.03 vs model 4) and 0.764 in women (p=0.44 vs model 1, p=0.07 vs model 4). 52% of women had scores ≥ 20 (sensitivity 82%, specificity 57%), 23% had ≥ 29 (se 44%, sp 84%). 52% of men had scores ≥ 13 (se 71%, sp 60%), 20% had ≥ 20 (se 36%, sp 85%).
Conclusion: Model 1 including age, waist, BP and HTM and the scored model are good ways to predict AGT in both sexes. No blood samples are needed.
Cut points and scores

		Q1 / scores	Q2 / scores	Q3 / score	Q4 / scores	Q5 / scores
Men	Age	41,3-45,4 / scores 0	45,5-48,5 / scores 7	48,6-54,5 / scores 7	54,6-60,7/ scores 7	60,8-66,1 / scores 11
	Waist	68-86 / scores 0	87-91 / scores 0	92-95 / scores 3	scores 3	96-102 / 103-155 / scores 10
	sBP	98-118,5 / scores 0	119-126 / scores 0	126,5-135 / scores 3	135,5-146 / scores 3	146,5-207,5 / scores 9
	HTM					scores 3
Women	Age	41,0-45,0 / scores 0	45,1-49,0 / scores 0	49,1-54,8 / scores 3	54,9-59,8 / scores 4	59,9-66,3 / scores 5
	Waist	58-75 / scores 0	76-81 / scores 8	82-86 / scores 12	87-94 / scores 17	95-134 / scores 22
	sBP	90-115 / scores 0	115,5-123 / scores 0	123,5-134 / scores 5	134,5-148 / scores 7	148,5-224,5 / scores 15
	HTM					scores -2

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Confirmatory thresholds of the glucose parameters and the effect of their application in a screening programme. ADDITION, Denmark.

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Background and Aims: Both WHO and ADA require confirmatory blood glucose (BG) testing on separate days to establish the clinical diagnose Diabetes Mellitus in screening programmes. It is likely that some people with a diabetic BG value have measures of BG so abnormal that the level of the measure itself predicts confirmed diabetes. The aims are therefore to identify the levels of such confirmatory thresholds and to analyse the consequences of application of confirmatory thresholds in a screening programme.
Materials and Methods: 432 people with a diabetic BG value were identified in the screening procedures of the ADDITION study, Denmark. The screening programme was stepwise: 40-69 year old people from 121 general practises were contacted with a risk chart. The high-risk responders underwent screening with random blood glucose (RBG) and HbA1c. People with RBG>5.4 mmol/l or HbA1c>6.0% underwent fasting blood glucose (FBG) and subsequently OGTT if FBG>5.5 mmol/l or HbA1c>6.0%. If RBG, FBG or BG 2 hours after OGTT (2hBG) were diabetic, a confirmatory test was performed on another day. Confirmatory thresholds were identified as the exact level of the glucose parameter, above which all individuals had confirmed diabetes (specificity=100%). The number of tests that could have been saved if the thresholds were applied were calculated. BG values were measured with HemoCue analysers in capillary blood samples.
Results: 304 fasting tests and 128 OGTT's were performed to identify 346 people with confirmed diabetes.

First Diabetic Value	n	Conf. DM	Conf. threshold	Sens.	Saved tests. Single. Fast.	Saved tests. Stepwise. OGTT	Saved Tests. Fast.	Saved Tests. OGTT
RBG	74	68	RBG > 12.0	82%	55	1	55	1
			HBA1C > 6.5	90%	60	1	+7	+1
FBG	305	244	FBG > 7.2	28%	68	1	68	1
			RBG > 10.0	10%	24	1	+7	+1
			HBA1C > 7.3	11%	27		+4	+0
2hBG	53	34	2hBG > 13.9	21%	1	6	1	6
			RBG > 10.6	6%	0	2	+0	+2
			HBA1C > 6.7	2%	2	0	+1	+0
			FBG*	-	-	-	-	-
ALL	432	346	HbA1c > 7.3	24%	82	0	-	-

* No individuals had FBG < 6.1 mmol/l

Application of a confirmatory threshold to each of the diagnostic tests could lead to 132 (31%) saved confirmatory tests (124 fasting and 8 OGTT's). By extracting all the information available in a stepwise model involving all the thresholds identified, the total number of tests that could be saved was 155 (36%) (143 fasting and 12 OGTT's) (overall sensitivity 45%).
Conclusions: The levels of the confirmatory thresholds and their corresponding sensitivities were dependent of the test leading to the first diabetic BG value.
Up to 45% of the people with confirmed diabetes could have been identified by application of confirmatory thresholds and 36% of all confirmatory tests in The ADDITION Study, Denmark could have been avoided (47% of the fasting tests and 9% of the OGTT's).
HbA1c contributed with very little additional information about confirmatory status after extraction of confirmatory information from the diagnostic tests.

PS 17
Beta Cell Function (I)

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Role of insulin and insulin-like growth factor-1 receptors in glucose-regulated preproinsulin gene expression probed by RNA interference and single cell promoter analysis.
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Background and Aims: Regulation of the preproinsulin gene by glucose has recently been shown to involve the release of insulin, and a consequent action of the hormone in *trans*. Here, we explore the relative contributions of the insulin and IGF-1 receptors, and of phosphatidyl inositol 3'kinase activity, in mediating the effects of glucose and insulin in MIN6 β-cells.
Materials and Methods: Cells were incubated for 48 h with 21-mer small interfering RNAs (siRNA) and the effects on insulin and IGF-1 receptor expression quantitated by western (immunoblot) analysis. Preproinsulin (PPI) promoter activity was measured by intranuclear microinjection of a firefly luciferase reporter construct encoding a 250 nucleotide fragment upstream of the human PPI promoter, and normalised to cytomegalovirus promoter activity driving *R.reniformis* luciferase expression.
Phosphatidylinositol-3,4,5-*tris*phosphate (PIP_3) accumulation was monitored by confocal imaging of the translocation to the plasma membrane of a general receptor of phosphoinositides-1 green fluorescent protein chimaera (Grp-1-EGFP).
Results: Normalised PPI promoter activity was increased 2.32 ± 0.43 fold (n = 6) by 6 h incubation at 30 vs 3 mM glucose, and PIP_3 levels increased by 84.6 ± 0.44 %. Both effects were mimicked by exogenous insulin (≥ 1 nM). Treatment with siRNA diminished insulin receptor protein levels by > 85 %, completely eliminated the effects of added insulin (1 – 20 nM), and reduced PPI promoter activity at both 3 and 30 mM glucose. By contrast, exogenously added IGF-1 (10 nM) was without effect on PPI promoter activity at either glucose concentration, and the silencing of IGF-1 receptor expression had no impact on the effects of glucose or insulin on PPI promoter activity.
Conclusion: Elevated glucose concentrations stimulate preproinsulin gene transcription in part through the activation of insulin, but not IGF-1 receptors, and the consequent stimulation of PIP_3 production. However, since exogenous IGF-1 failed to stimulate PPI promoter activity, these data imply that other, PIP_3-independent signalling pathways, are also involved in mediating the effects of the sugar.

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A dual action of insulin on its own secretion.
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Background and Aims: The autocrine effect of secreted insulin on β-cell function has for long been a matter of controversy. Even very recent studies disagree as to whether insulin inhibits or stimulates its own release. The aim of the present study was to study the effect of a wide range of insulin concentrations on β-cell function with special regard to the regulatory roles of phosphatidylinositol 3-kinase (PI3-kinase), isoforms of nitric oxide synthase (NOS) and cyclic AMP (cAMP) in the mechanisms of action of insulin
Materials and Methods: Isolated mouse islets were incubated for 90 min at a slightly stimulating level (8.3mM) with and without a wide range of insulin concentrations and different modulators of β-cell secretory activity. Insulin secretion was measured as C-peptide release (RIA) and islet NOS activities with a sensitive HPLC method. Islet NOS proteins were analysed by confocal microscopy and Western blots.
Results: A dose-response study showed that a low concentration of insulin (100pM) stimulated C-peptide release, whereas high concentrations (250-1000nM) were inhibitory. The inhibitory action of insulin was counteracted by the NOS inhibitor N^G –nitro-L-arginine dimethyl ester. The PI3- kinase inhibitor wortmannin (100nM) completely abolished the increase in C-peptide release stimulated by 100 pM insulin ($p<0.001$). This concentration of insulin did not not influence the activities of islet constitutive NOS (cNOS) or inducible NOS (iNOS). In contrast, in the presence of 250 nM insulin, C-peptide release was modestly inhibited from 395.6 ± 14.68 pg/islet/h (control) to 295.6 ± 28.41 ($p<0.01$), and cNOS activity was increased from 17.62 ± 1.61 pmol NO /mg protein/min to 24.94 ± 2.21 ($p<0.01$). Moreover, this high concentration of insulin induced the expression of iNOS protein as revealed by Western blot as well as by confocal microscopy and was associated with a high iNOS activity (13.98 ± 0.56 pmol NO /mg protein/min). Wortmannin abolished iNOS expression, did not influence the increased cNOS activity, but reversed the inhibitory effect of 250 nM insulin on C-peptide release. Furthermore, addition of the cAMP stimulating hormone GLP-1 (100nM) as well as cAMP (2mM dibuturyl-cAMP) itself in the presence of 250 nM insulin abolished the expression and activity of iNOS, suppressed cNOS activity to control levels, and greatly stimulated C-peptide release from 454.1 ± 22.58 to 816.1 ± 80.57 pg/islet/h ($p<0.01$) and to 944.0 ± 71.21 ($p<0.001$)
Conclusion: Our data suggest that insulin has a dual action on its own secretion, being stimulatory at low concentrations and inhibitory at high concentrations. The stimulatory effect is exerted through the PI-3 kinase pathway, whereas the inhibitory effect might be exerted through the NO pathway, involving a great increase in cNOS activity and, possibly elicited through PI3-kinase, a marked expression and activity of iNOS. This increase in islet NO production could be counteracted by raising the islet cAMP levels.

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Autocrine regulation of glucose metabolism in pancreatic islets.
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Background and Aims: It has been recently demonstrated that insulin stimulates in the islets the transcription of glucokinase and insulin genes, Ca^{2+} flux from endoplasmic reticulum, and exocytosis of insulin. In order to explore further the possible autocrine modulatory effect of insulin upon islet functions, we have studied its effect upon glucose metabolism in islets isolated from pancreases of normal hamsters.
Materials and Methods: We measured the production of $^{14}CO_2$ and ^3H_2O from D-[U-^{14}C]-glucose and D-[5-^{3}H]-glucose, respectively, in isolated islets (collagenase digestion) incubated with: a) 3.3 mM glucose alone, or plus addition of 5mU/ml insulin; b) 16.7 mM glucose alone, or with the alternative addition of 15 mU/ml insulin, insulin antibody (1:500), normal guinea-pig serum (1:500), 150 nM wortmannin, or 10 μM nifedipine. Insulin release was also measured (radioimmunoassay) in islets incubated with 3.3 or 16.7 mM glucose, with or without the addition of wortmannin (75, 150 and 300 nM).
Results: Insulin enhanced significantly $^{14}CO_2$ and ^3H_2O production with 3.3 mM ($p<0.001$), but not with 16.7 mM glucose. Addition of insulin antibody to the incubation media with 16.7 mM glucose decreased significantly both $^{14}CO_2$ and ^3H_2O production ($p<0.02$). A similar decrease was obtained when the islets were incubated with 16.7 mM glucose and wortmannin ($p<0.01$) or nifedipine ($p<0.01$). This latter effect was reverted by addition of 15mU/ml insulin to the incubation media ($p<0.01$). Addition of wortmannin decreased significantly the amount of insulin released in response to 16.7 mM glucose in a dose-dependent manner ($p<0.001$). Wortmannin induced a comparable decrease of glucose metabolism and insulin secretion.
Conclusion: Our results suggest that insulin exerts a physiological autocrine stimulatory effect upon glucose metabolism (oxidation and utilization) in intact islets, as well as on glucose-induced insulin release.

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Preliminary study of insulin like growth factor –1 gene therapy for streptozotocin-induced diabetic rats.
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Background and Aims: To investigate the effect of insulin like growth factor -1 (IGF-1) gene therapy on diabetic rats.
Materials and Methods: A cDNA fragment encoding mature IGF-1 molecule was amplified by PCR and identified by DNA sequence analysis. Recombinant transfer plasmid pCMV/IGF-1 was constructed by insertion of IGF-1 DNA into pCMV at its mutiple cloning sites. Diabetic rat models were prepared by introducing Streptozotocin. The treatment group(T group, n=7) was injected with 400μg pCMV/IGF-1 DNA each rat through intramuscular pathway. Control group(C group, n=7) was treated with similar amount of wild type pCMV DNA respectively. Normal nondiabetic group (N group, n=7) and DM group without either pCMV/IGF-1 or wild type pCMV (DM group, n=7) were also observed at the same time. Blood glucose level was measured routinely, and serum IGF-1 was determined by ELISA. Semi-quantitative RT-PCR was used to examine the variation of IGF-1 mRNA expression in livers, kidneys, and skeletal muscles. Tissue sections of livers, kidneys, heart and skeletal muscles were also assayed by

immunohistochemistry and observed by staining with haematoxylin and eosin for morphological studies.
Results: 1.It was found that when IGF-1 gene was introduced, the blood glucose level in T group was decreased from 29.16±0.74 mmol/L to 17.00±0.55 mmol/L. The blood sugar started to be reduced on the 5th day of pCMV/IGF-1 post injection. Optimal glycemic control (10.42±0.23mmol/L) could be seen on the 10th day of IGF-1 gene therapy, and this phenomenon was maintained for 14 days. On the 20th day of treatment, the glucose level raised again. At the end of the 4th week, the blood glucose went back to pretreatment level. 2.Accompanying with the glucose variation, serum concentration of IGF-1 in T group was significantly higher under the condition of pCMV/IGF-1 therapy versus basal (125.04±3.43 ng/ml vs 109.05±3.58 ng/ml). The increased serum IGF-1 could be shown from the first week of IGF-1 gene injection, and the highest level of IGF-1 was detected in the following 2 to 3 weeks with the concentration of 132.35±2.15 ng/ml. At the end of the 4th week, serum IGF-1 dropped down (118.25±5.25 ng/ml). 3.Statistic analysis showed that after IGF-1 gene therapy, the blood glucose in the treated rats was significantly lower than that of DM and C groups($P<0.05$), while serum IGF-1 was higher compared to DM and C group rats($P<0.05$). 4.Based on semi-quantitative RT-PCR assay, it was found that IGF-1 mRNA expression in livers and muscles of T group was higher than that of DM group. But IGF-1 gene in renal tissues had no difference between the two groups. 5.Immunohistochemical studies showed that in T group, a large amount of IGF-1 protein exited on the livers, heart and skeletal muscles except kidneys.
Conclusion: Our results suggested that IGF-1 gene therapy could influence the blood glucose of diabetic rats in an efficient way. The study lends support to the view that somatic gene therapy offers a potential approach to the glycemic control in diabetic mellitus.

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Induction of growth factors and transcription factors by streptozotocin treatment in pancreata of neonatal rats.
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Background and Aims: Treatment with streptozotocin (STZ) in neonatal rats triggers pancreatic regeneration. The mechanisms of events involved is this process are still unknown. Our aim was to model pancreatic β cell neogenesis *in vivo* by partial deletion of β cells in newborn rats and examine the temporal sequence of transcription factors involved in the differentiation process. Our previous results showed that protein and mRNA of Pdx-1, a homeodomain transcription factor largely restricted to β and α cells in newborn rats, was reduced in the STZ rats at day 2 ($p<0.05$) and increased at day 4 ($p<0.01$). Ngn-3, a marker of early precursors of the four pancreatic endocrine cells, was present until day 4 and remained at very low levels in sham animals. STZ treated animals had a slight increase by day 8. Although it is known that Ngn-3 activates Beta-2/Neuro D (involved in islet cell development and insulin gene transcription) during early stages of development, a significant decrease in mRNA was found in the STZ rats at day 4 and 8 ($p<0.05$).
Materials and Methods: Wistar rats (4 days old) were given a dose of STZ (70 mg/kg ip.) resulting in the destruction of 40-50 % of β cells within 72 hours. Rats were sacrificed at 1, 2, 4, 8, 12, 16, 20 and 40 days after STZ or citrate buffer treatment. Pancreata were dissected and fixed for immunohistochemistry (ICC) and examined for Isl-1. To study the expression of Pax-4 and FGF-1 and 7, total RNA was extracted from tissues and subjected to Northern blot analysis. Total RNA (20 µg) was loaded and 18S rRNA used to normalize loading and transfer. Statistical analyses were performed using two-way ANOVA, and Fisher's post-test.
Results: Isl-1, required for differentiation of all islet cells, showed a significant increase at day 16 in the sham group ($p<0.05$). In STZ rats, Isl-1 was significantly reduced by this time compared to sham in large and medium islets ($p<0.05$). Pax-4, which is involved in the differentiation of β and δ cells, was present in three isoforms. The 1.35 kb isoform was significantly reduced at day 2 and 16 and augmented at day 18 ($p<0.05$) in the STZ group. The 0.9 kb isoform showed a significant peak at day 14 in both groups ($p<0.05$), while the STZ group was significantly reduced at day 16 ($p<0.05$). The 0.7 kb isoform was significantly reduced at day 16 ($p<0.05$) in the STZ group. FGF signalling is required not only in pancreatic development, but also to control β cell function and maintenance of normal blood sugar levels. FGF-1 mRNA was present in two isoforms. The 4.4 kb isoform was significantly decreased at day 4 and increased at day 14 in the STZ treated rats comparing to the sham ($p<0.05$); while the 2.5 kb isoform was diminished at day 1 and 8 ($p<0.05$). FGF-7 was present in two isoforms, and no changes were found between treatments.
Conclusion: An orderly cascade of signals from these transcription factors induced β cell formation to replace the β cell loss caused by the STZ treatment mimicking pancreatic embryogenesis. The islet regeneration seen following β cell destruction by streptozotocin involves increased numbers of α, β, and δ cells, suggesting that changes in transcription factor expression are acting within islet cell precursors.

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Angiotensin II receptor expression and function in human islets of Langerhans.
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Background and Aims: Evidence exists for the presence of a local renin-angiotensin system (RAS) in the rodent, canine and human pancreas, but its physiological role has not been established. We have now investigated whether pancreatic β-cells express angiotensin II (Ang II) type 1 receptors (AT_1R) and whether Ang II influences β-cell function.
Methods: Expression of AT_1R mRNA and protein were detected by RT-PCR and Western blotting, respectively. Changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) were determined by microfluorimetry of fura-2 loaded cells, and insulin secretion was measured by radioimmunoassay.
Results: RT-PCR, using AT_1R-specific primers, amplified single products of 146 bp from rat and mouse islet cDNA, and 174 bp from human islet cDNA. AT_1R cDNA was also amplified from a pure insulin-secreting β-cell line (MIN6). An anti-AT_1R antibody recognised the 42.5kDa AT_1R protein in rodent and human islets and in MIN6 cells. Ca^{2+} microfluorimetry studies indicated that the Ang II analogue, Hypertensin (HT; 1-100nM), evoked a dose-related increase in $[Ca^{2+}]_i$ in human islets and MIN6 cells (islets: 50% of tolbutamide-sensitive cells were responsive to 1nM; 67% to 10nM; 100% to 100nM; MIN6: 22% of cells were responsive to 1nM HT; 44% to 10nM; 50% to 100nM). The stimulatory effects of HT on $[Ca^{2+}]_i$ were not affected by removal of extracellular Ca^{2+}, suggesting that HT-induced increases in $[Ca^{2+}]_i$ are dependent on calcium mobilisation from an intracellular store. The effects of HT on insulin secretion were examined in perifusion experiments. HT (100nM) caused a transient increase (6-8min) in basal (2mM glucose) insulin secretion from MIN6 pseudoislets and human islets (364.6±69.2% basal; 260.4±37% basal respectively, mean±SEM, n=4, p<0.05). HT also augmented glucose-induced insulin secretion transiently (6-8min) in pseudoislets and human islets (MIN6: 8mM glucose: 270.4±19.4% basal; +100nM HT: 931.4±227.7%, human islets: 8mM glucose: 284.2±25% basal; +100nM HT: 450±74%, mean±SEM, n=4, p<0.05).
Conclusion: These data indicate for the first time that rodent and human islets and pure β-cell populations (MIN6) express functional AT_1 receptors whose activation results in increased insulin secretion, most likely by increasing $[Ca^{2+}]_i$.

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Nicotine inhibited beta-cell function in rat and human pancreatic islets.
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Background and Aims: Epidemiological studies indicate that nicotine (smoking and snuffing) is a risk factor for type 2 diabetes. We hypothesized that nicotine exerts negative effects on beta cell function.
Materials and Methods: Isolated rat and human pancreatic islets were exposed to nicotine acutely or during 48 h culture conditions. Insulin secretion was tested during 60 min incubations at basal (3,3) and stimulatory (27 mmol/l) concentrations of glucose. The presence of nicotinic receptors was probed with ^{3}H-nicotine.
Results: Acute exposure to nicotine inhibited insulin release at basal glucose in rat islets by 41 % at 10^{-5} mol/l of the alkaloid. In human islets, nicotine inhibited the insulin response to glucose by 29 % at 10^{-6} mol/l and by 54 % at 10^{-4} mol/l of the alkaloid. Co-culture with nicotine at 10^{-6} mol/l and 10^{-4} mol/l for 48 h inhibited the insulin response to glucose both in rat and in human islets. Effects of nicotine were reversed by an overnight wash-out period. Likewise, co-culture of nicotine for 48 h with 10^{-10} mol/l alpha-bungarotoxin, a competitive nicotinic receptor antagonist, was reversed inhibitory effects of nicotine. High-affinity binding of ^{3}H-nicotine

in rat islets was observed in 10 min incubation. Such binding was abolished by prevoius exposure to alpha-bungarotoxin.
Conclusion: Both acute and longer term exposure to nicotine reversibily inhibits insulin secretion in rat and human beta-cells. Such effects are, in part at least, mediated by interation with intra-islet nicotinic receptors. Influence of nicotine on beta cell function could play a role for the nicotine-associated risk of type 2 diabetes.

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Transgenic insulin production in the gastric G cells: a model for gene therapy of diabetes.
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Background and Aims: Diabetes mellitus, a chronic group of disorders characterized by hyperglycemia, remains a major cause of premature disability and mortality in the world. As a first step in devising gene therapy strategies for treatment of diabetes we have chosen to utilize the gastrin-secreting G cells to produce secretagogue-responsive insulin secreting cells. The objective of our research is to engineer a transgenic animal model in which insulin secretion in response to food intake will ensure tight control of blood glucose levels. Our central hypothesis is that human insulin, produced by G cells under the regulation of the gastrin gene promoter, would be secreted in response to meal-associated stimuli. Specifically, insulin produced by and stored in the gastric G cells of G-InsKI mice is envisaged to be released in response to either hormonal regulators, including bombesin/GRP, or by luminal regulators, including aromatic amino acids, Ca^{2+} and tastants.
Materials and Methods: We have generated transgenic knock-in mice G-InsKi, in which the coding sequence of human insulin has been knocked into the mouse gastrin gene. The targeting construct G-InsKi was engineered to knock-in human insulin cDNA into the mouse gastrin gene. The targeting construct was electroporated into the germline-competent embryonic stem cells, and the clones that have undergone homologous recombination were identified by Southern blot analysis. Transgenic mice were generated with the use of ES cells positive for the presence of the modified InsKi allele. InsKi mice were maintained as heterozygotes for the presence of the modified allele to avoid creation of the gastrin-null phenotype.
Results: Insulin immunoreactivity was localized to endocrine cells of the antrum and pylorus/duodenum in knock-in mice, but not in control, wild type mice, whereas insulin immunostaining was observed in the pancreas of both transgenic and non-transgenic animals. Insulin cells in the antrum and pylorus/duodenum were predominantly at the base of the mucosa. Double labeling immunofluorescence showed that insulin immunoreactivity in the antrum and duodenum was localized to G cells, as visualized with mouse monoclonal gastrin antibodies. The Ins-Ki mice had a normal reproduction life and were healthy. The fasting level of blood glucose in G-Ins-Ki mice (105 ± 7.3) was significantly ($P<0.05$) lower than that of nontransgenic littermates (146 ± 17.1). We are currently exploring whether Ins-Ki mice have different susceptibility to the STZ induced diabetes as compared to the nontransgenic littermates.
Conclusion: We have developed an animal model, which allows assessing ability of the G cell-produced insulin to alleviate development of diabetes.

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The Tet-On system in transgenic mice: tissue-specific and doxycycline-inducible inhibition of the retinoid X receptor in pancreatic β cells.
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Background and Aims: To elucidate the function of retinoid X receptor (RXR) in the pancreatic β cell of adult animals, we generated double transgenic mice with tissue-specific and doxycycline-inducible expression of a dominant negative form of RXRβ (RXRβΔC2) in the pancreatic β cell using the Tet-On system.
Materials and Methods: The Tet-On system provides a powerful tool to analyze eukaryotic gene expression and function in transgenic mice. Because the system requires two transcriptional units for transactivator (rtTA) and for the target gene (Tet-O promoter), we firstly generated two lines of transgenic mice. In the Ins-rtTA transgenic mice, the expression of rtTA is restricted to their pancreatic β cells under control of the human insulin promoter. In Tet-O-RXRβΔC2 mice, RXRβΔC2 expression is regulated by the Tet-O promoter. The RXRβΔC2 that lacks 20 conserved C-terminal amino acids of ligand dependent transactivation domain is known to suppress the function of other nuclear receptors that dimerize with RXRs. Secondary, two lines of the transgenic mice were crossed and Ins-rtTA/Tet-O-RXRβΔC2 double transgenic mice were obtained. To test the effect of RXRβΔC2 in their pancreatic β cells, the double transgenic mice were treated with or without doxycycline and subjected to the analyses including intraperitoneal glucose tolerance test.
Results: The Tet-On system in this transgenic approach permitted a tissue-specific and doxycycline-inducible control of RXRβΔC2 expression in pancreatic β cells. In the double transgenic mice, overexpression of RXRβΔC2 in the pancreatic islets reduced the blood glucose level and increased the glucose responsive insulin secretion on the intraperitoneal glucose tolerance tests. The isolated islets from double transgenic mice treated with doxycycline showed significantly higher level of the glucose responsive insulin secretion. The quantitative RT-PCR analyses also showed higher activation of Glut2, glucokinase and UCP-2 genes in those isolated islets than in the islets without overexpression of RXRβΔC2.
Conclusion: The Tet-On system, which provides a tissue-specific and inducible expression of transgene in adult animals, is suitable to investigate the function of the eukaryotic genes that has not been well known in vivo. Our results suggest that the endogenous RXR heterodimers play inhibitory roles in the glucose responsive insulin secretion through direct or indirect suppression of the islet-specific genes, such as Glut2 and glucokinase.

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Beta Cell Function (II)

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Overexpression of the malate-aspartate shuttle member Aralar1 increases mitochondrial activation and insulin secretion in INS-1E beta cells.

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Background and Aims: The NADH shuttle system transports reducing equivalents from the cytosol to the mitochondrial respiratory chain and is essential for the coupling of glucose-metabolism to insulin secretion in pancreatic beta cells. The malate-aspartate shuttle, one of the NADH shuttles, is composed of the mitochondrial aspartate/glutamate carrier (AGC). Aralar1 and citrin are two isoforms of the AGC subfamily, but we show here that only Aralar1 is enriched in the beta-cell line INS-1E.
Materials and Methods: In the present study, we have examined the effects of Aralar1 overexpression in INS-1E cells. We prepared a recombinant adenovirus containing cDNA for human Aralar1, tagged in C-terminal with the small FLAG epitope. INS-1E cells were transduced with the AdCA-Aralar virus before measurements of mitochondrial membrane potential (rhodamine-123 fluorescence), 14C-glucose oxidation, cytosolic ATP levels in living cells expressing luciferase, and insulin secretion (radioimmunoassay).
Results: INS-1E cells transduced with AdCA-Aralar virus exhibited increased levels of Aralar1 protein revealed by immunoblotting. Immunolocalization studies showed that the overexpressed protein colocalized with the mitochondrial dye Mitotracker. Transduction of INS-1E cells with AdCA-Aralar caused a dose-dependent elevation of glucose-stimulated: (i) mitochondrial membrane hyperpolarization (ii) glucose oxidation (+33 %, $p<0.05$) (iii) and insulin secretion (+38 %, $p<0.01$). These effects were observed at stimulatory glucose concentrations (15 mM), but neither at basal nor at intermediate glucose or when the mitochondrial substrate pyruvate was used as a secretagogue. Glucose-induced ATP elevations were not different in cells transduced with AdCA-Aralar compared to controls.
Conclusions: The results indicate that Aralar1, a member of the malate-aspartate shuttle, can be rate limiting in glucose-induced insulin secretion. This study also indicates that the effects of Aralar1 overexpression on the secretory response involve the participation of factors other than ATP generated by the mitochondria.

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Cell-specific expression of the c-Jun NH2 terminal kinase Interacting Protein 1 (JIP-1/IB1) requires the silencing of Sp1 by the transcriptional repressor REST.

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Background and Aims: The *MAPK8IP1* gene encodes the human Islet-Brain 1 (IB1) protein or the murine c-jun (JIP-1) protein. The IB1/JIP-1 role in pancreatic β-cells has been shown. Indeed, a mutation (S59N) in the coding region was found to be associated with a rare monogenic form of type 2 diabetes. The restricted neuronal and β-cells expression of IB1/JIP-1 is controlled by REST, a transcriptional repressor expressed in most of tissues but not in neuronal and insulin-secreting cells. By silencing expression of IB1 in non-neuronal and non-β cells, REST is involved in the cell-specific expression of the *MAPK8IP1* gene. The goal of this study is to examine the molecular mechanism by which REST mediates the specific expression of IB1 in β-cells.
Materials and Methods: Binding activities of Sp1 and REST were analysed by electrophoretic mobility shift assay (EMSA). To measure the functional effect of sp1 and neuronal restriction silencer element (NRSE) elements, they were either each or both mutated within the IB1 promoter linked to a luciferase gene reporter. These constructs were transiently transfected in HeLa (REST-expressing cells) and in the mouse insulin-secreting βTC3 cells. To assess the influence of REST on Sp1 activity, REST was inactivated by using the REST dominant negative or trichostatin (TSA). The luciferase activity measurement was performed 48 h after transfection. Lastly, immunoprecipitation assay was performed to determine a potential interaction between REST and Sp1
Results: We identify on the IB1 promoter a conserved sp1-binding site located at 6 bp of the NRSE. This sp1-binding site is interacting with Sp1 factor as detected by EMSA. We hypothesize therefore that a functional and structural interaction is occurring between REST and Sp1. As expected, immunoprecipitation experiments detect a protein-protein interaction between Sp1 and REST. We next test the functional importance of this sp1 binding site in βTC3 cells. The high IB1 promoter activity drastically decreases when the sp1 element is mutated, indicating that this element is critical for the IB1 expression. In HeLa cells, the IB1 promoter activity is undetectable, and this activity is relieved upon REST is inactivated by using a REST dominant negative or TSA. When REST is inactivated, we show an increase of IB1 promoter activity, which is lost when sp1 element is mutated. These results indicate that the specific regulation of IB1 promoter by Sp1 is dependent to the REST activity.
Conclusion: In this study, we show that the cell-specific expression of IB1 mediated by REST requires the blocking of Sp1.

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PFK-2/FBPase-2 is an activator of glucokinase activity in insulin-producing cells.

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Background and Aims: The glucose phosphorylating enzyme glucokinase (GK) couples changes in the millimolar glucose concentration range to glucose metabolism in pancreatic beta cells. We could recently identify the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2) as new binding partner of GK. Pancreatic islets express the brain type PFK-2/FBPase-2 isoform in contrast to the cAMP-dependent liver isoform. It was the aim of this study to elucidate whether the interaction of GK with PFK-2/FBPase-2 affects the activity status of GK and concomitantly glucose metabolism in insulin-producing cells
Materials and Methods: Liver and islet isoforms of PFK-2/FBPase-2 were stably overexpressed in insulin-producing RINm5F-GK cells. PFK-2/FBPase-2 expression levels were verified by Northern and Western blot analyses. GK activities were measured by an enzyme-coupled photometric assay after pre-incubation with 2 or 10 mM glucose, 10 μM forskolin or anti-FBPase-2 antibody.
Results: PFK-2/FBPase-2 overexpression in RINm5F-GK cells resulted in a significant increase of the GK activity by 78% in cells expressing the islet isoform, by 130% in cells expressing the liver isoform and by 116% in cells expressing a cAMP-insensitive liver S32A/H258A double mutant form. The increase of GK activity was abolished by forskolin only in cells overexpressing the liver PFK-2/FBPase-2 isoform. This phenomenon can be explained by the fact that neither the islet isoform nor the liver mutant PFK-2/FBPase-2 contain a regulatory site for phosphorylation by a cAMP-dependent protein kinase. Through treatment with anti-FBPase-2-antibody the increase of GK enzyme activity was antagonized in RINm5F-GK cells overexpressing PFK-2/FBPase-2 irrespective of the isoform. An increase of the glucose concentration in the incubation medium from 2 to 10 mM in RINm5F-GK PFK-2/FBPase-2 cells had a significantly greater stimulatory effect on the glucokinase activity than in RINm5F cells overexpressing solely glucokinase.
Conclusion: The interaction of GK with PFK-2/FBPase-2 is apparently of physiological relevance for the activation of glucokinase activity in dependence on millimolar glucose concentrations. Thus this mechanism may contribute to the posttranslational regulation of GK enzyme activity and may open the perspective for a therapeutic targeting of this interaction in the therapy of type 2 diabetes mellitus.

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Leucine and β-ketoisocaproate stimulate pancreatic ß cells via distinct mechanisms.

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Background and Aims: We have previously reported that glucose inhibits leucine stimulation of pancreatic ß cell. The aim of this study is to test whether glucose also inhibits the actions of α-ketoisocaproate (KIC).
Materials and Methods: Isolated islets were pre-treated with high (HG) or low (LG) glucose before their cytosolic Ca^{2+}, insulin secretion and KIC oxidation was tested in the presence of leucine or KIC.

Results: Culturing rat islets in HG increased 1-^{14}C-KIC oxidation compared to culturing them in LG. Leucine caused insulin secretion (IS) in LG but not in HG rat islets, while KIC did so in both. Pre-treatment with HG for 40 min abolished leucine stimulation of IS by mouse islets and prevented the cytosolic Ca^{2+} rise without inhibiting IS and Ca^{2+} increment caused by KIC. When islets were pre-treated without glucose and glutamine, aminooxyacetic acid (AOA) markedly decreased KIC effects. When islets were pre-treated without glucose and with glutamine, AOA potentiated leucine effects, but attenuated KIC effects. AOA stimulated glutamine oxidation in the presence, but not the absence of BCH, a non-metabolized leucine analogue. Pretreatment with HG and glutamine partially reversed AOA inhibition of KIC effects. Glucose increased intracellular ATP and GTP while it decreased ADP and GDP in mouse ßHC9 cells. GDH activity of ßHC9 cell extracts was increased by leucine and attenuated by GTP, but potentiated by ADP.
Conclusion: Leucine and KIC stimulated ß-cells via distinct mechanisms. GDH is the sensor of leucine, while transamination plays an important role in KIC stimulation of pancreatic ß-cells.

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Clinicopathological characteristics of islet amyloid in Type 2 diabetes mellitus.

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Background and Aims: Type 2 diabetes is a metabolic disease characterised by both insulin resistance and insulin deficiency. However, the links between these two pathophysiologies are not clear. Histopathologically, islet amyloid is a prime hallmark of type 2 diabetes. Animal studies have suggested that islet amyloid is a consequence of insulin resistance and a cause of insulin deficiency. We conducted a multicentre autopsy study of patients with type 2 diabetes and provided clinicopathological evidence for this linking relationship of islet amyloid
Materials and Methods: Pancreases from 235 consecutive autopsies with type 2 diabetes were examined using immunohistopathological techniques. Islet amyloid deposits were detected by Congo red stain followed by polarising light microscopy and image analysis. Immunostains for insulin, amyloid P, cytokeratin 19, transforming growth factor-beta1 and a panel of inflammatory markers were performed to illustrate pancreatic beta cell, islet amyloid, ductal epithelium and inflammatory infiltrates. Relevant clinical data were retrieved from hospital's patient records.
Results: Islet amyloid was identified in 39.6% in the diabetic subjects. Clinically, the diabetic patients with islet amyloid had larger body mass index, higher HbA_{1c} level, higher blood pressure and were more frequently hypertensive than those without islet amyloid (all $p<0.05$). Histopathologically, islet amyloid was frequently accompanied by pancreatic fat infiltration (62.4%), hyaline arteriolosclerosis (100%), and fibrosis (76.3%). Histometrically, amyloid deposits occupied a mean islet area fraction of 36.2% and affected 2.6% to 91.8% of the pancreatic islets. Immunostains showed that islet amyloid was positive for amyloid P but negative for insulin. Significant loss of the insulin-secreting islet cells was observed in the type 2 diabetic patients with islet amyloid. However, the number of insulin-positive cells in pancreatic ducts and acini were significantly increased in the diabetic pancreases with islet amyloid. Cytokeratin 19-positive epithelial cells were populated in the exocrine acini, rather than the endocrine islets. Chronic inflammatory infiltrates in the diabetic pancreases with islet amyloid were dominated by CD68-, transforming growth factor-beta1-positive macrophages and CD45RO-, CD3-positive T- cells.
Conclusion: Islet amyloid was associated with insulin resistance, as reflected by obesity, pancreatic fat infiltration and high blood pressure. Islet amyloid was also related to insulin deficiency, as indicated by high HbA1c level, islet beta-cell loss, and aberrant insulin expression of dunctal and acinar cells. Therefore, islet amyloid is an important link between insulin resistance and insulin deficiency. In concert with islet amyloid, arteriosclerosis, chronic inflammation and fibrosis might accelerate the pancreatic failure. Weight management, tight metabolic and blood pressure control, and rational prescriptions may improve islet function by preventing amyloid deposition.

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Evaluation of the K_{ATP} channel-independent pathway of insulin exocytosis in two rodent models of Type 2 diabetes.

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Background and Aims: There is increasing evidence that glucose and other nutrient secretagogues can control insulin release independently from changes in K_{ATP} channel activity and in membrane potential, such a K_{ATP} channel-independent pathway (K_{ATP}-IP) working in synergy with the paramount classic K_{ATP} channel-dependent pathway (K_{ATP}-DP). In the present study we investigated the potential role of K_{ATP}-IP for the defective insulin secretion in STZ and GK rats, which represent two models of acquired and inherited type-2 diabetes.
Materials and Methods: STZ rats were adult Wistar rats injected with streptozotocin (100 mg/kg) on the day of birth. GK (Goto-Kakizaki) rats were originally obtained from Wistar rats by repeated selective inbreeding. Freshly collagenase-isolated islets from adult STZ, GK and non-diabetic Wistar (W) rats were incubated for 90 min at 37 C with test agent(s) in a control KRB medium (1 mmol/l Ca^{2+}, 5 mmol/l K^+), when K_{ATP}-DP plays a major role, or in a modified KRB medium (1 mmol/l Ca^{2+}, 0.250 mmol/l diazoxide to open K_{ATP} channels and 30 mmol/l K^+ to depolarize the membrane) in order to test K_{ATP}-IP. Insulin secreted was then measured by radioimmunoassay. Data were expressed relative to the basal release.
Results: D-glucose (17 mmol/l), L-leucine (10 mmol/l), 2-ketoisocaproate (KIC, 10 mmol/l) or the monomethylester of succinic acid (SAM, 10 mmol/l) significantly stimulated both K_{ATP}-DP and K_{ATP}-IP of insulin secretion in W islets. In both STZ and GK islets, the insulin riposte to either glucose or leucine was found severely decreased under conditions in which K_{ATP}-DP is the main component of B-cell secretory process. The stimulatory effect of these two secretagogues on K_{ATP}-IP was also found reduced, albeit to a lesser extent. The ability of KIC to stimulate K_{ATP}-DP was deficient, while its action on insulin output via K_{ATP}-IP was found well-preserved. In STZ islets, the stimulatory effect of SAM on both K_{ATP}-DP and K_{ATP}-IP was normal. By contrast, in GK islets, SAM stimulated normally K_{ATP}-IP, but failed to stimulate K_{ATP}-DP. Finally, in W, as well as in STZ and GK islets, L-glutamine (10 mmol/l) or the dimethylester of glutamic acid (GME) failed to affect K_{ATP}-DP, but stimulated K_{ATP}-IP to the same extent in the three groups of islets. Moreover, a similar enhancing action of glutamine (10 mmol/l) upon B-cell secretory riposte to leucine (10 mmol/l) through either K_{ATP}-DP (5-fold increase) or K_{ATP}-IP (2-fold increase) was found in W and diabetic (STZ or GK) islets.
Conclusion: Altogether, these data suggest that: 1) K_{ATP}-DP and K_{ATP}-IP of insulin secretion are controlled, at least partly, by different intracellular messengers, 2) K_{ATP}-IP is impaired in the B-cells of STZ and GK rats. Such an impairment could contribute to the lack of insulin secretory response to glucose and leucine previously documented in these two animal models of type-2 diabetes.

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Prolonged culturing increases early and late insulin release in KKAy-mouse islets which correlates with changes in islet protein profiles.

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Background and Aims: Early signs of type 2 diabetes include a reduction of first and second phase insulin release. These impairments accentuate and prolong postprandial hyperglycemia. In an attempt to find causes to the deranged insulin release we measured insulin secretion, oxygen tension (pO_2) and global protein patterns in islets isolated from KKAy-mice, an animal model for type 2 diabetes. The islet phenotypes were compared with and correlated to islet protein profiles.
Materials and Methods: Islets were isolated from 6 months old male KKAy-mice and cultured for 1, 5, 12 or 24 hours in RPMI 1640 containing 11 mM glucose. Individual islets from the four culture groups were perifused. During perifusion glucose was raised from 3 to 11 mM and insulin was measured with an ELISA. Islets cultured for 1 and 24 hours were also monitored by a Clark-microelectrode to detect glucose-induced changes in pO_2. Finally islets cultured for 1 and 24 hours were lysed to solubilize islet proteins. The islet lysates were applied on different chromatographic surfaces (weak cationic and strong anionic exchangers) and protein profiling was performed with surface enhanced laser/desorption ionization time of flight mass spectrometry (SELDI-TOF-MS). Differences in insulin secretion in the four culture groups were evaluated with ANOVA and Fisher's post hoc test. Differences in protein peaks were evaluated with Mann-Whitney U-test. Values are expressed as means ± SEM.

Results: Insulin release at 3 mM glucose was similar for all culture groups (9 ± 2 pmol*g^{-1}*s^{-1}). Glucose-stimulated insulin release was divided into early (0-10 min at 11 mM glucose) and late (10-30 min at 11 mM glucose) secretory responses. In islets cultured for 1 hour early and late secretory responses were 40 ± 17 and 37 ± 16 pmol*g^{-1}*s^{-1}, respectively. With increasing culture time both early and late insulin responses increased significantly. After culture for 24 hours the early and late secretory rates were 73 ± 10 and 77 ± 13 pmol*g^{-1}*s^{-1}, respectively. In contrast, glucose-induced reduction in pO_2 measured by comparing the pO_2 at 3 mM glucose with that observed 10 min after introducing 11 mM glucose was higher (33 ± 3 mmHg) for islets cultured for 1 hour compared to islets cultured for 24 hours (25 ± 3 mmHg). The mass spectrograms obtained from islets cultured for 1 and 24 hours showed several differences in islet protein profiles. In total 84 protein peaks were analyzed out of which 16 were differently ($p<0.05$) expressed in the two groups. The masses of these 16 peaks ranged from 2.4 to 73 kDa.
Conclusions: Culturing enhanced early and late glucose-induced insulin release in KKAy-islets. These secretory improvements were correlated to changes in protein expression, which may influence mitochondrial coupling explaining the lack of correlation between pO_2 and insulin release data. The differently expressed proteins will be identified and their relevance for impaired secretory and metabolic islet function verified.

PS 19
Beta Cells: Calcium^{2+} Regulation

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Molecular cloning of a novel sodium/calcium exchanger-NCEx from human insulinoma tissue.
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Background and Aims: Plasma membrane Na^+/Ca^{2+} exchangers play important roles in intracellular Ca^{2+} homeostasis and have been extensively studied in a variety of tissues. Two groups within the Na^+/Ca^{2+} exchanger superfamily have been characterized so far and consist of structurally and functionally distinct proteins, which are designated as Na^+/Ca^{2+} exchangers(NCX) and potassium-dependent Na^+/Ca^{2+} exchangers(NCKX). Recently, a novel Na^+/Ca^{2+} exchanger which was temporarily named as NCEx was cloned from a human insulinoma cDNA library by our group.In this study, we aimed at molecular cloning of NCEx and its possible roles in insulin secretion.
Materials and Methods: A CapFinder PCR cDNA library of human insulinoma was constructed. Expressed sequence tags (ESTs) were produced by using an ABI 3700 DNA Sequencer. The full-length cDNA cloning of NCEx was performed by using Autoassembler and DNA Strider 1.2 softwares and 5' RACE reactions. Tissue distribution of the human NCEx transcripts was studied using commercially available Multiple Tissue Northern. The location of NCEx transcripts in the pancreas was determined by in situ hybridization.The full-length cDNA encoding the NCEx protein was combined with expression vector pTet-on. The recombinant vector will be transfected into RIN-5F cell line. Insulin secretion of RIN-5F cells will be analyzed by using RIA method.
Results: Human NCEx encoded a protein of 584 amino acids that displayed 71%(417/584) homology to mouse Na^+/Ca^{2+} exchanger protein, but only shared ~25% homologies with NCX and NCKX families. The predicted topology of the human NCEx protein was very similar to that of NCX and NCKX families, beginning with a signal peptide, an extracellular loop, a cluster of five transmembrane spanning segments (M1 to M5), a long cytoplasmic loop, and a final hydrophobic cluster (M6 to M13). Furthermore, two Na-Ca-Ex domains which mediated Na^+/Ca^{2+} exchange were located in the NCEx molecule at amino acid positions 113-252 and 431-576 respectively. Northern blot analysis demonstrated abundant expression of NCEx in the pancreas and brain. It was also expressed at lower levels in prostate, testis, ovary, and lung. In the pancreas NCEx was predominantly expressed in the beta-cell of islet by using in situ hybridization.
Conclusion: In our study, we described a novel Na^+/Ca^{2+} exchanger-NCEx, which was cloned from a human insulinoma cDNA library and was predominantly expressed by pancreatic islet of Langerhans and brain tissue. It was proposed that NCEx was most likely invovled in the process of insulin secretion.

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Role of plasma membrane-related Ca^{2+}-ATPase-1 (PMR1) in islet β-cell Ca^{2+} homeostasis.
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Background and Aims: Recent studies have suggested that the mammalian homologue of the yeast and *C. elegans* Golgi Ca^{2+}-ATPase PMR1 (human gene nomenclature, ATP2C1) may have a role to play in higher organisms. Here we investigate the role of PMR1/ATP2C1 in controlling intracellular free Ca^{2+} concentrations in islet β-cells.
Materials and Methods: We have combined Ca^{2+} imaging with targeted-aequorins (Aqs) and „RNA interference" to monitor organelle Ca^{2+} concentration changes in INS1 β-cells depleted in PMR1.
Results: RT-PCR demonstrated the presence of mRNA encoding PMR1 in clonal β-cells (MIN6 and INS1), and in rat islets. Immunoblotting and immunocytochemical analysis of INS1 β-cells revealed that both endogenous and over-expressed PMR1 were widely distributed on intracellular membranes, including the ER, Golgi and insulin-containing secretory vesicles. After 48 h transfection with a small interfering RNA (siRNA) duplex corresponding to nucleotides 337-357 of rat PMR1 cDNA, expression of endogenous PMR1 was selectively reduced by ~ 80 % in these cells. Monitored using targeted Aqs, the rate of Ca^{2+} uptake into the secretory vesicles or the ER of permeabilised INS1 cells was inhibited

following PMR1 knockdown by ~ 30 % and ~ 40 %, respectively. In addition, the rate of Ca^{2+} influx into Ca^{2+}-depleted intact cells (0.07 ± 0.03 *vs* 0.44 ± 0.18 μM/s, control *vs* experimental, respectively; $p < 0.05$, $n = 4$) and the initial cytosolic Ca^{2+} concentration peak (1.25 ± 0.31 *vs* 3.24 ± 1.00 μM, $p < 0.01$, $n = 4$) were markedly increased by PMR1 silencing. This activation of Ca^{2+}-influx was completely blocked by nimodipine, an inhibitor of L-type voltage-gated Ca^{2+} channels.
Conclusion: PMR1 plays an important role in β-cells in both Ca^{2+} uptake by secretory vesicles and the ER, and in the control of Ca^{2+} entry by voltage-gated Ca^{2+} channels. Changes in PMR1/ATP2C1 activity could conceivably be involved in defective insulin secretion in some forms of Type II diabetes.

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Trafficking of α1D L-Type calcium channels regulated by intracellular free calcium concentration.
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Background and Aims: Chronic exposure of pancreatic β-cells to high concentrations of glucose impairs insulin secretory response to further glucose stimulation. This phenomenon is referred as glucose desensitization. It has been shown that glucose desensitization is associated with abnormal elevation of β-cell basal intracellular Ca^{2+} concentration. L-type Ca^{2+} channels play a central role in glucose induced insulin secretion in pancreatic β-cells. We hypothesize that changes in intracellular free calcium concentration regulate the total number of surface L-type Ca^{2+} channels via a mechanism involving protein trafficking.
Materials and Methods: Fura-2 fluorescent measurement was conducted to reveal the relationship between Ca^{2+} influx and basal Ca^{2+} concentration. In biotinylation experiment, Ins-1 cells were incubated in either control solution (1 mM Ca^{2+}) or the solution supplemented with 1 μM calcium ionophore A23187 for 30 minutes. A third group of cells was incubated in Ca^{2+} free solution supplemented with 10 mM EGTA and 1 mM BAPTA-AM for 30 minutes. After the treatments, surface proteins were biotinylated with Sulfo-NHS-SS-biotin and then incubated with neutravidin-linked beads. Bound proteins were eluted from beads and subjected to the Western blot. Immunofluorescent localization of α1D Ca^{2+} channels was determined by de-convolution microscopy.
Results: Glucose stimulation or membrane depolarization induced a nifedipine-sensitive Ca^{2+} influx, which was attenuated when the basal intracellular Ca^{2+} concentration was elevated. The L-type (α1D) Ca^{2+} channels were detected in both the plasma membrane and the cytoplasm by de-convolution immunofluorescent microscopy. The expression of surface α1D was decreased in the calcium ionophore treated cells compared to the controls. Cells in the low calcium condition expressed more α1D protein in the surface membrane than that of the controls. The pooled data shows that expression of α1D Ca^{2+} channel proteins (n =4) in the total membrane remained the same between the high calcium treated cells and the controls, whereas the channels in the surface membrane were significantly reduced in high calcium treated cells.
Conclusion: Depolarization induced Ca^{2+} influx is dependent upon intracellular free Ca^{2+} concentration. There is a population of α1D Ca^{2+} channels that is intracellularly located, which could be readily translocated into the plasma membrane. Biotinylation-Western blot results demonstrate an intracellular free Ca^{2+} regulated translocation of α1D Ca^{2+} channels between plasma membrane and intracellular locations. We have not detected the expression of α1C Ca^{2+} channels in Ins-1 cells with Western blot analyses.

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Ca^{2+}-induced Ca^{2+} release in pancreatic β–cells by inositol 1,4,5-trisphosphate receptor activation.
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Background and Aims: Although glucose-induced insulin secretion depends on Ca^{2+} influx through voltage-gated Ca^{2+} channels in the plasma membrane of the pancreatic β–cells, there is increasing evidence that intracellular Ca^{2+} stores play an important role in the regulation of insulin secretion. Glucose stimulation or the associated depolarization has been proposed to result in production of inositol 1,4,5-trisphosphate (IP_3), cyclic ADP ribose and nicotinamide adenine dinucleotide phosphate, which mobilize intracellular Ca^{2+} stores by different receptors. Moreover, influx of Ca^{2+} may trigger intracellular mobilization by Ca^{2+}-induced Ca^{2+} release (CICR). The aim of the study was to clarify the involvement of receptor activation in Ca^{2+} depletion of the endoplasmic reticulum (ER) after inhibition of the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA).
Materials and Methods: The effect of SERCA inhibition on the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) was studied in individual mouse, rat and human pancreatic β–cells as well as insulin-secreting clonal INS-1 cells. To ascertain maximal Ca^{2+} filling of the ER the β–cells were exposed to 20 mM glucose. Diazoxide (250μM) and methoxyverapamil (50μM) were also present to prevent voltage-dependent Ca^{2+} influx. The SERCA inhibition was made by exposing β–cells to cyclopiazonic acid (CPA) in Ca^{2+}-deficient medium supplemented with 2 mM EGTA.
Results: Caffeine, which is commonly used to trigger CICR via ryanodine (Ry) receptors, mobilised intracellular Ca^{2+} in INS-1 cells and this effect was prevented by pre-treatment with 100μM Ry for 45-60 min. However, in mouse, rat and human β–cells caffeine had no tendency to elevate $[Ca^{2+}]_i$ irrespective of the presence of the cyclic AMP-elevating hormone glucagon. Caffeine (20 mM) instead blocked the IP_3-mediated mobilization of Ca^{2+} from the ER in response to 100μM carbachol. Mouse, rat and human β–cells responded to CPA with slow leakage of Ca^{2+} from the ER, which was accelerated by gated release causing pronounced $[Ca^{2+}]_i$ transients. Whereas pretreatment with 100μM Ry had no effect on this gated release it was completely blocked by 20 mM caffeine, which did not interfere with the slow Ca^{2+} release.
Conclusion: These results indicate that the leakage of Ca^{2+} from the ER in pancreatic β–cells after SERCA inhibition is feedback-accelerated by CICR, which is mediated by IP_3 receptors.

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Activation of the calcium-sensing receptor regulates insulin secretion from human islets of Langerhans and MIN6 cells.
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Background and Aims: The extracellular calcium-sensing receptor (CaR) is the mechanism through which cells involved in the systemic regulation of Ca^{2+} recognise and respond to changes in extracellular Ca^{2+}. We have demonstrated that β-cells in human islets of Langerhans and the mouse insulinoma MIN6 line express the CaR. We have now used the calcimimetic compound A568 to investigate the effects of CaR activation on β-cell function.
Methods: Single cell Ca^{2+} microfluorimetry measurements were performed in Fura-2 loaded MIN6 cells or dispersed human islet cells. A perifusion system was used to measure the rate and magnitude of insulin secretion from human islets and from MIN6 cells configured as pseudoislets.
Results: In MIN6 cells A568 (1μM) in the absence of extracellular Ca^{2+} produced elevations in cytosolic calcium in 21/36 cells (58%) in 3 experiments. The velocity of the response to A-568 in the presence of 0.25mM $[Ca^{2+}]_o$ was significantly more rapid than the response to 0.25mM $[Ca^{2+}]_o$ alone ($p<0.0001$, $n=3$). Addition of A-568 in the presence of extracellular Ca^{2+} (0.25-2.0mM) evoked marked significant increases in cytosolic calcium ($p<0.05$). CaR activation also had marked effects on insulin secretion from human islets. A-568 (0.1μM) alone caused a small but significant stimulation of insulin secretion in the presence of 2mM glucose (194±35%control, $p<0.01$, $n=12$). Addition of extracellular Ca^{2+} (0.2-1.2mM) caused a further enhancement of secretion which was both rapid and transient with secretion declining to basal despite continued presence of the stimulus (0.2mM Ca^{2+}, 907±205% basal; 1.2mM Ca^{2+}, 3347±572%, $p<0.05$). The calcimimetic had direct effects on β-cells in islets since similar effects were observed in MIN6 cells configured as pseudoislets. Thus, A-568 (1μM) alone caused a small stimulation of secretion above basal (264±54% control, $p<0.01$, $n=9$), that was further enhanced ($p<0.0.5$) by the presence of extracellular Ca^{2+} (0.25-2.5mM).
Conclusion: These results demonstrate that CaR activation induces a marked but transient stimulation of insulin secretion from human and MIN6 cells, and suggest that signalling through the CaR may play an important regulatory role in the initiation of insulin secretory responses.

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Atypical mechanisms of Ca^{2+} release from the endoplasmic reticulum contribute to the cytosolic Ca^{2+} rise induced by depolarization in mouse ß-cells.

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Background and Aims: In pancreatic ß-cells, Ca^{2+} influx through voltage-dependent Ca^{2+} channels plays a prominent role in the depolarization-induced rise in free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). The contribution of Ca^{2+} release from the endoplasmic reticulum (ER) to this $[Ca^{2+}]_c$ elevation is largely debated and was investigated here.
Materials and Methods: $[Ca^{2+}]_c$ was measured by the fura-2 technique and the voltage-dependent Ca^{2+}current was recorded in the perforated mode of the patch-clamp technique. The expression of ryanodine receptors was studied by RT-PCR.
Results: Depolarization of single ß-cells by 45 mmol/l K^+ (in 10 mmol/l glucose + 0.1 mmol/l diazoxide) evoked two types of $[Ca^{2+}]_c$ responses: a monotonic and sustained elevation (~60% of cells) or a sustained elevation superimposed by a single large and transient $[Ca^{2+}]_c$ peak (TCP) ~60s after the onset of depolarization (~40%). TCP occurred only once during continuous depolarization, but was repeatedly activated by cycles of repolarization/depolarization. Simultaneous measurements of $[Ca^{2+}]_c$ and voltage-dependent Ca^{2+} current established that TCP did not result from a larger Ca^{2+} current. As the abolition of TCP by thapsigargin pretreatement indicated that it is caused by Ca^{2+} mobilization from the ER, we investigated the mechanisms of this mobilization. In other cell types, the ER releases Ca^{2+} upon activation of ryanodine receptors (RYR1 activated by depolarization and/or Ca^{2+}, RYR2-3 activated by Ca^{2+}) or IP_3 receptors (activated by IP_3 and/or Ca^{2+}). Whereas expression of the 3 RYR isoforms was prominent in control tissues (skeletal muscles for RYR1, cardiomyocytes for RYR2 and spleen for RYR3), mRNA of RYR1-2 was not found and mRNA of RYR3 was detected at low levels only in purified ß-cells. In a Ca^{2+}-free medium, attempts to activate RYR1 by 100 mmol/l K^+ and all RYRs by 10 mmol/l caffeine did not affect $[Ca^{2+}]_c$ in ß-cells, although similar protocols mobilized Ca^{2+} in skeletal and cardiac myocytes. 0.1 mmol/l ryanodine, a concentration that blocks all RYRs and efficiently suppressed the high K^+- or caffeine-induced Ca^{2+} mobilization in muscle cells, did not prevent the TCP elicited in ß-cells by 45 mmol/l K^+ in a Ca^{2+}-containing medium. Microinjection of ß-cells with heparin (a blocker of IP_3 receptors) suppressed acetylcholine-induced Ca^{2+} mobilization but did not prevent the TCP triggered by high K^+.
Conclusion: The TCP observed in 40% of β-cells in response to a depolarization-induced Ca^{2+} influx corresponds to a Ca^{2+}-induced Ca^{2+} release from the ER. Its mechanism is unusual as it does not involve ryanodine or IP_3 receptors.

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The timing for [Ca2+]i response to various glucose-concentrations show cell-specific profiles in the individual ß-cell.

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Background and Aims: That there are variations in the pattern of cytosolic free Ca2+ concentration ([Ca2+]i) among pancreatic ß-cells is well-known. Likewise varies the length of lag-time before onset of the [Ca2+]i response among the ß-cell population. The lag-time for [Ca2+]i/insulin response is of interest to characterise in view of the fact that early stages of type II diabetes mellitus are associated with an impairment of the first phase insulin release. We have previously shown that the [Ca2+]i response enclose a cell-specific lag-time for the single ß-cell when maximally stimulated. We have now studied whether the timing for the [Ca2+]i response is reproducible also when the cells are stimulated at various glucose-concentrations: 5.5, 8.3, 11.1 and 16.7 mM.
Materials and Methods: The early [Ca2+]i response was recorded from ob/ob mouse single ß-cells, by comparing the response from two consecutive exposures (10 min) of the same cell at the different glucose-concentrations (above). A rest period of 30 min at 3 mM glucose was included between the stimulations. Microfluorimetry with the fura 2/AM as probe was used.
Results: Correlation coefficient for onset of initial [Ca2+]i lowering, defined as the first value under an extrapolated base-line was, for 5.5 mM glucose; r=0.64, P<0.001 (86±23 vs. 64±10 s) for 8.3 mM glucose; r=0.44, P<0.05 (46±8 vs.43±10 s), for 11.1 mM glucose; r=0.69, P<0.01 (33±8 vs.47±16 s) and for 16.7 mM glucose; r=0.02 (18±4 vs. 19±4 s). Lag-time for [Ca2+]i rise, defined as the first value over the extrapolated baseline was, for 5.5 mM glucose; r=0.81, P<0.001(292±42 vs.303±30 s), for 8.3 mM glucose; r=0.81, P<0.001 (211±20 vs.257±29 s), for 11.1 mM glucose; r=0.77, P<0.001 (190±18 vs. 231±33 s) and for 16.7 mM glucose; r=0.70, P<0.001 (131±11 vs.159±15 s). Lag-times for nadir of the initial lowering and for maximum of the first peak were also compared within the pairs of stimulation, for each glucose-concentration tested. The timing of these events showed similar high degrees of correlations as the lag-times for initial [Ca2+]i lowering and lag-times for [Ca2+]i rise.
Conclusion: The results indicate that each single ß-cell is pre-programmed to react with a specific [Ca2+]i response within a certain time to a certain glucose-concentration. ß-Cells showing either a fast or a late response during the first glucose-stimulation reproduced their time pattern during the second stimulation. An individual lag-time may correspond to different function for ß-cells and may have importance for the impairment of the first phase insulin release.

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Acetylcholine restores glucose-induced insulin exocytosis in the diabetic GK islet by affecting post-cytosolic calcium signalling.

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Background and Aims: A markedly impaired insulin release in response to glucose (G) while responsiveness to others secretagogues is maintained, is present in the Goto-Kakizaki (GK) rat, a lean spontaneous model of type 2 diabetes. We and others have previously reported that besides its K^+channel-dependent effect, the glucose action via the K^+-independent pathway was also impaired in the GK β-cell. In the normal β-cell acetylcholine (Ach) potentiates glucose-stimulated insulin release by actions predominantly at a site distal to the elevation of $[Ca^{2+}]_c$. Accordingly we have now studied the location of the action of Ach and its interaction with the glucose pathway in GK islets.
Materials and Methods: Insulin secretion was determined from perifused freshly isolated islets. $[Ca^{2+}]_c$ was measured in parallel using the same fura-2 loaded islets. Double-stranded cDNA was synthesized starting from RNA extracted from 16 wk-old male W and GK islets and related cRNA was hybridized to Affymetrix RG-U34A rat array. Expression values for the genes were determined using Affymetrix Microarray Suite 5.0 and Affymetrix Data Mining Tool 2.0.
Results: We have first verified that the addition of Ach (1 mM) to perifused GK islets amplified (by ten times) their insulin response to 16.7 mM G with a clear return of the biphasic pattern of insulin release. In comparison the non-diabetic Wistar (W) islets exhibited only a two fold increase. Then we have demonstrated that: 1/ Ach elicited a first phase insulin release at 0 or 2.8 mM glucose in GK islet, while it has no significant effect in W; 2/ basal activity of total phospholipase C was not impaired in GK islets; 3/ Ach-induced inositol-phosphates production was normally enhanced in GK islets; 4/ SERCA-3 gene expression (Affymetrix microarray) was decreased in GK islets as compared to W, while IP3-receptor subtype3 gene expression was increased; 5/ Ach in the presence of 2.8 mM G, triggered a transient peak of $[Ca^{2+}]_c$ in the W islets which reflected mostly mobilization of Ca^{2+} from intracellular Ca^{2+} stores since it was suppressed by thapsigargin. In the GK islets, the Ca^{2+} response to Ach was not enhanced; 6/ inhibition of PKCs (Bim) did not affect the $[Ca^{2+}]_c$ nor the insulin release responses to Ach in GK islets; 7/ inhibition of PKAs (Rp-cAMP or H89) or adenylate cyclases (2-5 dideoxyadenosine), while not affecting the $[Ca^{2+}]_c$ response, significantly lowered the insulinotropic response to Ach (at low G) in GK islets.
Conclusion: At variance with W islets, Ach is a triggering signal for exocytosis at low G in GK islets and an hyperactive amplifying signal at high G. This is by a mechanism largely involving direct interaction with the insulin exocytotic machinery, since it reflects an enhanced efficacy of Ca^{2+} on exocytosis. Such a sensitization to Ach is independent of PKC pathway and is related to cAMP generation and the PKA pathway.

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Beta Cell: Signal Transduction

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ATP-dependent interaction of the cytosolic domains of Kir6.2 revealed by fluorescence resonance energy transfer.

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Background and Aims: ATP-sensitive potassium (K_{ATP}) channels couple intermediary metabolism to cellular electrical activity and play important roles in the regulation of insulin secretion. Thus, in pancreatic β cells, closure of K_{ATP} channels is largely responsible for the depolarization produced by elevated glucose concentrations. Whilst the N- and C- termini of the pore-forming subunit of the channel, Kir6.2, have been suggested to interact in an ATP-dependent manner, the dynamics of this putative interaction have not previously been explored in living cells.
Materials and Methods: We genetically fused the cytoplasmic N- and C-termini of the mouse Kir6.2 channel with cyan (ECFP) and yellow (EYFP) fluorescent protein, respectively. The interaction between these domains was then examined at different functional states of the channel expressed in living human embryonic kidney (HEK293) cells with the sulphonylurea receptor, SUR1, using fluorescence resonance energy transfer (FRET).
Results: Monitored in permeablized cells, FRET signals were increased by ATP ($K_{0.5}$ =1.78 mM), and this effect was recapitulated in living cells when cytosolic [ATP] was increased by muscarinic stimulation. Conversely, the FRET signal was markedly reduced by metabolic poisoning with sodium azide or the mitchondrial uncoupler, carbonylcyanide 4-trifluoromethoxyphenyl hydrazone.
Conclusion: These data imply that changes in ATP concentration in the millimolar range regulate K_{ATP} channel activity in living cells by altering the mobility and orientation of the cytosolic domains of Kir6.2.

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Functional coupling between Kir6.2 and SUR1 is prerequisite for [^{3}H]repaglinide, but not [^{3}H]glibenclamide, binding to pancreatic ATP-sensitive potassium (K_{ATP}) channels.

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Background and Aims: The pancreatic beta-cell ATP-sensitive potassium (K_{ATP}) channel is composed of four pore-forming subunits (Kir6.2) and four regulatory subunits (SUR1). Glibenclamide and repaglinide bind to SUR1 with high affinity and the N-terminus of Kir6.2 is involved in coupling sulphonylurea binding to closure of the channel pore. In the presence of MgATP, the binding site for potassium channel openers (PCOs) on SUR1 is allosterically coupled to the glibenclamide-binding site. In this study, we compare the interaction of glibenclamide and repaglinide with the Kir6.2/SUR1 channel by displacement of [^{3}H]repaglinide or [^{3}H]glibenclamide binding with the Kir6.2/SUR1 selective PCO, NNC 55-9216.
Materials and Methods: SUR1 was either expressed alone or co-expressed with Kir6.2 or with N-terminally deleted Kir6.2 (Kir6.2deltaN14) in HEK293 cells. Binding experiments were performed on isolated membranes at 37° C.
Results: Saturation analysis revealed a single high-affinity [^{3}H]glibenclamide binding site both when SUR1 was expressed alone (K_D=0.2 nM) or together with Kir6.2 (K_D=1.5 nM) or Kir6.2deltaN14 (K_D=1.9 nM). In contrast, repaglinide only showed high-affinity binding to SUR1 co-expressed with Kir6.2 (K_D=0.6 nM). The binding affinity of [^{3}H]repaglinide was 500-fold lower for SUR1 expressed alone or with Kir6.2deltaN14. NNC 55-9216 displaced ^{3}H-repaglinide (IC_{50}= 8.6 μM) and [^{3}H]glibenclamide (IC_{50}= 22 μM) binding to Kir6.2/SUR1 in the presence of MgATP. The ability of NNC 55-9216 to displace [^{3}H]glibenclamide binding was largely unaffected when SUR1 was expressed alone. In contrast, NNC 55-9216 produced no significant displacement of [^{3}H]repaglinide binding at concentrations up to 100 μM when SUR1 was expressed alone.
Conclusion: We conclude that the high-affinity [^{3}H]repaglinide site is allosterically coupled to the NNC 55-9216 binding site and its formation is critically dependent on functional coupling between Kir6.2 and SUR1.

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Glucose-induced translocation of K_{ATP} channel SUR1 and Kir6.2 subunits in β-cells from ob/ob mice.

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Background and Aims: The ATP-sensitive K^+ (K_{ATP}) channel plays an important role in the regulation of insulin exocytosis by coupling cell metabolism to electrical activity. In the pancreatic β-cell, the K_{ATP} channel consists of two subunits, sulfonylurea receptor 1 (SUR1) and inwardly rectifying K^+ channel 6.2 (Kir6.2), with 4:4 stoichiometry. The Kir6.2 subunits form the pore of the K_{ATP} channel and the SUR1 subunits are grouped around the pore formation. Two nucleotide binding folds in the cytosolic parts of the SUR1 subunit interact with adenine nucleotides, ATP and ADP. ATP has also been demonstrated to bind to the Kir6.2 subunit. These interactions regulate the activity of K_{ATP} channels. A rise in the ATP/ADP ratio results in the closure of the channel and leads to membrane depolarization, which in turn activates voltage-dependent Ca^{2+} channels. Ca^{2+} entry through the voltage-dependent Ca^{2+} channel triggers insulin exocytosis. The Kir6.2 and SUR1 subunits, like other membrane proteins, are likely to undergo dynamic regulation to maintain a certain number of functional K_{ATP} channels in the plasma membrane. However, it is not known how the Kir6.2 and SUR1 subunits change their locations in hyperglycaemia. The aim of the present work is to examine whether the SUR1 and Kir6.2 subunits can translocate in β-cells subjected to 1 h incubation with glucose, mimicking the initial phase of hyperglycaemia.
Materials and Methods: The different subcellular compartments in β-cells from ob/ob were separated using a linear sucrose gradient, where the SUR1 and Kir6.2 subunits as well as the plasma membrane marker syntaxin1A and insulin-containing granule marker synaptotagmin III were detected using Western blot analysis. Co-localization studies were done with immunocytochemistry, using insulin-, SUR1- and Kir6.2 antibodies subjected with FITC and Texas Red.
Results: Subcellular fractionation showed that the sucrose density increased linearly. Two peaks of protein concentration appeared in a linear sucrose gradient, indicating an adequate separation of the homogenized samples. Western blot analysis demonstrated that the SUR1 and Kir6.2 immunoreactivities were detected in both the plasma membrane and cytosolic fractions, identified by syntaxin 1A and synaptotagmin III antibodies, respectively, in cells treated with 3 mM glucose for 1 h. The incubation with 17 mM glucose for 1 h shifted the SUR1 and Kir6.2 signals to the plasma membrane fractions. Furthermore, immunocytochemical results showed that the Kir6.2 and SUR1 subunits did not localize in insulin-containing granules.
Conclusion: Taken together, our data suggest that the SUR1 and Kir6.2 subunits translocate from non-insulin containing vesicles in the cytosol to the plasma membrane during incubation with 17 mM glucose. This implies that the translocation of the SUR1 and Kir6.2 subunits in pancreatic β-cells may adapt the cells to hyperglycaemia by recruiting more functional K_{ATP}.

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B-cells from SUR1$^{-/-}$ mice exhibit slow waves of membrane potential.

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Background and Aims: In contrast to marked hypoglycemia seen in humans suffering from loss of SUR1-based K_{ATP} channels (PHHI), SUR1$^{-/-}$ mice survive and exhibit normal blood glucose concentration with mild glucose intolerance. The mechanism regulating insulin secretion in these animals is unclear.
Materials and Methods: Membrane potential (MP) was measured with either intracellular microelectrodes (ME) or with the patch-clamp technique; cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$) was assessed by fura-2 fluorescence.
Results: Patch electrode (PE) recordings from single, or small clusters, of SUR1$^{-/-}$ B-cells showed a depolarized MP with continuous spike activity.

However, $[Ca^{2+}]_c$, assumed to be regulated by changes in MP, oscillates. Surprisingly, B-cells exhibited slow waves in MP, measured with a ME, in $SUR1^{-/-}$ islets surrounded by small pieces of exocrine pancreas. The fraction of plateau phase (FOPP) was 26±4 % (n=7) in 15 mM glucose (15 G). The pattern of electrical activity changed characteristically in either 0.5 G or 25 G. Unexpectedly, in 0.5 G the FOPP was increased (39±2 %, n=3) seemingly due to a prolongation of bursts. The spike frequency during bursts in 0.5 G was considerably lower than in 15 G (243±23 APs/min, n=3 vs 437±36 APs/min, n=3, respectively). At 25 G the changes were marginal, the FOPP (25±4 %, n=5) was unaltered vs 15 G, but the spike frequency tended to increase as compared to 15 G (415±15 APs/min, n=4, vs 386±18 APs/min, n=4, respectively). As expected, neither tolbutamide (100 µM) nor diazoxide (100 µM) affected the MP of $SUR1^{-/-}$ B-cells. Disrupting cell metabolism with NaN_3 (5 mM, n=5) or H_2O_2 (1 mM, n=8) terminated the continuous spike activity measured with PEs. NaN_3 did not induce a hyperpolarizing current, the abrogation of spikes probably occurred via direct inhibition of Ca^{2+} channels as in $SUR1^{+/+}$ B-cells. In contrast, H_2O_2 hyperpolarized the plateau MP of $SUR1^{-/-}$ B-cells (measured with PE) by -19±5 mV (n=8). The hyperpolarizing current is inhibited by 20 mM TEA^+ (+16±4 mV, n=3) implying opening of K^+ channels.
Conclusion: The MP of $SUR1^{-/-}$ B-cells oscillates despite the lack of functional K_{ATP} channels generally considered to play a key role in normal B-cell oscillatory activity. We conclude a K^+ current different from the K_{ATP} channel hyperpolarizes $SUR1^{-/-}$ B-cells and may contribute to their regulation of insulin secretion.

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Carbon monoxide is a putative messenger for propagating Ca2+ signals between pancreatic β-cells.
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Background and Aims: It was recently proposed that nitric oxide (NO), derived from NO synthase containing nerves, could be important for co-ordinating the activity of the β-cells by precipitating transients of cytoplasmic Ca^{2+}.We have now studied whether carbon monoxide (CO), another gaseous messenger known to stimulate guanylate cyclase, has a similar action as NO.
Materials and Methods: Islets from obese (ob/ob) and lean C57BL/6J mice were used. Immunocytochemical demonstration of heme oxygenase (HO) and measurements of islet activities of HO with gas chromatography and nitric oxide synthase (NOS) with HPLC were performed. Single cells and small aggregates (< 10 cells) were prepared from isolated islets and the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]i$) was measured with a digital imaging technique. Blockade of the voltage-dependent Ca^{2+} channels with methoxyverapamil made it possible to examine $[Ca^{2+}]i$ transients due to intracellular release without background of periodic Ca^{2+} influx.
Results: A strong immunoreactivity for constitutive HO (HO-2) was found in pancreatic ganglionic cells as well as in the endocrine cells of the islets. Islets of ob/ob mice produced almost 1 nmol CO/mg protein/min, which is 6 times more than observed in islets from lean mice and 100-fold the rate found for NO derived from constitutive NOS (cNOS) in both types of islets. Continuous monitoring of $[Ca^{2+}]i$ in lean and ob/ob mouse β-cells revealed an increased (16 times) firing of transients in the β-cells of the ob/ob mouse with a rate of 0.2/min. The HO substrate hemin (0.1 and 1.0 mmol/l) promoted the appearance of the $[Ca^{2+}]i$ transients and the HO inhibitors Zn-protoporphyrin and Cr-mesoporhyrin (10 mmol/l) had a suppressive action both on the firing of the transients and their synchronization. Addition of CO gas (1-100 mmol/l) resulted in slow responses with generation of transients similar to those occurring spontaneously.
Conclusion: HO-derived CO is produced both in ganglionic cells and in islet endocrine cells of the pancreas. In the ob/ob-mice the β-cells are characterized by a greatly raised generation of CO and accelerated firing of cytoplasmic $[Ca^{2+}]i$ transients. The accumulated data suggest that CO is important for propagating synchronizing $[Ca^{2+}]i$ signals from one β-cell to another.

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External ATP has stimulatory and inhibitory effects on oscillatory Ca^{2+} signaling in pancreatic β-cells.
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Background and Aims: Pancreatic β-cells have an intrinsic Ca^{2+} rhythmicity, responding to glucose stimulation with slow oscillations depending on Ca^{2+} entry, sometimes superimposed with transients due to mobilization of Ca^{2+} from intracellular stores. These transients may provide a coupling force for synchronization of the slow Ca^{2+} oscillations. To understand how pulsatile release of insulin from the pancreas is generated, it is important to know how ATP and other factors, released from nerves and the β-cells themselves, may co-ordinate the β-cell rhythmicity within and among the islets.
Materials and Methods: Single cells and small aggregates (< 10 cells) were isolated from ob/ob mice and allowed to attach to coverslips during 2-5 days culture. Cytoplasmic Ca^{2+} was measured with digital imaging using the indicator fura-2. Images were sampled with a rate of 0.5 Hz, restricting the light exposure of the cells to the capture period.
Results: Addition of ATP (1 – 100 µmol/l) to a medium containing 3 mmol/l glucose resulted in a short-lived increase of cytoplasmic Ca^{2+}, absent in cells exposed to the sarcoendoplasmic reticulum Ca^{2+}-ATPase inhibitor cyclopiazonic acid (CPA). However, in β-cells oscillating in response to 20 mM glucose the addition of a wide range of ATP concentrations (0.01 – 100 µmol/l) resulted in an initial stimulation with a premature oscillation, seen also in the presence of 50 µmol/l CPA. At high concentrations of ATP (10 - 100 µmol/l) the premature oscillation was followed by suppression of Ca^{2+} to the basal level. In β-cells responding to the glucose stimulus with sustained elevation of Ca^{2+}, pulse additions (15 - 60 s) of the latter concentrations of ATP resulted in a temporary lowering followed by oscillations. Addition of ATP (0.1 -100 µmol/l) promptly triggered CPA- sensitive transients, which were superimposed on the oscillations (premature or ongoing). During prolonged exposure to micromolar concentrations of ATP the transients disappeared with maintenance of the slow oscillations.
Conclusion: Pulses of external ATP, at concentrations well below those reported to exist around glucose-stimulated β-cells, promptly trigger premature oscillations and transients of cytoplasmic Ca^{2+}. Prolonged exposure to 1 µmol/l or more of ATP has a suppressive action on the transients and at higher concentrations also on the oscillations. The results support the idea that the β-cells both receive and propagate a neuronal ATP signal important for synchronizing their Ca^{2+} rhythmicity.

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Pancreatic β-cells communicate via release of ATP.
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Background and Aims: Pancreatic β-cells respond to glucose stimulation with cytoplasmatic Ca^{2+} oscillations, sometimes superimposed with transients due to mobilisation of intracellular calcium. After the observation that the transients appear in synchrony in β-cells lacking physical contact, it was proposed that diffusible factors aid to the co-ordination of the Ca^{2+} oscillations resulting in pulsatile release of insulin. The present study was designed to explore whether ATP is a diffusible messenger for exchange of information between β-cells.
Materials and Methods: Single β-cells and small aggregates (< 10 cells) were prepared from collagenase-isolated islets of ob/ob-mice and allowed to attach to coverslips during 2 -5 days culture. After loading with fura-2 cytoplasmic Ca^{2+} was measured with digital imaging. Blockade of the voltage-dependent Ca^{2+} channels with 50 µmol/l methoxyverapamil made it possible to study Ca^{2+} transients induced by 20 mol/l glucose without the background disturbance of oscillations. The proportion of transients appearing in synchrony in cells lying up to 30 µm apart was referred to as the synchronization index.
Results: Spontaneous firing of Ca^{2+} transients (0.01 – 0.06/min) from a basal level was seen in glucose-stimulated β-cells exposed to methoxyverapamil. When glucagon (20 nmol/l) was introduced into the medium, there was a tenfold increase in the number of transients, which often appeared in synchrony in adjacent cells. Addition of ATP (0.01 –100 µmol/l), ADP (0.1 µmol/l) and 2-methylthio-ATP (0.01 µmol/l), but not of AMP (1 µmol/l) and UTP (1 – 10 µmol/l), resulted in prompt firing of a transient. During prolonged exposure to ATP both the generation and synchronization of the glucose-induced transients were suppressed. It was possible to induce transients during the suppressive phase by further raising the ATP concentration. After long-term exposure to 0.1 µmol/l ATP the

synchronization index decreased from 0.53 ± 0.08 to 0.11 ± 0.07; n =19 ; P < 0.001). The purinoceptor antagonists suramin (25 µmol/l), 2-deoxy-N-methyladenosine-3,5-bisphosphate (MRS 2179; 0.3 -30 µmol/l) and pyridoxalphosphate-6-azophenyl-2,4-disulfonic acid (PPADS; 10 - 30 µmol/l) or dephosphorylation of extracellular ATP with apyrase (2 U/l) markedly depressed spontaneous firing of Ca^{2+} transients. Moreover, inhibitors of exocytosis (100 nmol/l of adrenalin or somatostatin) suppressed the generation of Ca^{2+} transients by mechanisms additional to lowering of cytoplasmic cAMP.
Conclusion: The data indicate a role for ATP in the exchange of information between β-cells lacking physical contact. We propose that cytoplasmic Ca^{2+} transients, via stimulation of exocytosis, induce brief periods of ATP and ADP release responsible for generation of transients in adjacent β-cells by activation of $P2Y_1$ receptors.

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Acetylcholine enables the K_{ATP}-independent signaling pathways of glucose-stimulated insulin release (GSIR) in pancreatic β-cells hyperpolarized by diazoxide.
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Background and Aims: Potassium channel openers (PCO) improve insulin release (IR) in Type 2 diabetes (T2DM). This observation is counter intuitive in view of the widely accepted treatment of T2DM with SUR-1 agonists. Hyperpolarization of β-cell by PCO should block the K_{ATP}-dependent pathways for GSIR without compromising the K_{ATP}-independent signaling processes. Previous studies of the K_{ATP}-independent pathway were conducted in depolarized cells either in the presence of diazoxide combined with high K^+ or in the presence of SUR-1 agonists. The manipulations do in effect duplicate the physiological membrane depolarization caused by metabolic inhibition of the K_{ATP} channels. It remains thus unexplored whether the K_{ATP} channel independent signaling pathways do also operate in the hyperpolarized β-cells. We therefore studied GSIR in mouse β-HC9 cells hyperpolarized with 200 µM diazoxide in the presence of acetylcholine (Ach) and 0.1 mM IBMX, the latter to enhance cAMP dependent processes.
Materials and Methods: β-HC9 cells were encapsulated in microscopic agarose beads and perifused in bulk.
Results: Ach alone caused a brief monophasic burst of IR in the presence of diazoxide and IBMX. In the presence of 16.7 mM glucose, diazoxide and IBMX Ach stimulated biphasic IR but diazoxide blocked IR due to glucose plus IBMX when Ach was absent. Pretreatment with Ach and IBMX permits GSIR in a concentration-dependent manner in β-HC9 cells hyperpolarized with diazoxide. Verapamil or nimodepine did not affect the Ach-stimulated IR in hyperpolarized β-cells, but completely blocked GSIR in hyperpolarized cells enabled by Ach and IBMX.
Conclusions: These data suggest that K_{ATP}-independent signaling pathways of GSIR involving Ca^{2+}, protein kinase C and protein kinase A operate in hyperpolarized pancreatic β-cells and may be the basis of improved β-cell function during the treatment with PCO.

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Myosin 5a and actin network interactions are important for recruitment of new insulin granules for exocytosis.
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Background and Aims: The recruitment on new insulin granules for secretion requires the interaction between insulin granules and the cytoskeleton. The actin network locates immediately beneath the plasma membrane and is important not only for supporting cell structure, but also for mediating transport of vesicles, such as insulin granules, to the plasma membrane. The latter action is generally believed to be fulfilled by atypical myosin motor proteins. Here, we have elucidated the role of the actin network in insulin granule trafficking and subsequent exocytosis, as well as identified an important motor protein involved in this process.
Materials and Methods: Single-cell exocytosis was measured in clonal INS-1 cells as increases in cell capacitance. Granule trafficking was studied with live confocal imaging of insulin granules labelled with Lysotracker Red and/or an EGFP-phogrin chimeric protein. Immunocytochemistry was used for localisation studies.
Results: Inhibition of actin-myosin interactions by the myosin light chain kinase (MLCK) inhibitor ML-9 (30 µM) reduced single INS-1 cell exocytosis by 73 % (P<0.01; n=10), an action that could be fully counteracted by pretreatment with the actin-severing compound Cytochalasin D (10 µM; for 15 min). This compound itself exerted a profound 270 % stimulatory action on exocytosis (P<0.01; n=11). These data suggest a dual role for the actin network, acting as both a barrier and a guide for insulin granule transport. In support of this view, disruption of the actin network by cytochalasin D (confirmed by immunocytochemistry), failed to suppress insulin granule trafficking, whereas ML-9 significantly decreased the number of directed insulin granule translocation events by 90 % (P<0.01; n=6).
The unconventional Myosin 5a was identified in B-cells and INS-1 cells by Western blotting. Immunocytochemistry showed peripheral co-localisation of Myosin 5a and insulin. To study the role of Myosin 5a, an antibody directed against the granule-binding domain of the motorprotein was infused intracellularly into INS-1 cells using the standard whole-cell configuration of the patch-clamp technique. Infusion of the antibody inhibited insulin exocytosis in a use-dependent manner. Two minutes after infusion exocytosis was reduced by 32 % (p=0.23; n=5) and after 4 minutes by 74 % (P<0.01; n=5).
Conclusion: These data indicate a dual role for the actin network in granule trafficking. The actin network inhibits insulin granule to reach the plasma membrane by mere diffusion. On the other hand the actin network is dense and the insulin granules are dependent on myosin 5a-mediated transport through the network before exocytosis.

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Cytoplasmic dynein is not the principle molecular motor involved in retrograde movement of insulin-containing vesicles.
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Background and Aims: Retrieval of insulin-containing vesicles following exocytosis might influence the size of the readily-releasable and reserve pools in pancreatic β-cells. This process involves retrograde movements of vesicles from the plasma membrane towards the cell centre. We have explored the role of dynein, believed to be the principle retrograde motor in neurons, in vesicle transport in beta-cells.
Materials and Methods: INS-1 and MIN6 beta-cells were co-transfected with constructs encoding p50 (dynamitin) and enhanced green fluorescent protein (EGFP)-tagged phogrin or cytochrome c oxidase (subunit VIII) leader sequence fused to DsRed, prior to imaging. Movements of vesicles and mitochondria were monitored in real time using an UltraVIEW™ confocal microscope. Retrieval of vesicles following exocytosis at the vicinity of the plasma membrane was assessed by TIRF microscopy. Immunocytochemical analysis in p50- and empty vector-transfected cells was performed using appropriate primary and secondary antibodies. Glucose-stimulated release of co-transfected human growth hormone (hGH) was assayed using an hGH ELISA kit. Association of endogenous dynein with insulin-containing vesicles was assessed after

immunoabsorption of vesicles from cells infected with a phogrin-EGFP adenoviral construct.
Results: In this study we have overexpessed a dynactin subunit, p50, which is known to disrupt dynein-dynactin interactions and thereby inhibit retrograde movement of cargo membranes along microtubules. Correspondingly, inhibition of dynein function caused the collapse of the mitochondrial reticulum, and the formation of discrete mitochondrial units. Cytoplasmic dynein was readily detectable in whole cell homogenates from β-cells, but was undetectable in immunopurified vesicle proteins. Dynamitin overexpression reduced the frequency of the rare, long and rapid (> 2 μm/s) excursion of insulin-containing vesicles, but had no effect on movements < 2 μm/s or on the distribution and retrieval of the vesicles following exocytosis. In agreement with these results, p50 had no effect on the stimulation of human growth hormone release by 30 vs 3 mM glucose (4.07 +/- 0.54 fold and 4.22 +/-0.30 fold in p50- and empty vector-transfected cells, respectively).
Conclusion: The lack of a significant effect of the blockage of dynein-driven transport on vesicle movement suggests that another retrograde motor(s) is responsible for the retrieval of vesicles from the plasma membrane following exocytosis.

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Defective insulin release machinery and insulin granule acidification in chloride channel 3null (ClC3$^{-/-}$)mice.
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Background and Aims: Uptake of chloride ions (Cl^-) via ClC3 channels in the insulin granule membrane has been suggested to facilitate insulin granule acidification and in turn insulin secretion. Ablation of the ClC3 gene in mice (ClC3$^{-/-}$ mice) results in a phenotype with hippocampal degeneration and retinal blindness, but the effects on insulin release and glucose homeostasis have not been investigated in closer detail.
Materials and Methods: ClC3$^{-/-}$ mice and their wildtype littermates (wt) were challenged with glucose in vivo (11.1 mmol/kg i.p.). Insulin and glucagon release in isolated islets were measured in batch incubations. Single B-cell exocytosis was monitored as increases in cell capacitance. Semiliki Forest Virus-based ClC3 gene transfer was used for resue experiments in ClC3$^{-/-}$ islet cells. Insulin granule pH was monitored semi-quantitatively as changes in Lysosensor DND-189 fluorescence.
Results: Basal concentrations of plasma glucose and insulin did not differ between ClC3$^{-/-}$ and wt mice in vivo. 8 min after the glucose challenge, p-insulin increased by 96% in wt mice, whereas in the ClC3$^{-/-}$ mice, p-insulin remained unchanged (P<0.01; ClC3$^{-/-}$ mice vs. wt mice). Interestingly, in both groups glucose tolerance appeared intact, indicating a compensatory hypersensitivity to insulin in target tissues in ClC3$^{-/-}$ mice. In isolated ClC3$^{-/-}$ islets, insulin secretion elicited by glucose (20 mM) was decreased by 72% (P<0.001 vs. wt. Likewise, insulin release triggered by K^+ (50 mM) or glibenclamide (1 uM) amounted only ~10% of that observed in wt islets (P<0.001 and 0.01, for K^+ and glibenclamide, respectively). It was ascertained that these effects could not be attributed to a reduction in total insulin content in the ClC3$^{-/-}$ islets, which measured 90% of that in wt islets. Ca^{2+}-evoked exocytosis was overall reduced by 80% in ClC3$^{-/-}$ B-cells (P<0.001 vs. wt). ClC3 gene transfer using the Semiliki Forest Virus expression system, partially rescued exocytosis in the ClC3$^{-/-}$ B-cells, and averaged 61% of that in wt B-cells (P<0.001; non-transduced vs. transduced ClC3$^{-/-}$ B-cells). The reduction in exocytosis in ClC3$^{-/-}$ B-cells coincided with a reduced capacity for adjusting intragranular pH. After intracellular addition of the protonophore CCCP, ClC3$^{-/-}$ B-cells exhibited a slower timecourse of pH increase than wt B-cells (P<0.05).
Conclusion: ClC3 gene ablation results in a severe reduction in glucose-stimulated insulin secretion in vivo, as well as in isolated pancreatic islets. This effect is due to an inhibited capacity for Ca^{2+}-evoked exocytosis in the single B-cell, and coincides with a perturbed ability to modulate intragranule pH.

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Interactions of the chaperon protein CSP during insulin exocytosis.
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Background and Aims: Insulin exocytosis requires the interplay and remodelling of numerous protein complexes and their precise interaction is still poorly understood. The cystein string protein CSP, expressed on large dense core vesicles, is characterized by a variable N-terminus, a conserved J-domain followed by a linker region, the name-giving cysteine string and a C-terminal sequence, which varies among the different isoforms known. Notably the splice variant CSP2 lacks most of the C-terminus. We have previously demonstrated that the cysteine string protein CSP is required at a late step of insulin exocytosis. Its function implies an interaction between mainly the linker region and the C-terminus.
Results: Immunoprecipitation from permeabilized HIT-T15 insulinoma cells reveals a stimulation depending interaction between CSP and the SNARE-protein VAMP. Binding studies using recombinant and in-vitro translated proteins indicate that this interaction requires the C-terminus such as found in the isoform CSP1. To further characterize the molecular interactions of CSP in-situ during exocytosis we have employed chemical cross-linking in streptolysine-O permeabilized cells. Under these conditions CSP1 forms homodimeres as evidenced by the use of different epitope-tagged constructs and analysis by immunoblots. The formation of dimeres is enhanced in the presence of 10 micromolar free calcium as compared to 0.1 micromolar calcium. Dimerisation is furthermore strongly favoured by the lack of the C-terminus as found in CSP2. We are currently investigating the distribution and behaviour of the recently described isoforms CSP beta and gamma, which again differ mainly in their C-terminal part from CSP1.
Conclusion: Our results indicate that CSP interacts with the SNARE protein VAMP during insulin exocytosis and the availability of CSP for this interaction may be regulated CSP homodimerisation.

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Glucose-stimulated, pulsatile secretion from primary cultured pancreatic beta cells.
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Background and Aims: Pulsatile insulin secretion has been well documented in vivo, as well as in vitro from perfused pancreas and islets. At the cellular level, insulin secretion is hypothesized to occur in a regulated manner from secretory granules. Total internal reflection fluorescence (TIRF) microscopy allows direct observation of fluorescently labeled secretory granules near the plasma membrane, offering a means to image secretion from single living cells.
Materials and Methods: Primary cultured pancreatic beta cells were prepared from adult male rats using standard methods for islet isolation (collagenase infusion) and dispersion (dispase incubation with subsequent mechanical dispersion). Small clusters of cells (3-10 typically) were cultured in RPMI media (5 mM glucose) with 10% FBS. 16-24 hours after plating, cells were infected with adenovirus containing a construct for expression of either rat IAPP-EGFP or syncollin-GFP. Cells were imaged 36-48 hours following infection. For TIRF measurements, repetitive images were collected at approximately 5 Hz from cells continuously perfused with warmed (33 C) salt solution (3 mM Ca2+, 4 mM glucose). For stimulation, test solutions were locally applied.
Results: Clear examples of pulsatile secretion from individual cells within clusters occurred at glucose concentrations of 8, 12 and 20 mM. Not all cells responded to the glucose step, nor did all responding cells exhibit pulsatile secretion. The typical glucose response consists of several large (average 5, n=6), easily resolved pulses occurring within a few minutes of the glucose step (average ~4 mins, yielding and average frequency of 1.25 pulses/min). Single vesicle analysis of pulsatility is extremely time consuming, and we are developing automated algorithms. From preliminary analysis, approximately 15 vesicles (14 pulses from 3 cells) contribute to an average pulse.
Conclusion: Pulsatile secretion is directly observable from primary cultured pancreatic beta cells.

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Plasma membrane phosphatidylinositol 4,5-bisphosphate dynamics in individual insulin-secreting cells.

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Background and Aims: Phosphatidylinositol-4,5-bisphosphate (PIP_2) is increasingly recognized as an important messenger molecule in a variety of cellular processes, including insulin secretion. However, little is known about the distribution and dynamics of PIP_2 in insulin-secreting cells and the present study aimed at exploring these questions.
Materials and Methods: The phospholipase C (PLC)-delta1 pleckstrin homology (PH) domain fused to green fluorescent protein (GFP) was transiently expressed in insulin-secreting INS-1 cells. This PH domain binds with high affinity and specificity to PIP_2. Fluorescence was recorded with confocal and evanescent wave microscopy.
Results: Live confocal imaging revealed that in resting cells, the PH-GFP construct was predominantly found at the plasma membrane. Activation of phospholipase C with muscarinic or purinergic receptor agonists resulted in a dose-dependent, rapid drop of plasma membrane PH-GFP fluorescence and a reciprocal increase in the cytoplasm. The effects were reversed upon removal of the stimuli. Selective excitation of the plasma membrane with evanescent wave illumination facilitated the detection and quantification of the PH-GFP translocation. Using this technique it was found that depolarization with a high concentration of KCl resulted in a drop of membrane PIP_2 concentration (PH-GFP fluorescence decreased 21±2%; n=21). Most of this effect could be attributed to Ca^{2+}-activation of PLC, but a modest depolarization-induced decrease of the phospholipid was observed also in the absence of extracellular Ca^{2+} (8±1%; n=17). Cytoplasmic Ca^{2+} oscillations resulting from spontaneous or KCl-imposed rhythmic depolarizations were often associated with repetitive transient drops of membrane PIP_2 concentration.
Conclusion: Our data suggest that depolarization-induced Ca^{2+} oscillations in pancreatic β-cells may result in periodic activation of PLC and IP_3-mediated Ca^{2+} mobilization from the endoplasmic reticulum. The evanescent wave microscopy technique described here will be an important tool for further studies of the regulation of PIP_2 in individual pancreatic β-cells.

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Selective signaling via A- and B-type insulin receptors in insulin-producing cells involves different membrane microdomains.

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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 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. The insulin receptor (IR) exists in two isoforms as a result of alternative mRNA splicing that either lack (type A) or contain (type B) the 12 amino acids encoded by exon 11, which are located at the C-terminus of the α-chain of the receptor. While signaling via IR A-type/PI3 kinase Ia/p70s6 kinase and CaM kinase II up-regulates the transcription of the insulin gene, it requires signaling through the B-type isoform/PI3 kinase C2α-like and possibly Akt/PKB to up-regulate the β-cell transcription unit of the glucokinase gene (βGK). Little is known about the mechanisms that underlie IR isoform-specific signaling. One possibility to explain the differences in selective signaling is the different localization of the receptors in the β-cell plasma membrane and consequently the access to different adapter and effector proteins. The aim of this study was to evaluate the role of compartmentalization of the two IR-isoforms in different parts of the plasma membrane in selective insulin signaling in the pancreatic β-cell using insulin-promoter-driven DsRed expression (via IR-A) and βGK-promoter-driven GFP expression (via IR-B) as well as the distribution pattern of distinctly tagged IR isoforms in the β-cell plasma membrane as the read-out system.
Materials and Methods: The role of IR internalization was studied by dominant negative dynamin-2. Gradual cholesterol depletion of the plasma membrane and over-expression of caveolins were used to analyse whether different plasma membrane microdomains are involved in location and function of the IR isoforms. Western blot- and RT-PCR-analysis was used to study the expression of caveolin-1 and –2 in insulin-producing cell-lines. Digital fluorescence imaging and FRET analysis was used to study the distribution pattern of fluorescence-tagged IR isoforms.
Results: The IR isoform-dependent activation of the insulin-promoter (via IR-A) and the βGK-promoter (via IR-B) is I) not dependent on IR isoform-specific differences in receptor internalization, II) is dependent on signaling from cholesterol-sensitive membrane domains, III) is differently sensitive towards cholesterol depletion, and IV) is differently affected by the expression of caveolin-1 and –2.
Conclusion: Our data suggest the involvement of different plasma membrane microdomains in IR isoform-specific localization and signaling in the β-cell.

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Beta Cells: Glucagon

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Stimulation of glucagon release by cAMP in mouse pancreatic A-cells depends on Ca^{2+} influx through L-type Ca^{2+} channels.

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Background and Aims: Glucagon secreting A-cells are electrically excitable and generate spontaneous Na^{+}- and Ca^{2+}-dependent action potentials. Increase in intracellular Ca^{2+} initiate exocytosis of glucagon-containing granules. The aim of this study was to examine the mechanisms behind cAMP-dependent stimulation of glucagon secretion. Especially the involvement of the N- and/or L- type Ca^{2+} channels in basal and/or cAMP-stimulated exocytosis.
Materials and Methods: Exocytosis of secretory granules was measured as an increase in membrane capacitance. The whole-cell configuration of the patch-clamp technique was utilized to control membrane potential and to measure whole-cell currents.
Results: We studied the stimulatory effect of cAMP on rapid exocytosis by applying depolarizations from –70 mV to 0 mV of increasing duration (5-850 ms). Under control conditions, the increase in capacitance elicited by a 250 ms pulse was 213±48 fF (n=10). cAMP exerted a strong stimulatory effect on the exocytotic response (336±32 fF, n=27, $p< 0.05$). In the presence of cAMP, 50 μM of the L-type Ca^{2+}-channel antagonist nifedipine reduced the integrated Ca^{2+} current by 88±4% ($p<0.01$, n=7) and exocytosis by 90±15% ($P<0.01$, n=9). Application of 1 μM of the N-type Ca^{2+}-channel blocker ω-conotoxin-GVIA in the simultaneous presence of cAMP reduced the Ca^{2+}-current but contrary to the effect by nifedipine, the exocytotic response was not inhibited. A completely different pattern was obtained in the absence of cAMP. Under these experimental conditions the 60±1% ($p<0.01$, n=9) reduction in the Ca^{2+}-current by nifedipine did not affect the exocytotic response, whereas addition of 1 μM ω-conotoxin-GVIA caused a 58±9% ($p<0.01$, n=9) inhibition of the integrated Ca^{2+} current and a 88±12% ($p<0.05$; n=4) reduction of exocytosis. We were further interested in whether cAMP stimulates rapid exocytosis in the A-cell through the cAMP-sensor cAMP-GEFII. In the presence of the specific agonist for cAMP-GEFII, 8CPT-2Me-cAMP, the exocytotic response evoked by a train of ten depolarizations amounted 714±196 fF (n=6). This was not significantly different from control where the capacitance increase was 578±134 fF (n=6). Neither did application of Rp-cAMPS in continues presence of the agonist effect the exocytotic response.
Conclusion: We conclude that 1) cAMP-dependent stimulation of glucagon release depends on the influx of Ca^{2+} through L-type Ca^{2+} channels, whereas tonic release of glucagon is mediated by Ca^{2+} influx through N-type Ca^{2+}channels. 2) Different from the situation in insulin-secreting B-cells, cAMP-GEFII is either activated already under basal conditions or absent in A-cells.

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Hypersecretion of glucagon in mice with ablated R-type $Ca_V2.3$ calcium channels.

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Background and Aims: Glucose-induced electrical activity involves activation of voltage-gated Ca^{2+}-channels in the pancreatic B-cell, which is the trigger signal for insulin exocytosis. Mouse B-cells express different voltage-gated Ca^{2+}-channel subtypes, like L-type $Ca_V1.2$ channels and R-type $Ca_V2.3$ channels. $Ca_V1.2$ channels are closely associated with eliciting insulin exocytosis, but the specific role of the $Ca_V2.3$ channels is not fully elucidated.
Materials and Methods: $Ca_V2.3$ null mice ($Ca_V2.3^{-/-}$ mice) and their wildtype littermates (wt) were challenged in vivo by intraperitoneal glucose injections (11.1 mmol/kg). In vitro insulin and glucagon release were measured in batch incubations. Insulin and glucagon immunoreactivity were visualised by confocal immunocytochemistry. Whole-cell Ca^{2+}-currents and single B-cell exocytosis were investigated using the patch-clamp technique.
Results: In vivo glucose challenges (11.1 mmol/kg intraperitoneally) revealed a reduced glucose tolerance in $Ca_V2.3^{-/-}$ mice. Plasma glucose concentrations peaked 15 min after the glucose load and amounted 31.1± mmol/l in $Ca_V2.3^{-/-}$ mice, 23% higher than in wt ($P<0.05$; n=8).
Patch clamp experiments demonstrated that the voltage-gated whole-cell Ca^{2+}-currents in B-cells from $Ca_V2.3^{-/-}$ mice averaged 75% of that observed in wt mice ($P<0.05$). Depolarisation-evoked exocytosis in $Ca_V2.3^{-/-}$ B-cells decreased in proportion to the reduction in Ca^{2+}-influx (~~20%). Rates of exocytosis induced by intracellular dialysis of a Ca^{2+}/EGTA buffer were identical in both groups.
Confocal immunocytochemistry in intact and dissociated islet cells revealed that in wt mice, 80% of the cells stained positive for insulin. In the majority of the remaining cells (17% of total) glucagon immunoreactivity was detected. By contrast, in islet cells from $Ca_V2.3^{-/-}$ mice, 15% of the cell population stained positively for both insulin and glucagon. The the relative number of cells that revealed immunoreactivity for insulin or glucagon only, amounted 80% and a mere 2%, respectively.
In isolated $Ca_V2.3^{-/-}$ islets, glucose-stimulated insulin secretion was 18% reduced. Interestingly, glucose failed to suppress glucagon secretion in $Ca_V2.3^{-/-}$ islets. In wt islets, elevation of glucose from 1 to 20 mM reduced glucagon release from 36 to 20 pg/islet ($P<0.001$), whereas in $Ca_V2.3^{-/-}$ islets glucagon secretion was unaffected (43 and 41 pg/islet, in 1 and 20 mM glucose, respectively).
Conclusion: R-type $Ca_V2.3^{-/-}$ Ca^{2+} channels seem to play a role in the establishment of the glucagon-releasing A-cell lineage. The diabetic phenotype in $Ca_V2.3^{-/-}$ mice can be explained by a modest reduction in insulin secretory capacity, compounded by the failure of glucose to suppress glucagon release.

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AMPA receptor isoforms 2 and 3 mediate the stimulatory action of LY451646 on insulin and glucagon secretion in rats.

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Background and Aims: AMPA receptors belong to the class of ionotropic glutamate receptors and have been reported to modulate pancreatic hormone secretion. Here we have explored the expression profile of AMPA receptor isoforms and used the novel and highly potent AMPA receptor potentiator LY451646 to further investigate their effects on the regulation of insulin and glucagon secretion *in vitro* and *in vivo*.
Materials and Methods: RT-PCR was performed using standard protocols with total RNA isolated from human and rat islets. Insulin secretion was measured from batches of 5 islets isolated by collagenase treatment from male Wistar rats. Insulin was determined using SPA analysis. The *in vivo* efficacy of LY451646 was analyzed either in fasted or following an intravenous glucose tolerance test (IVGTT) in healthy male Wistar rats. Plasma levels of insulin and glucagon were measured by radioimmunoassay.
Results: Using RT-PCR, we found that AMPA receptor isoforms 2 and 3 are expressed in rat islets. Furthermore, performing restriction enzyme analysis with splice variant specific enzymes we found that both flip and flop splice variants of the AMPA receptor isoform 2 but only the flip variant of isoform 3 are expressed. A similar expression profile was determined in human islets, except that in addition the flip variant of AMPA receptor isoform 4 was identified. In rat islets, LY451646 induced glucose-dependent insulin secretion in a concentration dependent manner in the presence of 100 μM S-AMPA, whereas no stimulatory action was observed in the absence of S-AMPA. In an intravenous glucose tolerance test, LY451646 (0.1 and 0.5 mg/kg i.v.) significantly increased insulin secretion ($p<0.05$) at 3, 6, and 10 min after the i.v. glucose challenge (0.5 g/kg) in healthy rats. Plasma insulin levels were also significantly increased in fasted rats treated with 0.1-1 mg/kg LY451646 ($p<0.05$). Under these experimental conditions, plasma glucose concentrations were elevated by 19% (0.1 mg/kg; $p<0.05$) and 27% (1 mg/kg; $p<0.01$) 3 min after compound administration. It has previously been reported that activation of AMPA receptors leads to stimulation of glucagon secretion. To elucidate the mechanism behind the elevated insulin and glucose levels in the fasted rats after treatment with LY451646, we determined the plasma glucagon concentrations in these samples, which were increased between 70% (0.1 mg/kg; $p<0.01$) and 133% (1 mg/kg; $p<0.01$) at 3 min. The increased plasma glucagon concentrations most likely induced the elevation of plasma glucose concentrations under fasting conditions.
Conclusion: Our data suggest that activation of AMPA receptor isoforms 2 and 3 leads to enhanced secretion of both insulin and glucagon from the pancreatic islets.

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Adrenaline activates and glucose inhibits a store-operated depolarizing mechanism regulating glucagon secretion from the pancreatic α-cells.

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Background and Aims: Stimulus-secretion coupling underlying glucagon secretion is not well understood. There are diverging opinions about the possible involvement of K_{ATP} channels in nutrient inhibition of the release of this blood glucose-elevating hormone.
Materials and Methods: To clarify the mechanisms we have studied cytoplasmic Ca^{2+}concentration ($[Ca^{2+}]_i$) and membrane potential in individual mouse pancreatic α–cells, which were later identified by immunostaining.
Results: The secretagogue L–adrenaline increased $[Ca^{2+}]_i$ in α-cells causing initial mobilization of intracellular Ca^{2+}, followed by a late response due to activation of store–operated influx of the ion as well as depolarization with influx through voltage–dependent L–type channels. The α–cells express ATP regulated K^+ (K_{ATP}) channels, whose activation by diazoxide leads to hyperpolarization. The resulting inhibition of the voltage–dependent $[Ca^{2+}]_i$ response to adrenaline was reversed when the K_{ATP} channels were inactivated by tolbutamide. Like diazoxide glucose hyperpolarized the α–cells and inhibited the adrenaline–induced $[Ca^{2+}]_i$ signaling and concentrations as low as 3 mM had a pronounced stimulatory effect on Ca^{2+} sequestration in the ER. Release of Ca^{2+} from the ER after exposure to inhibitors of the sarco(endo)plasmic reticulum Ca^{2+} ATPase also resulted in activation of store–operated Ca^{2+} influx, depolarization and opening of the voltage–dependent Ca^{2+} channels.
Conclusion: The results do not support the involvement of K_{ATP} channels in the regulation of glucagon secretion but indicate that adrenaline stimulation and glucose inhibition of the α–cell involves modulation of a store–operated current which controls a depolarizing cascade leading to opening of L–type Ca^{2+} channels.

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Localization in human pancreatic islets of miniglucagon and of the enzymatic complex responsible for its processing from glucagon.

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Background and Aims: Miniglucagon, the COOH-terminal (19-29) fragment of glucagon, is produced through post-translational processing at the Arg_{17}-Arg_{18} dibasic site of the mother-hormone and displays original biological features, including a strong inhibitory effect on insulin secretion. Moreover, we know that the enzyme wich cleaves glucagon into miniglucagon, is a complex of two metalloproteases, NRD convertase (NRDc) and Aminopeptidase B (ApB) acting sequentially. This processing mechanism, at the root of the regulatory processes which implicate the glucagon/miniglucagon balance, takes place in different tissues including rat islets and liver and is mirorred in cell lines from those tissues, such as α pancreatic α-TC1.6 or hepatic Fao cell lines. Our aims were 1) for the first time, to analyze the presence and the localization of miniglucagon in human pancreatic islets ; 2) to analyze the presence and the localization of NRDc and ApB in the human pancreas, in comparison with what we observed in the rat.
Materials and Methods: Radioimmunoassays (RIAs) were used to detect the presence of glucagon and miniglucagon in extracts from human pancreas. Immunofluorescence and ultrastructural immunogold detection were performed using sections of human pancreas and ultrathin sections of human islets of Langerhans, respectively. The same primary antibodies recognizing insulin, the N-terminal epitope of miniglucagon, the central glucagon epitope, NRDc or ApB, were used in both types of experiments.
Results: Using RIA, we detected, for the first time, the presence of miniglucagon in human pancreas with proportions respective to glucagon similar to that observed in the rat. Using the confocal immunofluorescence technique, we observed that miniglucagon is present only in glucagon-secreting α-cells, in which glucagon and miniglucagon immunoreactivities, both punctuated, colocalize in the cytoplasm. Immunostaining of NRDc and ApB was visible in the cytoplasm of α-cells and, to a lesser extent, of other cell types (both endocrine and exocrine cells). Both enzymes appear, thus, to be more or less ubiquitous in the pancreatic tissue. On the other hand, they were always present together with their substrate glucagon. The immunogold electron microscopic technique allowed us to confirm that the miniglucagon colocalize with glucagon immunoreactivity in the electron-dense core of the mature α-secretory granules in which we also observed NRDc and ApB immunoreactivities.
Conclusion: As observed in the rat, miniglucagon is present in glucagon-containing α secretory granules of the human islets of Langerhans. The presence of NRDc and ApB in the same granules extend to the human species the observation made in rodents that these two metallo-proteases are responsible for the post-translational processing of glucagon into miniglucagon. These observations urge us to analyze in future studies possible variations in the expression of both enzymes, in view of the probable implication of the glucagon/miniglucagon balance in the physiopathology of human type 2 diabetes.

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Glucagon Receptor activates the Mitogene-activated protein kinase (p42/44 MAP kinases, ERK1/2) signaling cascade via a cyclic AMP/PKA-mediated pathway in pancreatic β cells.

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Background and Aims: In pancreatic β cells, the effects of secretagogues which use the cAMP/Protein kinase A (PKA) pathway are not restricted to the control of exocytosis of insulin secretory granules, but also extend their influence to regulation of gene transcription, cell proliferation and growth. We determined whether the glucagon receptor (GR), known to utilize the cAMP/PKA pathway, recruit in parallel downstream signaling components of the tyrosine-kinase receptors system thus increasing its repertoire of physiological effects on β cells.
Materials and Methods: We used the MIN6 pancreatic β cell line, insulin radioimmunoassay, western blotting using specific antibodies recognizing the active, phosphorylated forms of ERK and immunofluorescence confocal microscopy.
Results: we show that activated GR present in β cells are positively coupled to adenylate cyclase, lead to an increase in cAMP levels which favours in turn calcium uptake, the increased free cytosolic calcium level potentiating glucose-stimulated insulin release. We next investigated whether glucagon receptors activate the p42/44 MAP kinases (ERK1/2) concomitantly to the cellular events which trigger potentiation of insulin release and used pharmacological approaches to identify the signaling pathways leading from GR to ERK 1/2 activation. For the first time, we show that the GR present in β cells are positively coupled to the ERK 1/2 cascade. In the presence of stimulatory glucose concentrations, we observed that glucagon extends the duration of the glucose-induced ERK1/2 activation, while rapid kinetics of ERK1/2 activation by glucagon were observed in the absence of glucose. We also show that glucagon-induced MEK 1/2 and ERK 1/2 activation is mediated by the cAMP-PKA pathway and that an increase in the intracellular calcium concentration is required for activation of PKA and, subsequently, of MEK 1/2 and ERK 1/2 in the presence of glucose. In contrast, glucagon-induced activation of ERK 1/2 in the absence of glucose is cAMP-PKA dependent but independent from extracellular calcium influxes. Interestingly, we also found that, in both situations (presence or absence of stimulatory glucose), internalization of the GR through clathrin coated-pits formation is required for the ERK 1/2 cascade activation.
Conclusion: these new data now urge us to determine 1) the role of the glucose context and the subsequent variations of the intracellular calcium concentrations on the recruitment of key molecular components downstream the GR responsible for ERK1/2 activation ; 2) whether the glucagon effect on ERK1/2 is modulated by miniglucagon, the C-terminal glucagon fragment released together with glucagon, as observed on insulin release; 3) what precise repertoire of final physiological effects in β cells is triggered by glucagon through the activation of ERK1/2, such as induction of the expression of early response genes known to be implicated in β cell growth and differenciation.

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Beta Cell Function in Diabetes

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Neonatal beta-cell hyperactivity in nonobese diabetic (NOD) and lymphocyte-deprived NOD*scid* mice.

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Background and Aims: Despite extensive research, the pathogenesis of type 1 diabetes (T1D) remains unresolved. We hypothesized that anomalies of the islets of Langerhans, in addition to those of the immune system, are required for the development of the autoimmune reaction. In the spontaneous model of T1D, the NOD mouse, but also in NOD*scid* mice that lack functional lymphocytes and do not develop insulitis and diabetes, we previously reported, compared to various control strains, a transient hyperinsulinemia that appears after weaning (3 weeks of age) concomitantly with the first infiltrating macrophages and dendritic cells. We wondered whether this beta-cell hyperactivity was the result of previous glucose stimulation, due to possible alteration of maternal glucose homeostasis.
Materials and Methods: We studied various parameters of beta-cell distribution (insulin immunohistochemistry (IHC) and image analysis), and activity (insulin radioimmunoassay (RIA), RT-PCR for preproinsulin I and II and *in situ* hybridization for preproinsulin II), in relationship with glycemia and insulinemia in NOD, NOD*scid* and control C57BL/6 mice, from birth to 4 weeks of life.
Results: NOD*scid* neonates had significantly higher glycemia that NOD and C57BL/6 mice at 1 and 2 weeks of age, with slightly (but not significantly) increased insulinemia. At 4 weeks of age, insulinemia was higher NOD and NOD*scid* than control mice. Pancreatic insulin contents and mean islet size were similar in the 3 strains during the first month of life. However, NOD and NOD*scid* neonates had, at 1 day of age, twice as many very small islets (< 2000 pixels) as C57BL/6 mice. Using RT-PCR, significant increased expression of preproinsulin I and II was observed in both NOD and NOD*scid* mice between 1 and 2 weeks of life. *In situ* hybridization showed that 1-day-old NOD neonates expressed significantly higher levels of primary transcripts of preproinsulin II than age-matched C57BL/6 neonates but this difference disappeared during the first week of life. Finally, NOD and NOD*scid* mothers were treated with insulin (Ultratard, NovoNordisk, 1U/100g BW) during the last 2 weeks of gestation: at birth, insulin transcriptional activity was down-regulated in females NOD neonates but not in males nor in both sexes of NOD*scid* neonates.
Conclusion: Signs of beta-cell hyperactivity exist in NOD and lymphocyte-deficient NOD*scid* neonates. While periods of glucose intolerance in NOD mothers might stimulate beta-cell activity in their fetuses, in NOD*scid*, insulin resistance, possibly due to HPA axis disturbance, might play a role.

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Yearly lowering of insulin secretion capacity in Type 2 diabetic patients - a cross-sectional study.

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Background and Aims: It has been shown that insulin secretion capacity is reduced year by year in type 2 diabetes mellitus, however, it remains to be proven whether the reduction is common in all patients or not, and the secretion capacity may eventually be completely exhausted in type 2 diabetes. We cross-sectionally analyzed Japanese adults with type 2 diabetes for correlations between insulin secretion capacity and diabetes duration, as well as other factors influencing insulin secretion.
Materials and Methods: We studied 365 consecutive Japanese type 2 diabetic patients, excluding those with infection, liver disease, gastrectomy, positive GAD antibody, and unknown diabetic history, who were admitted to our hospital during the period from July 2000 to June 2002. Their mean age was 60.0±12.2 years old (17-84), age of onset 51.4±11.8 years old (17-82), duration after diagnosis of diabetes 8.6±8.8 years (0-46), BMI 24.4±4.0 (15.0-46.7), HbA1c 9.2±2.2% (5.2-16.8), FPG 175±54mg/dl (65-357), fasting serum C-peptide (CPR) 1.71±0.97ng/ml (0.00-6.94) (Mean±1SD). As markers of insulin secretion capacity we tested serum CPR increment around breakfast and glucagon i.v. load on admission. We statistically analyzed the association between each insulin secretion marker and duration after diagnosis or other clinical indicators including age and BMI using correlation coefficient and multivariate analysis.
Results: Increment of serum CPR values by glucagon test, as well as by meal load was reduced in relation to year after diagnosis of diabetes, however, no patients deteriorated in insulin secretion capacity. The increment of serum CPR by glucagon load was most significantly correlated with duration of diabetes (y=-0.0409x+2.60, r=-0.3300, p<0.0001) by simple regression analysis. Using this regression equation, CPR level was 1.78ng/ml at 20 years and 0.96ng/ml at 40 years, thereafter CPR was theoretically exhausted at 63.6 years after diagnosis. Every CPR value was positively correlated with BMI, and was negatively correlated with patient age, other than meal load CPR values. However, a multivariate analysis excluded the effects of patient age on yearly reduction of CPR values 6 min. after glucagon load. Moreover, with multivariate analysis of increment of glucagon load CPR values, diabetes duration was judged as the greatest contributor to yearly reduction of insulin secretion capacity among clinical indicators. With analysis using these CPR values, non-obese patients(BMI<25, n=212) more slowly decreased by year in insulin secretion capacity (y=-0.0351x+2.37, r=-0.3195, p<0.0001) compared with obese patients(BMI≥25, n=153; y=-0.0419x+2.85, r=-0.2945, p<0.0003).
Conclusions: 1) Insulin secretion capacity decreases year by year in type 2 diabetes, however it is not completely exhausted. 2) Among markers of insulin secretion, increment of CPR by glucagon load is most useful in evaluation of yearly lowering of the capacity. 3) Non-obese patients experience slower yearly lowering of insulin secretion capacity than obese patients.

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Non-glucose stimulated early insulin secretion is present even after long duration of Type 2 diabetes.

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Background and Aims: The progressive hyperglycaemia of type 2 diabetes mellitus (T2DM) is usually considered caused by loss of β-cell function. However, the nature of this loss in insulin secretion, and whether it is associated with long term glycaemic control, remains unclear. In a 10-year follow-up study of 41 subjects in the OCTOPUS-study, we evaluated glucose- and non-glucose stimulated insulin secretion in relation to average HbA_1c.
Materials and Methods: Insulin secretion was studied before and after a follow-up of 9.7 (1.1) (mean (SD)) years, with C-peptide measurements before and after 1 mg of glucagone i.v. (C-peptide test). In a subgroup of 8 subjects with poor glycaemic control during 10 years (average HbA_1c 9.0 (0.4)%) and 12 subjects with good control (average HbA_1c 7,4 (0.4)%) insulin secretion was also studied at the end of the 10-year follow-up period with a hyperglycaemic (+6 mmol/l) clamp that included the administration of 5 g aginine after 2h of hyperglycaemia and with measurements of fasting plasma glucose and C-peptide calculating insulin secretory index with the homeostasis model assessment (HOMA-B). Baseline data from the OCTOPUS study: age 59,3 (6,2) years, duration of diabetes 7,3 (3,1) years, BMI 26,7 (3,7) kg/m^2, HbA_1c 8,6 (1,5) %. All were Anti-GAD negative in the subgroup.
Results: The peak C-peptide value after glucagon decreased significantly during the 9.7 years follow-up from 1452 (502) to 841 (475) pmol/l, p<0,001. As expected, no first phase glucose-stimulated increase in C-peptide was observed during the clamp, and neither did we observe any significant increase in C-peptide levels during the first hour of the clamp. However, the injection of arginine increased C-peptide levels by 721 (584) pmol/l (p<0,001), with a peak 2-5 minutes after the injection. The increase correlated with arginine stimulated insulin secretion in a fasting state (r=0, 83). We observed no differences in loss of β–cell function, measured as C-peptide secretion, among the subjects with poor or good glycamic control: delta value of arginine stimulation: 580 (259) vs. 811 (719) pmol/l, delta value of glucagon stimulation: 488 (264) vs 369 (210) pmol/l, delta value of first hour of hyerglycaemia: 207 (100) vs. 174 (101) pmol/l and HOMA-B: 47,5 (20) vs. 48,1 (22) % (all ns). Furthermore, the decline in β–cell function, observed with the C-peptide test, was not correlated to average HbA_1c through the observation period.
Conclusion: Non-glucose stimulated early insulin secretion was still considerable after a mean duration of T2DM of nearly twenty years, while

glucose-stimulated insulin secretion could not be demonstrated. Glucagon-stimulated insulin secretion decreased significantly during 10-years of follow-up. We did not find any relationship between glycaemic control expressed as the average HbA_1c during 9.7 years and the reduction in insulin secretion. Our findings may suggest that the primary defect in insulin secretion after many years with T2DM is caused by impaired glucose signalling in the β-cells.

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Parasympathetic blockade attenuates differences in pancreatic polypeptide but not insulin secretion in Pima Indians versus Caucasians.

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Background and Aims: The hyperinsulinemia of type 2 diabetes may be vagally mediated. Compared with Caucasians, Pima Indians have a high risk of diabetes, hyperinsulinemia and elevated plasma pancreatic polypeptide (PP), a measure of the parasympathetic nervous system (PNS) drive to the pancreas.
Materials and Methods: To test if hyperinsulinemia is, in part, due to excessive vagal stimulation of the β-cell, we examined the effect of PNS blockade in 17 Caucasian [age 35±7y, body fat 23±7% (mean±SD)] and 17 Pima Indian males [age 28±8y, body fat 29±5%] with normal glucose tolerance. Each individual underwent 4 consecutive standardized liquid meal tests (64% CHO, 22% fat, 14% protein) during which a primed infusion of atropine was administered for 120 min at the following doses: 0, 2.5, 5 and 10 µg/kg FFM/h. Areas under the curve for early ($AUC_{0\text{-}30min}$) and total ($AUC_{0\text{-}120min}$) insulin (INS) and PP secretory responses were calculated. INS was adjusted for plasma glucose to compensate for the effect of atropine on the gastric emptying rate.
Results: Early INS and PP secretory responses were higher in Pima Indians compared to Caucasians (both p=0.01). Secretion of INS and PP was inhibited by atropine (both p<0.001). Increasing doses of atropine attenuated the ethnic difference in PP but not in INS (p=0.01, p=0.6, respectively for race*dose effect). Similar results were observed for total secretory responses.
Conclusion: Pima Indians have an exaggerated PNS drive to the pancreas compared to Caucasians. However, hyperinsulinemia in Pima Indians does not appear to be primarily due to increased vagal stimulation of the β-cells.

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Effect of nateglinide on beta-cell function in patients with mild Type 2 diabetes: a model analysis.

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Background and Aims: We recently developed a novel model-based approach to estimate parameters of *in vivo* ß-cell function in response to physiologic stimuli, such as a mixed meal. In this study, we tested whether nateglinide (Starlix), a rapid acting oral insulin secretagogue, improve ß-cell function in patients with mild type 2 diabetes (DM2).
Materials and Methods: Men and women (n=108) with DM2 (mean fasting glucose: 7.0-8.3 mM) on diet alone were randomized to nateglinide (30, 60 or 120 mg *tid*) or placebo for 24 weeks. Parameters of beta-cell function were derived by mathematical modelling of glucose and C-peptide responses to a standard mixed meal at baseline and 24 weeks. The model featured a glucose concentration-insulin secretion dose-response (parameters: secretion at 7 mM glucose, glucose sensitivity=slope of the dose-response), a secretory component proportional to the glucose concentration derivative (rate sensitivity), and a factor (potentiation factor) expressing potentiation of insulin secretion by prolonged hyperglycemia and incretins (AJP 283:E1159, 2002).
Results: Baseline demographic and metabolic characteristics were similar in the 4 groups. Treatment with nateglinide resulted in dose-dependent reductions in the mean post-prandial (PP) glucose response (-0.34±0.34, -0.80±0.28 and -1.26±0.32 mM, p<0.001, with 30, 60 and 120 mg, respectively) and, at the 120-mg dose, decreases in fasting glucose (-0.33±0.24 mmol/L, p=0.01) and HbA_{1c} (-0.39%, p<0.01). There were no significant effects of nateglinide on fasting insulin secretion (137±13 vs 126±10 pmol/min/sqm, week 0 *vs* 24, all doses), while total insulin output during the meal decreased slightly (66±6 *vs* 59±3 nmol/sqm, p<0.05, all doses). In contrast, secretion at 7 mM glucose increased in dose-dependent manner (164±15 *vs* 246±31 pmol/min/sqm, p<0.002, at the 120 mg dose); rate sensitivity was significantly enhanced at 24 weeks with the lowest nateglinide dose (0.42±0.09 *vs* 1.15±0.20, p<0.001), with no further stimulation at higher doses. Both glucose sensitivity and potentiation showed a trend towards increasing with increasing nateglinide dose, and were significantly greater than placebo at the 120 mg dose. By multiple regression, changes in rate sensitivity, glucose sensitivity, and potentiation all contributed to the observed changes in both absolute and incremental PP glucose concentrations.
Conclusion: Parameters of insulin secretory dynamics in response to a mixed meal show improvements with 24 weeks of treatment with nateglinide and predict the corresponding changes in glucose tolerance. These results highlight the potential value of these new *in vivo* indices of beta-cell function.

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Metformin ameliorates function and survival of pancreatic islets isolated from Type 2 diabetic patients.

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Background and Aims: Metformin (Met) is widely used for treatment of type 2 diabetes (T2D). Its main site of action is considered the liver, where it increases insulin action. In vitro studies, however, have suggested a possible action of Met on beta-cells.
Materials and Methods: We have directly tested this hypothesis by exploring the effect of therapeutic concentration of Met (2.4 µU/ml) in isolated pancreatic islets prepared from 4 multiorgan donors with Type 2 diabetes (T2D).
Results: Compared to islets from 4 matched non-diabetic donors, T2D islets had reduced insulin content (76±35 vs 118±29 µU/islet, p<0.05), impaired glucose-induced insulin secretion (2.9±1.7 vs 4.0±1.0% of insulin content, p<0.05, with absence of first-phase), increased apoptosis (ELISA, 1.8±0.3 vs 0.9±0.2 OD a.u., p<0.05), and enhanced activity of caspase 3 and 8 (respectively 0.17±0.03 vs 0.11±0.02 OD, and 0.19±0.04 vs 0.10±0.02 OD, both p<0.05). Moreover, mRNA expression (ratio over beta-actin mRNA) of catalase (0.8±0.2 vs 0.3±0.06, p<0.05) and GSH peroxidase (1.2±0.1 vs 0.6±0.06, p<0.05) was increased in T2D islets, suggesting enhanced oxidative stress. In addition, mRNA expression of AMP-activated protein kinase (AMPk) was lower in T2D islets than in Ctrl cells (0.33±0.06 vs 0.83±0.06, p<0.05). 24-hr incubation with Met was associated with increased insulin content (102±27 µU/islets) and improved glucose-induced insulin release (3.5±0.7% of insulin content, with partial restoration of first-phase). Apoptosis decreased to 1.2±0.2 OD, with concomitant reduction of caspase 3 and 8 activity (0.12±0.02 and 0.12±0.03 OD). In addition, normalization of the expression of catalase (0.2±0.02) and GSH peroxidase (0.5±0.01) occurred, and mRNA expression of AMPk increased (0.82±0.09).
Conclusion: These results demonstrate that isolated T2D pancreatic islets have several functional and survival defects, which can be corrected by therapeutic dose of metformin; the beneficial effects of the drug are likely to be mediated by an anti-oxidative mechanism. The possibility that these effects might be regulated through modulation of AMPk expression remains to be elucidated.

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The DPP IV resistant GLP-1 analogue, BIM 51077, improves diabetic control in ZDF rats.

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Background and Aims: Natural glucagon-like peptide 1 (GLP-1) has potent incremental properties with respect to glucose-induced insulin secretion, but its therapeutic usefulness is limited by its short biological half-life as a result of the action of dipeptidyl peptidase IV. BIM 51077, [$Aib^{8,35}$] hGLP-1(7-36 amide), is a peptide analogue with improved *in vitro* stability and full potency compared to GLP-1(7-36)NH_2 in both insulin secretion studies using isolated rat islets and *iv* glucose tolerance studies in fasted conscious rats. The aim of the present study was to examine the chronic anti-diabetic potential of BIM 51077 in diabetic male ZDF rats.

Materials and Methods: Male ZDF rats were obtained in a prediabetic state and at age 7-8 weeks, just before the normal onset of diabetes, they were provided with an intraperitoneally implanted osmotic minipump delivering either vehicle (physiological saline) or BIM 51077 at either 1.5 pmol.kg^{-1}.min^{-1} or 15 pmol.kg^{-1}.min^{-1} for a period of 28 days.
Results: The lower dose of BIM 51077 had no effect on any parameter. However, BIM 51077 at 15 pmol.kg^{-1}.min^{-1} produced a significant decrease in food intake relative to controls. Water consumption was used as a surrogate marker of the development of urinary glucose excretion. Water consumption of control rats increased throughout the study in line with the development of diabetes. This increase was markedly attenuated by BIM 51077. The suppression of the development of the diabetic state was also evident from a reduction in the fed blood glucose concentration, reduction in plasma fructosamine (control 4.83 ± 0.54 mmol.l^{-1}, BIM 51077 3.06 ± 0.22 mmol.l^{-1}, $p < 0.01$) and blood $HbA1_c$ (control 5.37 ± 0.40 % total Hb, BIM 51077 4.14 ± 0.34 % total Hb, $p < 0.05$). Oral glucose tolerance measured after 28 days treatment was also improved by treatment with BIM 51077.
Conclusion: The GLP-1 analogue, BIM 51077, was effective in preventing the development of diabetes in male ZDF rats. This finding together with the improved pharmacokinetics relative to natural GLP-1 as a result of its resistance to degradation by DPP IV suggests that BIM 51077 may have therapeutic utility.

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Inhibitors of the interaction between neuronal NO synthase and its protein inhibitor PIN corrects hyperinsulinic secretion of obese Zucker rats.
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Background and Aims: We have previously shown that pancreatic β-cells express a neuronal isoform of NO synthase (nNOS), which controls glucose-induced insulin secretion. We also demonstrated the presence of the protein inhibitor of nNOS, PIN, acting through blockade of nNOS dimerization. As nNOS pharmacological blockade results into an insulin secretory pattern in response to glucose similar to that occurring in prediabetic states, we wondered if the inhibition of the binding of nNOS to its inhibitor PIN might correct or improve pancreatic β-cells hyperresponsiveness to glucose in the Zucker fa/fa rat.
Materials and Methods: Chemical molecules inhibiting PIN-nNOS interaction were obtained by the screening of a chemical bank in an ELISA format. Molecules were then tested on isolated islets of Langerhans and the isolated rat perfused pancreas of obese and lean Zucker rats in the presence of 11.2 mM glucose.
Results: Two of the molecules obtained in the screening test, IDR 03 and IDR 04, were able to dose-dependently inhibit PIN-nNOS interaction in vitro (from 10 to 100 µM). On isolated islets from obese Zucker fa/fa rats, IDR 03 and IDR 04 decreased glucose-induced insulin secretion by respectively 17% and 21% at 10 µM and 63% and 49% at 100 µM. In the isolated perfused pancreas of obese Zucker fa/fa rats, mean integrated insulin response to a 20-min rise in glucose concentration from 4.2 to 11.2 mM averaged 1065 ± 385 ng x 20 min. Such a value was significantly reduced to 524 ± 94 and 464 ± 152 ng x 20 min ($p < 0.001$) by respectively IDR 03 and IDR 04 (10 µM), with a pattern and a magnitude very close to that observed in lean Zucker fa/+ rats (435 ± 47 x 20 min).
Conclusion: Chemical molecules, shown to inhibit PIN-nNOS interaction, are able in vitro to reestablish a normal secretory response to glucose in the hyperinsulinic obese Zucker rat.

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The lower fetal beta-cell mass is differently programmed by maternal protein restriction or general food restriction in the rat.
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Background and Aims: Malnutrition *in utero* may lead to glucose intolerance and diabetes later in life as revealed by epidemiological studies. In rats, both maternal Protein Restriction (PR) and General Food Restriction (GFR) reduce the foetal beta-cell mass and impair glucose tolerance in adults. As the beta-cell mass is determined by an adequate balance between differentiation (neogenesis), proliferation and apoptosis, we compared the impact of the 2 types of maternal food restriction on these processes in rat foetuses.
Materials and Methods: Pregnant Wistar rats were fed either a protein-restricted diet (8% instead of 20% protein throughout gestation, PR) or a generally food-restricted diet (50% during the last week of gestation, GFR). Offspring were analysed on the last day of gestation ($F_{21.5}$). Foetal pancreases were either fixed and embedded to analyse islet vascularisation, or digested to obtain islets which were cultured during 7 days. Pdx1 mRNA levels in islets were analysed by RT-PCR, islet cell proliferation was quantified by measuring BrdU incorporation into islet cell nuclei, and apoptotic rates were measured using the TUNEL method.
Results: Protein restriction did not affect the foetal islet number/cm^2, but impaired beta-cell proliferation ($p<0.05$) and enhanced beta-cell apoptosis ($p<0.05$) *in vivo*. Correspondingly, *in vitro*, PR also reduced the proliferation of islet cells by 50-60% ($p<0.01$) and enhanced their apoptotic rate by 40-50%($p<0.01$), without affecting mRNA levels for the transcription factor Pdx1. In addition, islet vascularisation *in vivo* was reduced by 25-30% in PR foetuses ($p<0.05$). In contrast,General Food Restriction impaired differentiation, as shown by a reduced islet number/cm^2 *in vivo* ($p<0.05$). The mRNA levels for Pdx1 were decreased by 25-30% in cultured islets from GFR foetuses ($p<0.05$) and in pancreatic buds from GFR foetuses at F_{17} ($p<0.05$). GFR affected neither proliferation nor apoptotic rates of foetal islets *in vitro*. Foetal islet vascularisation *in vivo* was unaffected by GFR.
Conclusion: Although the 2 types of foetal malnutrition lead to a reduced foetal beta-cell mass, the cellular and molecular mechanisms responsible for this reduction are different.

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Mitochondrial dysfunction and increased UCP2 expression following overexpression of β -cell cytosolic phospholipase A_2: loss of nutrient-induced insulin secretion.
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Background and Aims: Cytosolic phospholipase A_2 ($cPLA_2$) is expressed in β -cells where it catalyses the production of arachidonic acid (AA). Long term exposure to excess levels of fatty acids, including AA, is detrimental to cell function through increased expression of the mitochondrial uncoupling protein UCP2, which uncouples oxidative phosphorylation from ATP generation. We have created stably transfected MIN6 β -cells that constitutively overexpress $cPLA_2$ resulting in high levels of intracellular AA, and we have now studied the effects of this chronic excess of AA on β -cell function.
Materials and Methods: Changes in NAD(P)H autofluorescence and mitochondrial rhodamine 123 fluorescence in response to glucose were detected by fluorescence spectroscopy, and MIN6 cell UCP2 mRNA levels were quantified by real-time RT-PCR. Microfluorimetry was used to determine changes in intracellular calcium ($[Ca^{2+}]i$) in fura-2 loaded MIN6 cells. Insulin secretion from MIN6 cells formed as pseudoislets was measured in a perifusion system
Results: Non-transfected MIN6 cells showed a rapid and sustained increase in NAD(P)H autofluorescence in response to 25mM glucose, and this was reduced by ~95% in MIN6 cells overexpressing $cPLA_2$.This effect was

mimicked in non-transfected MIN6 cells by 20µ M FCCP, a mitochondrial uncoupler. Quantitative RT-PCR indicated that mRNA for UCP2 was increased in the $cPLA_2$ overexpressing MIN6 cells, and this could be prevented by 24 hour exposure to 100µM MAFP, a $cPLA_2$ inhibitor (control: 36± 3.2 copies UCP2/100 copies β -actin; transfected: 92± 10.1 copies; transfected + 100µM MAFP: 43± 0.9 copies, P<0.001 controls vs transfected; P<0.001 transfected vs transfected + MAFP). Glucose caused a decrease in rhodamine 123 fluorescence in control cells, but not in those overexpressing $cPLA_2$, consistent with the transfected cells being unable to maintain mitochondrial proton gradients as a consequence of UCP2 up-regulation. Calcium microfluorimetry measurements confirmed that $cPLA_2$ overexpressing cells were unable to respond appropriately to glucose (only 4% of transfected cells were glucose-responsive; P<0.001 vs controls), but overexpression of $cPLA_2$ had no effect on non-nutrient-induced increases in $[Ca^{2+}]i$ (control: 98% of cells responsive to 20mM KCl; transfected: 99% responsive, P>0.2). Glucose-induced insulin secretion from transfected cells was absent (109±15.2% basal; control: 326±24.9% basal, n=3, P<0.01), but 20mM KCl caused a sustained increase in insulin secretion from both cell populations (control: 831% basal after 20 min; transfected: 812% basal).
Conclusion: Our data indicate that excess AA results in severe impairment of the calcium and secretory responses of β -cells to nutrients, most likely through up-regulation of UCP2 and uncoupling of mitochondrial metabolism from ATP generation.

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Nutrient modulation of palmitoylated 24 kDa protein in rat pancreatic islets.
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Background and Aims: Nutrients such as glucose (glc) stimulate insulin release *via* ATP-sensitive potassium channel-dependent and -independent pathways. Molecular mechanism of the former pathway has been well delineated, however, little is known about the latter pathway, for which the role of protein acylation is implicated. Accordingly, identification of the target(s) of acylation was attempted in the pancreatic islets for the first time.
Materials and Methods: Freshly isolated rat islets were labeled with [^{3}H]palmitic acid for 1 h at 37°C and the whole cell lysate was analyzed by SDS-PAGE and 2 dimensional gel electrophoresis (2-DG). Immunoprecipitation of the labeled protein(s) by anti-SNAP-25 antibody was also performed. Intensity of the labeling was quantified by densitometry and statistical analysis performed.
Results: The labeling of the proteins by [^{3}H]palmitic acid was shown to be palmitoylation by the standard chemical analysis. Palmitoylation of 4 distinct bands (70 kDa, 37-50 kDa smear, 30 kDa, and 24 kDa doublet) was recognized and all of them were significantly attenuated upon labeling with high glc (> 11.1 mmol/l). A maximum glc effect was seen at 22 mmol/l and it was significantly greater (55% lowering) for the 24 kDa doublet than for other 3 bands (35% lowering each). Palmitoylation of the 24 kDa doublet was selectively attenuated by the mitochondrial fuels (20 mmol/l ketoisocaproate or a combination of 10 mmol/l glutamine and 2 mmol/l leucine) and an acylation inhibitor, cerulenin (> 30 µg/ml). High glc attenuation of the palmitoylation of the doublet was partially blocked by 20 mmol/l mannnoheptulose, a glucokinase inhibitor. A $t_{1/2}$ of the doublet (45 min) was significantly shorter than that of other 3 bands (> 120 min) upon pulse-chasing, irrespective of the presence or absence of high glc during the chase period. Pretreatment of the islets with 10 µmol/l cycloheximide for 1 h preferentially weakened the palmitoylation of the 24 kDa doublet. By 2-DG, the doublet was separated into acidic peptides. All of the 4 major palmitoylated bands were distinct from and not associated with SNAP-25.
Conclusion: We successfully demonstrated protein palmitoylation in the islet cells, and identified rapidly turning over 24 kDa acidic peptides distinct from SNAP-25. Application of nutrients causes cytosolic accumulation of (non-radioactive) LC-CoA in this cell type, which would lower the specific activity of [^{3}H]palmitic acid in the cytosol. This is expected to attenuate the labeling of any protein to a similar degree. Thus, a silimlar degree of suppression by high glc in the labeling of the 3 bands other than 24 kDa doublet is well explained by such a mechanism. A significantly greater attenuation of the 24 kDa doublet palmitoylation by nutrients and cerulenin is therefore most intriguing, which indicated existence of novel modulatory mechanism(s) for the palmitoylation of the doublet, though it remained to be elucidated. The data suggest functional role of the palmitoylated 24 kDa doublet in nutrient stimulation of insulin secretion.

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Epiregulin stimulates proliferation and insulin secretion in INS-1E and RINm5F insulinoma rat cell lines.
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Backgrounds and Aims: Growth factors play a key role in the proliferation of pancreatic beta cells. Epiregulin, a new member of the epidermal growth factor (EGF) family, has previously been shown to stimulate growth on several cell types (keratinocytes, fibroblastes). In this study, we determined if this growth factor could have a similar potent on insulinoma rat cell lines, INS-1E and RINm5F, and investigated its effects on insulin secretion. Further, the mechanisms by which epiregulin could act on these cell lines were analysed.
Materials and Methods: INS-1E and RINm5F cells were treated without or with various concentrations of epiregulin (0.5, 0.1, 1, 10, 20, 100 ng/ml) to determine cell proliferation by 5-bromo-2'-deoxyuridine (BrdU) incorporation. BrdU incorporation was measured by a cell proliferation ELISA assay. To determine the effect of epiregulin on insulin secretion in both cell lines, epiregulin alone or in combination with increasing glucose or arginine concentrations was added to the medium. Secreted insulin release and cellular insulin contents were measured by RIA. Because epiregulin binds to ErbB receptors, phosphorylation of the different subtypes of ErbB was detected by Western Blot after stimulation with epiregulin on both cell lines.
Results: INS-1E or RINm5F cell proliferation was stimulated in a dose dependent manner by epiregulin. 0.1 ng/ml resulted in optimal proliferation in both cell lines (+ 24 % and + 22%, respectively, compared to the control). Epiregulin was also tested for the effects on insulin secretion. In INS-1E cells, insulin secretion raised up 1.9-fold at 0.1 ng/ml epiregulin in the absence of glucose. The response to glucose-stimulation was also enhanced in combination with epiregulin. At 5.6 mM glucose, glucose-stimulated insulin release was increased up to 1.5-fold by 0.5 ng/ml epiregulin. At 11.1 mM, the stimulation of insulin secretion needs higher concentrations of epiregulin (100 ng/ml) to attempt an increase of 1.3-fold. Addition of 100 ng/ml epiregulin enhanced significantly insulin release by 1.4-fold at 16.7 mM glucose. Epiregulin stimulates also insulin secretion in RINm5F cells in a dose-dependent manner with a maximum at 0.1 ng/ml (2-fold). When RINm5F cells were exposed to increasing concentrations of arginine, insulin secretion was stimulated. Arginine-stimulated insulin secretion is also potentiated by epiregulin. At 10mM arginine, insulin release increased 2-fold with 100 ng/ml epiregulin and at 15 mM, the response was increased 2-fold by 10 ng/ml epiregulin. In INS-1E and RINm5F cells, epiregulin stimulates only the phosphorylation of EGFR suggesting that epiregulin activity is mediated by EGFR.
Conclusions: Epiregulin exerts a proliferative action and enhances significantly insulin secretion in both cell lines. Epiregulin activates downstream signalling by binding to EGFR. The enhancement of insulin secretion by epiregulin could provide a potent pharmacological tool to markedly enhance nutrient-stimulated insulin secretion.

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Impaired glucose-stimulated insulin release in GK rat is associated with a dysfunction of islet NO production.
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Background and Aims: The β-cell secretory dysfunction which characterizes type 2 diabetes in the GK rat is multifactorial in orgin and still far from elucidated. In view of recent observations showing that islet activity of nitric oxide synthase (NOS) is increased during hyperglycemia and that NO is a negative modulator of insulin release we now investigated a possible relation between the activity of the islet NOS-NO system and insulin secretion in the GK rat.
Materials and Methods: Isolated islets from GK rats and control Wistar rats were assayed (HPLC) for activities of constitutive NOS (cNOS) and inducible NOS (iNOS) both directly after isolation and after incubation at low (3.3 mmol/l) or high (16.7 mmol/l) glucose with and without addition of Glucagon-Like-Peptide 1 (GLP-1) (100 nmol/l). Insulin release from isolated islets and perfused rat pancreata was recorded with RIA. Morphological localization of cNOS and iNOS was performed immunocytochemically. iNOS protein was determined with Western blot.
Results: Biochemical assay of cNOS and iNOS in freshly isolated islets from GK and Wistar rats showed a moderately lower cNOS activity in GK

islets but no difference was noted immunocytochemically. Significant amounts of iNOS were not found either biochemically or immunocytochemically. Incubation of islets at low glucose (3.3 mmol/l) revealed the appearance of iNOS protein and a high iNOS activity (16.8 + 1.5 pmol NO/mg protein/ min) in GK islets. No iNOS was found in Wistar islets. There was no difference in insulin release at low glucose. In high glucose (16.7 mmol/l) Wistar islets displayed iNOS activity (17.0 + 1.3 pmol NO/mg protein/ min) although this was significantly lower than in GK islets (49.8 + 9.8 pmol NO/mg protein/ min) ($p<0.05$). cNOS activities were of similar magnitude in GK and Wistar islets. As expected GK islets secreted less insulin (1.70 + 0.19 ng/islet/ h) than Wistar islets (5.83 + 0.36 ng/islet/ h) ($p<0.001$) in high glucose. Addition of GLP-1 to the incubation medium almost abolished the expression of iNOS as well as the increased activity of iNOS in GK islets and restored the impaired insulin release to the same level as in Wistar islets. Moreover, the NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME) was found to partially restore the impairment of glucose-stimulated insulin release in isolated islets as well as in the perfused pancreas of the GK rat.
Conclusion: The results of present study suggest that the defective insulin response to glucose in the GK rats is, at least partially, explained by an increased disposition of the GK islets to express and activate the iNOS enzyme. This prospensity might possibly add to the diabetogenic susceptibility of their b-cells. Our data also suggest that GLP-1, and hence most likely the cyclic AMP system, counteracts the expression and activity of islet iNOS as well as restores the glucose-stimulated insulin release in the GK rat.

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The effect of diabetes on metabolic heterogeneity and oscillatory behaviorof individual cells in intact Islets of Langerhans.
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Background and Aims: The onset of diabetes type II is accompanied by degradation in the oscillatory pattern of insulin secretion. In order to understand the progression and manifestation of type II diabetes it is essential to determine the metabolic pathway responsible for insulin oscillations. Recent studies support the role of mitochondrial metabolic oscillation as an essential regulator of insulin secretion through the production of metabolic co-factors such as pyridine nucleotides, glutamate, and LC-CoA. As metabolic oscillations may be regulated both by intra- and intercellular signaling, we hypothesize that islet architecture may be an important parameter in controlling and synchronizing metabolic activity within the intact islet. Furthermore, we hypothesize that oscillatory behavior and synchrony will be modified by diabetes.
Materials and Methods: Using confocal microscopy, we recorded time-lapse images of mitochondrial membrane potential from individual islet cells in intact Islets of Langerhans from both diabetic and normal sandrats (*Psammomys obesus*) at two concentrations of glucose (6 and 12mM). We identified the beta cells from the other cell types according to their high NAD(P)H content using 2-photon excitation at 710 nm.
Results: The recorded mitochondrial membrane potential oscillations from cells in *P. obesus* islets are similar in frequency to previously recorded (3-8 min) oscillations of insulin secretion and oxygen consumption. Our data demonstrate that cells within the intact islet are metabolically heterogeneous in nature with respect to "regularity" (a measure of how well the mitochondrial membrane potential oscillations match a sinusoidal waveform), period (minutes/oscillation) and oscillatory "unity" (a measure of synchronization of phase and period). In all islets a. The majority of cells oscillate at periods of 3-6 min, while fewer cells oscillate with a period of 6-8 min., b. increasing glucose does not alter the oscillation frequency c. "unity" is greater at 12mM glucose compared to 6mM glucose ($p<0.05$; paired t-test). In the diabetic islets (compared to normal) a. the oscillatory period is virtually identical, b. glucose dependent recruitment of cells into clusters of cells with similar "unity" is less apparent., c. oscillating cells in diabetic islets demonstrate higher "regularity" at 6mm and 12mm glucose compared to normal islets($p<0.05$; paired t-test).
Conclusion: Our data demonstrate cellular metabolic heterogeneity within a single islet. This heterogeneity can be modified by both glucose and diabetic status.

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Impaired gene expression of exocytotic SNARE complex proteins in islets from Type 2 diabetic patients.
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Background and Aims: Exocytosis of insulin is critically dependent on the function of the SNARE (soluble N-ethylmaleimide attachment-protein receptor) complex proteins in the B-cells. Recently, we found decreased amounts of such proteins (e.g. VAMP-2, syntaxin-1A, SNAP-25, nSec1) in islets of rat models of type 2 diabetes, GK rats and *fa/fa* Zucker rats, with impaired insulin response to glucose. This investigation aims to study insulin release and expression of SNARE complex proteins in isolated islets of patients with type 2 diabetes and non-diabetic controls.
Materials and Methods: Isolated islets were obtained from patients with type 2 diabetes (n=2) and non-diabetic controls (n=2). Insulin secretion was determined by radioimmunoassay after batch incubation of isolated islets, and measurement of protein amounts by Western blot (WB). Islet gene expression was performed by microarray gene chip analysis (Human genome U133, Affymetrix).
Results: In islets from diabetic patients, insulin responses to 8.3 and 16.7 mM glucose were markedly reduced as compared to control islets (4.7 ± 0.32 and 8.4 ± 1.8 vs 17.5 ± 0.1 and 24.3 ± 1.2 mU/l, respectively; $p<0.001$ for both), and also insulin release at 10 mM arginine or 2 mM glibenclamide was diminished. In contrast, insulin responses to 100 nM GLP-1(7-37)amide at 16.7, but not 3.3 mM, glucose were similar in diabetes and control islets (33.7 ± 4.1 and 32.7 ± 4.2 mU/l, respectively). WB analysis of islet homogenates revealed decreased amounts of SNARE complex proteins in diabetes relative to control islets: syntaxin 1A (-77% of control levels), synaptophysin (-74%), SNAP-25 (-94%), and nSec1 (Munc18; -77%). According to the microarray analysis, gene expression was decreased in diabetes islets: syntaxin 1A (4-fold), synaptophysin (8-fold), SNAP-25 (4-fold), and nSec1 (6-fold).
Conclusion: Our data support the view that greatly decreased expression of islet exocytotic SNARE proteins, on RNA as well as protein levels, may play a role in impaired insulin secretion in patients with type 2 diabetes. It remains unclear, however, to which extent this defect is primary or due to e.g. glucotoxicity.

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Evidence that regulation by glucose of SNARE proteins in pancreatic islets of the rat include both transcriptional and translational events.
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Background and Aims: The glucose signal for insulin secretion is propagated by second messenger systems. While glucose regulation of early events in stimulus-secretion coupling are well known, there is less information about any direct effects on the process of exocytosis. SNARE-proteins are of key importance for exocytosis and lack of SNAP-25 has been implicated as a cause of diabetes.The aim of the present study was to investigate the effect of glucose on SNARE-proteins, and in particular SNAP-25 in pancreatic islets of the rat.
Materials 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. The mRNA levels of SNAP-25 including isoforms a and b were quantified by RT-PCR.
Results: The amount of protein after culture with 27 mM G was increased for SNAP-25 beeing 155±42% ($p<0.01$) of that after culture at 5.5 mM G. Corresponding effects for syntaxin was 131±26% ($p<0.05$) and Munc 18 139±47% (ns) vs 5.5 mM G. In contrast, culture in 27 mM G decreased VAMP-2 (93±9% of the amount after 5.5 mM G). mRNA levels of SNAP-25 increased significantly (156±11%) after high glucose culture corresponding to the protein results. Both the isoforms SNAP-25 a and b increased by 136±9 and 133±14% respectively. To investigate short-term effects islets were pre-cultured for 24 h in low glucose (5.5 mM G), and finally incubated for 1 h in 5.5 and 27 mM G. Also during these conditions 27 mM G significantly increased the amount of SNAP-25 protein by 172±29%.
Conclusion: The results indicate that glucose exerts effects on exocytotic proteins both at the transcription and the translation level. Furthermore, the balance rather than the absolute levels of different exocytotic proteins could determine the efficiency of the glucose-induced exocytotic process.

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Islet proteins implicated in culture-induced alterations of glucose-stimulated insulin release.

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Background and Aims: Glucose-stimulated insulin release (GSIS) from isolated cultured islets is affected by the glucose concentration during culture. If normal mouse islets are cultured at a high glucose concentration enhanced GSIS is observed. In an attempt to find causes contributing to the accentuated secretion we explored changes in global protein expression patterns of freshly isolated islets and islets cultured at 11 mM glucose.
Materials and Methods: Islets were isolated from 4 months old female C57BL/6J-mice and used directly or after 24 hours of culture in RPMI 1640 containing 11 mM glucose and supplemented with 10 % FCS. Islet protein samples were obtained by extraction and solubilization of proteins from approximately 200 freshly isolated or cultured islets. Subsequently, proteins were separated by two-dimensional gel electrophoresis (2-DE) using an optimized protocol where iso-electric focusing was performed on non-linear, immobilized pH gradient strips (pH 3.5-10) and the second dimension on 8-16 % gradient SDS gels. For visualization of the separated proteins a sensitive and reproducible silver-staining protocol compatible with mass spectrometry was developed and differential display analysis between gels from freshly isolated and cultured islets was performed. Selected protein spots were identified by excision, tryptic digestion and peptide fingerprinting by matrix assisted laser desorption/ ionization time of flight mass spectrometry.
Results: When 2-DE was performed on protein samples from freshly isolated and cultured islets highly reproducible (n=5) gel images were obtained containing 734 and 1074 spots, respectively. The image of freshly isolated islets was used as a master gel and reference map. Analysis revealed that 111 spots were differentially expressed in freshly isolated and cultured islets out of which 32 spots were present only in cultured islets and 62 spots only in freshly isolated islets (on/off proteins). So far, we have identified 41 spots corresponding to 13 protein entries. Among the differentially expressed proteins are molecular chaperones including GRP 78, endoplasmin (GRP 94), PDIA1, PDIA6, HSP; cytoprotective proteins including GRP 170; and energy producing enzymes including aconitase, ATP synthase. Conspicuous spots that were turned off in cultured islets include pancreatic amylase, which appeared as 6 spots. The prohormone convertase 2 (PC2), which was barely visible in freshly isolated islets, appeared as distinct spots on cultured islets.
Conclusions: Elevated expression of PC2 and molecular chaperones in islets cultured at high glucose indicates that increased insulin synthesis and content contribute to the enhanced GSIS observed in these islets. The disappearance of amylase may represent loss of exocrine cells.

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Studies on the expression and function of PAX4 in rat islets.

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Background and Aim: The paired homeodomain transcription factor PAX4 is expressed in the embryonic pancreas and subsequently becomes restricted to δ and β-cells while its presence in mature islets remains controversial. Recently, two independent studies have associated mutations in the *pax4* gene to Type 2 diabetes in the Japanese population suggesting an important role of this factor in regulating β-cell function and/or regeneration in adults. As an initial step towards understanding factors that may influence PAX4 expression and thereby impact endocrine cell mass, we have examined the response of the *pax4* gene to mitogens such as high glucose, activin A and betacellulin. Furthermore, to examine PAX4 involvement in β-cell function, this protein was overexpressed in isolated rat islets using adenovirus.
Material and Methods: Rat islets were exposed to either 2.5 or 30 mM glucose for 3 days. Islets were also treated with increasing concentrations of either activin A or betacellulin for 24 hours. Alternatively, islets were infected with a recombinant adenovirus containing a PAX4-IRES-GFP cassette and cultured for up to 6 days. PAX4 expression was confirmed by GFP co-expression and EMSA using a PAX4 DNA consensus sequence. Steady state mRNA levels for insulin, GK, PAX4, c-myc and cyclophilin were quantified by real time PCR.
Results: Low but consistent PAX4 mRNA levels were detected in freshly isolated rat islets. However, exposure to 30 mM glucose for 3 days resulted in a 10-fold increase in PAX4 mRNA levels as compared to control 2.5 mM glucose. As previously reported, a 4-fold increase in *c-myc* gene expression was also observed in islets. Although several putative c-myc binding sites were identified in the human PAX4 gene promotor, c-myc failed to induce a luciferase reporter construct harbouring this promotor in BHK cells. Increasing concentrations of either activin A or betacellulin resulted in a dose dependent induction of PAX4 mRNA reaching maximal values of 3.5 and 4 fold respectively at 0.5 nM. Insulin mRNA levels were increased 11-fold by glucose, whereas activin A or betacellulin were ineffective. Consistent with these findings, we show that adenovirus-mediated PAX4 overexpression in islets for up to 6 days had no marked effect on insulin mRNA levels or protein content. Strong PAX4/DNA binding activity was detected in infected cells confirming that a functional protein is expressed in islets. We are presently examining the effect of PAX4 on β-cell proliferation.
Conclusion: These results demonstrate that induction of PAX4 gene expression correlates with islet proliferation induced by high glucose, activin A and betacellulin. However, insulin gene transcription in mature β-cells does not appear to be controlled by PAX4.

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Islet Cell Differentiation

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Identification of genes expressed in a putative pancreatic islet cell progenitor cell line.
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Background and Aims: Embryonic Stem (ES) cells can be stimulated to differentiate into insulin-expressing cells but only at a low efficiency. Yet, transplantation of insulin-expressing cells derived from ES cells may represent a promising treatment for diabetic patients. To rationally identify the mechanisms involved in β cell differentiation we have developed several novel pancreatic cell lines from a line of transgenic mice (βGK-SV40Tag) that develop multiple types of pancreatic tumors in adulthood. One cell line (GKP4), generated from a periductal non-insulinoma, exhibits both a morphological and molecular phenotype that distinguishes it from β cell-derived tumors, which also occur in these mice. Because the GKP4 cells are able to spontaneously differentiate at a low frequency into insulin-expressing cells, we previously postulated that they are derived from an islet progenitor cell present in the adult animal. To explore the possibility that GKP4 cells come from an islet progenitor, and with the hope of identifying genes that may help to pinpoint islet progenitors within the pancreas, we have compared the gene expression profile of the GKP4 cells with a second cell line that has a β cell phenotype (GKP2).
Materials and Methods: We compared the gene expression profiles of GKP4 and GKP2 cells using the GeneChip® Murine Genome U74Av2 array from Affymetrix®.
Results: We identified 496 genes that are expressed at least 2.5 fold higher in GKP4 cells than in GKP2 cells. Among these we found markers of duct cells (*cytokeratin 7, Alcam,* and *carbonic anhydrase II*) and transcription factors known to play a role in endocrine cells differentiation (*Foxa2, Nkx2.2, NeuroD, Prox1* and *Brn4*), suggesting that GKP4 cells possess both a ductal and an endocrine progenitor phenotype. Others transcription factors (*Muscleblind, Klf9* and *Peg3*) were also identified in GKP4 cells and their expressions were confirmed in the developing pancreas at different stages of development. Interestingly, t*ransthyretin*, a hepatic gene that was previously reported to be present in the nestin-positive cells that can differentiate into insulin-expressing cells, is highly expressed in GKP4 cells. Using immunohistochemical staining, we found that t*ransthyretin* was present in the pancreas as early as embryonic day 10.
Conclusion: Together, these findings further suggest that GKP4 cells represent an islet progenitor cell line and helped identifying transcription factors (*Muscleblind, Klf9* and *Peg3*) that may play a role in pancreatic development and potential markers of islet progenitor cells, such as *transthyretin.*

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Crossing the germ layer: generating insulin-expressing cells from neural stem cells.
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Background and Aims: Stem cells have the potential to both divide indefinitely to produce large numbers of cells and to differentiate into a range of specialised cell types. These characteristics make stem cells ideal candidates for generating substitute tissue in transplantation therapies for diabetes mellitus. Neural cells in the brain share many characteristics of mature pancreatic beta cells despite originating from different embryonic germ layers. The aim of this work is to investigate the possibilty of differentiating neural stem cells found in the brain into insulin-secreting cells as a proof of concept of transdifferentiation between tissue types.
Materials and Methods: We have isolated stem cells from the forebrain of E14 rat embryos (14 days post conception) and expanded these cells in vitro under growth conditions specific for neural stem cell proliferation. Expanded cells were then exposed to retinoic acid (1μM) with db-cAMP (1mM) and nicotinamide (10mM) for 24-48 hours. Incubation medium was collected for C-peptide radioimmunoassay, since the presence of high concentrations of exogenous insulin in the growth medium precluded meaningful measurements of endogenous insulin. RT-PCR was performed on neural stem cell-derived cell (NSC) populations using primers specific for rat preproinsulin, pancreatic glucokinase, Kir6.2 and GLUT2. Fixed cells were screened by immunohistochemistry for known markers of pancreatic β-cell development. In parallel experiments, differentiated cells were loaded with Fura-2 for calcium (Ca) microfluorimetry analysis.
Results: Immunohistochemical analysis revealed the expression of transcription factors involved in β-cell development including PDX-1, nkx2.2 and islet1. RT-PCR indicated Kir6.2, GLUT2 and preproinsulin gene expression in NSCs. Furthermore, the preproinsulin mRNA is most likely translated, processed into the mature protein and secreted from these cells, since radioimmunassay revealed the presence of immunoreactive C-peptide in the culture media (58±5.3pM). Ca-microfluorimetry experiments confirmed that NSCs express at least some of the functional response elements of β-cells, since exposure to the sulphonylurea tolbutamide (100μM), caused an increase in intracellular Ca (47% of total cells were responsive; responses were 50% of those obtained with a pure β-cell line, MIN6). Furthermore, these cells were able to recognise and respond to glucose (20mM) with appropriate oscillatory Ca responses (73% of tolbutamide-sensitive cells were responsive; 52% of the response obtained with MIN6 cells).
Conclusion: Our results suggest that glucose and tolbutamide responsive, insulin expressing cells can be derived from foetal neural stem cells, indicating transdifferation across the germ layer. These observations imply that it may be possible to generate insulin-secreting cells by transdifferentiation from stem cells found in more accessible tissues such as liver.

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Nestin-positive duct stem cells in adult pancreas and differentiation into insulin secreting beta cells.
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Background and Aims: Stem cells in adult pancreas and their specific marker are poorly characterized. We hypothesized that pancreatic stem cells could evolve from the duct system in response to neogenic stimulation and may transiently express nestin during tissue regeneration.
Methods and Results: Following subtotal pancreatectomy, we found extensive formation of ductules consisting of nestin-positive epithelial cells with higher replicating ability in the neogenic foci. Nestin was highly expressed in the earlier stages of ductule morphogenesis and then regressed as the cells evolved towards differentiated pancreatic cell types. The neogenic ductules were isolated for the culture of nestin-positive duct stem cells. These nestin-positive duct cells were numerous and displayed extensive self-replication in the duct cell explants after 2-3 days of culture, thus depicted as nestin positive duct stem (NPDS) cells. As seen in the tissue of neogenic foci, NPDS cells were negative for cytokeratin-20 and vimentin, the marker for duct-epithelial and mesenchymal cells, respectively. Endocrine cells, mostly insulin cells with few glucagon and somatostatin cells, were present in the explants at day 2 as single cells or as small clusters adjacent to the NPDS cells, and formed islet-like masses at day 3 of culture, implying islet cell differentiation from NPDS cells. In addition, insulin secretion from these beta cells responded to glucose stimulation.
Conclusion: We suggest that NPDS cells could be generated from adult pancreas by neogenic motivations and they do differentiate into insulin-secreting-cells.

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Transcription factor GATA-6 is expressed in the endocrine and GATA-4 in the exocrine pancreas during fetal and postnatal development.
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Background and Aims: GATA-4 and GATA-6 are zinc finger transcription factors regulating gene expression, development and cell proliferation in a variety of tissues. They have been implicated in the development of several

endoderm derivatives, including epithelial cells in the yolk sac, lung and glandular gastric mucosa. Aim of the present work was to study the expression of GATA-4 and GATA-6 during pancreas development.
Materials and Methods: The expression of GATA-4 and GATA-6 was studied with immunohistochemistry, in situ hybridization and Northern analysis. Amphicrine pancreatic AR42J-B13 cell line was used to study the expression of GATA-4 and GATA-6 during the differentiation of these cells towards an endocrine phenotype.
Results: Expression of GATA-4 could not be detected in the embryonic day 10.5 pancreatic epithelial buds, although adjacent primitive gastric epithelium was clearly positive. At E15.5 GATA-4 expression was detected in the developing pancreatic acini, but not in the ductal or endocrine cells. Similar expression pattern persisted in the newborn and adult pancreas. Expression of GATA-6 was also undetectable in the early (E10.5) pancreatic buds. At E15.5, however, GATA-6 expression was evident in pancreatic endocrine and ductal cells, and in the adult it was found mainly in the islet beta cells. When amphicrine pancreatic AR42J cells were stimulated to differentiate along the endocrine lineage, the expression of GATA-6 was markedly upregulated whereas GATA-4 mRNA levels remained stable.
Conclusion: Our findings suggest that GATA-6 plays a role in the endocrine pancreas from fetal to adult life, whereas GATA-4 functions in the gene regulation of the exocrine pancreas. However, neither of these factors is expressed during the initial stages of pancreas development.

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Neogenesis of insulin-producing cells by adenovirus-mediated expression of beta-cell-associated transcription factors, differentiation/growth factors in pancreatic ducts.
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Background and Aims: Pancreatic beta-cell neogenesis is expected to provide a new therapy for diabetes. Some reports demonstrated that beta-cell-associated transcription factors and differentiation/growth factors are associated with endocrine neogenesis in vivo and in vitro. Recently we reported that adenovirus-mediated gene delivery of pdx-1 into pancreatic ducts induced proliferation of pancreatic ductal cells and neogenesis of insulin-producing cells [Gene Therapy. 2003;10:15-23]. In order to generate insulin-producing cells more effectively in mouse pancreas, we performed the adenoviral vector (AdV)-mediated gene delivery of other pancreatic beta-cell-associated transcription factors and differentiation/growth factors in addition to pdx-1.
Materials and Methods: We generated several AdVs expressing beta-cell-associated transcription factors and differentiation/growth factors. An empty AdV was used as a control. We administered these AdVs solution (10^9 PFU in 300µl of lactated Ringer's solution) into pancreatic ducts of 10-week-old male MCH/ICR mice by the retrograde intra common bile ductal (ICBD) injection. This technique (ICBD injection) is similar to the endoscopic retrograde cholangio-pancreatography, which has been already established as a safe procedure for humans. The mice were killed at 7-10 days after the injection, then we evaluated the degree of neogenesis of insulin-producing cells in the pancreases.
Results and Conclusion: We demonstrate here that a neogenesis of insulin-producing cells was observed mainly in the areas of proliferating pancreatic ducts to the same extent as the findings in our previous report. Although we have not yet evaluated the effect of this procedure on lowering blood glucose level in the present study, pancreatic beta-cell neogenesis induced by adenovirus-mediated gene delivery in pancreatic ducts is possible to provide a novel strategy for gene therapy for diabetes mellitus.

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Growth hormone and prolactin activate HNF-1α in INS-1 cells.
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Background and Aims: HNF-1α is a transcription factor, of which mutations have been linked to a subtype of maturity-onset diabetes of the young (MODY3), and could play a major role in insulin gene transcription and the maturation of pancreatic β-cells. Since previous studies have shown the activation of various hepatocyte transcription factors by growth hormone (GH), we speculate that the effects of GH on β-cells might also be mediated by the activation of HNF-1α. Thus the aim of this study was to investigate whether GH and its related peptide prolactin (PRL), the two well-known β-cell mitogens, activate HNF-1α using the differentiated insulin-secreting cell line, INS-1, which expresses receptors of these hormones and considered to be a good model to study their effects on β-cells.
Materials and Methods: Activation of HNF-1α was examined by electrophoretic mobility shift assay using the FLAT element, the specific DNA-binding site of HNF-1α present in the rat insulin I gene, as a probe. Transcriptional activity through the FLAT element was evaluated by the luciferase reporter gene assay. Implication of the HNF-1α activation in the action of GH and PRL was investigated using INS-1 cells overexpressing dominant-negative HNF-1α (DN-HNF-1α) under control of a doxcycline-dependent transcriptional activator. Using these cells, we examined the effects of the two hormones on insulin gene expression and on DNA synthesis, by Northern blotting and by thymidine incorporation assay, respectively.
Results: Both GH and PRL at 5 nM promoted the binding of HNF-1α to the FLAT element as well as its transcriptional activity after 12 h of incubation. These actions were abolished by the addition of herbimycin A, which blocked GH-induced JAK2 tyrosine phosphorylation. In the wild-type INS-1 cells, both hormones increased insulin mRNA expression after 6 h and stimulated DNA synthesis after 24 h of incubation. Overexpression of DN-HNF-1α resulted in a significant decrease in the insulin gene expression induced either by GH or by PRL, whereas the stimulation of DNA synthesis by these hormones remained unchanged by the DN-HNF-1α overexpression.
Conclusion: GH and PRL activate HNF-1α through JAK2 tyrosine phosphorylation in insulin-secreting cells. Stimulation of insulin biosynthesis, but not that of β-cell growth, by these hormones may be mediated by the activation of HNF-1α.

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Glucose-induced phosphorylation of PDX-1 by Caseine Kinase 2 (CK2).
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Background and Aims: The pancreatic and duodenal homeobox gene-1 (PDX-1) transcription factor is involved in the development of the pancreas as well as in the glucose-dependent regulation of the insulin gene expression. As we demonstrated previously a novel type of NLS within the third helix of the homeodomain of PDX-1 mediates its nuclear localization. Nuclear transport of PDX-1 can actively be induced by stimulating starved beta cells with high doses of glucose. The signalling events leading to activated PDX-1 are not fully understood. There is evidence that the PI-3 kinase is part of the signalling pathway but a PDX-1 kinase is not known.
Materials and Methods: Using extracts of phosphate-labeled, glucose-induced and starved MIN6 cells in pull-down experiments, GST-capture assays as well as recombinant enzymes we analysed the phosphorylation and DNA-binding of PDX-1.
Results: Here we give evidence that CK2 is able to phosphorylate the PDX-1 protein. Bacterially expressed GST-PDX-1, not able to bind to an oligonucleotide containing an A-box, does bind when phosphorylated with recombinant CK2. Treating PDX-1 with extracts of glucose-induced MIN6 cells also leads to phosphorylation and DNA-binding whereas treatment with extracts isolated from starved cells did not. Phosphorylation by recombinant CK2 or by extracts of glucose-induced MIN6 cell could be diminished by addition of heparin, a known inhibitor of CK2. Additionally we could show interaction of PDX-1 with the catalytic alpha-subunit of CK2 using a GST-capture assay.
Conclusion: The results suggest that CK2 is part of the glucose-dependent signalling pathway leading to activation of PDX-1.

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Effect of glucose on PDX-1 gene expression in isolated rat islets.
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Background and Aims: Pancreatic and duodenal homeobox gene-1 (PDX-1) is a transcription factor encoded by a Hox-like homeodomain gene. In human and other animal species, the embryonic development of the pancreas requires PDX-1. In adult subjects, PDX-1 is essential for normal pancreatic islet function. It is well known that glucose is the main physiological regulator of insulin gene. We have previously demonstrated

the effect of glucose on the expression of insulin gene in isolated rat islets, which proved the toxic effects of glucose in dose-dependent and time-dependent manner. The aim of this study is to investigate the effect of glucose on the expression of PDX-1 in isolated rat islets.
Materials and Methods: The isolated rat islets were incubated with glucose in the concentrations of 2.2mmol/l, 5.5 mmol/l, 11.1mmol/L, 16.7 mmol/l and 33.3mmol/l for 1, 4, 7 and 14 days, respectively. Total cellular RNA was extracted, and the expression of PDX-1 gene was detected by RT-PCR.
Results: Compared with normal glucose concentration (5.5 mmol/l), PDX-1 gene expression was suppressed by glucose of 2.2mmol/l, while the gene expression was stimulated by higher concentrations of glucose in dose-dependent manner in the first day. After incubation for 4 days, PDX-1 gene expression was suppressed significantly by glucose of 2.2mmol/l. PDX-1 gene expression was stimulated by glucose of 11.1 mmol/l, though there was no change in PDX-1 mRNA with 16.7 mmol/l glucose. However, PDX-1 mRNA was suppressed by 33.3mmol/l glucose significantly. After 7 days incubation with glucose PDX-1 gene expressions were suppressed markedly in various concentrations except for 5.5 mmol/L and 11.1 mmol/L. When islets were incubated with glucose as long as 14 days, all non-physiological concentrations of glucose possessed suppressive effect on PDX-1 gene expression in a dose-dependent manner.
Conclusion: Short-term exposure of isolated rat islets to superphysiologic concentration of glucose increases PDX-1 gene expression, whereas chronic exposure exerts suppressive action, indicating the glucose toxicity on β cells.

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Isolation and culture of PDX-1 positive pancreatic ductal cell derived from normal adult mouse and differentiation into insulin-producing cell.
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Background and Aims: Ductal cells of the adult pancreas include latent progenitor cells of islet endocrine cells which can be induced to differentiate into by the appropriate morphogen stimuli (referred to as neogenesis). A few normal pancreatic ductal epithelium derived from hamster, rat, guinea pig, dog, cow, rhesus monkey, and human have been successfully isolated and cultured, however, the isolation of mouse normal ductal cell has not been established.
Materials and Methods: Pancreas tissue of normal adult mouse (12-weeks) was digested with collagenase. After separation by passing them through stainless-steel filters, clumps of ductule fragments that included acinar cells, blood vessels and mesenchymal tissue were cultured in non-treated Petridish at 37°C, 5% CO2. These cell clusters were incubated with using serum-free DMEM/F12 medium containing cholera toxin.
Results: These colonies of proliferating cells grew in cobblestone patterns, which are known to be characteristics of pancreatic epithelial cell. This cell line proliferated best in 10% FCS and became nearly confluent over the next 7-12 days. We confirmed that this cell line did not contaminate islet endocrine cell, acinar cell and fibroblast by reverse transcribed PCR. Virtually all of these cells were immunopositive (results using anti-pancytokeratin antibody) in the cytoplasm. Furthermore, it was very interesting to note that most of them had moderate pancreatic and duodenal homeobox gene-1 (PDX-1) nuclear staining. Although there were relatively smaller amounts of PDX-1 immunoreactive protein than those in MIN6 cells (46kDa in both cells), mouse tumor-derived β-cell line, by Western blot analyses using anti-PDX-1 antibody, these ductal cells might be expected to have a latent ability to differentiate into pancreatic endocrine cells (referred to as transdifferentiation). We actually confirmed the insulin production in the 2 weeks with Matrigel, a commercial preparation of murine basement membrane, by immunofluorescent staining.
Conclusion: Thus, we believe that the establishment of cell line derived from normal mouse pancreatic ductal cells would be of value in the study of pancreatic ductal differentiation using genetic engineering model mouse.

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Lipids and Islet Function

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Transgenic mice with reduced β–cell cAMP levels develop severe diabetes when challenged with a high-fat diet.
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Background and Aims: Selective inhibitors of the cAMP-degrading phosphodiesterase (PDE) 3 have been shown to increase insulin release both *in vivo*, in mouse models and *in vitro*, in human and and rat islets. We have also demonstated that PDE3B, when overexpressed in clonal β-cells and rat pancreatic islets, lowers cAMP levels and inhibits insulin secretion stimulated either by glucose alone or in combination with GLP-1. Transgenic mice, generated in our laboratory, with a β-cell-specific, two-fold overexpression of PDE3B (RIP-P3B:2), exhibit reduced insulin secretory capacity, glucose intolerance and perturbed islet morphology, indicative of a pre-diabetic phenotype. In the present study, such mice have been challenged with a high-fat diet.
Materials and Methods: 2-month-old male RIP-P3B:2 mice and wildtype littermates (all on a C57Bl/6J background) were fed either a high-fat (58% fat) or standard (10.5% fat) diet. Weight gain and food intake were measured every week and once every four weeks blood samples were taken. After 7 and 10 weeks, respectively, intravenous glucose tolerance tests (IVGTT) were performed. Fasted mice (10 animals/group) were anaesthetized, 1 g D-glucose per kg bodyweight was injected intravenously into the tail vein and blood samples were taken.
Results: The RIP-P3B:2 mice gained markedly more weight compared to wildtype littermates when fed a high-fat diet. Already after four weeks, the mean weight of the transgenic mice was 35.9±1.4 g, whereas for wildtype mice 30.8±1.3 g ($p<0.01$). This difference was not due to increased food intake. After seven weeks of high-fat feeding, fasting plasma levels of glucose amounted to 19.6±2.3 mM in RIP-P3B:2 mice as compared to 16.3±0.7 mM in wildtype littermates. Fasting plasma insulin was also significantly higher (1.6±0.5 nM) in the transgenic mice than in wildtype controls (1.0±0.3 nM). In plasma of transgenic and control animals fed a standard diet, fasting values for glucose were 11.4±0.4 and 8.5±0.7 mM, respectively. Results from IVGTT performed both on transgenic and wildtype mice, fed the respective diets, indicate that whereas the insulin secretory response is impaired in wildtype mice fed the high-fat diet, the secretory response is equally impaired in RIP-P3B:2 mice fed either diet. Nevertheless, due to their hyperinsulinemic state, the peak value of plasma insulin is significantly increased in the transgenic mice fed with high fat. However, in spite of the strikingly high amounts of insulin secreted during the test situation, these animals remain hyperglycemic with an apparent incapability of normalizing the elimination of glucose from the circulation.
Conclusions: The limited, two-fold increase in PDE3B activity in β-cells of transgenic mice causes distinct metabolic perturbations, suggesting a pre-diabetic phenotype. When obesity is precipitated by high-fat feeding, such mice appear to rapidly develop full-blown diabetes.

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Enhanced sensitivity to dimethyl-glutamate is associated with hyperinsulinemia in islets from glucose intolerant and insulin resistant high fat diet-fed C57BL/6J mice.
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Background and Aims: Hyperinsulinemia evolves as a compensatory mechanism to maintain euglycemia in insulin resistant states. If this adaptive process fails type II diabetes mellitus will develop. It is unclear to which cellular cues pancreatic β-cells react in order to adaptively increase insulin secretion. To study these mechanisms, insulin secretion *in vivo* and *in vitro* in a model for insulin resistance was examined.
Materials and Methods: C57BL/6J mice were kept on a high fat diet (HFD; 58% on a caloric base; 11% in controls) for 12 weeks. Glucose homeostasis *in vivo* was assessed by repeated sampling of plasma glucose and insulin as well as by intravenous glucose tolerance tests (IVGTT). Insulin secretion *in vitro* was assayed by static incubations of islets; K_{ATP}-channel independent glucose sensing was examined in the presence of 35 mM KCl and 250 μM diazoxide. Glutamate, a proposed coupling signal in K_{ATP}-independent glucose sensing, was examined for its potential to mediate a hyperinsulinemic response.

Results: Basal fed plasma glucose and insulin levels rose gradually under the study period; at 12 weeks plasma glucose was 9.3 ± 1.3 vs. 6.1 ± 0.8 mM (P<0.01) in HFD-fed and control mice, respectively, while insulin levels were 11.2 ± 2.6 vs. 3.7 ± 0.4 ng/ml (P=0.028). Thus, the mice are glucose intolerant and exhibit insulin resistance with compensatory hyperinsulinemia. An IVGTT at week 10, revealed that first phase insulin secretion was attenuated in HFD-fed mice; in controls, insulin levels rose 3.5-fold while those in HFD-fed mice rose only 1.8-fold. Accordingly, plasma glucose levels were consistently higher in HFD-fed mice during the test. In static incubations (1 h) of control islets, insulin secretion rose dose-dependently (3-20 mM glucose) from 54 ± 9 to 483 ± 66 pg/islet/h; in islets from HFD-fed mice, basal secretion at 3 mM glucose was elevated (190± 66 pg/islet/h), while very little increase was seen at 20 mM glucose (591± 46 pg/islet/h). Moreover, under K_{ATP}-channel independent conditions, glucose provoked a 3.2-fold increase in insulin secretion in control islets but only a minimal increase in islets from HFD-fed mice. In control islets, 10 mM dimethyl-glutamate, a cell permeable form of the amino acid, potentiated insulin secretion at 15 mM by 26.5%. Remarkably, in islets from HFD-fed mice, this potentiation was increased to 537% (P<0.01 versus 15 mM glucose alone).
Conclusions: Based on these results we conclude that while HFD-fed mice exhibit basal hyperinsulinemia, a perturbation of fuel-stimulated insulin secretion exists. Seemingly, K_{ATP}-independent glucose sensing is affected. Because a hyperinsulinemic response in islets from HFD-fed mice could be elicited by dimethyl-glutamate, it is possible that an impaired capacity of β-cells to generate glutamate may contribute to the secretory deficiency that ultimately evolves in long-standing insulin resistance.

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Inadequate β-cell compensation for insulin resistance elicited by high-saturated-fat feeding leading to glucose intolerance during pregnancy: possible involvement of β-cell lipotoxicity.
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Background and Aims: Insulin resistance (IR), diminished insulin action in target tissues, predicts the development of type 2 diabetes. In non-diabetic subjects, a regulated negative feedback loop exists, such that changes in insulin sensitivity are compensated by inverse changes in insulin secretion. However, β-cell compensation for IR is impaired during the progression to type 2 diabetes. During the last trimester of gestation, maternal lipid metabolism switches to a catabolic state concomitant with the development of maternal IR that facilitates channelling of glucose to the developing fetus. Maternal leptin levels are elevated, which would be predicted to stimulate lipid oxidation by muscle and β cells. Insulin hypersecretion compensates for peripheral IR and glucose tolerance is maintained. High-saturated fat feeding, like pregnancy, elicits IR, with compensatory insulin hypersecretion. Our aim was to delineate whether high-fat feeding adversely impacts on the regulatory loop between insulin action and secretion
Materials and Methods: We investigated the effect of increased dietary saturated fat on insulin secretion and glucose tolerance in pregnant rats in relation to changes in maternal leptin and lipid levels. Pregnant rats were transferred to the diets at day 1 of gestation. Controls were pregnant rats on standard diet. Maternal insulin secretion and glucose tolerance were assessed after an intravenous glucose challenge (0.5 g glucose/kg body weight) in conscious, unrestrained late (19 day) pregnant rats.
Results: In late-pregnant rats, pregnancy-induced IR was exacerbated by high-saturated-fat feeding (a significant 82% (P 2.6 fold (P<0.001), while, consistent with augmented lipid oxidation, triglyceride and fatty acid levels were lowered (by 48% and 73%; P<0.05). Impaired insulin sensitivity was not accompanied by adequate compensatory insulin hypersecretion and the rate of glucose disappearance after intravenous glucose challenge was almost halved (43% decrease; P<0.001) by high-saturated-fat feeding, resulting in marked glucose intolerance.
Conclusion: Failure of insulin hypersecretion to compensate for peripheral IR may precipitate the development of gestational diabetes, with adverse consequences for both mother and offspring. Our studies suggest that excess dietary saturated fat during pregnancy, by promoting excessive lipid utilisation, imposes a major challenge to the endocrine pancreas that could ultimately precipitate the development of diabetes.

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Inhibition of diglyceride lipase activity and lipolysis in rat islets inhibits insulin secretion.
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Background and Aims: Lipids are thought to serve as coupling factors in K_{ATP}-independent glucose sensing in the pancreatic β-cell. We have previously demonstrated that β-cells harbour lipase activities, one of which is the hormone-sensitive lipase. Whether β-cell lipases are critical for glucose-stimulated insulin secretion by providing lipid-derived signals from endogenous lipids stores, e.g., triglycerides, is not known. Therefore, using a broad lipase inhibitor (orlistat), we examined whether inhibition of lipase activity impacts insulin secretion.
Materials and Methods: Insulin secretion was assayed by static incubations and perifusions of rat islets in the presence and absence of orlistat. Glycerol release from islets or isolated adipocytes was used as an index of lipolysis; enzyme activity in islets and adipocytes towards a synthetic diglyceride substrate was determined.
Results: Glycerol release from islets increased from 12.9±3.6 to 22.6±3.0 pmol/islet/h when glucose was raised from 2.8 to 16.7 mM; this increase was completely abolished by 200 μM orlistat. In isolated rat adipocytes, the cAMP-raising agent forskolin increased glycerol release by 6-fold; 200 μM orlistat blocked this increase by 28 % (P<0.001). Diglyceride lipase activity in islets was 254±131 μU/islet; 200 μM orlistat reduced this activity to 38±12 μU/islet. Also in adipocytes, orlistat significantly blocked diglyceride lipase activity. These observations demonstrate that orlistat is an appropriate tool for probing the importance of lipase activity for β-cell function. Insulin secretion in isolated rat islets rose from 138±68 to 819±249 pg/islet/h as glucose was raised from 2.8 to 16.7 mM; 2.5 μM forskolin potentiated secretion at 16.7 mM glucose by 5-fold. Orlistat dose-dependently inhibited insulin secretion both in the presence and absence of forskolin. At 16.7 mM glucose and 2.5 μM forskolin, secretion was inhibited by 68% (P<0.001) in the presence of 200 μM orlistat. When 1 mM palmitate was added to the same conditions, the inhibitory action of orlistat on insulin secretion was abrogated. During perifusion of islets, insulin secretion rose 7-fold as glucose was raised from 2.8 to 16.7 mM. In the presence of 200 μM orlistat, the first phase of insulin secretion, defined as suprabasal $AUC_{insulin}$ from minute 14 to the peak insulin value at minute 18, was unaffected. In contrast, the second phase of insulin secretion, defined as suprabasal $AUC_{insulin}$ from minute 19 to 40, was attenuated in the presence of orlistat (P<0.05).
Conclusions: Our observations show that β-cell lipase activity is involved in the process whereby glucose stimulates insulin secretion. The second phase insulin secretion appears to be preferentially affected, which suggests that lipids generated by a lipase are coupling factors in K_{ATP}-independent glucose sensing, thus further emphasizing the important role of β-cell lipid metabolism in glucose-stimulated insulin secretion.

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Mechanisms of fatty acid inhibition of insulin gene expression in isolated rat islets.
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Background and Aims: Chronically elevated fatty acid levels have been proposed to impair pancreatic beta-cell function. Previously, we and others have shown that prolonged exposure of isolated rat islets to supraphysiologic levels of fatty acids decreases insulin mRNA levels. However, the mechanisms of these effects are largely unknown. The aims of this study were to determine whether the mechanisms of palmitate and oleate inhibition of insulin gene expression 1) are transcriptional or post-transcriptional, and 2) involve de novo ceramide synthesis.
Materials and Methods: Isolated rat islets were cultured in the presence of 2.8 mM glucose or 16.7 mM glucose in the absence or presence of palmitate or oleate (0.1 to 0.5 mM, complexed to 0.1 mM BSA). Insulin and glyceraldehyde-3-phosphate dehydrogenase mRNA levels were measured by ribonuclease protection assay. Insulin mRNA decay was assessed after addition of actinomycin D (5 μg/ml) to block transcription. Isulin promoter activity was measured in transient transfection experiments by infecting islets with adenoviruses encoding firefly luciferase under the control of the rat insulin 1 promoter (RIP1-Luc) or the CMV promoter (CMV-Luc). Ceramide content in islet lipid extracts was determined by the diacylglycerol kinase assay and thin layer chromatography.

Results: Both oleate and palmitate dose-dependently decreased insulin mRNA levels in the presence of 16.7 mM glucose (ANOVA, n=4, P<0.05) to 71.5+/-11 and 76+/-8 % of control levels, respectively. Palmitate did not significantly affect insulin mRNA half-life at either 2.8 (27.2+/-3.1 vs. 27.7+/-1.5 h, n=3) or 16.7 (39.0+/-2.0 vs. 40.8+/-3.0 h, n=3) mM glucose. Glucose stimulated luciferase activity in a dose-dependent manner over 24 hours in islets infected with the RIP1-Luc adenovirus (ANOVA, n=4, P<0.01). Both palmitate and oleate decreased glucose-stimulated luciferase activity in a dose-dependent manner (ANOVA, n=4, P<0.05), to 44.3+/-0.7 and 35.8+/-13.3 % of control levels, respectively. Intracellular ceramide content was increased 4.6+/-1.1 (n=8, P<0.01) and 4.2+/-1.1 (n=5, P<0.05) fold after 72h of culture with palmitate or oleate, respectively. The palmitate-, but not the oleate-induced increase in ceramide was significantly reduced in the presence of the inhibitor of de novo ceramide synthesis myriocin (2.4+/-0.6 fold increase, n=4, NS). Furthermore, the palmitate-induced decrease in insulin mRNA levels (54+/-9% of control, n=6, P<0.01) was largely prevented in the presence of myriocin (83 +/- 15% of control, n=5, NS).
Conclusion: Fatty acids decrease insulin mRNA levels in isolated islets by inhibiting insulin promoter activity. The effect of palmitate involves, at least in part, de novo ceramide synthesis.

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Effects of palmitate on insulin and glucagon secretion in normal Wistar rat and diabetic Goto-Kakizaki rat islets
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Background and Aims: Lipotoxicity contributes to pancreatic beta-cell dysfunction. To assess the potential link of acute and chronic exposure to saturated fatty acid (palmitate) to the expression of SNARE proteins in isolated rat pancreatic islets and evaluate the mechanisms of insulin and glucagon release in normal and type-2 diabetic state, we performed the study in isolated islets of the Wistar (W) and Goto-Kakizaki (GK) rat, an animal model of hereditary type 2 diabetes.
Material and Methods: Insulin and glucagon release were determined by radioimmunoassay in batch incubation of isolated GK and control W rat islets exposed to 0.2 mM palmitate for 1 day or 3 days in RPMI 1640 culture medium containing 5.5 mM glucose. To assess the role of palmitate on the expression of exocytotic SNARE complex proteins, syntaxin-1A, VAMP-2, SNAP-25 and nSec1, Western blots were performed in W and GK rat islet homogenates.
Results: After 1 day culture, palmitate stimulated basal insulin release at 3.3 mM glucose 4.6-fold to 16.2 ± 6.1 µU/islet per h (n=6; p<0.001), but did not affect insulin reponses to 16.7 mM glucose and 10 mM arginine in W islets. In GK islets, however, palmitate not only enhanced basal insulin release 3.6-fold to 9.7±1.5 µU/islets per h (n=4; p<0.01), but also tended to increase insulin response to 16.7 mM glucose and 10 mM arginine (16.8±3.9 and 10.0 ±3.2 µU/islet per h, respectively, n=4; p=0.053). After 3 day-exposure, palmitate exposure reduced insulin response to 16.7 mM glucose in W islets (by 69%, n=4; p<0.001) compared to control group. In contrast, insulin release at 16.7 mM glucose increased 1.8-fold in GK islets (n=4; < 0.05).
Interestingly, arginine-induced glucagon release was reduced in W islets exposed to palmitate both for 1 day (by 57%, n=5; p=0.06) and 3 days (by 77%, n=5; p<0.001). In GK islets, arginine-stimulated glucagon release was unaffected by palmitate.
Western blots showed significant reduction of syntaxin-1A, VAMP-2, SNAP-25, nSec1 by 31-44% (p<0.01 for all), whereas actin levels were increased to 189% (p<0.001) comparing GK islets with W islets after 1 day control culture. In W islets, 1 day- or 3 day-exposure to palmitate did not alter the expressions of the SNARE proteins but increased actin levels (p<0.05). However, in GK islets 3 day-exposure to palmitate caused a further reduction of SNAP-25 and nSec1 (p<0.01) but actin levels did not change.
Conclusions: Short-term exposure to palmitate enhanced basal insulin release in W and GK islets, as well as stimulated insulin responses to glucose and arginine in GK islets. After 3 days of palmitate, insulin responses were suppressed in W islets but slightly enhanced in GK islets. Glucagon release was reduced by palmitate in W islets but not in GK islets. The lipotoxic effects by palmitate on hormone secretion in W islets were not accompanied by decreases in SNARE complex protein levels. GK islets appeared less sensitive to further impairment of hormone release due to palmitate. Increased actin levels may play a role in impairment of islet hormone release.

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Islet proteins implicated in glucose- and lipid-induced metabolic and secretory alterations identified by correlating islet phenotype with islet global protein patterns.
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Background and Aims: Elevated blood glucose and lipid levels are implicated in the development of metabolic and insulin secretory perturbations associated with obesity and type 2 diabetes. In an attempt to find causes contributing to the deranged islet metabolism and secretion we have measured glucose-induced changes in insulin secretion and oxygen tension (pO_2) in normal mouse islets cultured at varying concentrations of glucose in the presence or absence of oleate. Global islet protein expression patterns were also measured in such islets. The islet phenotype was correlated to changes in protein patterns.
Materials and Methods: Islets were isolated from 4 months old male C57BL/6J-mice and cultured for 24 hours in RPMI 1640 containing 3 mM glucose, 3 mM glucose and 0.5 mM oleate, 11 mM glucose or 11 mM glucose and 0.5 mM oleate. After culture islets were perifused individually and glucose-induced changes in insulin release or pO_2 were measured when the glucose concentration was increased from 3 to 11 mM. Insulin and pO_2 were measured by ELISA and a Clark-microelectrode, respectively. Islet protein samples were also prepared and applied on different chromatographic surfaces (weak cationic and strong anionic exchangers). Protein profiling was performed with surface-enhanced laser/desorption ionization (SELDI) time of flight (TOF) mass-spectrometry (MS). Differences between groups were evaluated with Student's t-test and Mann-Whitney U-test. Data are expressed as means ± SEM.
Results: Insulin release at 3 mM glucose was similar in the four islet culture groups (10 ± 2 pmol*g^{-1}*s^{-1}). Glucose-stimulated insulin release (GSIS) in islets cultured at 3 and 11 mM glucose was 16 ± 5 and 281 ± 77 pmol*g^{-1}*s^{-1} (p<0.01), respectively. Including oleate during culture improved GSIS in islets cultured at 3 mM glucose to 129 ± 60 pmol*g^{-1}*s^{-1} (p<0.05) but didn't affect islets cultured at 11 mM glucose (265 ± 68 pmol*g^{-1}*s^{-1}). Corresponding glucose-induced decreases in pO_2 were 13 ± 2 and 38 ± 4 mmHg (p<0.01) for islets cultured at 3 and 11 mM glucose, respectively, and 20 ± 3 and 33 ± 4 mmHg (p<0.05) for islets cultured at 3 and 11 mM glucose in the presence of oleate. Based on these differences in phenotype we compared mass spectrograms obtained from islets cultured at 3 mM glucose and at 3 mM glucose and oleate. A total of 68 peaks were analyzed out of which 2 were significantly (p<0.05) increased/decreased. Also, mass spectrograms obtained from islets cultured at 3 mM glucose and 11 mM glucose were compared. In this comparison 166 peaks were analyzed out of which 4 were significantly (p<0.05) increased/decreased. The masses of these 6 peaks ranged from 3.8 to 27 kDa.
Conclusions: Differences in glucose-induced changes in secretion and metabolism observed in islets cultured at low or high glucose in the presence or absence of oleate could be correlated to significant changes in peaks of corresponding mass spectrograms obtained by SELDI-TOF-MS. These peaks will be identified and their relevance for glucose- and/or lipid-induced impaired secretory and metabolic islet function verified.

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Downregulation of hormone-sensitive lipase may serve to protect islets from lipotoxicity.
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Background and Aims: Lipid accumulation in β-cells during high-fat (HF) feeding may be involved in inducing a defective insulin secretion due to lipotoxicity. Much attention has been paid to study the role of exogenous lipids for β-cell dysfunction. It is, however, possible that dysregulation of the lipases responsible for the hydrolysis of intracellular triglyceride pools may result in inappropriate storage of acylglycerols that could lead to secretory defects associated with development of type 2 diabetes. Hormone-sensitive lipase (HSL) is one lipase that is expressed and active in β-cells, but its importance during the development of type 2 diabetes is not known.
Materials and Methods: To assess the role of HSL in β-cells during development of lipotoxicity, we investigated two mouse models; C57BL/6J mice fed a high-fat diet and transgenic mice with specific overexpression of HSL in β-cells. In this way it was possible to study the effect of exogenous fatty acids and, in the HSL transgenic model, the effect of increased intracellular fatty acids caused by increased hydrolysis of the triglyceride pool. HSL protein expression and accumulation of islet triglycerides were studied in correlation to islet performance as judged by GSIS.

Results: Long-term (10 months) HF feeding resulted in down-regulation of β-cell HSL to represent only 25±4 % of the expression level found in control mice. This finding was paralleled by increased accumulation of triglycerides in the islets (60±12 vs. 28±2 pmol/islet, P<0.01) and blunted GSIS. In islets with overexpression of HSL, diglyceride lipase activity was increased from 52±11 to 846±243. Also lipolysis, measured as release of glycerol, was increased from undetectable in wild-type islets to 88±36 pmol/islet/30 min. Islet triglyceride levels were slightly, but not significantly, decreased in HSL transgenic islets (12±3 vs. 16±4 pmol/islet), while GSIS was blunted both in vivo and in isolated islets from the HSL transgenic mice.
Conclusion: In conclusion, we demonstrate that in mice fed a HF diet, islet HSL was downregulated. The decreased HSL expression presumably reflects an adaptation to the increased flux of fatty acids into the islets, serving to reduce the hydrolysis of the triglyceride pool and thus reducing the amount of fatty acids entering lipotoxic pathways. In the opposite situation, overexpression of HSL in β-cells resulted in lipotoxicity and disturbed β-cell function. Increased HSL action was reflected by increased lipolysis and increased lipase activity suggesting that increased turnover of the triglyceride pool may result in lipotoxicity. Further studies will attempt to elucidate the precise mechanisms whereby HSL regulates β-cell lipid metabolism and explore HSL as a target for development of new drugs for treatment of the islet dysfunction that characterize type 2 diabetes.

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Differential regulation of beta-cell function and gene expression patterns by PPRAgama overexpression and PPARgamma activation.
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Background and Aims: PPARγ plays an essential role in energy storage and adipogenesis. Upon by ligand-activation, PPARγ heterodimerizes with retinoid X receptor (RXR), recruits cofactors, and binds to responsive DNA elements. Thereby the transcription of target genes is activated. While most studies have focussed on the role of PPARγ in insulin action, some reports have suggested that its activity regulates pancreatic β-cell function. PPARγ thiazolidinedione agonists, troglitazone and rosiglitazone, have been reported to enhance glucose-induced insulin secretion both in native β-cells and INS-1 cells. Troglitazone also restores normal β-cell function by preventing intracellular fat deposition, mitochondrial damage, impaired glucose-stimulated insulin secretion, and β-cell destruction in Zucker Diabetic Fatty rats. In addition, PPARγ has been proposed to improve glucose sensing by regulating the expression of Glut2 and glucokinase. The present study is aimed to elucidate whether thiazolidinediones modulate β-cell function through PPARγ-dependent or -independent mechanisms.
Materials and Methods: We have developed a strategy to evaluate the correlation between pharmacological actions of thiazolidinediones and PPARγ activity in insulin-secreting cells. We employed the tet-on system and established two INS-1 derived cell lines that allow the inducible expression of either wild type PPARγ or a naturally occurring PPARγ mutation in the ligand binding domain (P467L). Patients carrying this mutation (P467L), which functions in a dominant-negative manner, manifest severe hyperglycemia and insulin resistance.
Results: Doxycycline-induced expression of either wild-type PPARγ or the dominant-negative mutant PPARγP467L was confirmed by Northern and Western blot analysis. Graded overexpression of PPARγ stepwisely increased the expression of β-cell-specific tanscription factor Pdx-1, whereas induction of PPARγP467L or treatment with rosiglitazone was without effect. Quantitative Northern blotting also revealed a typical synergistic effect of PPARγ overexpression in combination with rosiglitazone on Fatty acid translocase (FAT/CD36) mRNA levels, which were increased by several hundred-fold. In contrast, the expression of lipogenic genes was unaltered. Overexpression of PPARγ reduced the mRNA levels of SREBP-1c, a transcription factor implicated in β-cell gluco-lipo-toxicity. The RXR agonist, 9-*cis*-retinoic acid enhanced the basal (2.5 mM), but inhibited glucose-stimulated insulin secretion by 30% only when combined with rosiglitazone and PPARγ overexpression. The mRNA levels of Glut2 and glucokinase were not altered by PPARγ induction, rosiglitazone, and 9-*cis*-retinoic acid, either separate or combined.
Conclusions: Our cellular models should help to elucidate the molecular mechanism underlying the actions of PPARγ and thiazolidinediones on β-cell function. They will also serve to evaluate the impact of altered lipid metabolism on β-cell function.

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Perifused islets of thiazolidinedione pretreated db/db mice show enhanced insulin secretion.
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Background and Aims: Thiazolidinediones are known to improve peripheral insulin sensitivity by activating the peroxisomal proliferator-activated receptor-γ (PPAR-γ). Recently it was shown that the thiazolidinedione rosiglitazone also stimulates insulin secretion in the perfused pancreas of healthy rats. The aim of the present study was to investigate insulin secretion from isolated pancreatic islets of rosiglitazone pretreated diabetic db/db mice. Furthermore the islet morphology and insulin content of treated and untreated mice were determined.
Materials and Methods: 6-week-old male db/db mice were treated twice daily with 30mg/kg rosiglitazone over a period of 14 days. Blood glucose and insulin levels were measured at day 0, 3, 7, 10, and 14. Islets were isolated from 8-week-old animals using a modified collagenase digestion method. The isolated islets were perifused with Krebs Ringer buffer using a 12-channel perifusion system.
Results: Plasma glucose levels rose continuously over the 2-week period from 404±36 mg/dl to 600±19 mg/dl in untreated mice. In rosiglitazone treated mice the glucose concentration dropped from 399±35 mg/dl at day 0 to 256±13 mg/dl at day 7 of the treatment and remained at this level during the whole study. Isolated islets from 8-week-old non-treated animals were small and showed a rough surface. In contrast, islets from rosiglitazone treated mice were significantly larger, perfectly round, and had a smooth surface. The islet yield was 76/animal in untreated compared to 244/anmial in rosiglitazone treated mice. The determination of the insulin content/islet is currently under investigation. Glucose (16.7 mM) induced insulin secretion from the perifused islets of 8-week-old rosiglitazone-treated mice was approximately 10-fold higher ($p<0.05$) than from the untreated animals. Both, the islets of treated and untreated mice showed a biphasic insulin secretion pattern.
Conclusion: 1) Rosiglitazone therapy in db/db mice helps to maintain the pancreatic islet structure, yield in isolation, and glucose induced insulin secretion. 2) This can be a direct effect of the compound or a consequence of lessened glucotoxicity, or both.

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Diabetes induced with multiple low doses of streptozotocin is associated with generation of H_2O_2 and deficient antioxidant enzyme responses in pancreatic islets of C57BL/6 mice.

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Background and Aims: Multiple low doses of streptozotocin (MLD-STZ) induce type-1 diabetes in male mice of susceptible strains, whereas female mice are resistant. The reactive oxygen species (ROS), superoxide radical (O2-.), hydrogen peroxide (H2O2), and hydroxyl radicals (.OH) are involved in inflammatory reactions and are implicated as mediators of ß-cell destruction. Physiologically, ROS are intercepted by different antioxidative enzymes: superoxide dismutase (SOD) catalyzes O2-. to H2O2. Incomplete depletion of H2O2 by catalase (Cat) and glutathione peroxidase (GPx) facilitates generation of highly toxic .OH through the Fenton reaction. Since islets are equipped with relatively low levels of antioxidative enzymes they may succumb to attacks by ROS that are generated by MLD-STZ and activate the transcription factor nuclear factor (NF)-kB, which is involved in gene activation of proinflammatory cytokines.
Materials and Methods: In isolated islets, analyses of H2O2 generation by the fluorescence method with scopoletine and mRNA expression of Cat, GPx, and SOD by semiquantitive RT-PCR.
Results: *In vitro*, STZ stimulated (P< 0.05) dose-dependently H2O2 generation in islets of male but not of female mice. MLD-STZ upregulated H2O2 generation in islets *ex vivo* of male but not of female mice. The *ex vivo* analyses of the mRNA expression of antioxidative enzymes in islets of both genders demonstrated constitutive levels of Cat, GPx, and CuZnSOD. In male mice, MLD-STZ upregulated (P< 0.05) the mRNA expression only of Cat (P< 0.05), whereas in female mice all three antioxidants were increased (P< 0.05).
Conclusions: Male C57BL/6 mice develop MLD-STZ diabetes, because they fail to respond to ROS by upregulation of several antioxidative enzymes, whereas their diabetes-resistant female counterparts do so. ROS are not only mediators of ß-cell destruction, but can activate NF-kB, which is involved in gene activation of proinflammatory cytokines and chemokines, which in turn, generate ROS. Since this laboratory found that MLD-STZ increased the activity of NF-kB in islets of male but not of female mice, this effect may augment the potential of proinflammatory cytokines due the downregulation of the anti-inflammatory cytokines interleukin (IL)-4 and IL-10. Protection from MLD-STZ diabetes may depend on sufficient antioxidative responses on the one hand and on a bias towards anti-inflammatory cytokine profiles on the other hand.

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Altered monocyte cyclooygenase expression in non-obese diabetic mice.

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Background and Aims: Monocytes and T cells infiltrate the islets in the destructive process leading to Type 1 diabetes mellitus (T1DM). Non-obese diabetic (NOD) mice are an animal model of human T1DM. Activated monocytes express cycloxygenase (COX), a key enzyme in prostanoid metabolism, which is expressed in two isoforms, COX-1 (believed constitutive) and COX-2 (inducible). The aim of this study was to investigate: a) quantitative COX mRNA levels (both COX-1 and COX-2) both basal and in response to non-specific antigen stimulation with lipopolysaccharide (LPS) in NOD mice, and b) whether these COX mRNA responses to LPS are altered in diabetes.
Materials and Methods: $CD11b^+$ monocytes were isolated from splenocytes of male and female diabetic mice (n= 10; 5 female: 5 male; age range 14-37 weeks), non-diabetic mice (n=38, 22 female: 16 male; age range 5-30 weeks) as well as female C57BL/6 control mice (n=7; age 5 weeks) and examined for COX-1 and COX-2 mRNA using real time quantitative RT-PCR (Taqman).
Results: We found no difference in basal COX-1 and COX-2 mRNA levels between diabetic and non-diabetic NOD mice and controls. Following LPS COX-1 mRNA levels decreased in both female (p=0.0052) and male (p=0.0038) non-diabetic NOD mice, as well as controls (p=0.0095) irrespective of their age, which was surprising, while COX-2 mRNA levels increased in all groups irrespective of age, as expected. These observations demonstrate an isoform switch in COX mRNA response to LPS with COX-1 decreasing and COX-2 increasing. This isoform switch was detected after 5 and 10 weeks of age in female and male NOD mice respectively, as well as in controls by 5 weeks of age. The isoform switch was altered in diabetic NOD mice in that COX-1 failed to respond to LPS (p=0.36), though, COX-2 mRNA levels increased (p=0.03). In contrast to diabetic NOD mice who had no COX-1 mRNA response to LPS, non-diabetic NOD mice of similar age showed a decreased response (p=0.043). While diabetic NOD mice had a smaller COX-2 mRNA response to LPS compared with non-diabetic NOD mice (p=0.017).
Conclusion: In conclusion, we show quantitatively for the first time that following non-specific antigen stimulation with LPS there is an isoform switch of monocyte COX mRNA in NOD mice. This isoform switch develops at a young age in NOD and C57BL/6 control mice. We also show for the first time that this monocyte COX isoform switch is altered in diabetes. Diabetic compared to non-diabetic NOD mice showed a reduced response of both COX-1 and COX-2. This alteration in monocyte responses in mice implicates a defect in innate effector cells in the pathogenesis of diabetes, since we have identified a similar defect in human diabetes.

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Characterization of dendritic cells in diabetes mellitus.

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Background and Aims. In type 1 diabetes (T1D), β cells are destroyed by an autoimmune process involving dendritic cell (DC)-mediated activation of autoreactive T cells. Altered DC properties have been proposed for their preference to drive an immunogenic rather than tolerogenic T cell response. However these studies are conflicting and the physiological relevance is unclear since *in vitro*-generated DC were examined. To date, natural DC have not been well characterized in diabetes. In this study, we examined DC subsets in their natural state to gain a clearer understanding of their role in the etiology of diabetes.
Materials and Methods. Whole blood was directly labeled with a panel of monoclonal antibodies and analyzed by 4-color flow cytometry to determine the frequency and phenotype of natural DC in subjects with new onset T1D (<1 yr post-diagnosis or C-peptide positive), long term T1D (>10 yrs post-diagnosis), type 2 diabetes (T2D), type 1.5 diabetes (T1.5D) and non-diabetic controls. The rate of activation by DC cultured in various stimuli was also examined by flow cytometry. Enriched DC were examined for their ability to stimulate T cells.
Results. The total number of DC per liter of blood was similar in each group, however the percentage of DC constituting the total leukocyte population was higher in long term T1D (p<0.05) and lower in T2D (p<0.01) than controls. The proportion of myeloid DC ($CD123^{lo}$ $CD11c^+$ DC) was higher than lymphoid DC ($CD123^{hi}$ $CD11c^-$ DC) in each group. Between groups, the DC population comprised a similar percentage of lymphoid DC. In contrast, the proportion of myeloid DC was significantly higher in long term T1D compared to T2D (p<0.01) and T1.5D (p<0.01). Each DC subset was in a similarly low activation state in all groups, as defined by similar expression of the CD83, 4-1BB ligand, CD80 and CD86 molecules. DC from each group were effective stimulators of both allogeneic and autologous T cells. Preliminary findings suggest that DC in T1D may have an increased ability to upregulate costimulator molecules.
Conclusion. These findings provide new insight into an increasingly important role for myeloid DC during the pathogenesis of T1D.
Juvenile Diabetes Research Foundation, Canadian Institute of Health Research

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Importance of the cellular immune status for development of diabetes in the LEW.1AR1-*iddm* rat.

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Background and Aims: The LEW.1AR1-*iddm* rat is a new animal model of type 1 diabetes mellitus (T1DM), which arose spontaneously within a MHC-congenic inbred LEW.1AR1 (*RT1^{r2}*) colony in 1997 at Hannover Medical School. In contrast to the BB rat this model shows no T-cell lymphopenia or other obvious defects of the cellular immune system. It was the aim of this study to characterise the role of the cellular immune system for development of T1DM by adoptive transfer on the basis of an immunocompetent recipient.
Materials and Methods: Immune cells were isolated from lymph nodes and spleen from diabetic LEW.1AR1-*iddm* rats and the diabetes resistant LEW.1AR1 background strain. After activation by ConA up to 7 x 10^6 cells were injected into the tail vein of LEW.1AR1-*iddm* rats and LEW.1AR1 rats 3 weeks after birth. The diabetes incidence was monitored by blood glucose measurements and histological examination of the pancreas.
Results: Adoptive transfer of ConA activated immune cells from diabetic donors into diabetes prone LEW.1AR1-*iddm* rats significantly increased the incidence of diabetes by 100 %. On the other hand the transfer of cells from diabetic donors failed to induce diabetes in the LEW.1AR1 background strain. The transfer of immune cells from the diabetes resistant LEW.1AR1 background strain into LEW.1AR1-*iddm* rats reduced the incidence of diabetes by 50 %. FACS analyses of the ConA activated cells revealed that immune cell preparations comprised 70 % T-cells, 3 % B-cells and 3 % NK-cells. Pancreatic islets from diabetic rats, which had received immune cells through adoptive transfer from diabetic LEW.1AR1-*iddm*donors showed a typical infiltration by T-lymphocytes, B-lymphocytes and NK cells. The time course of pancreatic beta cell destruction after adoptive transfer was comparable to that after spontaneous development of diabetes in LEW.1AR1-*iddm* rats.
Conclusion: The data provide evidence that autoreactive T-cells confer beta cell destruction in the LEW.1AR1-*iddm* rat. More importantly the LEW.1AR1 background strain contains regulatory T-cell subsets, which could suppress the autoimmune T-cell response in LEW.1AR1-*iddm* rats. Perspectively, the LEW.1AR1-*iddm* rat might be an interesting model to study the regulatory dysfunction leading to T1DM and to develop novel protective strategies for immunotherapy.

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Zn2+-enriched drinking water prevented spontaneous diabetes in NOD mice.

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Background and Aims: Type 1 diabetes in man results from the destruction of the insulin-producing beta cells. T cell-dependent inflammatory immune reactions are considered to mediate the damage. It is assumed that reactive oxygen species (ROS) are ultimate mediators in the destructive process both in human as well as in the spontaneous and experimentally induced diabetes models of laboratory animals. In the spontaneous non obese diabetic (NOD) mouse, a massive infiltration of the pancreatic islets with lymphoid cells occurs weeks before hyperglycemia at the age of about 12 weeks. The pro-inflammatory cytokines IFN-γ and TNF-α play a critical role in the pathogenesis, whereas the anti-inflammatory cytokines IL-4 and IL-10 operate counterregulatory. It is known that ROS can stimulate pro-inflammatory cytokine production and that these cytokines generate ROS. In NOD mice, the diabetes prevalence is higher in females than in males. Previously, we reported that Zn2+-enriched drinking water (Zn2+) prevented diabetes induced with multiple low doses of streptozotocin (MLD-STZ) in mice. It is concluded that Zn2+ may exert its protective effect through the antioxidant metallothionein (MT), which was upregulated in pancreatic islets by Zn2+ and which is known to be a potent scavenger of the highly reactive hydroxyl radicals (·OH), the most toxic species of the group of ROS. Here, we studied, whether Zn2+ also protects from spontaneous diabetes in NOD mice.
Materials and Methods: NOD breeding pairs received either Zn2+ or control water, the offsprings were also divided into two groups receiving either Zn2+ or control water.
Results: Zn2+ protected NOD mice from diabetes. The protective effect was more pronounced when both the breeding pairs and their offsprings received Zn2+. In females, the percentage of euglycemic mice was duplicated from 25% up to 56.5%. Zn2+ was also protective in male NOD mice - although less effective - and increased the percentage of euglycemic mice from 69.6% up to 94.1%.
Conclusion: Since Zn2+ was shown to significantly induce MT in islets of three different mouse strains, we hypothezise that Zn2+ inhibited ß-cell destruction by inducing MT to scavenge ·OH as mediators of ß-cell damage. It remains to be analyzed whether Zn2+ affects the insulitis in NOD mice. Since zinc ions protected from diabetes induced with both MLD-STZ and alloxan in mice as reported from this laboratory as well as from spontaneous diabetes in the NOD mouse - present data - , and in experimental protocols applied by other investigators in rat and mice, it is suggested to study, whether supplementation with zinc ions can at least retard diabetes manifestation in individuals at risk for type 1 diabetes.

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Protective effect of neonatal oral administration of Diapep277 on diabetes Type 1 in bio-breeding diabetes-prone rats.

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Background and Aims: Diabetes Type 1 is an autoimmune disease that leads to the destruction of insulin-producing ß-cells in the islets of Langerhans in the pancreas. The identity of the target self-antigen is not known. Several self-antigens have been reported to be involved in activating T-cells to destroy the islets. Hsp60 is one of those self-antigens. Heat shock proteins are found in all life forms and are highly conserved. Hsp60 (the human equivalent of bacterial hsp65) has been implicated in autoimmunity through molecular mimicry, based on the high homology with hsp65 of microorganisms leading to recognition of the hsp60 and consequent reaction of the immune system. DiaPep277 is composed of a 24-amino-acid sequence of hsp60. NOD mice spontaneously developing diabetes manifest progressive T-cell reactivity to p277 beginning at the onset of insulitis. The objective of the present study was to test whether it was possible to affect diabetes development by neonatal oral administration of DiaPep277 in two models of diabetes; the BB-DP rat model and BB-DP rats on a hydrolysed casein (HC) diet, which gives partial protection against diabetes.
Materials and Methods: Neonatal BB-DP rats received orally either DiaPep277 in saline or saline on day 4, 5, 6, and 7 of life (300 µg/rat/day). After weaning, rats received either standard laboratory chow or HC diet.
Results: The group that received DiaPep277 and standard chow showed a lower diabetes incidence (69% DiaPep277 versus 86% in control). The DiaPep277 and HC diet group showed an even lower incidence (31% Diapep277 + HC compared to 53% in control + HC). As previously reported, groups that received HC diet showed a delay in onset of diabetes (from the age of 60 days in the control groups to an average 80 days in the HC groups). Analysis of the intestinal flora of the animals showed a significant increase in *Eubacterium sp.* in the HC groups. No differences were found between the groups receiving DiaPep277 or saline.
Conclusion: While HC diets could have their protective effects by changing the composition of intestinal microflora, DiaPep277 could have its effect through induction of oral tolerance.

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Pioglitazone delays diabetes onset in the non-obese diabetic (NOD) mouse.

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Background and Aims: Thiazolidinediones (TZDs) acts as perixosome proliferator activated receptor-gamma (PPAR-) agonists to be used as insulin sensitizer for the treatment of type 2 diabetes. In addition,it has anti-inflammatory and immuno-modulatory properties that make them of interest for preventing immune process in the islets in type 1 diabetes. The aim of this study was to explore the effects of pioglitazone on the prevention of insulitis and diabetes in female NOD mice.
Materials and Methods: Female NOD/Lt mice at 4 weeks of age were divided into 3 groups and fed a regular diet with or without pioglitazone.Pioglitazone was given as 0.01% or 0.04% food admixture from 4 to 30 weeks of age. Animals were monitored weekly for glycosuria from age 10 weeks onwards. Diabetes was diagnosed when two successive blood glucose levels were ≥16.9mmol/l. Pancreases were removed from non-diabetic mice at 12 weeks of age to assess insulitis by histology.

Results: At 30 weeks of age, the diabetes incidence was 80% (20/25) in the control group,60.9% (14/23) in the 0.01% pioglitazone treated group and 60% (15/25)in the 0.04% pioglitazone treated group (P>0.05). The lower diabetes incidence was found in 0.01% pioglitazone treated group at 14 weeks of age [0% (0/23) vs 16.0% (4/25), P=0.045], and in 0.04%pioglitazone treated group at 19 weeks of age [12.0% (3/25)vs 36.0% (9/25), P=0.049], compared to corresponding control groups respectively. The insulitis score was not different between control group (n=5) and 0.01% (n=4) or 0.04% (n=7) pioglitazone treated groups at 12 weeks of age (1.99±0.75 vs1.01±0.68, P=0.079; and 1.19±0.84, P=0.118), but significantly lower in a total treated group (1.12±0.75 vs1.99±0.75, P=0.049).
Conclusion: Pioglitazone, to some extent, lessens insulitis severity and delays diabetes onset in female NOD mice, resulting from its action as an inhibitor of pro-inflammatory genes.

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Graft failure in association with engraftments of islet grafts in the liver of mice is mediated by INF-γ.
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Background: Currently, islets isolated from 2-3 cadaveric donor pancreases are required for the treatment of single recipient with insulin-dependent diabetes mellitus by islet transplantation (Tx). Thus, the number of clinical islet Tx is limited due to the inability of affording adequate number of donor islets. In the present study, we hypothesize that INF-γ might play a significant role in engraftments of islet grafts in the liver, which is the site of clinical islet Tx, leading to graft failure.
Materials and Methods: Isolated syngeneic islets were transplanted into the liver of streptozotocin (180 mg/kg)-induced diabetic mice (C57BL/6). The non-fasting plasma glucose levels were monitored by 60 days after Tx when IPGTT was performed, followed by the morphological study of the grafts.
Results: When 400 and 200 islets were grafted into the liver of diabetic wild-type mice, 100 (6/6) and 0% (0/10) of recipients, respectively, became normoglycemic (<200 mg/dl) after Tx. Morphologically, well-granulated and degranulated b cells of islet grafts were seen in the liver of mice receiving 400 and 200 islets, respectively. Thus, diabetic mice receiving 200 islets remained hyperglycemic after Tx. In marked contrast, when 200 islets were grafted into INF-γ-deficient diabetic mice, all mice (n=5) became normoglycemic by 14 days after Tx. When human recombinant INF-γ (50,000 units) was administered ip for 7 times from day 0 to 6 after Tx, INF-γ-deficient diabetic mice receiving 200 islets did not become normoglycemic. When diabetic wild-type mice receiving 200 islets and treated with anti-INF-γ antibody (ip, 100 μg/injection) for 3 times at day 0, 2 and 4 after Tx, 4/5 mice became normoglycemic, while all mice (n=4) receiving 200 islets and treated with the control antibody (rat IgG1k) remained hyperglycemic. IPGTT revealed that the plasma glucose levels of wild-type hyperglycemic mice (n=4) receiving 200 islets and treated with control antibody were 440.5±75.8 (mean±SD), 633.3±81.3 and 456.0±164.8 mg/dl at 0, 30 and 120 minutes, respectively, after the glucose injection (1g/kg) and those of normoglycemic mice (n=4) treated with anti-INF-γ antibody were 70.0±8.0, 324.3±6.5 and 188.0±10.6 mg/dl, respectively. The plasma glucose levels of normoglycemic mice (n=4) receiving 400 islets without the treatment were 66.5±4.1, 413.8±18.5 and 259.8±18.7 mg/dl at 0, 30 and 120 minutes, respectively.
Conclusion: These findings clearly show that INF-γ plays a significant role in engraftments leading to graft failure of intrahepatic islet grafts and indicate that the treatment targeted at INF-γ may facilitate to improve engraftments resulting in successful islet transplantation from one donor to one recipient. The effects of anti-INF-γ-antibody on the outcome of intrahepatic islet allografts are currently under investigation.

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Cellular and antibody-mediated rejection of intrahepatic islet xenografts from rat to mouse is prevented by a blockade of co-stimulatory signals with anti-ICOS antibody in conjunction with CTLA4Ig.
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Background and Aims: Cellular and humoral immune responses are responsible for islet xenograft rejection although the precise mechanisms remain unknown. Recently, an inducible co-stimulator (ICOS) has been found as the third member of CD28 families. ICOS is expressed on activated, but not resting T cells and may participate in a variety of immunoregulatory functions. The purpose of the present study was to determine whether blockade of ICOS pathway by a specific antibody in conjunction with that of CD28 by CTLA4Ig has any beneficial effect on prevention of rat islet xenograft rejection in mice.
Materials and Methods: 500 Lewis rat islets were transplanted into the liver via the portal vein of STZ (180mg/ kg) diabetic C57BL/6 mice. The non-fasting plasma glucose levels were monitored before and after transplantation (Tx). Rejection was considered to have occurred when the two consecutive plasma glucose levels exceeded 200 mg/dl after Tx. ICOS expression of infiltrating cells into the liver in association with rejection was examined by flowcytometry. Furthermore, production of anti-rat antibody in mice receiving islet xenografts was determined by flowcytometry.
Results: Rat intrahepatic islet xenografts were rejected in STZ diabetic mice without any treatment at 7.5±0.7 days (mean±SD, n=12) after Tx. Morphologically, islet grafts infiltrated with mononuclear cells were found in the liver. FACS analysis revealed an expansion of CD8 T cells in the liver in association with rejection and that ICOS expression became up-regulated on CD4 T cells as well as expanded CD8 T cells. Anti-rat IgM, IgG1 and IgG2a were detected in recipient mice at the time of rejection. The treatment with anti-ICOS Ab (JMAb-51, 100 μg ip, day 1, 3, 5) alone did not affect the outcome of islet xenograft rejection including graft survival days, CD8 T cell expansion and anti-rat antibody production. The mean graft survival time (MST) in mice treated with CTLA4Ig (50 μg ip, day 0, 2, 4) was 16.2±11.9 days (n=10) after Tx. The expansion of CD8 T cells in the liver was seen at the time of rejection, and however, anti-rat antibody was not identified. In marked contrast, the MST of the grafts in diabetic mice treated with anti-ICOS antibody in conjunction with CTLA4Ig, was significantly prolonged to 50.5±41.7 (n=10) and 50% of the mice were normoglycemic at 30 days after Tx. Morphologically, intact islet grafts with well-granulated β cells were found in the liver. In the mice accepting islet xenografts, neither the expansion of CD8 T cells in the liver nor the production of anti-rat antibody was seen.
Conclusion: These findings clearly demonstrate that cellular and humoral immune responses are responsible for islet xenograft rejection from rat to mouse and that the blockade of ICOS as well as CD28 pathways leads to acceptance of intrahepatic islet xenografts by inhibiting both cellular and humoral immune responses to xenografts.

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Redox status and immunologic function in Type 1 diabetes families.
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Background and Aims: An abnormal redox status clusters in type 1 diabetes (T1DM) families and the intracellular thiol redox status seems to modulate immune function. We aimed to investigate the relationship between familiar oxidative stress and immunologic features. We measured oxidative markers, pro-inflammatory cytokines, soluble cytokine receptors, and subsets of blood lymphocytes from low-risk (without islet autoimmunity), non-diabetic first-degree relatives of patients with T1DM compared with matched controls.
Materials and Methods: Oxidative markers were erythrocyte glutathione (RBC GSH), plasma (P) and RBC malondialdehyde (MDA), haemolysis, plasma advanced oxidation protein products (AOPP) and thiols (P SH). Serum cytokines included interleukin-1 β (IL-1 β), IL-6, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α); soluble cytokine receptors sIL-2R and sIL-6R. Lymphocyte subsets were identified by combinations of the following clusters of differentiation: CD4, CD8, CD23 or low affinity IgE receptor, and CD25 or IL-2 receptor. We recruited 39 T1DM patients with duration of disease from 4 months to 41 years (mean 18±11 y), 76 non-diabetic first-degree relatives (44 parents and 32 siblings), and 95 age-sex-matched healthy subjects.
Results: Circulating AOPP were increased, whereas P SH were decreased not only in patients with T1DM, but also in relatives nothwistanding their low immunologic risk as assessed by two islet antibodies (anti-GAD65 antibodies, GADA, and islet-cell antibodies, ICA). RBC GSH was lower than normal only in T1DM patients, who showed increased erythrocyte fragility. IFN-γ was not detectable in serum of all subjects but one. IL-1 β was above the minimum detectable concentration in 21 controls, 15 T1DM, and 27 relatives without differences among groups. IL-6 and TNF-α did not differ in T1DM and relatives in comparison with age-sex-matched control subjects. Serum sIL-2R and sIL-6R were increased in T1DM than in controls. Monocyte percentage and absolute count were lower both in T1DM and their relatives than controls. Lymphocyte phenotype from patients with T1DM highlighted decreased counts of CD4CD8 and CD23CD25 double positive (DP) cells. A significant decrease in CD25 single positive (SP) and CD23CD25 DP cells was observed also in T1DM relatives vs control subjects. In T1DM, GADA levels were associated (R 0.6, p=0.01) positively with sIL-6R, negatively with duration of diabetes and CD23CD25 DP cells. Plasma creatinine correlated positively (R 0.6, p<0.001) with both sIL-2R and TNF-α. In the whole study group, we found a correlation (R 0.5, p<0.001) of CD23CD25 DP cells with blood counts of monocytes, CD4CD8 DP cells, CD25 SP cells, basal haemolysis, and plasma levels of thiols.
Conclusion: Our study reports first evidence that the oxidative stress observed in T1DM families is related to some immunologic hallmarks (decreased peripheral numbers of monocytes as well as cells bearing a CD4CD8, CD23CD25 DP, and CD25 SP phenotype) suggestive of different immunoregulatory mechanisms. It remains to be elucidated the course of events culminating in the loss of physiological immune homeostasis and disease pathology in T1DM families.

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The correlation between islet autoimmunity and basal C peptide in young adults with Type 2 diabetes mellitus.
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Background: As recently shown, it is difficult to establish the diabetes type without measuring the islet antibodies in young subjects with phenotypic features of type 2 diabetes. Particularly in this group of patients, if islet immunity is present, beta-cell failure is very likely to develop, and the diabetes type is labelled slow-progressing type 1 diabetes or LADA.
Aims: The aim of the study was to establish the clinical and immunological characteristics of subjects with diabetes diagnosed in the young adult age, considered at the onset as type 2 diabetes patients.
Materials and Methods: Fasting C peptide, HbA_{1c}, islet cell autoantibodies (ICA) and antibodies to glutamic acid decarboxylase (GADA) were measured using ELISA in 318 patients, labelled at onset as having clinical type 2 diabetes, with a diabetes duration of 5 years or less. Positivity of GADA and ICA was defined by values higher than the 97th percentile in 48 age-matched healthy non-diabetic adults (1.8 for GADA and 0.75 for ICA).
Results: The biochemical characteristics of the subjects are summarized in the table below.

*Age (yrs)	47 (21-55)
Sex (% Female)	49.7
*Diabetes duration (yrs)	2.8 (0.3-4.2)
*C peptide (ng/ml)	1.96 (0.27-5.43)
*GADA	1.81 (0.53 – 3.53) %
GADA positive	50.6
*ICA	0.5 (0.25-0.69)
% ICA positive 2.9	
*BMI	30.1 (22.1-40.2)
*HbA_{1c} (%)	8 (5.6-13.6)
% on insulin 22.1	

*Means and 5th-95th percentiles

In 51.3 percent of cases, at least one antibody was positive, while in 2.2 percent both antibodies were present in significant titer. With two exceptions, all ICA-positive subjects were also positive for GADA. In 4.4 percent of cases fasting C peptide levels were below 0.25 ng/ml (3rd percentile among non-diabetic controls), that being suggestive of decreased insulin secretion. Diabetic subjects with low basal C peptide had also significantly lower BMI and waist circumference (25.4 vs. 30.8 and 85.5 vs. 107.3 cm, respectively, p<0.0001) and higher HbA_{1c} (10.1 vs.8.4%, p=0.05) than those with normal fasting C peptide, but no significant differences in GADA and ICA positivity and distribution were noted when compared to the latter. Patient testing positive for diabetic antibodies had lower fasting C peptide concentrations (2.1 vs. 2.4 ng/ml) but the difference did not achieve significance, due to the small sample size.
Conclusion: Type 2 diabetes in young adults is a heterogeneous disorder. Patients with low C peptide concentrations, positive autoantibodies, and low BMI and waist circumference most probably belong to the LADA category.

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Novel reagents for the assay of IAA idiotypes. Towards the standardisation of diabetes-predictive IAA measurement.
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Background and Aims: The measurement of insulin autoantibodies (IAA) in childhood diabetes is currently performed using radiobinding assays which merely detect antibody and titrate it, and therefore cannot be fully standardised. In order to standardise and quantify IAA, partition assays employing idiotype-specific reagents are needed to measure the mass of a single IAA idiotype. The aim of this study was to raise a monoclonal antibody specific to a well characterised IAA idiotype.
Materials and Methods: Serum containing human insulin-specific, threonine B30-dependent (h)IAA and polyclonal IA resulting from insulin injection was affinity-purified with protein-G sepharose. The purified fraction was used to immunise BALB/c mice following tolerisation with anti-human γ to optimise detection of the insulin binding site. Immunogen-specific hybridomas and clones were selected using enzyme-linked immunosorbent assay (ELISA) and a panel of monoclonal antibodies was tested for its ability to distinguish (h)IAA, diabetes-related (d)IAA and IA. Standard deviation scores (SDS) were calculated from a panel of control IAA negative sera.
Results: Three murine monoclonal antibodies designated mA, mB and mC were examined. mA bound (h)IAA immunospecifically (127.5 SDS), but neither control IAA negative sera (0.5 SDS) nor (d)IAA (2 SDS), suggesting restriction to a private (h)IAA idiotype. mB and mC, in contrast, bound all three serum types with SDS 6.5 ± 6.46 and 5.0 ± 5.0 respectively, suggesting recognition of a public idiotype on the IgG molecule . (h)IAA distinguishes human from porcine insulin at the amino-terminal of the B chain. The restriction of mA to an idiotype capable of such resolution was further supported by the ability of human recombinant insulin to displace the reaction where Lispro insulin (Lys/Pro switch at B28/29) could not.
Conclusion: We report for the first time a novel monoclonal reagent specific for an IAA idiotype. Such reagents will provide the basis for idiotype-specific partition assays (radioimmunoassays) with which to quantify diabetes-predictive IAA in a standardised format.

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Identification of the insulin epitope B9-23 for the diabetes-susceptible allele HLA-DQ9, despite the presence of an acidic residue at position 9 of the epitope.

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Background and Aims: The aim of this study was the testing of the sequence insulin B13-21 as a putative epitope for the allele HLA-DQ9 (A1*0301/B1*0303) that has appreciable population frequency in the Japanese, where it predisposes to type 1 diabetes mellitus, while it is relatively rare in Caucasian papulations.
Materials and Methods: Binding experiments were performed using insulin peoptides B12-21 and B12-22, as well as positive control peptides for the alleles HLA-DQ8 and –DQ9, using lymphoblastoid cells that express the specific proteins. Experiments of molecular simulation of HLA-DQ9 associated with peptides were made using the coordinates of the DQ8-insulin B10-23 complex.
Results: The insulin sequence (**E**ALYLVCG**E**, anchoring position in bold letters) binds strongly to DQ8, but not to DQ9. By contrast, the addition of the relevant amino acid to each end of the nonamer core, i.e. B12-22: V**E**AL**Y**LVCG**E**R, brings about equally strong binding of this peptide to DQ8 as well as DQ9. Molecular simulation of DQ9 shows that the 11-mer peptide can bind quite well into the groove, because of interactions of the flanking residues with the DQ9 molecule and the very good fitting of residues at p1, p4 and p6, which counteracts the energetically unfavorable placing of an acidic residue at p9.
Conclusions: The relative paucity of DQ8 in the Japanese, in combination with the widespread representation of insulin protective allele VNTR III in the same population, constitutes, at least in part, the explanation for the low frequency of diabetes in Japan. The differences in DQ9 recognition of the major insulin B chain epitope may lead to altered antigen presentation or T cell recognition of insulin, and predict that T cell clones may be present in DQ9 patients with T1D which recognize extended versions of this epitope sequence.

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Echovirus 16 and islet autoantibodies.

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Background and Aims: Enterovirus infections, specially those caused by Coxsackie B, have been implicated in the development of type 1 diabetes. The possible role of other enteroviruses serotypes in the pathogenesis of this disease has also been reported. The aim of this study is to determine whether the emergent infection by echovirus 16 occurring in Cuba during 2000, was related to the presence of type 1 diabetes islet associated autoantibodies.
Materials and Methods: The presence of ICA, GADA, IA2 antibodies and neutralizing antibodies (NtAb) to echovirus 16 were determined in sera from 38 children and adolescent infected during the epidemic and 80 controls, matched in sex, age, local residence and time of sample collection.
Results: The occurrence of a large-scale echovirus 16 epidemic was associated to the appearance of humoral autoimmune markers of progression to type 1 diabetes, especially for ICA and GADA. In the convalescent stage, ICA seroconversion was demonstrated in 92.1% (35/38) of sera and in case of GADA 28.9% (11/38). None of the serum samples of the 80 uninfected subjects presented ICA, while only one was GADA positive. ICA and GADA frequency during the convalescent stage was higher in relation to the acute stage ($p<0,0005$) and non-infected subjects ($p<0,0001$). ICA presence was correlated with the presence of NtAb to echovirus 16. A strong positive correlation was found between the NtAb to echovirus 16 and ICA levels in both acute and convalescent stage ($r=0,91$; $p<0,0001$, $r=0.55$; $p=0.0003$ respectively).
Conclusion: Our findings show the first evidence of an association of echovirus 16 with the presence of antibodies related to type 1 diabetes (ICA and GADA). Echovirus 16 infection may be capable of inducing a process of autoimmune beta cell damage and confirm the hypothesis that enterovirus infections are important risk factors for the development of type 1 diabetes.

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Antithymocyte globulin in the treatment of Type 1 diabetes of recent onset: first results of a controlled randomized study.

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Background and Aims: Destruction of beta-cells in Type 1 diabetes is mediated by autoreactive T-lymphocytes, the subtypes of which have not been fully identified so far. Different immunosuppressive protocols have been tested in attempts to slow down the loss of beta-cell mass, including chemically defined immunosuppressive substances and monoclonal antibodies. While the later affect only a single activation passway, polyclonal anti-T-cell globulin (ATG) contains antibodies against several different T-cell surface molecules and signal peptides. ATG-Fresenius S acts predominantly on activated T-cells, does not cause a complete T-cell depletion and may have the ability to prevent the beta-cell loss with acceptable side effects.
Materials and Methods: We report on the preliminary results of a randomized placebo controlled single-blind study comparing the effect of ATG therapy (ATG Fresenius S 9 mg/kg + 3 additional doses of 3 mg/kg) in addition to intensified insulin therapy (Group 1) versus placebo with intensified therapy (Group 2) in Type 1 diabetes of recent onset . The main inclusion criteria are: clinical diagnosis of Type1 diabetes within the last 4 weeks, C-peptide level post glucagon stimulation of at least 0.3 pmol/ml and age 15-35 years. By February 2003, 11 patients had been randomized (7 into Group 1 and 4 into Group 2) with a mean follow-up of 13.7 (range 2-26) months. We compared the rate of diabetes remission, C-peptide levels and selected immunological parametes (anti-GAD, IA2, AIA and ICA).
Results: Two complete clinical remissions (insulin independence)occurred in Group 1 (1 for 3 months and the other continuing for more than 6 months) and none in Group 2. At the last follow-up, insulin requirements showed a tendency to decrease in 5 subjects in Group 1 and in 1 subject in Group 2. Pre-study vs last follow-up insulin does; Group 1: 0.50 ± 0.27 vs 0.40 ± 0.26 U/kd/day, Group 2: 0.41 ± 0.08 vs 0.49 ± 0.10 U/kg/day). Mean $\pm$ SD post-glucagon C-peptide levels in Groups 1 and 2 were 0.40 ± 0.33 and 1.8 ± 0.9 pmol/ml, respectively. Humoral markers of autoimmunity have remained unchanged in both groups so far. Adverse events in Group 1 included fever (4 subjects), mild symptoms of serum sickness (3 subjects) and transitory peripheral vein irritation (5 subjects).
Conclusion: Preliminary results of this pilot study suggest a positive effect of ATG therapy on C-peptide production and insulin requirement in early stages of clinical Type 1 diabetes. More patients need to be included to confirm this results.

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Exocrine tissue contamination during human pancreatic islet preparation: influence on macrophage chemotaxis and chemokines release.

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Background and Aims: The variable success rates observed in clinical islet transplantation have been attributed, in part, to the difficulty in obtaining high-quality purified islet preparation. The mean preparation purity of human pancreatic islets intended for transplantation reaches only 50 to 70%. In this study, we investigated the influence of exocrine tissue contamination on macrophage chemotaxis and chemokine released during human pancreatic islet preparation.
Materials and Methods: 24 hours after culture, the supernatants of 10,000 human islets were tested in a chemotactic chamber for chemotactic activity towards human monocyte-derived macrophages during 90 min. fMLP was used as control. Three grades of islet purity were tested: grade I with a purity higher than 75%, grade II with a purity between 40 to 75% and grade III with a purity lower than 40%. Chemokine release in the supernatant of islet preparations was evaluated by CCL-5 (RANTES) and CCL-3 (MIP-1a) determination using ELISA tests.
Results: The chemotactic activity of islet preparations in grade I was comparable to culture medium (1.3 ± 0.4, n=7). In grade II, the macrophage chemotaxis increased significantly from 1.8 ± 0.2 to 3.1 ± 0.2 ($p<0.001$, n=7) and from 3.9 ± 0.1 to 4.4 ± 0.2 in grade III preparations ($p<0.01$, n=7). In grade I, CCL-5 and CCL-3 secretion was 28.9 ± 12.2 pg/ml and 8.6 ± 7.6 pg/ml respectively. CCL-5 and CCL-3 secretion increased in grade II from 81.9 ± 8.7 to 131.2 ± 7.5 pg/ml ($p<0.05$, n=3) and from 34.1 ± 8.2 to

492.06±31.6 pg/ml (p<0.001, n=7) respectively and up to 198.9±6.8 pg/ml and 910.79±32.5 pg/ml in grade III preparations (n=7).
Conclusion: In conclusion, the purity of the islet preparation strongly influences the chemokine release and the macrophage chemotaxis and consequently probably islet rejection. These results suggest that the use of more highly purified islet preparations will improve the outcome of islets transplantation.

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The effect of acute rejection on pancreatic graft function.
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Background and Aims: Along with surgical complications, acute rejection (AR) is the most frequent cause of pancreatic graft loss in the early post-transplant period. Our study was designed to determine to what extent AR affects late pancreatic graft function.
Methods: In the 1994-2002 period, standard intravenous glucose tolerance test (IVGTT) was performed in 122 long-term normoglycemic pancreas recipients (fasting glycemia ≤ 6 mmol/l) to calculate coefficient of glucose assimilation (K_G). AR was diagnosed on the basis of clinical manifestations (combined, in all cases, with histological findings) or only on the basis of per-protocol histological evaluation. AR classification developed by Drachenberg.
Results: IVGTT was performed, in the first post-transplant year, in 105 pancreas recipients; of this number, 20 were treated for a rejection episode. Despite fasting normoglycemia and normal glycosylated hemoglobin levels, 7 patients showed an abnormal response (DIA) to IVGTT (K_G < 0.8 %/min); of this number, 2 (29%) had a history of AR. Impaired glucose tolerance (IGT, 0.8 ≤ K_G < 1.2 %/min) was diagnosed in another 30 subjects; of these, 10 (33%) had a history of AR. 68 patients had normal glucose tolerance (NGT, K_G ≥ 1.2 %/min). AR was demonstrated in only 8 (12%) of them. Between post-transplant years 2 and 5, DIA was present in 8 patients; of this number, only 1 (12%) had a history of AR. Among 13 IGT patients, AR was previously diagnosed in 3 (23%). Among 24 pancreas recipients without IGT, AR was established in 4 (17%). On overall evaluation of the last tests of the 122 patients (with mean post-transplant times of 2.0 ± 2.4 [SD] years), AR was present in the history of 4 out of 19 (21%) recipients with a DIA response to IVGTT; 8 out of 27 (30%) with IGT, and in 10 out of 76 (13%) with NGT. The individual groups (DIA vs. IGT vs. NGT) did not differ among themselves in free fasting immunoreactive insulin (9.1 ± 4.7 vs. 12.9 ± 10.5 vs. 15.2 ± 9.9 mU/l) or in maximum and baseline rates of insulin production during IVGTT (3.7 ± 2.1 vs. 7.2 ± 10.0 vs. 5.8 ± 4.1). In the group with a history of AR (n = 22) K_G was 1.3 ± 0.5 %/min, without AR (n = 100), next K_G 1.4 ± 0.53 %/min (NS). No marked difference was even seen between the groups in the predefined insulin levels. Only in the first post-transplant year we noticed an increased incidence of AR in patients with IGT (IGT vs. NGT; p = 0.02). AR experienced at a late date had no significant effect on the endocrine graft function.
Conclusion: Early diagnosis of rejection is largely aided by per-protocol biopsies in the first post-transplant year. Early diagnosed and treated AR need not have adverse effects on future pancreatic graft function.
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Beta Cell Apoptosis

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Discovery of gene networks regulating cytokine-induced apoptosis in insulin-producing INS-1 cells.
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Background and Aims: Locally released cytokines contribute to β-cell dysfunction and apoptosis in type 1 diabetes mellitus. In vitro exposure of insulin-producing INS-1E cells to the cytokines IL-1β+IFN-γ leads to a significant increase in nitric oxide (NO) production and apoptosis. To characterize the genetic networks implicated in β-cell apoptosis, we performed a time-course microarray analysis of cytokine-induced genes in INS-1E cells.
Materials and Methods: INS-1E cells were exposed in duplicate to IL-1β (10 U/ml) + IFN-γ (100 U/ml) for six different time points (1, 2, 4, 8, 12 and 24h) with or without the iNOS blocker LMA (1 mM). RNA was extracted and analyzed with the U34A Affymetrix rat oligonucleotide array containing 5000 known genes and 3000 ESTs. Gene expression was considered as modified if the duplicates averaged 2.5-fold change in at least one of the six time-points. Based on their temporal pattern of variation, the cytokine-regulated genes were classified into 15 clusters by the K-means method. These genes were further classified into 14 different groups according to their putative function.
Results: After respectively 12 and 24h exposure to cytokines, the percentage of apoptotic cells were 11 ± 1% (P<0.05 vs controls-12h) and 23 ± 1% (P<0.001 vs controls), while it was only 4 - 5% in control cells. LMA did not protect against cytokine-induced apoptosis (cytokines+LMA-12h, 9 ± 2%; cytokines+LMA-24h, 18 ± 2%). The microarray analysis identified 700 genes as cytokine-modified. Changes in the expression of genes related to metabolism, signal transduction and transcription factors at all time points indicate β-cell attempts to adapt to the effects of continuous cytokine exposure. Forty-six percent of the genes modified by cytokines after 12-24h were NO-dependent. The distribution of NO dependent/independent genes is approximately 50% in most of gene clusters, with the following exceptions: a. In the metabolism cluster 60% of the genes are NO-dependent. The most NO-dependent metabolism subgroups are amino acids (67%), lipids (57%) and ATP (87%); b. In the cell cycle cluster 71% of the genes are NO-dependent; c. In the cytokine and chemokine and MHC-related genes clusters, respectively 87% and 100% of the genes are NO-independent.
Conclusion: The present time course microarray and cluster analysis provides a detailed picture of the different gene clusters involved in the β-cell progression from initial functional stimulation to late inhibition and apoptosis. It also identified >300 novel cytokine-induced genes, several of them with potential functional relevance.

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Sequential nitrosylation, p38MAPK activation and apoptosis in RINm5F β-cells in response to nitric oxide.
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Background and Aims: Nitric oxide (NO) is thought to be involved in the autoimmune destruction of β-cells and it is known to induce apoptosis in RINm5F β-cells, although the molecular mechanisms remain unclear. One group of proteins closely associated with the control of apoptosis is the mitogen activated protein kinases (MAPKs). Here we determine the time course of apoptosis in response to NO and use antibodies specific for the activated (phosphorylated) forms of JNK, ERK and p38 MAPKs, to establish if NO changes MAPK activity and, if so, if the changes precede the onset of apoptosis. We have also monitored a potential signalling mechanism of NO, S-nitrosylation of protein thiols, over time for comparison with the temporal profiles of MAPK activation and apoptosis.
Materials and Methods: For all methods, RINm5F cells were cultured for up to 24h with or without 250μM of the NO donor, S-nitrosoglutathione (SNOG). Apoptosis was assessed at different time points by caspase activation, using CaspACE™ or phosphatidylserine externalisation, using FITC-labelled Annexin V. Western blots of lysates from 0.5, 1.5 or 2h time points were probed using anti-active JNK, ERK or p38 antibodies (Promega). For the nitrosylation assay, lysates were collected at 15-30

minute intervals for up to 3h. S-nitrosothiol levels were then assayed by the mercuric ion-mediated liberation of NO by water and measurement of the resultant nitrous acid by its reaction with sulphanilamide and N-1-naphthylethylenediamine to form a coloured product with peak absorbance at 540nm.
Results: Increases in apoptosis were first seen following 2h exposure to SNOG (% apoptosis (±SEM): CaspACE™: Control 2.44% (±0.76); +250µM SNOG 7.55% (±1.4), p=0.046. PS externalisation: Control 0.09% (±0.09); +250µM SNOG 2.02% (±0.40), p=0.009; n=9; 1-way ANOVA with Tukey's). A strongly immunoreactive band corresponding to p38 was detected in cells exposed to SNOG for 1.5h. This activity was transient, decreasing to control levels after 2h. No change was detected in the activity of ERK or JNK at any time, versus controls. Significant increases in S-nitrosylation were detected spectrophotometrically by 15min; levels peaked at 1h and had returned to control levels after 3h exposure to SNOG (A_{540}: untreated cells at 0h: 0.034 (±0.0006), + SNOG @ 15min: 0.086* (±0.006), + SNOG @ 1h: 0.094* (±0.003) *p<0.001 vs control; n=6).
Conclusion: SNOG induced a transient activation of p38 MAPK at a time point (1.5h) that preceded the first detectable increase in apoptosis at 2h. ERK and JNK activities were not responsive to SNOG and are therefore unlikely to be involved in NO-induced apoptosis in RINm5F cells. The transient S-nitrosylation of proteins, first detected at 15min and peaking at 1h, also preceded the detected change in p38 activity and the onset of apoptosis. This temporal sequence allows for the possibility that the S-nitrosylation of particular proteins and p38 activation may be causal in the initiation of NO-induced apoptosis. Work is ongoing to determine if this is so.

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Beta-cell expression of IGF-I prevents inflammatory response in a transgenic mouse model of autoimmune diabetes.
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Background and Aims: Transgenic mice expressing human IFN-ß in ß-cells (RIP/hIFNß) show functional alterations in islets and increased susceptibility to develop insulitis. To obtain a new model of type 1 diabetes, the inflammatory effect of multiple very low doses of streptozotocin (STZ) (15 and 20 mg/Kg) were examined in these mice. Transgenic mice expressing IGF-I in ß-cells recover ß cell mass after STZ treatment. Here we also studied whether IGF-I expression may counteract autoimmune diabetes in IFN-ß expressing mice. To this end, double transgenic mice expressing both IFN-ß and IGF-I in ß-cells were obtained.
Materials and Methods: Two-month-old male control, IFNß transgenic, IGF-I transgenic and IFNß/IGF-I double transgenic mice (CD-1 genetic background) were treated with very low doses of STZ (15 or 20 mg/ Kg bw) for 5 consecutive days.
Results: Transgenic mice expressing IFN-ß in ß-cells showed mild infiltration of the pancreatic islets. Nevertheless, these mice were normoglycemic and normoinsulinemic and presented normal fertility and viability throughout adulthood. However, IFN-ß transgenic mice developed high lymphocytic infiltration of the islets, with presence of CD4+ and CD8+ T cells, when treated with 15 or 20 mg/Kg of STZ. These mice presented ß-cell destruction by apoptotic mechanisms and showed a chronic increase in glycemia and hypoinsulinemia. These STZ treatments did not induce diabetes in control mice. After STZ treatment, the expression of IGF-I in ß-cells of double transgenic mice IFNß/IGF-I counteracted insulitis. STZ-treated double transgenic mice were normoglycemic and normoinsulinemic and presented similar pancreatic insulin content and ß cell mass to control healthy mice.
Conclusion: These results indicate that transgenic mice expressing IFN-ß in ß cells treated with very low doses of STZ may be a new animal model of type 1 diabetes in which to assay new therapies. Furthermore, our studies suggest that local expression of IGF-I may protect ß-cells against inflammatory responses and prevent autoimmune diabetes.
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Transgenic mice expressing IGF-II in ß-cells develop diabetes after streptozotocin treatment.
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Background and Aims: Insulin-like growth factor I and II (IGF-I and IGF-II) are growth-promoting polypeptides with powerful mitogenic and antiapoptotic effects. IGF-II induces ß-cell proliferation and differentiation *in vitro* and is involved in the regulation of islet growth and differentiation. We have recently shown that transgenic mice expressing IGF-I in ß-cells (RIP-I/IGF-I) recover ß-cell mass after induction of experimental diabetes. Here we examined whether local expression of IGF-II in pancreatic ß-cells of transgenic mice may also counteract cytotoxicity and diabetes after treatment with streptozotocin.
Materials and Methods: Two-month-old male RIP-I/IGF-II transgenic and control mice (C57Bl6/SJL genetic background) were treated with 5 consecutive doses of 40 mg/Kg of STZ to induce experimental diabetes. Metabolic parameters and morphometric analyses of pancreas were performed in these mice.
Results: Similarly to control mice, transgenic mice expressing IGF-II in ß-cells showed hyperglycemia and overt diabetes after STZ treatment. These mice were hypoinsulinemic, developed polydipsia and polyphagia and altered glucose tolerance test. Two months after STZ treatment, quantitative morphometric analysis of pancreas of transgenic mice showed strong reduction of ß-cell mass. This was parallel to reduced pancreatic insulin content. In contrast to IGF-I transgenic mice, IGF-II transgenic mice did not recover the altered parameters and did not survive longer than 4 months after STZ treatment.
Conclusion: These results indicate that local expression of IGF-II in ß-cells of transgenic mice does not counteract type 1 diabetes. They also suggest that IGF-I and IGF-II may act through different mechanisms in pancreatic ß-cells *in vivo*.
This study was supported by CICYT SAF99-0094 and Fundació La Marató de TV3.

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The role of STAT5 in the anti-apoptotic effect of GH on insulin producing cells.
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Background and Aims: Type 1 diabetes is characterized by an autoimmune destruction of the β-cells. The proinflammatory cytokines IL-1β, IFN-γ and TNFα exert cytotoxic effects on the β-cells leading to β-cell death. GH is found to stimulate both proliferation and insulin production in β-cells in vitro. Activation of STAT5 is shown to be important for both the GH-induced proliferation and the anti-apoptotic effect of GH on cytokine-induced cytotoxicity in β-cells. The aim of this study was to evaluate if activation of SOCS3 (Suppressor of Cytokine Signaling-3) or $Bcl\text{-}_{XL}$ may be involved in the anti-apoptotic effect of GH.
Materials and Methods: We used the insulin producing INS-1E cell line which is a sub clone of INS-1 cell line, but reported to be more glucose-responsive. A dominant negative variant of STAT5 (mSTAT5aΔ749, which inhibits both STAT5a and STAT5b) and a constitutive active form of STAT5b (mSTAT5b1*6) were introduced by adenovirus-mediated gene-transfer (400 PFU/cell). Apoptosis was induced by a mixture of IL-1β (40 pg/ml), IFN-γ (50 ng/ml) and TNFα (0,5 ng/ml). Apoptosis was measured by a Cell Death Detection Elisa^{PLUS} kit (Roche), which detects fragmented DNA released to the cytoplasm. Messenger RNA for $Bcl\text{-}_{XL}$ and SOCS3 were measured by real-time RT-PCR using LightCycler (Roche).
Results: We found that apoptosis induced by a mixture of IL-1β, IFN-γ and TNFα was inhibited by the addition of GH. A dominant negative variant of STAT5a abolished the anti-apoptotic effect of GH in the INS-1E. On the other hand, the constitutive active variant of STAT5b exerted a protective effect on the cells. We found that the expression of SOCS3 mRNA was increased by treating the cells for 4 hours with either cytokines (7.15 fold ± 0.36, p<0.0001) or GH (2.76 fold ± 0.13, p<0.0001). The combination of cytokines and GH resulted in an additive increase (12.01 fold ± 0.54, p<0.0001). The mRNA expression of the anti-apoptotic $Bcl\text{-}_{XL}$ was increased by GH (1.57 fold ± 0.06, p<0.0001). (n=4)
Conclusions: These results show that STAT5 is an important mediator of the anti-apoptotic effect of GH. This effect may be mediated by the

increased expression of Bcl-$_{XL}$ and the SOCS3. Overexpression of SOCS3 in INS-1 cells was previously shown to prevent cytokine-induced apoptosis and the present findings suggest that increased SOCS3 expression may contribute to the anti-apoptotic effect of GH. Increased expression of Bcl-$_{XL}$ by GH may also contribute to this effect.

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Sphingosine-1-phosphate signaling and beta-cell survival.

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Background and Aims: A bioactive phospholipid, sphingosine-1-phosphate (SPP) can act both as an intracellular second messenger and a receptor ligand. Isolated pancreatic islets and INS-1 cells were studied to determine a role for SPP in insulin release and β-cell survival.
Materials and Methods: Expression of mRNA for EDG receptors in rat islets, mouse islets and INS-1 insulinoma cells was determined by RT-PCR. Sphingosine kinase (SPHK) converts sphingosine to SPP. SPHK activity and SPP synthesis was determined after treatment of ^{32}P-prelabeled INS-1 cells. Apoptosis was determined by annexin V binding.
Results: The endothelial differentiation gene-encoded G protein-coupled (EDG) receptor class (EDG-1 through -8) includes a family of receptor isoforms (EDG-1, -3, -5, -6, and -8) that bind SPP with high affinity and specificity. Rat islets contain the mRNA for EDG-1, -2, -3, -5, -6, and -7 receptors, but not EDG-8 receptors. Mouse islets also expressed EDG-4 mRNA. INS-1 cells showed mRNA expression for EDG-1, -2, -3, -5, -6 but not EDG-7 or -8. In isolated rat islets, extracellular SPP caused a concentration-dependent (0.1 - 10 μM) inhibition of cyclic AMP production in response to glucagon-like peptide 1 (GLP) (0.1 μM). SPP also induced a concentration-dependent decrease in glucose (G) (8 mM) plus GLP-1 stimulated insulin secretion; basal insulin release was unaffected. Long-term 7-day islet G (11 mM) stimulation elicited a marked down-regulation of EDG-1 mRNA levels relative to basal 5.5 mM G values. Islets cultured for 7 days at 11 mM G had more than 2-fold ($P<0.05$) higher relative SPHK mRNA expression than control islets at 5.5 mM G; this paralleled an increase in SPHK activity of 17-fold ($P<0.05$). SPHK activity and SPP synthesis were determined in INS-1 cells treated with interleukin-1β (IL) and tumor necrosis factor α (TNF) for 8.5 h. The cytokines increased endogenous SPP production in intact cells by 151±15% ($P<0.05$). The cytokine effect was partially inhibited by cycloheximide. There was little or no release of [^{32}P]SPP from INS-1 cells. IL increased relative islet SPHK mRNA levels 6-fold ($P<0.02$) after 1 h compared to control values, and INS-1 cells treated with IL for 8.5-22 h showed SPHK activity was increased almost 2-fold in cell lysates. Apoptosis in INS-1 cells after 48 h IL/TNF/interferon γ treatment was reduced by the presence of exogenous SPP (10 μM). Transfection of cells with the SPHK gene also affected apoptosis. Caspace 3 activity appears to modulate the onset of apoptosis and may be a site of regulation by SPP.
Conclusion: Thus, pancreatic islets and INS-1 cells express signaling mechanisms consistent with SPP affecting β-cell biology inside and out. EDG receptors transduce an inhibitory signal to the adenylyl cyclase - cyclic AMP pathway and insulin release, whereas endogenous SPP has the potential to affect β–cell growth and survival.

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Elevated intracellular cAMP level suppress high glucose-induced apoptosis in isolated islets of rat pancreas.

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Background and Aims: High glucose-induced apoptosis has been implicated in loss of β–cells of pancreatic islets in animal models of type 2 diabetes. A previous study has shown that phosphodiesterase (PDE) inhibitors suppress nitric oxide (NO) production and can protect islets against autoimmunity. But there was no report about the relationship of intracellular cAMP and apoptosis of pancreatic islets in high-glucose condition. We examined effects of cAMP-elevating agents on the high glucose-induced apoptosis of isolated rat islets.
Materials and Methods: Isolated rat islets were cultured in RPMI-1640 media with various glucose concentrations (5.5, 11.1, 17, and 28 mM), 5×10^{-6} M forskolin, 2×10^{-4} M 3-isobutyl-1-methylxanthine (IBMX), 1×10^{-5} M cilostazol, and 2×10^{-5} M H-89. Islet apoptosis was measured by sandwich enzyme-immunoassay using antihistone antibody.
Results: Apoptosis was minimal at 11.1 mM glucose concentration and increased at higher glucose concentrations (1.17±0.07 A.U. (arbitrary unit) at 5.5 mM, 1.00±0.01 A.U. at 11.1 mM, 1.10±0.04 A.U. at 17 mM, and 1.30±0.10 A.U. at 28 mM glucose concentrations). 28 mM glucose concentration showed a significantly higher apoptosis than 11.1 mM glucose ($p<0.05$). In 28 mM glucose concentration, forskolin suppressed apoptosis of isolated islets (1.30±0.25 vs 0.88±0.03 A.U., $p<0.01$). IBMX (1.30±0.25 vs 0.92±0.03, $p<0.05$) and cilostazol(1.30±0.25 vs 0.96±0.04, $p<0.05$) also reduced islet apoptosis in 28 mM glucose. Inhibition of apoptosis mediated by forskolin treatment was completely prevented when islets were cocultured with the protein kinase A (PKA) inhibitor H-89 ($p<0.05$).
Conclusion: These results clearly show that high glucose-induced apoptosis of rat islet is attenuated by cAMP-elevating agents. And intracellular cAMP appears to act via PKA when preventing the apoptosis in islet in rat pancreas.

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Differential effects of repaglinide, nateglinide and glibenclamide on apoptosis in human beta cells in vitro.

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Background and Aims: Loss of β-cell mass and function constitutes a concern to the application of sulfonylureas for the treatment of type 2 diabetes. Previous studies have shown that the sulfonylureas tolbutamide and glibenclamide induce apoptosis in β-cell lines and rodent islets. Therefore, we investigated the effect of the new insulin secretagogues repaglinide, nateglinide and of the sulfonylurea glibenclamide on β-cell apoptosis in human islets.
Materials and Methods: Human islets from four organ donors were cultured onto extracellular matrix coated plates and exposed to glibenclamide, repaglinide or nateglinide at 5.5 mM glucose. Apoptosis was studied using the TUNEL assay. The doses of the three compounds were chosen according to detected maximal effects- i.e. efficacy.
Results: Exposure of human islets for 4 h to 0.1 and 10 μM glibenclamide induced a 2.38- and 2.47-fold increase of β-cell apoptosis, respectively, whereas repaglinide (10 and 1000 nM) did not change the number of apoptotic β-cells. At low concentration (10 μM), nateglinide did not induce β-cell apoptosis, however, at high concentration of 1000 μM it induced a 1.49-fold increase in the number of apoptotic β-cells. Prolonged exposure for 4 d of the islets to the secretagogues induced β-cell apoptosis. The increase was of 4.1- and 4.4-fold at 0.1 and 10 μM glibenclamide, 2.37- and 3.8-fold at 10 and 1000 nM repaglinide, and of 3.2- and 4.6-fold at 10 and 1000 μM nateglinide, respectively. Glibenclamide at 0.1-10 nM (doses which were less efficient on insulin secretion) did not induce β-cell apoptosis after 4 h incubation, as well as 0.1 nM after 4 d incubation. However, 1 and 10 nM Glibenclamide for 4 d induced a 1.8-fold increase in β-cell apoptosis, respectively.
Conclusion: Glibenclamide induced β-cell apoptosis in human islets. Repaglinide and low concentration of nateglinide did not induce β-cell apoptosis after a 4-h exposure. Thus, glibenclamide may precipitate the decrease in β-cell mass observed in patients with type 2 diabetes. In contrast, exposure of β-cells to repaglinide or nateglinide for the duration of their circulating half-life (1.5-1.8 h) may preserve β-cell mass.

PS 30
IAPP and Islet Inflammation

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Islet amyloid polypeptide immunoreactivity in amyloid: mass analysis of microdissected islet deposits.

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Background and Aims: We previously reported that N-proIslet amyloid polypeptide (N-proIAPP) is the major IAPP species in human beta cells cultured at high glucose. It was also identified by immunohistochemistry in frozen sections of pancreatic tissue from patients with type 2 diabetes, while no C-proIAPP-immunoreactivity was detected. We have now performed mass analysis and Western blot of microdissected islet amyloid to further define the molecular nature of this IAPP immunoreactivity.
Materials and Methods: Frozen sections from amyloid containing pancreata of type 2 diabetes patients were mounted on membrane-coated slides in preparation for microdissection; the control islets were stained with methylene blue and the amyloid-containing islets with Congo red, before they were microdissected using a Leica Laser Microdissection System. Samples consisting of 5-10 pooled islets were solubilised and submitted to SDS-PAGE followed by Western blot, or to Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS).
Results: Western blot analysis of microdissected islet amyloid revealed the presence of multiple IAPP-immunoreactive bands none of which was recognized by an antibody to the N-terminal flanking peptide of proIAPP. MALDI-TOF analysis indicated a predominant peak at 3908 Da, which corresponds to mature human IAPP. No masses corresponding to N- or C-proIAPP were detected. Control islets displayed prominent signals at m/z 3485 and 5817, which represent the masses of, respectively, glucagon and insulin.
Conclusion: MALDI-TOF-MS and Western blot analysis of microdissected islet amyloid both detect mature IAPP, but do not confirm the presence of masses consistent with the IAPP-precursors. These results indicate major - but yet unknown- mass modifications of the N-proIAPP immunoreactivity present in human islet amyloid.

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Mapping islets in the human pancreas indicates preferential localization of amyloid-containing islets in the periphery of the gland.

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Background and Aims: Islet amyloid occurs in more than 90 percent of type 2 diabetes patients where amyloid-containing (A+) islets are more abundant than in age-matched controls. The percent of A+ islets varies considerably among patients, for as yet unknown reasons. An unequal distribution has been reported within the human pancreas, while a more uniform distribution was noticed in transgenic mice developing islet amyloid. We investigated the topography of A+ islets in the pancreas from type 2 diabetes patients.
Materials and Methods: Whole tissue sections were made from the dorsal and ventral head, body and tail regions of six pancreata from type 2 diabetic patients; sections were stained with the neuroendocrine marker synaptophysin and the amyloid marker Congo-red, and the head regions were further identified by pancreatic polypeptide (PP) immunocytochemistry. Sections were scanned, tissue images captured in order to map the islets, and image analysis was used to determine the amyloid and synaptophysin surface areas.
Results: The degree of islet amyloidosis varies markedly among these six organs (5 to 85% A+ islets) but is comparable when dorsal head, body and tail regions are analysed within the same organ. The ventral PP-rich head region exhibits consistently a markedly lower % A+ islets than the dorsal PP-poor head region ($p<0.05$). When body and tail regions were investigated for their respective distribution of A+ islets, the abundance of A+ islets was significantly higher in the peripheral zone of the sections than in the central zone ($p<0.05$).
Conclusion: Amyloid containing islets are heterogeneously distributed in the pancreas of type 2 diabetic patients. In the body and tail regions, amyloid-containing islets are preferentially located in the periphery of the gland. In the ventral head region containing the PP-rich islets, fewer islets contain amyloid than in the other regions. Understanding the basis for these differences in topography should help to clarify the mechanisms leading to increased amyloid formation in type 2 diabetes.

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Novel mutation in the islet amyloid polypeptide (IAPP) gene promoter confers enhanced in the transcriptional activity through a new CRE-like binding site.

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Background and Aim: Over-expression of IAPP has been implicated in islet amyloidogenesis, which is a characteristic feature of human type 2 diabetes (T2DM). A mutation in the IAPP promoter at position –132 (G to A) has been described in 9.7% of T2DM subjects from Spain. Previous studies showed a 2-fold increase in the IAPP promoter activity, when this new mutant sequence was used compared to the wild-type form. Moreover, the IAPP mutant promoter activity was enhanced (2-fold increase) after incubation with forskolin or dexamethasone. The aim of this study was to investigate the interaction between nuclear proteins and mutant sequence motifs and to identify the transcription factors involved in regulation of the transcriptional activity in the IAPP gene in this region by electrophoretic mobility shift assay (EMSA).
Material and Methods: Experiments were performed from MIN6 cells in presence of 5.5 or 22.7mmol/l glucose, 11.2 mmol/l mannoheptulose, 11.2mmol/l 6-deoxy-glucose, 0.6mmol/l diazoxide, 100 µmol/l verapamil, 10µmol/l forskolin and 10µmol/l dexamethasone. Gel shift assays were performed with the mutant and wild- type oligonucleotides derived from the position –138 to-122 of human IAPP promoter. They were labeled with T4polynucleotide kinase and (g-32P) ATP. Nuclear proteins were incubated in 40mM HEPES (pH=7.9), 200mM KCl, 0,5mM dithiothreitol, 0,2mM EDTA, 5% Ficoll, and 3µg poly (dI-dC). Labeled oligonucleotide was added to the mixture and incubated at room temperature. Supershift assay, 1µl anti CREB, anti CREM, anti CBP, anti cJun, anti Fos was added before the addition of the probe and was incubated for 2h. As a control of supershift a specific CREB peptide was used. The DNA-protein complexes were separated on a 5% polyacrilamide gel, resolved at 20mA, vacuum dried and exposed to x-ray film at -70C.
Results: The EMSA snalysis showed at least two protein-DNA complexes forms in wild-type or mutant oligonucleotide. However, in wild-type oligonucleotide a very low signal was detected, compare with the mutant oligonucleotide. These bands were enhanced in mutant oligonucleotide after forskolin or dexametasone treatment but were unchanged after verapamil, diazoxide, 6-deoxy-D-glucose and mannoheptulose stimulation. A super-shifted band was detectable when anti-CREB antibody was used.
Conclusions: These results of EMSA demonstrate that a –132bp G-to-A mutation in the promoter region of the IAPP gene contributes to increase the transcriptional activity through of new CRE-like regulatory element.

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Long term rosiglitazone and metformin treatment reduce the severity of islet amyloid deposition but do not prevent its formation.

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Background and Aims: Islet amyloid deposition in type 2 diabetes is associated with reduced ß-cell mass and function. In human islet amyloid polypeptide (hIAPP) transgenic mice, a model of islet amyloid deposition, increased dietary fat intake is associated with islet amyloid formation and the development of obesity and insulin resistance. Since obesity and insulin resistance are in turn associated with increased ß-cell secretory demand, we hypothesized that anti-diabetic agents that decrease ß-cell secretory demand would decrease the extent of islet amyloid deposition.

Materials and Methods: Six to eight-week old non-diabetic hIAPP transgenic mice were treated for 12 months with rosiglitazone (ROSI, 1.5 mg/kg/d) or metformin (MET, 1 g/kg/d) to reduce ß-cell secretory demand, glyburide (GLY, 10 mg/kg/d) to increase ß-cell secretory demand, or control (CON). Mice were fed a high fat (9% w/w) diet throughout the study. At 12 months, mice were sacrificed and islet amyloid prevalence (% islets containing amyloid) and severity (% islet area occupied by amyloid), islet area and ß-cell area were quantified. In addition, body weight and abdominal (Abd) and subcutaneous (SQ) white adipose tissue (WAT) weights were quantified, the latter two to obtain the ratio of Abd WAT / SQ WAT (Abd/SQ) as a measure of body fat distribution.
Results: ROSI treatment resulted in no change in body weight compared to CON but led to a reduction in Abd/SQ, consistent with a redistribution of body fat. In contrast MET treatment resulted in lower body weight than the other three groups but no change in Abd/SQ, consistent with a lack of redistribution of body fat. The prevalence of islet amyloid and the severity of islet amyloid deposition, as well as islet area and ß-cell area were all significantly reduced with both ROSI and MET. When the effect of ROSI to decrease Abd/SQ and the effect of MET to decrease body weight were adjusted for, ROSI and MET's effects on the severity of islet amyloid deposition, islet area and ß-cell area were no longer significant. However, the prevalence of islet amyloid remained significant for both treatments. GLY treatment was not associated with any significant differences from CON.
Conclusion: By their action to reduce abdominal fat mass, through redistribution of body fat (ROSI) or reduction of body weight (MET), both ROSI and MET decrease the severity of islet amyloid deposition, islet area and ß-cell area. However, as the prevalence of islet amyloid remained significant even after adjusting for Abd/SQ or body weight respectively, this suggests that decreasing ß-cell secretory demand reduces the severity of amyloid deposition but does not prevent islet amyloid formation.

	ROS (n = 16)	METI (n = 15)	GLY (n = 10)	CON (n = 9)
Body weight (g)	54.1 ± 2.4	42.4 ± 2.5 *	52.2 ± 3.0	54.5 ± 3.2
Abd / SQ	1.64 ± 0.10 *	1.94 ± 0.11	2.03 ± 0.13	2.14 ± 0.14
Amyloid Prevalence (%)	11.7 ± 4.3 #	5.6 ± 2.6 #	41.1 ± 10.6	48.1 ± 12.2
Amyloid Severity (%)	0.71 ± 0.63 †	0.02 ± 0.01 †	5.33 ± 2.32	8.87 ± 4.71
Islet area (µm²)	27488 ± 3373 #	25510 ± 4378 #	46890 ± 4198	47983 ± 7611
ß-cell area (µm²)	18414 ± 2716 #	16675 ± 3370 #	29273 ± 2477	27955 ± 3833

Mean ± SEM; * p<0.05 vs other 3 groups; # p<0.05 ROS, MET vs CON, GLY; p<0.05 ROS, MET vs CON

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The importance of subcellular catalase localisation for the protection of insulin-producing RINm5F cells against reactive oxygen species (ROS) and pro-inflammatory cytokine mediated toxicity.
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Background and Aims: Pancreatic islets and insulin-producing tissue culture cells are known for their extremely low antioxidant enzyme expression level and their susceptibility against reactive oxygen species (ROS) and NO. The stable overexpression of antioxidant enzymes results in an improved protection against ROS and pro-inflammatory cytokines, which are important mediators of beta cell destruction. Here, we analysed the protective effect of targeted catalase overexpression in the mitochondrial compartment of insulin-secreting RINm5F cells.
Materials and Methods: Catalase was stably overexpressed either in mitochondria (MitoCat) or peroxisomes (Cat) of RINm5F cells. After incubation with H_2O_2, menadione, hypoxanthine-xanthine oxidase (HX/XO), IL-1ß or with a cytokine mix (IL-1ß, TNF-a, IFN-γ) the remaining viability of the cells was determined by the MTT assay.
Results: The overexpression of catalase in both MitoCat and Cat cells resulted in a 20fold increase of enzyme activity *vs.* control cells. Mitochondrial and peroxisomal catalase overexpression significantly protected the cells against ROS toxicity. MitoCat cells showed a significantly better protection against menadione and HX/XO toxicity than Cat cells (EC_{50} menadione: 20 µM *vs.*13 µM; EC_{50} HX/XO: 5.4 mU *vs.*3.5 mU). In comparison to Cat RINm5F cells the MitoCat cells showed no enhanced protection against H_2O_2 mediated toxicity. The mitochondrial overexpression of catalase resulted also in an increased protection of these cells against a 72 h cytokine incubation (remaining viability ILß-1: 81 % *vs.* 72 %, cytokine mix: 78 % vs. 70 %).
Conclusion: In this study we could show, that the protective effect of catalase overexpression against toxicity of ROS and pro-inflammatory cytokines could be further enhanced by mitochondrial targeting of this enzyme. The results emphasise the central role of mitochondria in the radical mediated destruction process of insulin-producing cells. The inactivation of H_2O_2 through catalase localised in mitochondria together with the intrinsic mitochondrial MnSOD can result in an optimal protection of this important cellular compartment and in a better protection of the whole ß-cell.

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Does the Ca^{2+} -binding protein secretagogin play a role in the pathogenesis of Type 1 diabetes?
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Background and Aims: In Type 1 Diabetes (T1D) the insulin producing ß-cells are selectively destroyed during mononuclear cell infiltration of the islets of Langerhans with concomitant release of proinflammatory cytokines. One of these, IL-1ß, can inhibit insulin synthesis and release and induce apoptosis *in vitro* and *in vivo*. Exposure of diabetes prone BB (DP-BB) rat islets to IL-1ß *in vitro* down-regulates the Ca^{2+}-binding protein secretagogin. Secretagogin is a neuroendocrine and islet of Langerhans specific protein influencing calcium influx and cell proliferation, but the exact function is unknown. Secretagogin has high similarity to the Ca^{2+}-binding protein, calbindin D-28, which when over-expressed, can inhibit cytokine induced ß-cell apoptosis. In humans the secretagogin gene (*SCGN,* 6p22.1-22.3) is located telomeric of the HLA region. The aims of our study were to investigate: the expression of secretagogin during development of diabetes in DP-BB rats, the effect of over-expression of secretagogin in RIN-5F cells and to screen the human *SCGN* gene for polymorphisms associated to T1D.
Materials and Methods: DP-BB, diabetes resistant BB (DR-BB) rats were syngeneically transplanted with 200 neonatal islets under the kidney capsule at day 30 of age. Transplants were removed 7, 12, 23, 37, 48 and 174 days after transplantation or at onset of diabetes (n=3-6 in each group) and protein expression was analysed by 2 dimensional gel electrophoresis and mass spectrometry. RIN-5F cells stably over-expressing secretagogin were exposed to different combinations and concentrations of IL-1ß, IFN-α and TNF-α. Released nitric oxide (NO) and insulin were measured as well as cell viability by the MTT assay. The human *SCGN* gene was screened for polymorphisms by Single Stranded Conformational Polymorphism (SSCP). Five identified polymorphisms were verified by sequencing and a Danish T1D family collection of 253 families were genotyped by a Mutagenically-Separated PCR-assay (MS-PCR). Linkage to T1D were analysed by the transmission disequilibrium test (TDT-test).
Results: In the islet transplants from DR-BB rats no differences were observed in the expression level of secretagogin, whereas at onset of diabetes in the DP-BB rats lower expression was observed (ANOVA, p=0.007). In transfected RIN-5F cells significant lower NO (282 vs. 420 percentage of control) were released after cytokine exposure compared to control cells (n=6, p=0.015). No differences were observed in insulin release and cell viability. None of the identified polymorphisms were found linked to T1D, but one of the polymorphisms showed significantly distorted transmission of the alleles to unaffected offspring.
Conclusion: The expression of secretagogin is lower at onset of diabetes in DP-BB rats and over-expression of secretagogin in RIN-5F cells results in lower NO production in response to cytokine exposure. Genetic studies in humans for one of the identified polymorphisms showed significantly distorted transmission to un-affected offspring. Taken together, this is suggestive of a ß-cell protective effect of this gene.

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Onset and progression of infiltration in the islets of Langerhans of the spontaneously diabetic LEW.1AR1/Ztm-*iddm* rat, a new animal model of T1DM.
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Background and Aims: The LEW.1AR1/Ztm-*iddm* rat is a new animal model of type 1 diabetes mellitus (T1DM). It was the aim of this morphological study to investigate the onset and time course with respect to beta cell destruction and pancreatic islet infiltration as the morphological correlate of the autoimmune process.

Materials and Methods: Pancreases from 45-60 day old animals were analysed immunohistochemically, ultrastructurally and morphometrically for the migration of subsets of immune cells in the islets and of beta cell death through TUNEL staining.
Results: At day 45 the islets of diabetic prone animals did not yet reveal any signs of infiltration and beta cell damage. The beta cells were densely granulated as under control conditions. At day 50 a quarter of pancreases revealed an infiltration of the islets starting in the periphery and mainly composed of macrophages and some lymphocytes, though the animals were still normoglycaemic. Many beta cells were apoptotic. At day 55 the immune cell migration process in the islets had progressed towards the center of the islets accompanied by an immune cell increase with a predominance of cytotoxic CD8+-lymphocytes and a massive increase of beta cell apoptosis. At day 60, 60 % of the rats had developed overt diabetes with hyperglycaemia and hypoinsulinaemia, with heavily infiltrated islets and with a high degree of beta cell loss through apoptosis. Two to five days after diabetes manifestation all beta cells within the islets were lost and the infiltration of immune cells in the pancreas disappeared. Other organs including thyroid and salivary glands were not affected.
Conclusion: At day 50 the migration of immune cells, macrophages as the first and T lymphocytes as second immune cell population, started to infiltrate the islets resulting in a complete loss of beta cells through apoptosis within one week after manifestation of diabetes. The morphological pattern of immune cell infiltration and apoptotic beta cell loss is characteristic for an autoimmune mediated disease process. Thus the LEW.1AR1/Ztm-*iddm* rat is an interesting new animal model suitable for the elucidation of the mechanisms underlying the development of autoimmune diabetes relevant also for the situation in humans.

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MHC class II expression by pancreatic beta-cells in autoimmune diabetes.
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Background and Aims: Insulin dependent diabetes mellitus (IDDM) is caused by an antigen-specific, T cell-dependent destruction of pancreatic beta-cells. Up to now, in the most widely used model for human IDDM, the non obese diabetic (NOD) mouse, MHC class II has not been detected in insulin producing beta-cells of the pancreas, suggesting that beta-cells are not a direct target of autoreactive CD4+ T-cells.
Materials and Methods: We employed single cell multiplex RT PCR in combination with single cell immunofluorescence to analyze MHC class II expression by pancreatic beta-cells of NOD mice at different stages of progression to IDDM
Results: We demonstrate here for the first time that pancreatic beta-cells from NOD mice express the I-Ag7 protein as well as the corresponding mRNA. Moreover, we demonstrate that the frequency of MHC class II mRNA expressing beta-cells is drastically increased during the progression to overt diabetes. At 3 weeks of age (no insulitis), beta-cells expressed I-Ag7 mRNA at very low frequency (5%). At 6 weeks of age (periinsulitis), expression of MHC class II was upregulated (37% I-Ag7+). In mice of 9 and 11 weeks of age (invasive insulitis) 49% and 69% of analyzed beta-cells were MHC class II positive, respectively. Finally, in mice with overt diabetes, as much as 77% of beta-cells scored positive for I-Ag7. In contrast, beta-cells from 11 week old NOD/SCID mice express MHC class II at the same low frequency (7%) as beta-cells from insulitis-free NOD mice of 3 weeks of age. The kinetics of upregulation during diabetogenesis resembled the kinetics of Fas- and TNFR2 upregulation (Walter et al., Eur. J. Immunol. 30:1224) inasmuch the statistically most important transition is that from 3 to 6 weeks of age, that is, the transition from the absence of any infiltration to periinsulitis. These findings underline the impact the lymphocytic infiltrate already has on pancreatic beta-cells at early stages of the disease. Although still free of invasive infiltration, beta-cells undergo important changes in receptor expression triggered “at distance” by cytokines released by the infiltrating cells surrounding the beta-cell mass.
Conclusion: In conclusion, beta-cells may have the capacity to directly interact with autoreactive CD4+ T-cells and may therefore be involved in the pathogenesis of type-1 diabetes.

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Human Obesity

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Impact of childhood obesity in glucose metabolism.
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Background and Aims: The prevalence of obesity in children in Greece has increased dramatically in recent years. Obesity in adults is associated with insulin resistance/hyperinsulinemia which in turn is implicated in most of the obesity-related metabolic and cardiovascular complications. The aim of this study is to investigate how early obesity associated alterations in glucose metabolism occur.
Material and Methods: 194 schoolchildren of 6-12 years old were studied. Data concerning height (H), weight (W), birth height (BH) and birth weight (BW), systolic (SBP) and diastolic (DBP) blood pressure were collected. They were grouped according to BMI (Cole 2000 criteria) into: normal weight (N:104), overweight (OW:56) and obese (OB:34). Total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, Lp (a) and Apo (a) and Apo (b) were measured. All children underwent a 2h OGTT test (1,75 g gluc/kg body weight); fasting and 2h glucose and insulin levels were determined (FG, 120G, INS0 and INS120’ respectively). IR-HOMA, an index of insulin resistance was calculated. For statistical analysis ANOVA and x^2 and Pearson correlation were used.
Results: OB as well as OW children, compared to N, had higher FG (x ±SDmg/dl: N: 90± 9, OW:93±8 OB:91±7 p<0,005) and 120G (x±SDmg/dl: N:90±14,OW:96±14, OB:96±16 p<0,005) and INS0 (x ±SD IU/dl: N:6± 4, OW:10±5, OB:8 ±5 p<0,005) and INS 120 levels (x ±SDIU/dl: N:24±12, OW:35±23, OB:30±22 p<0,005). IR-HOMA values were also significantly higher in both groups compared to N (x ±SD: N: 1.5±1.1, OW:2.4±1.2, OB:1.7±1.1 p<0.005), independent of gender and age. There was no difference among groups in lipid parameters, however there was a significant correlation between insulin, both fasting and 2h and TG levels (r=0203, p=0.014 and r=02107 p=0.015 respectively). Further, the OW and OB children had higher systolic BP (x±SD mmHg: N: 91±15, OW:97±14, OB:100 ±18 p<0,005) compared with N but there was no difference in DBP. There was also no difference in BH and BW.
Conclusions: Obese but also overweight children, had significant alterations in glucose metabolism (higher fasting and post-load blood glucose levels with concomitant hyperinsulinemia and insulin resistance) and higher systolic BP than normal weight children. The presence of insulin resistance /hyperinsulinemia at such early age may play an important role in the future development of impaired glucose tolerance and type 2 diabetes. Therefore, the effective prevention of childhood obesity is of critical importance.

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Increased oxidative stress is associated with insulin resistance in obese subjects.
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Background and Aims: Oxidative stress has been linked to insulin resistance and several clinical trials with anti-oxidants have demonstrated improved insulin sensistivity in insulin-resistant and diabetic patients. However the direct relationship of oxidative stress with insulin resistance in obese subjects have not been as yet studied. In the present study, we measured the plasma levels of 8-epi-PGF2α, which is considered to be the most reliable marker of oxidative stress, in obese and nonobese subjects to clarify whether there is direct relationship between oxidative stress and insulin resistance in obese subjects.
Material and Methods: This study comprised 16 subjects with obesity (BMI≥25.0) and 18 age-matched nonobese (BMI‹25.0) subjects. None of the subjects had diabetes mellitus, hypertension, hyperuricemia or smoking history. There were 5 patients with hyperlipidemia in obese subjects. Five had hyperlipidemia in nonobese subjects. Plasma levels of total 8-epi-PGF2α were measured using a commercially available enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). The serum levels of free fatty acids (FFA) were measured by an automated enzymatic method and serum levels of vitamine E (α- and γ-tocopherol) were measured by HPLC. Insulin resistance was evaluated by the euglycemic hyperinsulinemic clamp technique using an artificial pancreas (Nikkiso

STG-22, Tokyo, Japan). The mean amount of glucose given during the last 30 min was defined as the glucose infusion rate (GIR). Body fat distribution was evaluated by abdominal CT scans taken at the umbilical level.
Results: Obese subjects had significantly higher plasma concentrations of 8-epi-PGF2α than nonobese subjects ($p<0.05$). The plasma levels of 8-epi-PGF2α were significantly correlated with BMI and visceral fat area in all (obese and nonobese) subjects (BMI; $r=0.588$, $p<0.01$, visceral fat area; $r=0.496$, $p<0.01$). There was also a significant correlation between the plasma levels of 8-epi-PGF2α and GIR in all ($r=-0.722$, $p<0.01$) and obese subjects ($r=-0.680$, $p<0.01$). In all subjects, the plasma levels of 8-epi-PGF2α were significantly correlated with fasting serum levels of insulin ($r= 0.460$, $p<0.01$). No significant correlations were observed between the plasma levels of 8-epi-PGF2α and serum levels of FFA ($r=0.296$, $p=0.10$) or vitamin E (α-tocopherol; $r=-0.302$, $p=0.08$, γ-tocopherol; $r=-0.185$, $p=0.29$) in all subjects.
Conclusion: The present study showed that the plasma levels of 8-epi-PGF2α are significantly increased and correlated with insulin resistance in obese subjects. These findings suggest that obesity is an important factor for enhanced oxidative stress and that, this oxidative stress triggers the development of insulin resistance.

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The circulating mononuclear cells in the obese is in a pro-inflammatory state.
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It has previously been shown that plasma concentrations of pro-inflammatory cytokines, TNFα and IL6, and the inflammatory marker, CRP, are elevated in the obese. We have now investigated whether the peripheral blood mononuclear cell (MNC) is also in a pro-inflammatory state when compared with that of normal subjects. MNC were prepared from fasting blood samples of 8 obese (BMI=37.7± 5.0) and 8 lean (BMI=23.8 ± 1.9) subjects. Nuclear extracts, RNA, and whole cell homogenates were prepared from the MNC. Nuclear Factor κB (NFκB) binding to nuclear extracts was measured by EMSA. It was found to be significantly elevated by 28% ($p<0.05$) in the obese. Nuclear homogenates, on the other hand, showed the Rel-A (p65) of the NFκB to be slightly but not significantly higher in obese subjects. The inhibitory subunits of NFκB, IκB-κ and IκB-β, were measured in the total cell homogenate and showed a significant decrease in IκB-β by 48% ($p<0.001$) but a moderate and non-significant increase in IκB-α. Real time RT-PCR revealed elevated levels of mRNA for MIF by 200%, IL-6 by 315%, TNF-a by 87% and MMP-9 by 240%. These data show for the first time that MNC in the obese is in a pro-inflammatory state with increased transcription of pro-inflammatory genes induced by NFκB. The higher NFκB binding activity in the obese is likely due to lower quantities of IκB-β. These data also suggest that the increase in the concentration of pro-inflammatory cytokines and mediators in plasma may receive a contribution from circulating MNC more quite apart from the adipose tissue which has been shown to secrete TNF-α and IL-6.

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Prolonged reduction of hyperinsulinemia improves insulin sensitivity in non-diabetic, insulin resistant obese individuals.
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Background and Aims: Chronic hyperinsulinemia and insulin resistance frequently occur in healthy non-diabetic obese subjects and are a predictor of subsequent development of type 2 diabetes mellitus. Hyperinsulinemia is thought to be a compensatory mechanism to overcome the defect in insulin action, although chronic physiologic hyperinsulinemia per se can induce insulin resistance in non-diabetic subjects. The aim of the present study was to examine whether the amelioration of hyperinsulinemia is associated with increased insulin sensitivity in non-diabetic, insulin resistant obese individuals.
Materials and Methods: Six healthy, non-diabetic obese subjects (3 females and 3 males), ranging in age from 21 to 59 years (mean= 41±6) and BMI from 28.8 to 36.4 kg/m^2 (mean= 32.8±1) were studied. The mean percent fat mass (by bioimpedance) was 36±7%. All subjects received an oral glucose tolerance test (OGTT) and a two step euglycemic hyperinsulinemic (40 and 160 mU/m^2 per minute) clamp, followed by the continuous subcutaneous infusion of octreotide (Sandostatin), a somatostatin analog, at the rate of 240 μg/24 h for 4 days. The OGTT and the clamp were repeated on days 3 and 4, respectively, of the octreotide infusion. Each subject served as his own control and consumed a weight-maintaining diet throughout the study.
Results: Mean fasting plasma glucose (PG) conc before and during the Sandostatin infusion were 98±4 and 106±4 mg/dl, respectively (P= NS). PG conc during the OGTT averaged 134±2 before and increased to 150±7 mg/dl after Sandostatin ($P<0.05$). The area under the curve (AUC) of the PG during the OGTT increased from 160±3 to 180±8 after Sandostatin ($P<0.05$). Following Sandostatin, the fasting plasma insulin (PI) conc decreased from 7±0.6 to 5±0.4 μU/ml ($P=0.03$) and mean PI conc during the OGTT decreased from 44±4 to 22±2 μU/ml ($P<0.01$). The AUC for PI was reduced from 55±5 to 26±2 after Sandostatin ($P<0.01$). Plasma glucagon (59±8 vs 59±12 ng/L) and growth hormone (0.5±0.2 vs 0.2±0.1 ng/ml) levels were not statistically different. The insulin sensitivity index (ISI) during the OGTT increased by 33%, from 5.4±0.4 to 7.2±0.2 ($P=0.03$). During the first insulin clamp step (physiologic hyperinsulinemia), the M value increased by 31%, from 4.2±0.2 to 5.5±0.2 mg/kg per min ($P<0.001$); no change in the M value was observed during the second insulin clamp step (maximal insulin stimulation) (9±0.3 and 9±0.2, P=NS).
Conclusion: Sustained reduction of fasting and postprandial plasma insulin concentrations in normal glucose tolerant, insulin resistant obese individuals ameliorates the defect in insulin-mediated glucose disposal. Thus, the "compensatory" hyperinsulinemia, not only serves to maintain glucose tolerance, but represents a self-perpetuating cause of the insulin resistance. These results suggest that long-acting somatostatin analogs may represent a therapeutic option for the prevention of type 2 diabetes in selected, at risk individuals.

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Obese subjects display an impaired muscle lipid oxidative capacity after weight loss.
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Background and Aims: Recent studies support the concept that a low resting energy expenditure and a low ability to oxidize fat are risk factors for weight gain. Formerly obese subjects have been shown to have a decreased fat oxidation in the fasting state, postprandially and during exercise. However, the cellular mechanisms responsible for this decrement in muscle lipid oxidation after weight loss is not completely defined at molecular level.
Aims: The aim of the present study was therefore to discern if changes in the major genes encoding for protein enzymes involved in fat oxidation play a role in the reduction of lipid oxidation and if they are related to the triglyceride muscle content.
We investigated the expression of genes of oxidative and lipid synthesis pathways by RT-PCR: PPARα, Acyl-CoA Oxidase (ACO), Carnitine Palmitoyl Transferase 1B (CPT1B), Acetyl-CoA Carboxylase (ACAC) β and Fatty Acid Synthase (FAS) in skeletal muscle biopsies from obese patients before and after biliopancreatic diversion (BPD).
Methods: Ten morbidly obese subjects (BMI= 49.04 ± 3.19 kg/m^2) were observed before and 18 ± 2 months after BPD. Body composition was determined by ^{3}H-water. Euglycemic hyperinsulinemic clamp was performed before and after weight loss. The rates of glucose and lipid oxidation were estimated in respiratory chamber, muscle triglyceride (TG) concentration was also measured. Insulin, glucose and free fatty acids (NEFA) were assayed in plasma; mRNA was measured by RT-PCR in muscle biopsies and normalised to β-actin content.
Results: After BPD treatment the patients had lost about 30% of their initial body weight, reducing their FM from 46% to 29% and also their FFM from 71 kg to 53 kg. Moreover, they shown a significant decline in plasma insulin, glucose, NEFA levels and significantly improved their insulin-sensitivity as assayed by clamp studies (2.1 ± 0.3 vs 7.3 ± 0.2 mg×Kg/min; $p < 0.001$). After weight loss we observed an increase in glucose oxidation (140.0 ± 28.3 vs 112.0 ± 31.1 mg/min; $p<0.02$) and a decline in lipid oxidation (188.4 ± 22.1 vs 295.5 ± 42.3 mg/min; $p<0.0001$). Moreover the muscle TG content resulted significantly decreased after BPD (12.2 ± 1.4 vs 4.2 ± 0.2 μmol/g; $p < 0.05$). A clear reduction in PPARα and CPT1B mRNA content was found in muscle biopsies obtained after weight loss.
Conclusions: Our results suggest the presence of a defect of both peroxisomal and mitochondrial oxidative pathways at muscular level that may contribute to the reduced fat oxidation in formerly obese subjects.

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Relationship between serum resistin concentrations and peripheral tissue insulin sensitivity in obese and non-obese experimental model of insulin resistance.

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Background and Aims: Resistin is a novel adipocyte derived hormone which has been shown to induce insulin resistance in obese mice and may link of obesity to the development of type 2 diabetes. Recently these findings were questioned by findings that resistin mRNA expression in adipose tissue and plasma resistin levels were lower in subjects with type 2 diabetes and obese rodents. In this study we investigated the relationship between serum resistin levels and muscle and adipose tissue insulin sensitivity in obese (SHROB/Koletsky rats) and non-obese (hereditary hypertriglyceridemic rats; HHTg) experimental models of insulin resistance.
Material and Methods: The experiments were carried out in adult male HHTg and SHROB non-fasting rats weighing 365±15 and 624±48 g. respectively ($p<0.001$). Normotrglyceridemic Wistar rats and SHROB lean siblings were used as controls. All animals were fed a standard laboratory diet. R0esistin concentrations were measured (Linco Res., St Charles, MO, USA).
Results: Triglyceridemia was elevated both in HHTg rats (2.78±0.07 vs Wistar controls 1.32±0.15 mmol/l, $p<0.001$) and SHROB obese animals (3.05±0.24 vs 1.08±0.15 mmol/l, $p<0.001$). Glucose intolerance measured after glucose load was higher in HHTg than in Wistar rats and in SHROB than in SHR lean rats but was not significantly different between HHTg and SHROB rat strain (AUC: HHTg 893±31 vs SHROB 906±56 mmol/l, N.S.). Deterioration of the sensitivity of skeletal muscle (m. soleus) to insulin action as evaluated by in vitro ^{14}C-glucose incorporation into glycogen (HHTg: 150±38 vs SHROB 138±28 nmol glucose/g tissue, N.S.) was of similar degree in HHTg and SHROB rats. Soleus muscle triglyceride content was elevated markedly in SHROB rats compared with HHTg rats (9.99±1.48 vs 4.23±0.32 mmol/g w.wt., $p<0.001$). ^{14}C-glucose incorporation into epididymal adipose tissue lipids under the basal conditions (without insulin) was lower in both HHTg rats and SHROB than in controls by approximately 30%. Insulin stimulated glucose incorporation showed similar tissue resistance in both nonobese and obese experimental groups (HHTg: 31.65±2.84 vs SHROB: 25.67±2.41 nmol/mg protein/120min, N.S.). Serum resistin levels was increased threefold in SHROB rats as compared to SHR lean controls (62.66±2.85 vs 21.79±2.55 ng/ml, $p<0.001$). In contrast, no difference in the serum levels of resistin was found in HHTg rats (22.27±4.09 ng/ml) and Wistar controls (18.65±2.01ng/ml, N.S.).
Conclusion: Results indicate that in obese model of insulin resistance circulating concentrations of resistin were increased in parallel with reduced muscle and adipose tissue insulin sensitivity and support hypothesis that resistin may provide the link between obesity and insulin resistance. In contrast, similar degree of tissue resistance to insulin action in HHTg rats was not associated with elevated serum levels of resistin. Further studies are needed to explore if markedly elevated lipid accumulation in muscles in obese animals may participate in elevation of plasma resistin levels.

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TNF α, its receptors and insulin resistance in obese subjects. The comparison between the preperitoneal and subcutaneous fat deposition.

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Background and Aims: To clarify the association of the insulin resistance, tumor necrosis factorα (TNFα) and its receptors (sTNFR-1,2) in preperitoneal and subcutaneous fat deposition type of obese subjects.
Materials and Methods: Ultrasonography was performed on 194 persons who visited Futaba Kosei Hospital for standard health check up. Among the individuals, 40 persons who had a body mass index (BMI) greater than 26kg/m^2 and 10 age-matched healthy non-obese subjects who were chosen at random were included in this study. Subjects with a history of pharmacological treatment for hypertension or hyperlipidemia were excluded. The thickness of subcutaneous and preperitoneal fat layers were measured directly on the screen using electronic callipers. The maximum thickness of preperitoneal fat (P-fat) and the minimum thickness of subcutaneous fat (S-fat) were used as representative markers.Abdominal wall Fat Index (AFI) was calculated as the P-fat / S-fat ratio.The obese subjects were divided into 2 groups according to AFI. We determined high-AFI group (male≥1.0 and female≥0.7) is preperioneal fat deposition subjects and low-AFI group ((male<1.0 and female<0.7) is subcutaneous fat deposition subjects.Plasma TNFα, sTNFR-1,2, serum insulin, fasting plasma glucose, uric acid, triglycerides (TG), total cholesterol and HDL-cholesterol were compared between obese and non-obese subjects.
Results: In obese subjects, plasma TNFα(17.6±6.0 vs 8.3±1.8pg/ml, $p<0.05$), sTNFR-1(1052±412 vs 816±179 pg/ml, $p<0.05$), serum insulin(10.5±4.7 vs 5.5±1.1 uU/ml, $p<0.05$) acid and TG(141±59 vs 78±44 mg/ml, $p<0.05$) were significantly higher than those in non-obese subjects, respectively. HDL-cholesterol(53±14 vs 80±23 mg/ml, $p<0.05$) was significantly lower than non-obese group. However, in obese subjects, sTNFR-2(2585±851 vs 2322±669, pg/ml, NS), fasting blood glucose(109±11 vs 94±6 mg/ml, NS) and uric acid(5.1±1.4 vs 4.4±1.0 mg/ml, NS) were not significantly higher than those in non-obese subjects. In preperitoneal fat deposition subjects, plasma TNFα(21.6±4.1 vs 8.5±1.8 pg/ml, $p<0.05$), sTNFR-2(2877±997 vs 2322±669 pg/ml, $p<0.05$) HOMA-IR(2.62±1.4 vs 1.3±0.3 mg/ml, $p<0.05$) and TG(171±42 vs 78±44 mg/ml, $p<0.05$) were significantly higher than those in non-obese subjects. However, in subcutaneous fat deposition subjects, plasma TNFα, sTNFR-2 HOMA-IR and TG were not higher than those in non-obese subjects. In both the healthy non-obese and obese subjects, the plasma TNFα levels exhibited a significant positive correlation with BMI, AFI, P-fat , but no correlation with S-fat. The plasma TNFα levels also were correlated significantly with the level of sTNFR-1,2 .
Conclusion: The insulin resistance was observed together with lipid profile alteration in obese subjects. Furthermore, the results suggest that TNF α is associated with the insulin resistance of the obese subjects, and that the elevation in circulating sTNFR-2 is due to its increased expression especially in the preperitoneal adipocytes.

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Central fat and circulating fatty acids but not skeletal muscle triglyceride are independent predictors of whole-body insulin sensitivity in men.

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Background and Aims: Body lipid depots, including total and abdominal fat mass, circulating lipids and skeletal muscle triglyceride content (SMT), have all been found to predict insulin sensitivity in man. The relative influence of each lipid compartment is less clear. Recently, agents which elevate or lower circulating fatty acids have been found to affect insulin sensitivity with varying effects on body adiposity and SMT. We examined whether circulating fatty acids, total and abdominal fat mass and SMT were independent predictors of insulin sensitivity and whether there were relationships between the lipid compartments.
Materials and Methods: 59 non-diabetic Caucasian males (age 45.4 ± 15.6 y, BMI 29.1 ± 3.7 kg/m^2, fasting plasma glucose 5.5 ± 0.7 mmol/l) underwent body composition assessment (DEXA) and percutaneous biopsy of vastus lateralis for assessment of SMT. Indirect calorimetry was performed in the basal state for assessment of substrate oxidation. Fasting circulating non-esterified fatty acid (NEFAs), total cholesterol and triglyceride (TG) levels were measured. Euglycaemic-hyperinsulinaemic clamp (50 mU/m^2/min) was performed, with stable glucose infusion rate (GIR) used to assess whole-body insulin sensitivity. Associations between continuous variables were investigated by simple and multiple regression analyses.
Results: Insulin sensitivity (GIR) was related to central fat (r=-0.59, p<0.0001) and less strongly to total body fat (r=-0.34, p<0.01). GIR was also related to fasting NEFAs (r=-0.47, p<0.001) and weakly related to SMT (r=-0.27, p<0.05). Fasting NEFAs were related to central fat (r=0.45, p<0.001) but not SMT (r=0.17, p=0.2). Total cholesterol had similar associations to GIR and central fat as NEFAs, but total cholesterol and NEFAs were not associated with each other (r=0.18, p=0.17). There was a trend for serum TG to be related to GIR (r=-0.27, p=0.06). SMT was related to central fat (r=0.27, p<0.05). Basal fat oxidation related positively to fasting NEFAs (r=0.46, p<0.01) but was not related to GIR (r=0.08, p=0.64). Multiple regression analyses showed central fat and NEFAs to be independently associated with GIR, accounting for ~40% of the variance. SMT was not an independent predictor of GIR.
Conclusion: When central fat is measured accurately, it is the strongest predictor of insulin sensitivity and the association of muscle triglyceride and insulin sensitivity may be dependent on their association with central fat. Circulating fatty acids, although closely correlated with central fat, remained independent predictors of insulin sensitivity. This suggests that lipolytic regulation as well as the mass of central fat are important in modulating insulin sensitivity.

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Normal secretion and action of the gut incretin hormones glucagon like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) in young insulin resistant men with low birth weight (LBW).

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Background and Aim: Low birth weight (LBW) is associated with insulin resistance, disproportionately defective insulin secretion and an increased risk of Type 2 diabetes later in life. We recently found evidence of impaired insulin secretion in response to oral - but not intravenous - glucose ingestion indicating defective secretion or action of the gut incretin hormones in young men with LBW. Accordingly, decreased secretion and/or action of the incretin hormones glucagon like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) has been reported in patients with overt Type 2 diabetes.
Our aim was to study the secretion and action of GLP-1 and GIP in young men with LBW.
Methods: 24 Caucasian men aged 19–21 years with a birth weight within the lowest 10 percentile of the normal range and 25 age matched men (controls) with a birth weight in the upper normal range were included. All subjects were born at term and none had any family history of diabetes. The study included 3 days. One day a standard meal test was given to study incretin hormone secretion. On two separate days, either GLP-1 or GIP was infused in constant rates (60 pmol/kg/h and 240 pmol/kg/h respectively) for 120 min to obtain supraphysiological levels during a hyperglycaemic clamp (p-glucose 7 mM), in order to study incretin hormone action. The concentrations of GLP-1, GIP, insulin and C-peptide were measured frequently during each study day.
Results: The mean birth weight was 2785 g in the LBW group and 3951 g in the control group (p< 0,0001). The men with LBW had significantly higher fasting p-glucose (p=0,04), near significantly higher waist to hip ratio (p=0,06) and were on average 5 cm shorter (p=0,02). There were no differences in fasting levels of incretin hormones. During the meal test subjects with LBW showed significantly elevated levels of glucose areas under the curve ($AUC_{glucose}$ p<0,0016), as well as elevated insulin AUC (p<0,006) and elevated C-peptide AUC (p<0,02). Neither GIP nor GLP-1 levels differed significantly at any time points before or after the test meal, and AUC for both GLP-1 and GIP were subsequently similar. Glucose disposal was 20 % and 9 % decreased during GLP-1 and GIP infusion respectively in LBW-subjects compared with controls (p < 0,016 and p < 0,28). However, plasma insulin as well as plasma C-peptide concentrations (absolute values and AUC´s) were similar at all time points during both GLP-1 and GIP infusion, demonstrating normal action of these incretin hormones on pancreatic insulin secretion in subjects with LBW.
Conclusions: The study support the notion of an important role of the intrauterine environment on glucose homeostasis as illustrated by elevated plasma glucose levels and reduced glucose disposal rates indicating insulin resistance in young men with LBW. Nevertheless, the study does not support the idea that a disproportionately reduced pancreatic insulin secretion in subjects with LBW may be due to early defects of neither the secretion nor the action of the incretin hormones GLP-1 or GIP

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A model to identify individuals likely to be insulin resistant.

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Background and Aims: Insulin resistance has been associated with both type 2 diabetes mellitus (T2DM) and cardiovascular disease. The euglycemic insulin clamp method offers an accurate measure of insulin resistance; however, it is too expensive and intrusive for widespread application in a clinical setting. In light of recent findings that lifestyle and pharmacological therapy can prevent or delay the onset of T2DM, we sought to develop a model based on physical examination and biochemical measurements which could be used to identify insulin resistant candidates for T2DM preventive treatment.
Materials and Methods: We analyzed cross-sectional data on 1308 non-diabetic subjects from 19 different sites participating in the European Group for the Study of Insulin Resistance (EGIR), 140 non-diabetic subjects in San Antonio, Texas (of whom 97 were Mexican Americans), and 560 non-diabetic subjects from the Pima Indian Reservation in Arizona. Each individual's measured glucose disposal rate (M) was divided by their lean body mass (LBM) to develop a continuous dependent variable which was categorized into the most resistant quartile versus others in the pooled data set. This resulted in 7.5% of EGIR, 22.1% of San Antonio, and 72.0% of the Pima subjects being defined as insulin resistant.
Results: The best model we developed was: $p = 1/(1+e^{(6.7173-0.1028*BMI-0.00402*FPI*FPG)})$ Where p = the probability of being IR, BMI = body mass index (kg/m^2), FPI = fasting plasma insulin (pmol/L), and FPG = fasting plasma glucose (mmol/L). Each coefficient of this model was significant at p < 0.001. The area under the receiver

operating characteristics curve (AROC) was 0.920. We attribute this high AROC to the wide range of insulin resistance measures resulting from including subjects who were European Caucasian, an ethnic group known to be relatively insulin sensitive and other subjects who were from the Pima Indian tribe known to be insulin resistant. When stratified by race, the AROCs were 0.806, 0.869, and 0.883 for Caucasians, Mexican Americans, and Pimas respectively. At a cut point corresponding to a prevalence of 25%, the sensitivity of the pooled model is 74% and the specificity is 93%. The model was validated in two other studies: the AROC using the model score to predict the lowest quartile of insulin sensitivity (measured by euglycemic insulin clamp) among 136 subjects from South Carolina was 0.911. It was 0.900 among 485 subjects from San Francisco (measured by SSPG method).
Conclusion: The product of fasting insulin and fasting glucose adjusted for body mass index provides a reasonably accurate surrogate measure for insulin resistance measured by insulin clamp.

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Assessment of peripheral insulin sensitivity from an oral glucose test by a new labelled oral minimal model.
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Background and Aims: An Oral Minimal Model (OMM) was recently proposed to estimate insulin sensitivity (S_I) during an oral test. S_I measures both the ability of insulin to enhance glucose utilization as well also inhibit glucose production. It would be important to segregate these two action components, so as to measure peripheral insulin sensitivity ($S_{I\ per}$) which takes into account insulin action on glucose disposal only. The aim here is first, to develop a Hot Oral Minimal Model (HOMM) to estimate S_{Iper} during an oral test and, second, to validate HOMM measurement against both a meal and an hot-IVGTT measurement obtained in the same subjects.
Materials and Methods: Thirty six normal subjects (20 males and 16 females; age= 46.9 ± 4.0 years, body weight = 79.6 ± 2.2 kg) had both a [1-^{13}C]-labelled meal, consisting of a 10 kcal/kg, 45% carbohydrate, 15% protein, 40% fat, and a labelled IVGTT consisting of a bolus of 0.33 g/kg of glucose and 0.132 g/kg of 6,6-$^2H_2$-glucose.
a) HOMM was identified on [1-^{13}C]-glucose and insulin data, thus providing estimates of both $S_{I\ per}$ and glucose rate of appearance in plasma (Ra).
b) During the meal protocol 3-^{3}H-glucose was infused at variable rate in order to clamp the ratio between 3-^{3}H-glucose and exogenous (from meal) glucose concentration. Under these experimental conditions Ra of exogenous glucose can be estimated in a virtually model-independent fashion (reference Ra, Ra^{ref}). Ra^{ref} was considered the known input of the classic intravenous hot minimal model (IVHMM), which allowed to estimate a reference insulin sensitivity index ($S_{I\ per}^{\ ref}$).
c) IVHMM was identified on 6,6-$^2H_2$-glucose IVGTT data providing $S_{I\ per}$ ($S_{I\ per}^{\ IVGTT}$).
Results: HOMM $S_{I\ per}$ was similar to $S_{I\ per}^{\ ref}$ and $S_{I\ per}^{\ IVGTT}$ ($S_{I\ per}$ = 10.19 ± 1.06 10^{-4} dl/kg/min per µU/ml [mean ± SE]; $S_{I\ per}^{\ ref}$ = 10.32 ± 0.94 10^{-4} dl/kg/min per µU/ml; $S_{I\ per}^{\ IVGTT}$=9.31 ± 1.18 10^{-4} dl/kg/min per µU/ml) and also well correlated with $S_{I\ per}^{\ ref}$ (r=0.82, p<0.0001) and $S_{I\ per}^{\ IVGTT}$ (r=0.77 p<0.0001). Finally HOMM Ra profile agrees well with Ra^{ref}.
Conclusion: A new labelled oral minimal model has been proposed to asses peripheral insulin sensitivity during an oral test. The model was validated by comparing its S_I measurement with that provided by both a meal model-independent technique and the labelled IVGTT method.

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Insulin action is differently determined by hyperinsulinemic clamp parameters and by HOMA index.
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Background and Aims: HOMA index is suggested to be inversely related to hyperinsulinemic clamp variables in the evaluation of insulin sensitivity. The aim of this study was to compare HOMA indexes with clamp parameters in different disorders of insulin action.
Materials and Methods: We examined 40 Type 2 diabetic patients (DM) treated by oral agents, 20 patients with insulinoma (INS), 16 with primary hyperaldosteronism (PHA), 12 with high renin hypertension (HRH) and 30 healthy persons (C) by hyperinsulinemic isoglycemic clamps. All groups were of comparable age and body mass index. The mean glycated hemoglobin was 8.0±0.7% in patients with diabetes. In all persons diagnosis was supported by clinical and biochemical examination and the presence of insulinoma and of the adrenal tumor was later confirmed by finding at surgery. Hyperinsulinemic isoglycemic clamps were performed on Biostator (mode 1:7) using insulin infusion rate 1 $mU.kg^{-1}.min^{-1}$. Metabolic clearance rate of glucose (MCRG) and insulin sensitivity index (MCRG/I) were compared with HOMA index calculated according to formula (glucose[$mmol.l^{-1}$] . insulin[$mU.l^{-1}$] /22.5). The results were expressed as means±SD and the differences between the groups were tested by Wilcoxon rank/sum test. Spearman correlation was used to find relationship between the variables of insulin action, serum cholesterol, triglyceride concentrations and systolic or diastolic blood pressure.
Results: The basic results of separate groups are shown in the Table.

Variables of insulin action in different groups

	DM	INS	PHA	HRH	C
IRI ($mU.l^{-1}$)	28[a] ±24	42[a]±26	18±7	24[c]±19	16±12
MCRG ($ml.kg^{-1}.\ min^{-1}$)	3.7[a]±2.0	9.2±3.3	4.4[a]±2.2	6.0[b]±3.1	8.8±3.5
MCRG/IRI ($ml.kg^{-1}.\ min^{-1}$ per $mU.l^{-1}$ x 100)	3.6[a]±2.8	7.4[c]±2.8	5.2[a]±2.0	5.8[b]±4.2	10.6±5.8
HOMA	11.6[a]±7.5	6.3[a]±2.9	4.0[c]±1.2	5.1[b]±2.1	3.4±1.9

Statistical significance as compared to controls: [a]p<0.001, [b]p<0.01, [c]p<0.05.
BMI was very strongly associated with the insulin sensitivity index in the whole cohort of subjects (r=-0.70, p<0.001). Serum cholesterol and triglycerides were inversely related to insulin sensitivity in Type 2 DM and in healthy persons but not in INS, PHA or HRH. Significant relationship was observed between MCRG/I and HOMA in healthy persons (r=-0.66, p<0.0001), Type 2 DM (r=-0.68, p<0.0001) and in HRH (r=-0.69, p<0.02) but not in patients with INS or PHA. Greater insulin resistance was found by clamp in PHA than in INS (p<0.01) whereas HOMA index was significantly higher in INS than in PHA patients (p<0.01).
Conclusion: Our findings indicate that HOMA index does not offer the same information as glucose clamps because calculated parameters may be differently influenced by dominantly impaired either peripheral or hepatic insulin action. The evaluation of HOMA index has to be done with a caution in patients with different pathogenesis of impaired insulin action.
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Waist circumference predicts metabolic risk factors in adults, but not in young children (The EarlyBird Diabetes Study).
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Background and Aims: Insulin resistance underlies the development of diabetes and the metabolic syndrome. Visceral fat mass is the principal contributor to metabolic risk in adults, for which girth is a useful surrogate. Centile charts for waist circumference have recently been published for use in children, but their relationship to health risk has not been established. We have assessed the value of waist circumference in the prediction of insulin resistance and triglycerides in young children.
Materials and Methods: EarlyBird is a non-intervention prospective cohort study of 300 randomly selected young children, entering school between 2000 and 2001 (mean age 4.9 years) and their parents. It aims to identify which children develop insulin resistance, and why. BMI, skinfold thickness at five sites (triceps, biceps, subscapular, suprailiac and para-umbilical), circumferences (waist, hip, midarm), insulin resistance (HOMA-IR) and triglycerides were measured in the children. BMI, waist circumference, HOMA-IR and triglycerides were measured in the parents.
Results: 1) Waist and BMI both predicted insulin resistance in the parents (mothers r= 0.62 and r= 0.64, p< 0.001 respectively; fathers r= 0.57 and r= 0.53, p< 0.001). 2) The addition of waist to BMI significantly improved the prediction of insulin resistance in the parents (mothers R^2 change= 0.01, p= 0.05; fathers R^2 change= 0.04, p< 0.001). 3) The same measures, however, were poorer predictors of insulin resistance in children (girls waist: r= 0.31, p< 0.001, BMI: r= 0.24, p= 0.01; boys waist: r= 0.21, p= 0.01, BMI: r= 0.15, p= 0.06). 4) The combination of BMI, height and hip circumference best predicted insulin resistance in the boys, but together explained only 9%

of the variance. In the girls, the combination of BMI, height, and triceps thickness explained 18% of the variance in insulin resistance. 5) The addition (or substitution) of waist circumference or waist/ height to these models did not improve their R^2 value. 6) Waist circumference and BMI were equally predictive of triglycerides in the parents (mothers r (for both) = 0.36, p< 0.001; fathers r (for both) = 0.42, p< 0.001) and neither measure provided any additional information once the other was known owing to their high co-correlation (r= 0.92, p< 0.001). 7) In the boys the best predictor of triglycerides was BMI (r= 0.27, p< 0.001). The addition of waist circumference to BMI did not provide any further information to the prediction (partial r= -0.05, p= 0.53). 8) In the girls, none of the anthropometric measures were significantly associated with triglycerides.
Conclusion: Waist circumference can be used to identify adults with metabolic risk factors (insulin resistance and triglycerides). However, its poorer clinical performance in children questions the value of measuring girth in addition to BMI at this age.

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Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebral vascular disease, peripheral arterial disease or abdominal aortic aneurysm. Findings from the SMART cohort.
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Background and Aims: Patients with the metabolic syndrome are at increased risk for developing vascular morbidity and mortality. Previous studies have shown the increasing prevalence of the metabolic syndrome in various asymptomatic populations. Despite its great attention, limited or no information is available about the prevalence of the metabolic syndrome in patients with manifest atherosclerotic vascular disease. Aim of this study is to determine the overall and gender specific prevalence of the metabolic syndrome and its components in patients with different manifestations of atherosclerotic vascular disease.
Materials and Methods: Cross-sectional survey of 1248 patients, aged 18 - 80 year, entering the Second Manifestations of ARTerial disease (SMART) study between 1 January 1999 and 1 July 2002. This study comprised patients with coronary heart disease (CHD) (n=566), cerebral vascular disease (CVD) (n=284), peripheral arterial disease (PAD) (n=289) or abdominal aortic aneurysm (AAA) (n=109). The metabolic syndrome was defined by Adult Treatment Panel III (≥ 3 of the following abnormalities): abdominal obesitas, hypertriglyceridemie, low HDL cholesterol, high blood pressure, hyperglycemie.
Results: The prevalence of the metabolic syndrome in patients with manifest atherosclerotic vascular disease was 45%. PAD patients had the highest prevalence (54%). The prevalences in CHD, CVD and AAA subjects were 40%, 44% and 47%. Overall, women had a higher prevalence than men (54% vs. 42%). The most common combination of metabolic abnormalities was hypertriglyceridemia, high blood pressure and low HDL-cholesterol (50%). Age did not influence the prevalence of the metabolic syndrome; OR 1.0 (95% CI 0.99-1.02).
Conclusion: Our results demonstrate a high prevalence of the metabolic syndrome in patients with manifest atherosclerotic vascular disease. Screening for metabolic syndrome in patients with high-risk for new vascular incidents, may identify patients with even higher vascular risk and may direct anti-atherosclerotic treatment in order to prevent new vascular incidents in the same or another vascular bed.

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Non-alcoholic fatty liver disease represents insulin resistance regardless of obesity in non-diabetic subjects.
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Background and Aims: The presence of non-alcoholic fatty liver disease (NAFLD) in patients with diabetes and obesity is known to be associated with metabolic abnormalities. But the meaning of NAFLD in non-obese, non-diabetic subjects is not well known. We evaluated the associations between metabolic abnormalities and non-alcoholic fatty liver disease (NAFLD) measured by ultrasonography in non-obese, non-diabetic subjects.
Materials and Methods: We examined 779 Korean adults above 30 years old (274 men, 505 women) participating in medical check-up in Health Promotion Center. All were non-obese (BMI < 30 kg/m^2), negative hepatitis B and C serology, and non-alcoholic (< average daily alcohol intake 2 drinks/d). A standard interview, physical exam, and biochemical study was conducted. An experienced operator carried out ultrasound liver studies.
Results: . 370 subjects had NAFLD (47.3%). The frequency in men was higher than that in women (57 vs 42%, p<0.05). The frequency in normal weight group (BMI < 25 kg/m^2) was lower than that in overweight group (BMI > 25 kg/m^2) (32.3% vs. 65.5%, p<0.05). BMI, waist circumference, body fat, systolic pressure, plasma concentration of aspartate aminotransferase, alanine aminotranferase, total cholesterol, triglyceride, the ratio of triglyceride to HDL-cholesterol, insulin resistance index ($NOMA_{IR}$), and the presence of impaired fasting glucose, hypetension were significantly increased in subjects with NAFLD compared to control group (p<0.05, respectively). After multiple regression analysis, waist, alanine aminotranferase, $HOMA_{IR}$, triglyceride to HDL-cholesterol ratio, aspartate aminotransferase, and systolic pressure were associated with severity of non-alcoholic fatty liver disease. Odd ratios of insulin resistance in mild, moderate, and severe NAFLD were 5.7 (CI: 3.6-8.8), 6.9 (CI: 4.6-10.3), 14.7 (CI: 6.8-32.0).
Conclusion: These results suggest the presence of NAFLD represents insulin resistance regardless of obesity in non-diabetic subjects and may be another feature of metabolic syndrome.

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A pathophysiological link between insulin resistance and fatty liver-associated metabolic syndrome.
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Background and Aims: The patients with type 2 diabetes mellitus, obesity and insulin resistance often accompanies nonalcoholic fatty liver disease (NAFLD). The histological spectrum of NAFLD includes fatty liver alone to nonalcoholic steatohepatitis (NASH) with inflammation and fibrosis. To clarify a pathophysiological link between NAFLD and metabolic abnormalities, we comprehensively investigated the relationship among liver pathology, liver function tests, insulin resistance and metabolic abnormalities in patients with NAFLD.
Materials and Methods: We measured anthropometrics and metabolic variables in 97 patients with NAFLD. Seventy-nine patients met the American Diabetes Association criteria for type 2 diabetes. Steatosis, inflammation and fibrosis of the liver were histologically graded from score 0 to 4 in 41 patients. Indexes of insulin resistance were determined using the homeostasis model assessment methods (HOMA) and quantitative insulin sensitivity check index (QUICKI). Metabolic clearance rates (MCR) were measured by hyperinsulinemic euglycemic clamp methods. Individuals were defined as having metabolic syndrome by WHO criteria.
Results: (1) Hepatic steatosis score correlated with serum levels of alanine aminotransferase (ALT) (p=0.00234), insulin sensitivity indexes (MCR, p=0.0164; HOMA, p=0.0395; QUICKI, p=0.0395) and plasma levels of leptin (p=0.0486) and plasminogen activator inhibitor-1 (p=0.0197). (2) Incidence of metabolic syndrome was higher in patients with steatosis in more than 25% of lobular parenchyma involved (p=0.0048). Hepatic steatosis score was higher in patients with metabolic syndrome than without metabolic syndrome (2.5±1.1 vs. 1.5±1.1, p=0.0210). (3) On the other hand, hepatic fibrosis score correlated with inflammation (p<0.0001), but not with steatosis. (4) There were no relationship between severity of fibrosis and the components of metabolic syndrome. (5) Serum levels of ALT, but not aspartate aminotransferase (AST), correlated with insulin sensitivity indexes (MCR, r=-0.291, p=0.0300; HOMA, r=0.414, p=0.0006; QUICKI, r=-0.301, p=0.0125). (6) As hepatic fibrosis developed to bridging fibrosis, serum AST levels were elevated (p=0.0020). AST levels also correlated with markers for hepatic fibrosis, such as N-terminal propeptide of type III procollagen (r=0.846, p<0.0001) and type IV collagen 7S domain (r=0.650, p=0.0052).
Conclusion: Steatosis of the liver predicts insulin resistance and risk for cardiovascular disease, while inflammation and fibrosis predict liver cirrhosis and hepatic failure as seen in NASH. We propose an entity of 'fatty liver-associated metabolic syndrome (FLAMS)' with predominant steatosis and less fibrosis in the liver and with increased risk for atherosclerosis, as a pre-NASH or an early stage in NASH. Elevated ALT level predicts FLAMS, while AST level does advanced NASH.

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Sensory nerve inactivation by Resiniferatoxin improves insulin sensitivity in male obese Zucker rats.

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Background and Aims: Recent studies have suggested that sensory nerves influence insulin secretion and action in normal rodents. The present study investigated the effects of resiniferatoxin (RTX) inactivation of sensory nerves (desensitization) on oral glucose tolerance, insulin secretion and whole body insulin sensitivity in the glucose intolerant and insulin resistant obese Zucker rat.
Materials, Methods and Results: Following RTX treatment, fasting plasma insulin was significantly reduced ($p<0.0005$), and oral glucose tolerance was significantly improved ($AUC_{0\text{-}120\ min}$: 980±29 vs. 860±27 mM•min in RTX treated rats, $p<0.005$). Pancreas perfusion showed that baseline (at 7mM glucose) insulin secretion was significantly lower in RTX treated rats (1.37±0.17 vs. 0.54±0.17 pmol/min in RTX treated rats, $p=0.01$). Both 1st and 2nd phase insulin secretory responsiveness (defined as % increase to 20 mM glucose above baseline insulin secretion) was significantly enhanced in RTX treated rats (First phase: 269±39 vs. 701±75 % in RTX treated rats, $p<0.005$; Second phase: 333±52 vs. 727±65 % in RTX treated rats, $p<0.005$). However, in stimulated isolated pancreatic islets insulin secretion was unaffected. At the peak of spontaneous insulin resistance in the obese Zucker rat (i.e. 14-15-week old rats) whole body insulin sensitivity was substantially improved following RTX treatment as evidenced by higher glucose infusion rates (GIR) required to maintain euglycemia during a hyperinsulinemic-euglycemic (5 mU/kg•min) clamp ($GIR_{60\text{-}120min}$: 5.97±0.62 vs. 11.65±0.83 mg/kg•min in RTX treated rats, $p=0.003$).
Conclusion: Oral glucose tolerance, insulin secretion and especially insulin sensitivity were all improved in obese Zucker rats following RTX desensitization. These data strongly suggest that sensory nerves play an important role in the regulation of integrated glucose homeostasis.

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Glucose allostasis.

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Background and Aims: It is generally believed that in healthy individuals with normal glucose tolerance (NGT) normoglycemia can always be maintained by compensatorily increasing glucose-stimulated insulin secretion (AIR) in response to decreasing insulin action (M) and vice versa. This has been mathematically described by a hyperbolic relationship and interpreted to indicate glucose homeostasis with glucose concentration remaining constant along the hyperbola (= constant disposition index).
Materials and Methods: We analyzed M and AIR in healthy Pima Indians (N = 413) and Caucasians (N = 60) with NGT. We mathematically controlled for the inevitable variability in appropriateness of beta cell compensation.
Results: Keeping everything else constant, a higher demand on the beta cell (due to reduced insulin action) was positively associated with glycemia in cross-sectional analyses. Longitudinally, with increasing beta cell demand glycemia increased (and vice versa) over a wide range of almost 10 mg/dL for fasting and 40 mg/dL for 2-hour plasma glucose concentrations. Prospectively, subjects with higher beta cell demand to begin with were more likely to become “diabetic” than those with lower beta cell demand.
Conclusion: This confirms what should be obvious based on theoretical reasoning alone: the chronic stimulus (increased glycemia) responsible for the chronic compensation (increased AIR) can not be fully removed or there would be no further compensation. That means, the compensation, although physiologically normal and appropriate, can not possibly be successful in completely restoring the original state as long as insulin resistance is present. It is this persistent increase in glycemia - among other factors - that mediates the ongoing increase in insulin secretory function. For this physiologic adaptation to the chronic stressor insulin resistance, we propose to use the term “glucose allostasis”. Allostasis (= stability through change) ensures the continued homeostatic response (= stability through staying the same) to acute stress (glucose load) at some cumulative costs to the system. With increasing severity and over time, however, the allostatic load (increase in glycemia) will have pathological consequences such as development of type 2 diabetes.

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Protein expression of AMPK α, β, γ isoforms and effect of insulin on AMPK activity in skeletal muscle from obese Type 2 diabetic subjects.

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Background and Aims. Altered function of the AMP-activated protein kinase (AMPK) has been suggested to play a role in insulin resistance in type 2 diabetes. We recently reported that failure of insulin to activate muscle glycogen synthase (GS) in type 2 diabetic subjects was associated with increased phosphorylation at the NH2-terminal sites (Ser7 and 10). AMPK phosphorylates GS at Ser7 in vitro, and may be involved in impaired glycogen synthesis in type 2 diabetic subjects.
Materials and Methods. We measured basal protein expression of the seven known AMPK subunits (α1, α2, β1, β2, γ1, γ2 and γ3), and activity and/or phosphorylation of AMPK and acetyl CoA-carboxylase (ACC) in skeletal muscle biopsies obtained from type 2 diabetic and control subjects in the basal and insulin-stimulated state of euglycemic-hyperinsulinemic clamp studies.
Results. Basal protein expression of all AMPK subunit isoforms (α1, α2, β1, β2, γ1, γ2 and γ3) in obese type 2 diabetic subjects were similar to that of well-matched healthy subjects. In addition, α1 and α2 associated AMPK activities in skeletal muscle (in vitro measured activity), phosphorylation of AMPK α subunits at Thr172 (in vitro measured activity), and phoshorylation of ACC at Ser221 (indicator of endogenous AMPK activity) showed no difference between the two groups, and was not regulated by physiological concentrations of insulin.

Conclusions. These data suggest that impaired insulin action on muscle glycogen synthesis in obese type 2 diabetic subjects is unlikely to be caused by altered function of AMPK, and that pharmacological activation of the AMPK system is a feasible treatment in type 2 diabetes.

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The pathogenesis of diabetes involves a defective amplification of the late phase insulin response to glucose by GIP – regardless of aetiology and phenotype.

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Background and Aims: The effect of the insulinotropic incretin hormone, glucagon-like peptide-1 (GLP-1), is preserved in typical middle aged, obese, insulin resistant type 2 diabetic patients, whereas a defective amplification of the "late phase" plasma insulin response (20-120 min) to glucose by the other incretin hormone, glucose-dependent insulinotropic polypeptide (GIP), is seen in these patients. The aim of the present investigation was to evaluate plasma insulin and C-peptide responses to GLP-1 and GIP in 5 groups of diabetic patients with aetiology and phenotype distinct from the obese type 2 diabetic patients.
Materials and Methods: We studied (6 in each group): 1) patients with diabetes mellitus secondary to chronic pancreatitis, 2) lean type 2 diabetic patients (BMI < 25 kg/m^2), 3) LADA-patients, 4) diabetic patients with mutations in the *HNF-1α* gene (MODY3), and 5) newly diagnosed type 1 diabetic patients. All participants underwent three hyperglycaemic clamps (2 hours, 15 mmol/l) with continuous infusion of saline, 1 pmol GLP-1 (7-36)amide/kg body weight/min or 4 pmol GIP pmol/kg body weight/min.
Results: The early phase (0-20 min) plasma insulin response tended to be enhanced by both GIP and GLP-1 compared to glucose alone in all 5 groups. In contrast the late-phase (20-120 min) plasma insulin response to GIP was attenuated compared to the plasma insulin response to GLP-1 in all 5 groups. Glucose infusion rates required to maintain the hyperglycaemic clamp were not significantly different between GIP and GLP-1 clamps in the "early-phase", whereas significantly higher infusion rates were required during the "late phase" of the GLP-1 stimulation compared to the GIP stimulation.
Conclusion: Lack of GIP amplification of the late phase plasma insulin response to glucose seems to be a consequence of diabetes mellitus, characterising most, if not all, forms of diabetes

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Decreased insulin sensitivity is an initial primary defect in impaired glucose tolerance, however, both progressively decreased insulin sensitivity and insulin secretion are important factors in the development and progression of Type 2 diabetes mellitus.

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Background and Aims: Both insulin resistance and decreased insulin secretion have been known to be defects causing type 2 Diabetes Mellitus (T2DM). However progressive pancreatic betacell dysfunction has been suggested to be a more important factor in the development of T2DM.. We investigated to see whether insulin secretory defect is a crucial factor in the development and progression of T2DM
Materials and Methods: We performed oral glucose tolerance test and measured plasma immunoreactive insulin and glucose to calculate Acute Insulin Response (AIR, $\Delta insulin_{(30min-0min)}/\Delta glucose_{(30min-0min)}$), HOMA%β and HOM_{IR} in the subjects with normal glucose tolerance (NGT,n=18), the subjects with impaired glucose tolerance (IGT,n=30), type 2 diabetic patients with moderate hyperglycemia (Moderate T2DM, fasting plasma glucose <11.1 mM) (N=117), and type 2 diabetic patients with severe hyperglycemia (severe T2DM, Fasting plasma glucose >/= 11.1 mM) (N=89).
Results: HOMA%β (61.82+/- 6.68 vs 74.12+/-9.76) and AIR (1.09+/-0.28 vs 1.28+/-0.22) were not different in between NGT and IGT. However, $HOMA_{IR}$ was significantly higher in IGT than NGT (2.11+/-0.20 vs 1.1+/-0.12, p<0.0006). HOMA%β (46.53+/-3.69) and AIR (0.25+/-0.03) in moderate T2DM were much lower than in IGT (p=0.0015, p<0.0001 respectively). $HOMA_{IR}$ was also higher in Moderate T2DM than in IGT (3.20+/-0.21, p=0.0044) but statstical power for difference was lower compared to those of both HOMA%β and AIR. Severe T2DM was significantly defected in both HOMA%β and AIR (16.45+/-3.77, 0.08+/-0.03 and p<0.0001, p<0.0047 respectively compare to moderate T2DM). $HOMA_{IR}$ was also significantly higher in severe T2DM than in moderate T2DM (4.20+/-0.48 vs 3.20+/-0.28, p=0.0331). Statistical power was not much bigger compared to HOMA%β and AIR.
Conclusion: Decreased insulin sensitivity may be an initial pathogenetic cause in the progression of IGT from NGT. Both defect in insulin sensitivity and insulin secretion are crucial pathogenetic factors in T2DM but our data suggested that decreased insulin secretion is a more important factor in the development and progression in T2DM

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Defective insulin secretion rather than insulin resistance the major factor in pathogenesis of post-transplant diabetes mellitus.

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Background and Aims: With the dramatic increase in the number of organ transplants, the prevalence of Post-Transplant Diabetes Mellitus (PTDM)is increasing. However, the exact pathogenesis of this disorder is not yet fully understood. This study was carried out to assess the relative role of insulin resistance and defective insulin secretion in the developement of this disorder.
Material and Methods: Forty one non-diabetic renal allograft recipients formed the material of the study. They were subjected to an 75gm oral glucose tolerance on the day 90 after transplantation along with estimation of the fasting serum insulin levels. Based on the results of the OGTT they were categorized as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or post-transplant diabetes mellitus (PTDM). The insulin resistance (HOMA-R) and insulin secretion (HOMA-B)were calcualated using the HOMA model.
Results: Of the 41 subjects 27 had NGT, 2 had IGT and 12 had PTDM. The incidence of PTDM was 29.2% amongst this group. The three groups were comparable with respect to age, FPG, cumulative doses of steroids and cyclosporine. However,the PTDM group had significantly higher BMI, WHR,serum cholesterol and LDL cholesterol levels. The insulin resistance was comparable among all the three groups while the insulin secretion was significantly lower in the PTDM group (table1).
Conclusions: Defective insulin secretion rather than insulin resistance plays a major pathogenic role in the developement of PTDM

Demographic & Biochemical Data

Parameter	NGT (n=27)	IGT (n=2)	PTDM (n=12)	Statistical Significance
Age (years)	26.92 ± 5.67	25.0 ± 7.07	30.25 ± 8.35	ns
BMI(kg/m2)	23.40 ± 1.67	30.02 ± 7.26	27.43 ± 5.39	p<0.01
WHR	0.95 ± 0.05	1.03 ± 0.09	0.99 ± 0.9	p<0.05
Cumulative steroid dose (gm)	1.96 ± 0.72	1.99 ± 0.38	1.98 ± 0.19	ns
Cumulative Cyclosporine dose (gm)	31.09 ± 2.39	38.59 ± 12.7	35.43 ± 6.31	ns
FPG (mg/dl)	82.14 ± 12.78	85.0 ± 14.14	85.91 ± 13.85	ns
cholesterol (mg/dl)	205.18 ± 17.93	214.0 ± 22.62	224.5 ± 28.49	p<0.05
Triglycerides (mg/dl)	136.59 ± 10.22	156.0 ± 19.79	145.33 ± 16.91	ns
LDL (mg/dl)	140.64 ± 18.81	147.80 ± 20.08	159.85 ± 26.30	p<0.05
HDL (mg/dl)	37.22 ± 3.76	35.0 ± 1.41	35.58 ± 2.60	ns

Insulin Kinetics

	NGT (n=27)	IGT (n=2)	PTDM (n=12)	Statistical Significance
Fasting serum insulin (mIU/ml)	39.02 ± 26.06	48.26 ± 12.34	13.39 ± 12.80	p<0.05
Insulin Resistance (HOMA-R)	7.45 ± 4.93	7.49 ± 2.51	6.21 ± 4.61	ns
Insulin Secretion (HOMA-B)	1153.08 ± 884.13	2347.8 ± 124.8	366.18 ± 246.34	p<0.05

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Levels of β-cell dysfunction and insulin resistance differ between Europeans and North Americans with rcently-dagnosed dabetes in te ADOPT study.

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Background and Aims: The phenotype of type 2 diabetes varies around the world with differing contributions from insulin resistance and insulin secretion (β-cell dysfunction).
Materials and Methods: To determine whether these two parameters differ between North America and Europe, we examined baseline data from Caucasian subsets with recently-diagnosed (< 3 years) diabetes from North America (n = 1,756) and Europe (n = 2,008) participating in the ADOPT study. ADOPT is a randomised, double-blind clinical trial designed to determine whether the response of drug-naive patients with type 2 diabetes to initial monotherapy differs between rosiglitazone, metformin and a β-cell secretagogue (glibenclamide).
Results:

	North America	Europe	*P*
Age	55.9	58.5	< 0.0001
% Male	56.4	60.9	0.005
BMI (kg/m^2)	33.0	30.9	< 0.0001
Waist circumference (cm)	108.0	103.7	< 0.0001
HbA_{1c} (%)	7.3	7.2	0.0006
Fasting plasma glucose (mmol/l)	8.5	8.4	0.02
Fasting plasma insulin (pmol/l)	167.6	135.1	< 0.0001
Fasting proinsulin/insulin (%)	29	39	< 0.0001
HOMA %S	38.1	47.4	< 0.0001
HOMA %B	69.8	65.7	< 0.0001

* represent geometric mean, otherwise all data expressed as means

The North American cohort included less males, was younger, and more obese including an increase in central adiposity. Glycaemic differences were small but, in keeping with their increased central adiposity, the North American cohort was more insulin resistant as determined by HOMA %S and reflected in elevated fasting insulin levels. In contrast, the European cohort had worse β-cell function as quantified by HOMA %B accompanied by a higher proinsulin to insulin ratio. When adjusted for demographic characteristics and measures of obesity, HOMA %S and the proinsulin to insulin ratio remained significantly different but not HOMA %B.
Conclusions: We conclude that there are differences in levels of insulin resistance and β-cell dysfunction in North Americans and Europeans with recently diagnosed diabetes which are partly, but not completely, explained by differences in adiposity. The ADOPT study will examine whether these differences influence the response to the initial pharmacologic treatment of type 2 diabetes.

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Primacy of multiple pancreatic beta-cell dysfunctions in postprandial hyperglycaemia of Type 2 diabetes mellitus.

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Background and Aims: To establish the defects of insulin secretion and action in Type 2 diabetes mellitus (T2DM) after ingestion of a mixed meal.
Materials and Methods: 31 T2DM patients [17 obese (Ob) with BMI 32.4±0.8 kg/m^2; 14 non-obese (N-Ob) with BMI 25.7±0.3 kg/m^2)] on oral drugs and fair glycaemic control (HbA_{1c} 7.44±0.13%) were studied for 7 h after ingestion of mixed meal (793 Kcal, 60% CHO, 15% lipids, 25% proteins) along with 24 age, gender and BMI matched nondiabetic control subjects (C) (13 N-Ob, 11 Ob).
Results: Plasma glucose (PG) was greater in T2M vs C (area under curve, $AUC_{0\text{-}7h}$, 82.3±2.2 vs 41.4±5.4 g/dl/7h, p<0.05) regardless of BMI. Plasma insulin (IRI) was lower in T2DM vs C ($AUC_{0\text{-}7h}$ 78.2±8.0 vs 124.8±14.3 nmol/L/7h) (p<0.01) both in N-Ob and Ob T2DM vs C (p<0.01). Insulin deficiency was more pronounced in early phase (0-2h) in T2DM vs C ($AUC_{0\text{-}2h}$, N-Ob 17.8±3.8 vs 32.5±3.9, Ob 19.9±2.2 vs 53.5±6.0 nmol/ml/2h, p<0.05) than in a late phase ($AUC_{2\text{-}7h}$, N-Ob 46.2±9.1 vs 54.2±7.5, p=NS Ob 69.9±7.3 vs 116.1±17.5 nmol/L/2h, p<0.05). Plasma C-Peptide (CP) was also lower in T2DM vs C, but to a greater extent in N-Ob ($AUC_{0\text{-}7h}$ 594±49 vs 888±56 nmol/L/7h, p<0.05) as compared to Ob ($AUC_{0\text{-}7h}$ 452±31 vs 468±57 nmol/L/7h, p=NS). PG, IRI and CP were used to model insulin secretion and action (Diabetes Care 24:539, 2001; Am.J.Physiol.270:E522, 1996). Total insulin secretion was severely impaired in an early phase (0-2h) in T2DM vs C (N-Ob 4±0.5 vs 13±2 nmol/L, Ob 7±1 vs 15±2 nmol/L)(p<0.05), but not in late phase (2-7h). Beta-cell sensititivity (ability of beta-cell to secrete insulin depending on PG) was severely impaired in early phase in T2DM vs C ($AUC_{0\text{-}2h}$ N-Ob 11±1 vs 163±38, Ob 17±2 vs 124±25 min^{-1}/[$mmol_{PG}$/$pmol_{IRI}$], p<0.05). Insulin sensitivity (OGIS) was lower in T2DM (N-Ob 342±16, Ob 340±11 ml/min/m^2) vs C (N-Ob 511±14, Ob 418±100 ml/min/m^2) (p<0.05). Obesity reduced insulin sensitivity in C, but did not further impair the already severe insulin resistance in N-Ob T2DM. The adaptation index (insulin sensitivity x insulin secretion) was lower in T2DM vs C (N-Ob 6±1 vs 16±2, Ob 12±1 vs 17±1, nmol/min/m^2) and in N-Ob vs Ob T2DM (p<0.05). Hepatic insulin extraction was greater in Ob T2DM vs Ob-C (78±2 vs 67±3 ml/min) (p<0.05).
Conclusions: Postprandial hyperglycaemia in T2DM results from both insulin deficiency and insulin resistance. The latter is more severe in Ob-T2DM than in Ob-C (effect of Ob) but similar in N-Ob and Ob T2DM (hyperglycaemia masks the effect of Ob on insulin resistance). Because the insulin resistant Ob-C are normo-tolerant, whereas post-prandial hyperglycaemia develops in T2DM, inability to secrete appropriately insulin in an early phase after meal ingestion, not insulin resistance, is the key factor of post-prandial hyperglycaemia in T2DM.

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Association of insulin-stimulated adipose tissue glucose uptake with regional fat mass and whole-body insulin sensitivity in patients with newly diagnosed Type 2 diabetes.

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Background and Aims: We have earlier shown that insulin-stimulated adipose tissue glucose uptake in abdominal subcutaneous and intra-abdominal region is inversely associated with regional fat mass in nondiabetic men.
Materials and Methods: In the current study we measured insulin-stimulated glucose uptake in abdominal subcutaneous and intra-abdominal adipose tissue, and in skeletal muscle in patients with newly diagnosed type 2 diabetes (DM; n = 44, age 58 ± 1 years, BMI 29.4 ± 0.6 kg/m^2) and in nondiabetic subjects (nonDM; n = 34, age 39 ± 2 years, BMI 27.0 ± 0.7 kg/m^2) using [^{18}F]-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and positron emission tomography. Fat masses were measured using magnetic resonance imaging.
Results: Intra-abdominal fat mass was higher (2.3 ± 0.1 vs 1.9 ± 0.1 kg, P < 0.05) and glucose uptake rate lower (17.0 ± 0.9 vs 22.9 ± 2.2 μmol/kg·min, P < 0.05) in diabetic than in nondiabetic subjects. However, glucose uptake rates (μmol/min) in the whole intra-abdominal depot were similar between the groups (37.5 ± 2.5 vs 31.9 ± 1.8 μmol/min, DM vs nonDM). Subcutaneous fat mass and glucose uptake rates were similar between diabetic and nondiabetic subjects. Regional glucose uptake rate in intra-abdominal adipose tissue was inversely correlated with the corresponding fat mass in diabetic subjects (r = - 0.44, P < 0.01), and in nondiabetic subjects (r = - 0.79, P < 0.0001). In abdominal subcutaneous adipose tissue, a correlation of glucose uptake rate with fat mass was found in nondiabetic subjects (r = -0.68, P < 0.05) but not in diabetic subjects. Intra-abdominal adipose tissue glucose uptake correlated with skeletal muscle glucose uptake (r = 0.34, P < 0.05 and r = 0.49, P < 0.01; DM and nonDM), and with whole-body glucose disposal (r = 0.35, P < 0.05 and r = 0.61, P < 0.001; DM and nonDM). In abdominal subcutaneous adipose tissue, a correlation of glucose uptake rate with skeletal muscle glucose uptake and whole-body glucose disposal was found in nondiabetic subjects (r = 0.48, P < 0.01 and r = 0.58, P < 0.001, respectively) but not in diabetic subjects.
Conclusion: In intra-abdominal adipose tissue depot, insulin-stimulated glucose uptake per whole depot was similar in DM and in nonDM subjects. Glucose uptake rate per mass unit was inversely associated with fat mass. In subcutaneous adipose tissue, this association was found in nondiabetic but not in type 2 diabetic patients. Although intra-abdominal adipose tissue glucose uptake correlated also with skeletal muscle glucose uptake and

whole-body glucose disposal, glucose uptake rates per mass unit are partly explained by the mass of intra-abdominal fat depot.

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Acute hyperglycaemia episodes increase TNFα plasma level in patients with Type 2 diabetes mellitus.

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Background and Aims: Several studies have reported the presence of chronic inflammation in patients with type 2 diabetes. Abnormalities include increase plasma level of Il-1, Il-6, and TNFα, which may be associated with accelerated development of vascular damage (atherosclerosis). This immune activation could occur as a result of metabolic disturbances, especially hyperglycaemia. We studied the role of acute hyperglycaemia (expressed as a decrease of anhydroglucitol plasma level) in regulation of TNFα plasma level in patient with type 2 diabetes mellitus.
Material and Methods: 56 patients aged 41 to 77 years with type 2 diabetes were included into study. In each patient metabolic control parameters (fasting and postprandial glycemia, anhydroglucitol, HbA1c) as well as TNFα were analyzed in serum. Anhydroglucitol (AG) was used as an indicator of short (1-2days), retrospective glucose excursion. Then patients were included into one for following groups: (1) with insufficient short-term and long-term metabolic compensation (high HbA1c and AG level), (2) with insufficient short-term and satisfactory long-term metabolic compensation (low HbA1c and high AG level), (3) with sufficient short-term and long-term metabolic compensation (low HbA1c and AG level).
Results: Circulating concentrations of TNFα were increased in all investigated group of patients regardless of metabolic compensation. The highest level was observed in patients with insufficient short-term and long-term compensations; 67,1 pg/ml (6,0-274,4). Analyzed the influence of acute hyperglycemia in patients with comparable, sufficient long-term metabolic compensation (HbA1c<6,5%), we noted that patients with episodes of hyperglycemia 1-2 days before investigation (expressed as decrease of AG level) were characterized by significant increase of circulating level of TNFα in comparison with group characterized by sufficient metabolic compensation; 48,5 pg/ml (6,5-272,8) vs 39,5 pg/ml (9,5-150) respectively.
Conclusions: Acute hyperglycaemic episodes increase circulating TNFα concentration. This suggests a causative role of hyperglycemia in the immune activation in patients with diabetes mellitus.

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Correlation of anthropometric measures and insulin sensitivity with intramyocellular lipids in healthy and Type 2 diabetic Asian Indian males.

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Background and Aims: Intramyocellular lipid (IMCL) content of soleus muscle has been shown to correlate with insulin sensitivity in Caucasians. We analysed IMCL content of the soleus muscle of healthy and type 2 diabetic Asian Indian males and correlated it with anthropometric parameters, fasting blood glucose, lipid profile, and insulin sensitivity.
Materials and Methods: In this case control study, thirty-eight males (19 with type 2 diabetes and 19 healthy controls, matched for age, smoking, and alcohol intake) were evaluated with anthropometry [body mass index (BMI), percentage of body fat (% BF), waist-to-hip ratio (W-HR), and skin folds at 4 sites] and oral glucose tolerance test for blood glucose and insulin. IMCL was measured by proton nuclear magnetic resonance spectroscopy of the soleus muscle. Insulin sensitivity was calculated using homeostatic model assessment (HOMA).
Results: Type 2 diabetics had a higher mean %BF (32.3 vs. 28.5, p<0.02), % BF/BMI ratio (1.35 vs. 1.21, p<0.02), fasting serum insulin (171.7±64.1 vs. 121.2±41.3, p<0.007) and were more insulin resistant by HOMA (8.3±3.8 vs. 4.2±1.5, p<0.0002) as compared to healthy subjects. The mean IMCL content was higher in type 2 diabetics (20.3±13.5) compared to healthy subjects (15.2±8.2), but it was statistically not significant. IMCL content correlated significantly with age (r=0.49, p<0.05), and W-HR (r=0.48, p<0.05) in healthy subjects, and BMI (r=0.76, p<0.05) and waist circumference (r=0.64, p<0.05) in diabetic subjects. No significant correlation between IMCL content and insulin sensitivity was observed in the pooled data.
Conclusions: IMCL content tended to be higher in type 2 diabetics as compared to control, and correlated with obesity, particularly abdominal obesity, in both groups. Unlike Caucasians, however, no correlation was observed between insulin sensitivity and IMCL in Asian Indian males.

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Acute starvation impairs insulin resistance in Type 2 diabetic patients.

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Background and Aims: Although long-term body weight loss is well known to improve insulin resistance, the effects of acute energy restriction in type 2 diabetic (T2DM) patients have not yet been described. Whilst energy restriction is widely used in clinical practice, there is poor knowledge of the short-term metabolic effects. Objectives: 1. To describe metabolic pattern of 60 hr. starvation in T2DM subjects with regards to endocrine changes. 2. To assess whether insulin sensitivity is increased or decreased after short-term starvation.
Methods: 10 obese T2DM patients (6 males, 4 females; BMI 36,98±7,50 kg/m2; aged 54,5±5,02 years) treated with insulin and/or OHA's were studied. Patients with endocrinopathy or disease incongruent with starvation were excluded as well as those on betablockers or ACE-inhibitors. After admission to hospital we performed a baseline isoglycemic double-step (60 and 120 mIU/kg/m2) clamp. Then the subjects were starved for 60 hours. Plasma levels of glucose, lactate, insulin, and free fatty acids (FFA) were sampled every 2 hours. Indirect calorimetry (N-waste as urea and ammonia) was performed every 12 hr and levels of contraregulatory hormones and amino acids determined every 24 hr. Second clamp was performed after 60 hr. of starvation at the same glucose level as previously.
Results: The subjects lost on average 2.2±0.47 kg, out of this 1.10±0.46 kg represented fat mass. The insulin level dropped from 18.46±8.40 to 8.5±5.12 IU/l (p<0.01) whilst glycaemia remained constant (9,49±2,34 vs. 9,84±3,03 mM, NS). Level of FFA did not increase significantly whilst the plasma concentration of 3-hydroxybutyrate increased from 0,17±0,16 to 0,90±0,30 mM (p<0,05). Overall insulin-mediated glucose disposal was significantly impaired after starvation (for 60 mIU/m2/min: 2,62±1,11 vs. 1,91±0,73 mg/kg/min, p<0,05), mainly because the oxidative component thereof was significantly reduced (0,24±0,12 vs. 0,00±0,01 mg/kg/min, p<0,01). Non-oxidative glucose disposal during 120 mIU/m2/min clamp phase decreased non-significantly after starvation.
Conclusion: Acute starvation in T2DM patients impairs glucose oxidation, probably due to Randle's mechanism.

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Adipokines in diabetic and non-diabetic obese subjects: insulin resistance more closely correlates with resistin than leptin, adiponectin, and interleukin-6.

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Background and Aims: Adipose tissue regulates insulin sensitivity at least in part by secretion of adipokines such as resistin, adiponectin, leptin, and interleukin-6 (IL-6). The relative importance of these adipokines in the insulin resistance of type 2 diabetes (T2DM) is unclear. The aim of this study was to determine the differences in adipokine levels in obese non-diabetic subjects and type 2 diabetic subjects matched for BMI.
Materials and Methods: Plasma levels of resistin, adiponectin, leptin, IL-6, and insulin were measured by commercially available assays. Statistical analyses were performed by analysis of variance followed by Dunnet's t-test for comparison between groups. Regression analysis was used to correlate adipokine levels with insulin resistance as determined by homeostasis model of insulin resistance index (HOMA-R). OGTT to exclude diabetes in obese subjects was interpreted using WHO criteria. Data are expressed as mean ± SEM.
Results: Description of study population is shown in the table (*a* and *b* represent p<0.05 and p<0.001, respectively).

	Obese subjects	Diabetic subjects
Number (males)	33 (14)	24 (12)
Age (years)	46.7 ± 1.1	51.9± 1.0[a]
BMI (kg/m^2)	33.9± 1.4	36.6± 1.2
Insulin (mIU/l)	13.6± 1.7	17.4± 1.6[a]
Glucose (mmol/l)	5.2± 0.1	10.7± 0.7[b]
HOMA-R	3.7± 0.4	8.9± 1.3[b]

No significant differences were found in resistin (30.3±5.9 vs. 29.2±3.3 ng/ml) , leptin (28.9±4.13 vs. 23.8±3.8 ng/ml) , and IL-6 levels (1.58±0.17 vs. 1.89±0.6 pg/ml) in non-diabetic versus diabetic obese subjects, respectively. Adiponectin was significantly higher in non-diabetic subjects (8.5±0.7 vs. 6.6±0.5 µg/ml, p<0.05). Significant gender differences were found for resistin (19.4±3.3 vs. 38.6± 3.7 ng/ml, p=0.007) , leptin (11.6±2 vs. 39.4±3.6 ng/ml, p<0.001), adiponectin (6.53±.5 vs. 8.7±0.8 µg/ml, p=0.026), and IL-6 levels (1.35±0.14 vs. 2.04±0.18 pg/ml, p=0.004) in males and females, respectively. Resistin positively correlated with log HOMA-R (r=0.519, p<0.001). Unlike other measured adipokines, correlation between resistin and log HOMA-R remained significant even after correction for age, BMI, and gender (r=0.48, p<0.001).
Conclusion: These data indicate that resistin correlates with insulin resistance more closely than other examined adipokines and this correlation is independent of gender and BMI. Significant gender differences exist in adipokine levels. When diabetic and non-diabetic subjects matched for BMI were compared, overt T2DM was accompanied by a decrease in adiponectin but no significant changes in resistin, leptin, and IL-6 levels in the studied group.

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Inverse association of adiponectin with alanine but not asparate transaminase in young healthy men independently of BMI or leptin.
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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized condition that may progress to end-stage liver disease. Most patients with NAFLD have no signs and symptoms of liver disease and mildly elevated serum levels of ALT, AST or both are the most common and often the only laboratory abnormality found in patient with NAFLD, which is associated with obesity, type 2 diabetes, dyslipidemia and insulin resistance.
Materials and Methods: We examined associations of ALT and AST with serum leptin, adiponectin and highly sensitive CRP in addition to components of insulin resistance syndrome in 198 male college students aged 18 years.
Results: ALT showed positive associations with BMI (r=0.51), serum leptin (r=0.31),serum insulin(0.22) and homeostasis model assessment of insulin resistance (r=0.22)(all p≦0.001). In addition, ALT were positively associated with TG(r=0.35), LDL cholesterol (r=0.27), apoB (r=0.39), and systolic blood pressure(r=0.21)(all p<0.01). Further, it was negatively associated with HDL cholesterol (r=-0.18), LDL particle size (r=-0.21), and serum adiponectin (r=-0.28)(all p≦0.01) whereas ALT was not related to fasting plasma glucose, apo AI and CRP. After adjustment for BMI associations of ALT with CRP (r=0.16) in addition to TG (r=0.16), LDL cholesterol (r=0.29), apoB (r=0.35), and leptin (r=0.21) were significant. Relationships were smaller for AST and significance disappeared after adjustment for BMI. Multiple regression analysis revealed that ALT was significantly and independently associated with adiponectin , apoB and percent body fat.
Conclusion: Serum ALT levels were associated with many components of the insulin resistance syndrome in young healthy men. Adiponectin, apoB, and percent body fat emerged as significant and independent predictors of ALT levels, which are considered to represent a nonspecific marker of a spectrum of hepatic diseases.

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Portal serotonin infusion enhances net hepatic glucose uptake but blunts nonhepatic glucose uptake.
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Background and Aims: Net hepatic glucose uptake (NHGU) is enhanced and nonhepatic glucose uptake (nonHGU) is blunted by portal vs peripheral glucose delivery, a phenomenon termed the portal signal. The portal signal appears to be neurally mediated, but the exact mechanism is unknown. The neurotransmitter serotonin (5-hydroxytryptamine, or 5HT) is released by the gut and enters the portal vein postprandially, and evidence suggests that peripheral administration of 5HT or its precursor 5-hydroxytryptophan reduces blood glucose in a manner independent of insulin release. Therefore, the aim was to determine whether intraportal 5HT delivery can enhance NHGU during peripheral glucose infusion and, if so, what dose(s) is(are) effective.
Materials and Methods: Arteriovenous difference and tracer ([3-^{3}H]glucose) techniques were used in conscious 42-h-fasted dogs surgically prepared ~16 d before study. Each experiment consisted of equilibration (-120 to -30 min), basal (-30 to 0 min), and experimental (EXP; 0-270 min) periods. During EXP, somatostatin, 3-fold basal intraportal insulin, basal intraportal glucagon, and peripheral glucose (to double the hepatic glucose load) were infused. In one group of dogs (SER, n=8), saline was infused intraportally from 0-90 min, and during 90-150, 150-210, and 210-270 min,

5HT was infused at 10, 20, and 40 μg·kg^{-1}·min^{-1}, respectively. In the other group (SAL, n=8), saline was infused intraportally from 0-270 min.
Results: Arterial plasma insulin concentrations during EXP were 123±10 and 133±8 pmol/l in SAL and SER, respectively, and glucagon concentrations remained basal in both groups. The hepatic glucose loads during EXP averaged 284±13 and 285±16 μmol·kg^{-1}·min^{-1} in SAL and SER, respectively. Arterial blood 5HT concentrations remained basal in SAL but increased from 0.5±0.1 to 1.2±0.3, 1.9±0.3, and 2.5±0.5 μg/ml in SER during 90-150, 150-210, and 210-270 min, respectively. The liver showed no net uptake of 5HT in SAL but exhibited net uptake of 7.6±5.3, 30.7±13.7, and 47.7±26.4 μg·kg^{-1}·min^{-1} in SER during 90-150, 150-210, and 210-270 min, respectively. NHGU and nonHGU differed between groups during the last 2 h of study:

NHGU and nonHGU (μmol·kg^{-1}·min^{-1})

	0-90 min	90-150 min	150-210 min	210-270 min
$NHGU_{SAL}$	12.4±2.3	14.9±2.7	13.4±2.1	15.1±1.8
$NHGU_{SER}$	13.2±3.0	16.4±2.4	19.0±2.4*	22.0±2.9*
$nonHGU_{SAL}$	31.7±0.9	43.9±5.1	55.1±5.6	66.2±8.6
$nonHGU_{SER}$	26.1±5.7	31.6±9.4	35.1±7.6*	34.7±7.7*

Values are mean±SE; *P<0.05 vs SAL (ANOVA with post hoc evaluation by univariate F tests)

Glucose R_d in SER was reduced 27% and 37% during 150-210 and 210-270 min, respectively (P<0.05 vs SAL).
Conclusion: NHGU was significantly enhanced but nonhepatic glucose uptake was blunted during intraportal 5HT infusion at 20 and 40 μg·kg^{-1}·min^{-1}. This suggests that 5HT might be involved in bringing about the portal signal.

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Differential effects of a modified insulin (HIM2) on muscle, fat and liver.

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Background and Aims: We have previously shown that a modified insulin (HIM2), administered as a brief infusion into the portal vein to mimic first phase insulin, resulted in higher circulating plasma levels and was more effective in lowering blood glucose than the same dose of human insulin (HUM). The present study investigated activities and clearances of HIM2 in liver, muscle and adipose tissue after infusing HIM2 and HUM at rates that produced approximately equivalent plasma insulin levels in conscious overnight fasted dogs.
Materials and Methods: After a 40 min control period, somatostatin was infused along with a basal amount of glucagon. Concomitantly, HUM (n=6, 600 and 1200 μU/kg-min) or HIM2 (n=6, 233 and 778 μU/kg-min) was infused via a peripheral vein at each dose level for periods of 2h each.
Results: Arterial plasma glucagon remained basal in both groups while arterial plasma insulin rose from a baseline of 8±1 to 21±6 and 62±17 μU/ml in HIM2 and 8±1 to 22±2 and 82±5 μU/ml in HUM. Euglycemia was maintained by glucose infusion in both groups. Hepatic insulin clearance of HIM2 was 3.2±0.9 and 6.1±0.7 ml/kg-min in the 2 infusion periods while for HUM it was 7.4 ±0.7 and 10.6±1.8 ml/kg-min respectively. Whole body insulin clearance was 13.2 and 14.4 ml/kg-min for HIM2 and 26.8 and 25.5 ml/kg-min for HUM. Net hepatic glucose output (NHGO) fell from a baseline of 1.6±0.2 to 1.3±0.3 and 0.4±0.3 mg/kg-min in response to HIM2 and fell from 1.9±0.7 to 0.0±0.3 and -1.5 ±0.5 mg/kg-min in response to HUM. The glucose infusion rates required to maintain euglycemia in HIM2 were 3.3±0.7 and 7.7±1.2 mg/kg-min while in HUM they were 9.8±1.0 and 18.8±1.9 mg/kg-min. Plasma NEFA fell from 722±92 to 264±40 and 112±13 μmol/l in HIM2 and from 475±52 to 191±28 and 77±11 μmol/l in HUM.
Conclusions: Hepatic and total body clearance of HIM2 was approximately 50% that of HUM. Insulin-like activity of HIM2 appeared to be less at liver and muscle relative to HUM. That does not seem to be the case in adipose tissue however. These results suggest that a modified insulin can be efficacious with differential tissue effects compared to human insulin.

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Both glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) contributes to the insulinotropic effect at basal and postprandial glucose levels in healthy subjects.

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Background and Aims: Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are both incretin hormones regulating postprandial insulin secretion. Their relative importance in this respect under normal physiological conditions is unclear, however, and the aim of the present investigation was to evaluate this.
Materials and Methods: Eight healthy male volunteers (mean age: 23(range 20-25) years; mean body mass index: 22.2 (range 19.3-25.4) kg/m^2) participated in studies involving stepwise glucose clamping at fasting plasma glucose levels and at 6 mmol/l and 7 mmol/l. Physiological amounts of either GIP (1.5 pmol/kg/min), GLP-1 (7-36)amide (0.33 pmol/kg/min) or saline were infused for three periods of 30 minutes at each glucose level, with one hour "wash-out" between the infusions. On a separate day a standard meal test (566 kcal) was performed.
Results: During the meal test, peak insulin concentrations were observed after 30 minutes and amounted to 223±27 pmol/l. Glucose + saline infusions induced only minor increases in insulin concentrations. GLP-1 and GIP infusions induced significant and similar increases in insulin secretion at fasting glucose levels and at 6 mmol/l. At 7 mmol/l further increases in insulin secretion were seen, with GLP-1 effects exceeding those of GIP. Insulin concentrations at the end of the 3 infusion periods (60, 150 and 240 minutes) during the GIP clamp amounted to 53±5 pmol/l, 79±8 pmol/l and 113±15 pmol/l, respectively. Corresponding results were 47±7 pmol/l, 95±10 pmol/l and 171±21 pmol/l, respectively, during the GLP-1 clamp. Total and intact incretin hormone concentrations during the clamp studies were higher compared to the meal test, but within physiological limits. Glucose infusion alone significantly inhibited glucagon secretion, which was further inhibited by GLP-1 but not by GIP infusion.
Conclusion: during normal physiological plasma glucose levels, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide contribute nearly equally to the incretin effect in humans.

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Neither endogenous nor exogenous glucagon stability is affected by dipeptidyl peptidase IV inhibition *in vivo*.

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Background and Aims: Glucagon has a short plasma half-life (< 3 min) *in vivo*, but little is known about the mechanisms involved. *In vitro* studies have shown that micromolar concentrations of glucagon can be degraded by dipeptidyl peptidase IV (DPP IV), but it is unknown whether DPP IV plays any physiological role in glucagon metabolism. The aim of this study was to assess the role of DPP IV in glucagon metabolism *in vivo* using valine-pyrrolidide to inhibit DPP IV under basal conditions and during exogenous glucagon infusion.
Materials and Methods: Anaesthetised pigs (n = 5) received 2 infusions of glucagon (1 pmol/kg/min), one in the absence and one in the presence of valine-pyrrolidide (300 μg/kg; a dose previously shown to inhibit plasma DPP IV activity by > 95% for at least 2 hours). Glucagon concentrations were analysed using 3 well-characterised region-specific radioimmunoassays.
Results: Basal glucagon concentrations differed (ANOVA, $P < 0.001$) according to the assay, reflecting the differing cross-reactivity with other endogenous proglucagon products (C-terminal, 8 ± 5 pmol/l [pancreatic glucagon]; N-terminal, 27 ± 6 pmol/l [pancreatic glucagon + oxyntomodulin]; processing-independent, 57 ± 7 pmol/l [pancreatic glucagon + oxyntomodulin + glicentin]). Incremental arterial glucagon concentrations were similar during infusion of glucagon alone when determined by processing-independent (Δ plateau concentration 44 ± 5 pmol/l) and C-terminal assays (53 ± 7 pmol/l), but were greater (ANOVA + *post hoc* test, $P < 0.01$) than those determined by N-terminal assay (32 ± 2 pmol/l). Neither endogenous (9 ± 6, 29 ± 7 and 50 ± 8 pmol/l; C-terminal, N-terminal and processing-independent assays respectively), incremental glucagon concentrations during the glucagon infusion (43 ± 1, 30 ± 1 and 43 ± 2 pmol/l; C-terminal, N-terminal and processing-independent assays)

nor incremental areas under the glucagon curve (1434 ± 261 vs 1307 ± 83, 928 ± 17 vs 908 ± 98 and 1479 ± 240 vs 1352 ± 147 pmol/l x min; glucagon alone vs glucagon + valine-pyrrolidide and C-terminal, N-terminal and processing-independent assays, respectively) were affected by DPP IV inhibition. The plasma half-life and metabolic clearance rate were unchanged by valine-pyrrolidide when determined by processing-independent (3.2 ± 0.2 vs 3.1 ± 0.5 min and 15.0 ± 0.9 vs 16.6 ± 1.1 ml/kg/min) and C-terminal assays (3.1 ± 0.4 vs 3.2 ± 0.2 min and 17.1 ± 1.9 vs 18.4 ± 0.5 ml/kg/min), but although there appeared to be a small trend towards an increase in stability determined with the N-terminal assay (2.6 ± 0.3 vs 2.9 ± 0.4 min and 30.5 ± 4.1 vs 25.7 ± 0.4 ml/kg/min), these changes were not significant.
Conclusion: Glucagon undergoes limited N-terminal degradation *in vivo*, but this is not significantly changed by valine-pyrrolidide. This suggests that, in contrast to its major role in the metabolism of other members of the glucagon-secretin family of peptides (glucagon-like peptides -1 and -2, and glucose-dependent insulinotropic polypeptide), DPP IV plays little, if any, role in glucagon metabolism *in vivo*.

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An anticipatory rise in GLP-1 is necessary for scheduled meal consumption.
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Background and Aims: Because eating relatively large meals may be a necessity of life in many environments, an important adaptation for minimizing the impact of the postprandial elevations of fuels is to anticipate the meal by initiating responses that lessen postprandial hyperglycemia. Examples of these anticipatory responses are well established in animals and include pre-meal insulin secretion, alteration of the metabolic rate, and elevation of body temperature. Glucagon-like peptide 1 (GLP-1) is an insulinotropic gut hormone that regulates glucose homeostasis and food intake. Because of its role as an incretin, we hypothesized that GLP-1 is involved in the sequence of physiological changes that prepares an animal for the elevated fuels associated with a large meal.
Materials and Methods: In Experiment 1, male rats were conditioned to consume all of their daily calories within the same 4-hour period each day. They were implanted with indwelling vena cava catheters to allow for repeated blood sampling with minimal stress. Blood samples were obtained at 10-min intervals beginning 2 hr prior to the time of their meal. In Experiment 2, another group of rats was administered the GLP-1 receptor antagonist, Exendin (desHis-1, Glu-9; 20 µg, iv), or vehicle, 30 min prior to the onset of the observed GLP-1 peak. To assess the role of the GLP-1 peak in anticipatory ghrelin secretion, in Experiment 3, a separate group of rats was given Exendin as described above, and plasma ghrelin was measured.
Results: In Experiment 1, plasma insulin peaked at 15 min prior to meal onset, plasma ghrelin peaked 10 min prior to meal onset, and plasma GLP-1 displayed a large peak 1 hr prior to meal onset that returned to baseline after 10 min. These results were replicated with a separate cohort of rats that was maintained on a different light:dark cycle. In Experiment 2, Exendin-treated rats ate significantly *less* food at all time points throughout the 4-hr meal compared to control rats. In Experiment 3, Exendin-treated rats displayed a significantly greater rise in ghrelin prior to the meal compared to controls.
Conclusion: These data suggest a novel role for GLP-1 as a necessary component in the complex set of changes that occurs in anticipation of a large meal.

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Direct and indirect effects of amino acids on hepatic glucose metabolism in humans.
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Background and Aims: The direct (substrate mediated) and indirect (hormone mediated) effects of amino acids (AA) on hepatic glucose metabolism were examined for 6 hours in healthy men. The protocols were: (i) CON+S (n=7): control conditions with somatostatin (S) to inhibit endogenous hormone release resulting in fasting plasma concentrations of AA, insulin (~28 pmol/l) and glucagon (~65 ng/l), (ii) AA+S (n=7): AA infusion-fasting insulinemia-fasting glucagonemia, (iii) GLUC+S (n=6): fasting AA-fasting insulinemia-hyperglucagonemia (~99 ng/l) and (iv) AA-S (n=5): AA infusion without S resulting in AA-induced hyperinsulinemia (~61 pmol/l)-hyperglucagonemia (~147 ng/l).
Materials and Methods: Net glycogenolysis was calculated from liver glycogen concentrations using ^{13}C nuclear magnetic resonance spectroscopy. Total gluconeogenesis (total GNG) was calculated by subtracting net glycogenolysis from endogenous glucose production (EGP) which was measured with [6,6-$^2H_2$]glucose. Net GNG was assessed with the ^2H_2O method.
Results: During AA+S and GLUC+S, plasma glucose increased by ~50% (P<0.01) due to an equivalent rise in EGP. This was associated with a 53-% (P<0.05) and a 65-% increase (P<0.01) of total and net GNG during AA+S, whereas net glycogenolysis rose by 70% (P<0.001) during GLUC+S. During AA-S, plasma glucose remained unchanged despite nearly-doubled (P<0.01) total GNG.
Conclusions: Conditions of postprandial amino acid elevation stimulate secretion of insulin and glucagon, but do not affect glycemia despite markedly increased gluconeogenesis. Only diminished insulin secretion unmasks the direct gluconeogenic effect of amino acids and increases plasma glucose which could contribute to the postprandial hyperglycemia of insulin resistant states.

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Skeletal muscle is the major site of lactate release during hypoglycemia in humans.
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Background and Aims: In humans, lactate is considered the predominant substrate for the increased gluconeogenesis during hypoglycemia, which is largely attributable to its increased release into the systemic circulation. Skeletal muscle has been shown to be the primary source of lactate in postabsorptive humans and accounts for most of the increased systemic lactate release during a hyperinsulinemic euglycemic clamp, but is not considered to play a major role in lactate release during hypoglycemia. This view is based on human net balance studies finding only a modest net release of lactate by skeletal muscle during hypoglycemia. However, no inferences can be drawn from these studies regarding the contribution of skeletal muscle to systemic lactate release since they do not take into consideration simultaneous muscle lactate uptake and release.
Materials and Methods: We therefore used a combination of tracer techniques (13 C lactate) and net balance measurements (radial artery and forearm deep venous catheters) to determine systemic lactate uptake (SLU) and release (SLR), and muscle lactate net balance, fractional extraction (MLFx), uptake (MLU) and release (MLR) in 6 postabsorptive healthy subjects during a 2 hour hyperinsulinemic euglycemic clamp (~5.0 mM) followed by a 90 min hypoglycemic clamp (~2.9 mM).
Results: During the final 30 min of the euglycemic and the hypoglycemic clamp, plasma insulin was similar (~250 pM) but plasma glucagon, epinephrine, norepinephrine, growth hormone and cortisol were all greater during the latter (all $p < 0.03$). Although arterial lactate concentrations were comparable during both clamps (1.27 ± 0.24 vs 1.13 ± 0.18 mM, $p > 0.3$), SLU (21 ± 2 vs 17 ± 1 µmol/kg/min, $p < 0.02$) and SLR (22 ± 2 vs 17 ± 1 µmol/kg/min, $p < 0.02$) were greater during hypoglycemia. Forearm blood flow (6.7 ± 0.6 vs 4.1 ± 0.5 ml/100cc/min, $p < 0.01$) and muscle net release of lactate (2.1 ± 0.5 vs 0.3 ± 0.1 µmol/100cc/min, $p < 0.02$) were also greater during hypoglycemia. The increased muscle net release of lactate was due to increased MLR (2.9 ± 0.5 vs 1.5 ± 0.3 µmol/100cc/min, $p < 0.02$) and reduced MLFx (11 ± 2 vs 25 ± 3%, $p < 0.01$) so that despite greater muscle lactate delivery (9.1 ± 2.8 vs 4.9 ± 1.4 µmol/100cc/min, $p < 0.05$) MLU remained unchanged (0.7 ± 0.1 vs 1.1 ± 0.2, $p > 0.13$). Extrapolations of forearm muscle data to the whole body indicate that during hypoglycemia MLU accounted for a lesser (18 ± 3 vs 34 ± 9%, $p < 0.08$) and MLR for a greater proportion (64 ± 12 vs 42 ± 8%, $p < 0.05$) of SLU and SLR, respectively.
Conclusion: We conclude that contrary to the present view, skeletal muscle is the major site of lactate release during hypoglycemia in humans.

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Regulation and counter-regulation of lipolysis in vivo: different roles of sympathetic activation and insulin.

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Background: To get further information on the regulation of lipolysis in vivo the effect of increasing sympathetic nerve activity via lower body negative pressure (LBNP, -20mmHg) was studied in eleven healthy human subjects.
Materials and Methods: Subcutaneous and muscle microdialysis as well as blood flow measurements were performed in the postabsorptive state and during an euglycemic hyperinsulinemic clamp.
Results: LBNP for 30 min in the postabsorptive phase resulted in a ~50% increase ($p<0.005$) in the interstitial-arterial concentration difference (AI) for glycerol in adipose tissue whereas no such effect was registered in muscle. Blood flow in adipose tissue and forearm remained unaltered. During euglycemic hyperinsulinemic conditions (p-insulin 645 ± 62 pmol/L) both interstitial adipose tissue and arterial concentrations of glycerol was reduced.
LBNP resulted in an increase in AI glycerol similar to that seen in the postabsorptive state (~50%, $p<0.05$). Muscle glycerol was not changed by either insulin or LBNP. Glucose infusion rate during the clamp was significantly decreased during LBNP (7.82 ± 0.88 vs 8.67 ± 1.1 ml/kg-min, $p<0.05$).
Conclusions: We conclude that the symphatetic nervous activation by LBNP results in an increased lipolysis rate in adipose tissue both in postabsorptive phase and during insulin infusion, whereas muscle glycerol output was not affected either by LBNP or insulin. The data suggest that 1. lipolysis is regulated differently in muscle and adipose tissue and, 2. postabsorptive lipolysis is mainly regulated by insulin and, 3. sympathetic nervous activation effectively inhibits the antilipolytic action of insulin by inducing insulin resistance.

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The effects of age and gender on post-prandial glucose metabolism.

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Background and Aims: Glucose tolerance decreases with age.The present studies sought to determine the mechanism of this deterioration and whether it differs in men and women.
Materials and Methods: To address this question, 44 healthy elderly (70 ± 1.0 years) men (EM) and 33 healthy elderly (72 ± 1.1years) women (EW) ingested a mixed meal containing [^{13}C] glucose while [6,6-$^{2}H_2$] glucose and [6-^{3}H] glucose were infused intravenously to provide independent assessments of the rates of meal appearance (Mra), endogenous glucose production (EGP) and glucose disposal (Rd). Results were compared to those observed in 13 young (25 ± 1.1 years) men (YM) and 16 young (22 ± 1.0 years) women (YW).
Results: Postprandial (area above basal) glucose ($p<0.001$), insulin ($p<0.05$) and C-peptide ($p<0.001$) concentrations were higher in both EM and EW than YM and YW respectively. Glucagon and cortisol concentrations did not differ. Post-prandial growth hormone concentrations were lower ($p<0.001$) in the elderly women than young women but did not differ in the men. The higher post-prandial glucose concentrations in the EM than YM were due to a smaller increase in Rd ($p<0.01$) and a lesser percent suppression of EGP ($p<0.01$) with the differences being most marked during the first 2 hrs after meal ingestion. Mra, if anything, was lower ($p<0.05$) in the EM than YM. On the other hand, the higher post-prandial glucose concentrations in the EW than YW were solely due to a lower ($p<0.05$) Rd during the first two hours (when the rate of rise of glucose differed) after meal ingestion. Mra and percent suppression of EGP did not differ between EW and YW. Of note, post-prandial glucose and C-peptide (but not insulin) concentrations were greater in both ($p<0.001$) EW than EM and YW than YM respectively. The higher post-prandial glucose concentrations in the EW than EM were due to higher ($p<0.05$) Mra; Rd and EGP did not differ between groups. In contrast, the higher post-prandial glucose concentrations in YW than YM were due to lower ($p<0.01$) Rd during the first two hours (when the rate of rise of glucose differed); Mra and EGP did not differ between groups.
Conclusion: In summary, the cause of the age related deterioration in post-prandial glucose tolerance differs in elderly men and women. Whereas post-prandial glucose disposal was decreased in both elderly men and women, suppression of endogenous glucose production was only impaired in elderly men. Furthermore, despite an age-related deterioration in glucose tolerance, elderly women continued to have higher postprandial (but not fasting) glucose concentrations than elderly men. We conclude that both age and gender influence post-prandial glucose metabolism albeit via difference mechanisms.

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Quantification of the human insulin concentration in adipose and muscle tissue of healthy subjects by means of the no-net- flux calibration technique and open-flow microperfusion.

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Background and Aims: Investigation into the insulin concentration of interstitial fluid (ISF) within peripheral tissues has been hampered by having no direct access to this fluid compartment. The novel sampling technique of Open-Flow Microperfusion (OFM) allows direct access to ISF in the tissues by means of macroscopically perforated double lumen catheters. We quantified the concentration of human insulin in the ISF of adipose and muscle tissue of healthy subjects under hyperinsulinaemic conditions making use of OFM and calibration with the No-Net-Flux protocol.
Materials and Methods: A constant i.v. infusion of human insulin (1 $mU \cdot kg^{-1} \cdot min^{-1}$) was given to 9 healthy subjects with blood glucose clamped at 5 mmol/L. Four macroscopically perforated double lumen catheters (OFM) were inserted into the subcutaneous adipose tissue of the abdominal region and the medial quadriceps muscle and constantly perfused with five solutions, each containing Krebs-Ringer solution and serum but different concentrations of human insulin (1 – 36 mU/L) around the expected interstitial level (≤ 60 % of serum). ISF insulin concentrations were derived from linear regression analysis applied to OFM insulin data from perfusate and recollected perfusate (No-Net-Flux calibration technique).
Results: High fractions of ISF in the samples (range of 41-81 % in muscle and 29-68 % in adipose tissue catheters) allowed reliable regression analyses of OFM insulin data. Derived ISF insulin concentrations in muscle tissue (median 15 mU/L, range 9 – 41 mU/L) and adipose tissue (median 13 mU/L, range 6 – 19 mU/L) were significantly lower ($p = 0.008$, Wilcoxon Signed Ranks Test) than serum concentrations (median 61 mU/L, range 53 – 86 mU/L). There was no significant difference between insulin levels in both tissues ($p > 0.139$).
Conclusions: Our results demonstrate that in the hyperinsulinaemic state the interstitial concentration of insulin in healthy subjects' peripheral tissues is approx. 25 % of that in serum. Thus, the observed ISF-to-serum insulin gradient provides experimental evidence that transcapillary exchange of insulin is limited in human skeletal muscle and adipose tissue.

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Expression profiling in skeletal muscle of young Zucker diabetic fatty rats: implications for a role of stearoyl-CoA desaturase 1 in insulin resistance.

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Background and Aims: To investigate early tissue-specific events in the development of insulin resistance in skeletal muscle, we studied differentially regulated genes in young male Zucker diabetic fatty (ZDF) rats.
Materials and Methods: Expression profiles from skeletal muscle and white adipose tissue of two age-matched groups (6 and 7 wks, respectively) of ZDF rats (insulin sensitive lean controls [Gmi, +/?] and insulin resistant obese animals [Gmi, fa/fa]) were determined by using Affymetrix™ microarrays. Relevant metabolic blood serum parameters were determined in all animals. Individual microarray data were confirmed by quantitative real-time PCR. Additionally, functional analyses were performed.
Results: Obese insulin resistant animals (6 and 7 wks old), in contrast to the lean insulin sensitive controls, exhibited the expected clinical serum parameters of insulin resistance (hyperinsulinemia, dyslipidemia). Employing stringent statistical conditions, our microarray analysis revealed a surprisingly low number of regulated genes / ESTs in skeletal muscle of both age-groups, compared to white adipose tissue (21 / 22 versus 216 /104, respectively). Of the few genes regulated in muscle, rat stearoyl-CoA desaturase isoform 1 (SCD1) exhibited the strongest regulation and an increased expression (5.9 to 10.6-fold, respectively) in insulin resistant animals. SCD1 is rate-limiting in the synthesis of monounsaturated fatty acids and catalyzes the introduction of a double-bond at the delta-9 carbon position of stearoyl- and palmitoyl-CoAs. To investigate whether the observed overexpression of SCD1 also reflects differential enzymatic activities, long-chain acyl-CoA (LCACoA) patterns along with SCD1 expression levels in skeletal muscle of lean and obese ZDF rats (8 wks) were determined. We observed an altered pattern of LCACoAs with increased levels of palmitoleoyl-CoA (16:1) and a highly upregulated expression of SCD1 in obese insulin resistant animals, compared to the age-matched lean control group.
Conclusions: We conclude that early stages of insulin resistance in skeletal muscle of ZDF rats involve the regulation of only a small number of genes. As the strong upregulation of SCD1 expression was the major change observed, altered patterns of LCACoAs with increased levels of palmitoleoyl-CoA (16:1) seem to be a direct result of high SCD1 expression in obese insulin resistant ZDF rats. The degree of saturation of body lipids and LCACoAs are increasingly thought to affect insulin signaling. Thus, elevated SCD1 expression likely plays a role in the pathogenesis of insulin resistance.

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The effect of peroxisome proliferator-activated receptor alpha (PPAR-α) knockout on insulin sensitivity as studied by the hyperinsulinemic-euglycemic clamp technique.

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Background and Aims: PPAR-α is an important regulator of lipid metabolism required for lipid oxidation during fasting. Chronic PPAR-α activation decreases circulating lipids and tissue lipid content in rodents with subsequent improvement of insulin sensitivity. In contrast, some studies reported that PPAR-α knockout mice are partially protected against high fat diet-induced insulin resistance. The aim of our study was to clarify the role of PPAR-α in the development of high fat diet induced-insulin resistance using PPAR-α knockout mice (PPAR -/-) and wild type controls (WT).
Materials and Methods: Male PPAR -/- and WT mice fed by normal chow or high fat diet (HFD, 40% fat, fed for 12 weeks) were studied at the age of 20 weeks. Biochemical and hormonal measurements and hyperinsulinemic-euglycemic clamp were used to quantify the severity of insulin resistance.
Results: Both WT and PPAR -/- mice on HFD gained significantly more weight relative to groups on chow diet. Independent of diet PPAR -/- mice had higher circulating triglycerides and fatty acids than WT. Both PPAR -/- and WT on HFD displayed an increase in insulin levels (WT vs. WT-HFD, mean±SEM: 0.8±0.1 vs. 1.6±0.1 ng/ml, $p<0.05$; PPAR -/- vs. PPAR -/- HFD: 0.8±0.1 vs. 3.1±1.2 ng/ml, $p<0.05$) and decrease in adiponectin levels relative to chow-fed groups. Hyperinsulinemic-euglycemic clamp performed in non-fasting state demonstrated that HFD caused a 30% reduction in whole body glucose uptake in both wild type and PPAR -/- mice relative to chow-fed groups (WT vs. WT-HFD: 234±7 vs. 154±5 μmol/kg/min, $p<0.05$; PPAR -/- vs. PPAR -/- HFD: 246±7 vs. 171±3 μmol/kg/min, $p<0.05$) indicating whole body insulin resistance. Similarly, glucose uptake measured directly in the muscle and white adipose tissue was also reduced in HFD-fed groups. Suppression of endogenous glucose production during the clamp was markedly blunted in both WT and PPAR-/- HFD-fed mice indicating liver insulin resistance. The magnitude of HFD-induced changes in whole body and tissue insulin sensitivity was comparable in PPAR-/- and WT mice suggesting that PPAR-/- mice developed similar level of insulin resistance as wild type mice.
Conclusion: Our data show that PPAR-α knockout does not protect against HFD-induced insulin resistance as measured by hyperinsulinemic-euglycemic clamp in non-fasted state. We suggest that the inability of PPAR -/- mice to utilize fatty acids leads to preferential use of glucose as a fuel during fasting. This fact, rather than increased insulin sensitivity, may explain why glucose disposal in PPAR -/- mice under fasting conditions might be higher than in wild type mice as described previously.

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Influence of PKC-dependent Shp2 phosphorylation on ERK1 and ERK2 in mouse embryonic fibroblasts.

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Background and Aims: Protein kinases C and protein phosphatases are both of particular interest regarding negative regulation of insulin actions. As previously shown, the cytoplasmic protein-tyrosine phosphatase Shp2 is phosphorylated on serine residues 576 and 591 by PKC isoforms α, β1, β2 and η. Physiological effects of these phosphorylations are not yet determined. Since Shp2 activates the ras/mitogen-activated protein (MAP) kinase pathway we tested whether serine residues 576 and 591 are involved in mitogenic signals.
Materials and Methods: [^{3}H]Thymidine incorporation was measured in Shp2 knock out mouse embryonic fibroblasts (MEF), stably overexpressing human wild type Shp2 or the double mutant Shp2-S576/591A. MAP kinase phosphorylation in stably transfected MEF after stimulation with 100 nM TPA, 10 nM PDGF or 100 nM insulin (10 min.) was analyzed by Western blotting.
Results: In Shp2-S576/591A transfectants [^{3}H]thymidine incorporation was reduced by 60 % compared to vector controls and wildtype Shp2 MEF. Adding protein kinase C inhibitor bisindolylmaleimide decreased [^{3}H]thymidine incorporation in wild type Shp2 transfectants by 50 % and in Shp2-S576/591A transfectants by 34 %. Stimulation with TPA, PDGF or insulin led to a reduced phosphorylation of the MAP kinases ERK1 and ERK2 in Shp2-S576/591A transfectants compared to vector controls and wildtype Shp2 MEF.
Conclusion: Shp2-S576/591A significantly reduce mitogenic activity in MEF compared to Shp2 wild type. Because the PKC inhibitor bisindolylmaleimide decreases cell DNA synthesis of Shp2 wild type expressing cells to a similar degree as the double mutant Shp2-S576/591A, one can suppose that mitogenesis in MEF is at least partially mediated by PKC-dependent phosphorylation of Shp2 on serine residues 576 and 591. The decreased phosphorylation of ERK1 and ERK2 in Shp2-S576/591A transfectants confirms that Shp2 mediates the mitogenic signal upstream of the MAP kinases and emphasizes the importance of Shp2 serine residues 576 and 591 to activation of MAP kinase signalling.

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Differential role of insulin in the Nitric Oxide (NO) production and Plasminogen Activator Inhibitor-1 (PAI-1) release in fibroblasts from insulin resistant individuals. Insights into the signaling pathway.

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Background and Aims: Insulin resistance is associated to both increased plasma PAI-1 and decreased NO availability. This might contribute to accelerated atherosclerosis in insulin resistant states. Insulin can stimulate both NO and PAI-1 release in a variety of cell types. However, in order for PAI-1 to be increased in insulin resistant states, one has to postulate that, in these conditions, pathways leading to insulin stimulated PAI-1 synthesis are still insulin sensitive while pathways leading to NO production are impaired. We determined insulin effect on both NO and PAI-1 release in fibroblasts from individuals with different degrees of insulin resistance.
Materials and Methods: Six fibroblast strains were cultured from skin biopsies obtained from 3 insulin sensitive (IS, clamp M>7mg/Kg/min) and 3 insulin resistant (IR, clamp M<5mg/Kg/min) volunteers matched for age and BMI. On each strain, we measured, in separate experiments, insulin stimulation of NO synthesis (conversion of ^{3}H-arginine into ^{3}H-citrulline) and PAI-1 release (ELISA).
Results: Insulin stimulated PAI-1 release was not different in fibroblasts from IS and IR individuals (54±6 vs 43±5 ng/ml and 100±11 vs 88±9 ng/ml, at 10 and 100 nM insulin respectively, p= n.s.). Conversely, the effect of insulin (100nM) on NO release was significantly less in fibroblast from IR as compared to IS individuals (respectively 0.85±0.09 vs 1.25±0.14 nmoles/min/mg protein, p<0.05). To gain insight into the signaling pathways leading to insulin stimulated PAI-1 release, we repeated the experiments in the presence and in the absence of Ly2940029 (an inhibitor of phosphatidylinositol 3-kinase [PI3-K]) or of PD98059 (an inhibitor of mitogen-activated protein kinases [MAPK]). After exposure to Ly2940029, insulin (100nM) induced PAI-1 secretion was decreased in both fibroblasts from IS and IR individuals, by 70% ± 6 and 65% ± 5, respectively (both p<0.05 vs control). Exposure to PD98059 was also followed by decreased insulin induced PAI-1 release in both cell strains (both by > 65% as compared to control, p< 0.05). This shows that insulin stimulated PAI-1 synthesis in both cell strains is due to PI3-K activation followed by MAPK activation.
Conclusion: We conclude that insulin ability to stimulate PAI-1 release is preserved in cells from IR individuals in which NO release is resistant to insulin stimulation and that MAPK activation plays a central role in insulin stimulation of PAI-1 release in cells from both IR and IS individuals. Thus, in the insulin resistance syndrome, hyperinsulinemia might be one the culprit for the observed increase in PAI-1 levels while insulin resistance can account for impaired insulin induced vasodilation.

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Diverse regulation of delta-6 desaturase in dietary-induced and/or genetically fixed insulin resistance.

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Background and Aims: Our previous studies have shown that insulin resistance is associated with different fatty acid (FA) profile in insulin target tissues, possibly due to an impairment of the desaturation pathway. Thus, the aim of our study was to measure enzyme activity of and gene expression for the key desaturation enzyme, i.e. the delta-6 desaturase in liver of rats with either a high sucrose diet-induced or hereditary fixed hypertriglyceridemia and insulin resistance.
Materials and Methods: The control Wistar (C) and the hereditary hypertriglyceridemic (hHTg) rats were fed for 21 days a standard rat chow. In addition, another group of normal rats was fed for the same time interval the high sucrose diet [63 cal % of sucrose (HS)]. The enzyme activity of delta-6 desaturase was determined radiometrically in a microsomal fraction using the 1-^{14}C-linolenic acid as substrate. The relative abundance of mRNA for the delta-6 desaturase was measured by the Northern blot technique using a specific cDNA probe. Fatty acid composition of total phospholipid fraction in liver was determined by capillary gas chromatography after TLC separation.
Results: In harmony with a raised index of delta-6 desaturase (as calculated from liver fatty acid profile as a ratio of n-6 polyunsaturated fatty acids metabolites to the linoleic acid), a higher activity of the delta-6 desaturase was found in liver of rats fed the high sucrose diet (HS: 89.7+1.5; C: 62+0.7 pmol/mg/min; p<0.01). However, these changes were not accompanied by appropriate changes in the hepatic mRNA levels for delta-6 desaturase. In contrast, a reduced activity of delta-6 desaturase in liver of hHTg rats (hHTg: 13.07 +0.7; C: 62+0.7; pmol/mg/min; p<0.01) was associated with a similar decrease in the abundance of delta-6 desaturase mRNA (hHTg: 0.05+ 0.007; C: 0.21+0.047; arbitrary units; p<0.001). These changes were paralled by a decrease of the delta-6 desaturase index based on fatty acid composition in total phospholipid fraction in liver.
Conclusions: Our results have shown that 1) the high sucrose-induced insulin resistance goes with a higher activity of, and the 2) hereditary hypertriglyceridemia/ insulin resistance associates with a lower activity of and gene expression for the delta-6 desaturase. Thus, a diverse regulation of the aforementioned key desaturation enzyme seems to participate in the abnormal fatty acid profile of both, the diet-induced and the genetically fixed insulin resistance.

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Induction of a metabolic syndrome relies on timing of high fat feeding and brain melanocortin system blockade.

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Background and aims: Obesity is associated with the development of a metabolic syndrome characterized firstly by an insulin and leptin resistance. In a rat model for diet induced obesity (blockade of the brain melanocortin system by a 14-day icv infusion of SHU9119 combined with a high fat diet - HF, fat = 60% of energy), we previously observed that, despite exaggerated hyperleptinemia in SHU9119-treated HF rats relative to rats fed a high carbohydrate diet (HC, CHO = 60% of energy), plasma insulin and adiponectin levels were comparable among diet groups. The present study investigated whether these secretion profiles of adipose and pancreatic hormones are influenced by the duration of adaptation to HF feeding before SHU9119 treatment.
Materials and Methods: Male Wistar rats (n=64) were either adapted to HF feeding for 2 months prior to the onset of SHU9119-infusion (LT), or were switched from the HC to the HF diet at the onset of SHU9119 infusion (ST).
Results: Following 14-day SHU9119 treatment, early light phase plasma leptin levels were not different among groups (44.4 ± 7.7 ng/ml in LT and 36.5 ± 5.3 ng/ml in ST rats). Baseline plasma adiponectin levels were significantly higher in LT (7.9 ± 0.9 mg/ml) than in ST rats (5.0 ± 0.4). Interestingly, plasma insulin levels were markedly higher in ST (33.0 ± 7.4 ng/ml) than in LT (8.3 ± 1.1 ng/ml) rats. Thus, despite comparable increases in food intake, plasma adiponectin was 36 % lower, whereas plasma insulin was 400% higher in ST relative to LT rats.
Conclusion: This dramatic increase in plasma insulin concentration in ST rats might indicate severe insulin resistance as a consequence of acute HF exposure and low brain melanocortin activity.
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Prevention of obesity and insulin resistance by glucokinase expression in skeletal muscle of transgenic mice.

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Background and Aims: In type 2 diabetes, glucose phosphorylation, a regulatory step in glucose utilization by skeletal muscle, is impaired. Since glucokinase expression in skeletal muscle of transgenic mice increases glucose phosphorylation, we examined whether these mice can counteract the obesity and insulin resistance induced by a high-fat diet.
Materials and Methods: Transgenic mice expressing glucokinase in skeletal muscle were fed a high-fat diet for 12 weeks. Effects on body weight, food intake, glucose tolerance and insulin sensitivity were analysed.
Results: When fed this diet, control mice became obese while transgenic mice remained lean. Furthermore, high-fat fed control mice developed hyperglycemia and hyperinsulinemia (a 3-fold increase), indicating that they were insulin resistant. In contrast, transgenic mice were normoglycemic and showed only a mild increase in insulinemia (1.5-fold). They also showed improved whole-body glucose tolerance and insulin sensitivity and increased intramuscular concentrations of glucose 6-phosphate and glycogen. A parallel increase in uncoupling-protein 3 mRNA levels in skeletal muscle of GK-expressing transgenic mice was also observed.
Conclusion: These results suggest that the rise in glucose phosphorylation by glucokinase expression in skeletal muscle leads to increased glucose utilization and energy expenditure that counteracts weight gain and maintains insulin sensitivity.

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Transgenic mice overexpressing phosphoenolpyruvate carboxykinase in adipose tissue are more sensitive to diet-induced insulin resistance.
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Background and Aims: When glucose supply to adipose tissue is limited, glycerol 3-P originates from pyruvate through glyceroneogenesis. A regulatory enzyme of this pathway is phosphoenolpyruvate carboxykinase (PEPCK). Since PEPCK overexpression in transgenic mice leads to obesity without insulin resistance, here we examined whether adipose overexpression of PEPCK counteracts the insulin resistance induced by a high fat diet.
Materials and Methods: For the experimental induction of insulin resistance, control and homozygous transgenic male mice overexpressing PEPCK aged 4-months were fed a high-fat diet.
Results: After 6 weeks on this diet, transgenic mice gained 40% of initial body weight whereas control mice gained only 20%. However, food intake was similar in both groups. Surprisingly, whereas blood glucose levels were similar in control and transgenic mice, the latter were strongly hyperinsulinemic. Moreover, transgenic mice were more glucose intolerant and presented a higher degree of whole-body insulin resistance than control mice. Circulating leptin levels increased to the same extent in transgenic and control mice and serum free fatty acids were not higher in transgenic mice. In contrast, circulating adiponectin levels were decreased in transgenic mice.
Conclusion: These results suggest that PEPCK overexpression exacerbates the obesity and insulin resistance induced by a high-fat diet probably through the decrease of the adipose-specific factor, adiponectin.
This study was supported by European Union (EuroDiabetesGene QLRT-1999-00674).

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Impairment of the insulin-induced activation of the constitutive nitric oxide synthase via the phosphatidylinositol 3-kinase pathway in platelets from obese subjects.
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Background and Aims: Human platelets present a constitutive nitric oxide synthase (cNOS), that is activated by insulin with production of nitric oxide (NO). NO, acting on guanylate cyclase, increases platelet concentrations of cGMP, which reduces platelet aggregation in response to agonists.We previously demonstrated that both insulin and NO are less effective in inducing cGMP increase and anti-aggregation in platelets from obese subjects than in platelets from healthy controls.Since NO mediates the insulin-induced anti-aggregating effect, the resistance to NO in obesity could explain the resistance to insulin. An impairment of the insulin-induced activation of cNOS in obese subjects has not been demonstrated so far.Aim of this study is to clarify: i)whether insulin activates cNOS in human platelets via the intracellular signalling pathway of phosphatidylinositol 3-kinase (PI-3K); ii)whether the insulin-induced activation of cNOS in platelets is impaired in human obesity.
Materials and Methods: We studied 5 healthy male volunteers (age 36.8±2.5 years, BMI 22.25±0.38, HOMA 2.3±0.03) and 5 male obese subjects (age 39.6±2.87 years, BMI 32.54±0.60, HOMA 5.6±0.04). Platelet cNOS activation was measured as L-citrulline production after incubation with L-arginine, since citrulline and NO are produced by cNOS equimolecularly. Experiments were carried out in washed platelets incubated with: i)control buffer; ii)2 nmol/l human regular insulin for 5 min, both without and with a 20 min pre-incubation with wortmannin, a PI-3K inhibitor.
Results: NO production (expressed in pmol citrulline/10^8 platelets) rose from 0.082±0.01 to 0.24±0.01 (p=0.0001) in platelets from healthy controls, an effect blunted by wortmannin (0.078±0.01 with wortmannin alone and 0.077±0.01 with insulin+wortmannin, ns), and rose from 0.049±0.01 to 0.1024±0.01 (p=0.0001) in platelets from obese subjects, also in this case an effect blunted by wortmannin (0.049±0.01 with wortmannin alone and 0.052±0.01 with insulin+ wortmannin, ns). Delta values between basal and insulin-induced NO production were 0.157±0.03 and 0.054±0.01 pmol citrulline/10^8platelets in control and obese subjects, respectively (p=0.012).
Conclusion: Insulin activates platelet cNOS via the PI-3K pathway, a phenomenon impaired in obese subjects. Thus, in human obesity, the reduced ability of insulin to anti-aggregate platelets is attributable not only to the impaired NO ability to increase cGMP (i.e resistance at the guanylate cyclase level), but also to the impaired insulin ability to produce NO (i.e. resistance at the cNOS level). These results show that platelet hyperactivation in obese subjects is attributable to a multi-step resistance to physiological modulators.

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Metabolic adaptations of three inbred strains of mice (C57BL/6, DBA/2 AND 129Sv) in response to a high fat diet.

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Background and Aims: The advent of transgenic and gene targeted animal models has proven to be a powerful and increasingly popular scientific tool to study the molecular and cellular mechanisms underlying metabolic and endocrine disorders, such as obesity and Type II diabetes. However, there are now many examples that demonstrate that the background strain of these genetically engineered animals has a profound influence on the phenotype exhibited. While awareness of these isogenic or "inbred phenotypes" is increasing, systematic metabolic characterization is lacking. The aim of this study was to examine metabolic parameters of three commonly used inbred strains of mice (C57BL/6, DBA/2 and 129/Sv) in response to a low (7% w/w) and a high fat (60% w/w) diet for 7 weeks.
Materials and Methods: Male mice (10 weeks) were randomly assigned to 2 diet groups and fed *ad libitum*. At 6 weeks, calorimetric and spontaneous physical activity measurements were carried out while, in week 7 glucose kinetic studies were performed.
Results: Basal endogenous glucose production (EGP) was not different between the strains and was not affected by a high fat diet (C57BL/6: 55.6 ± 10.0 vs 46.3 ± 4.5, DBA/2: 56.7 ± 6.6 vs 38.3 ± 4.6, 129/Sv: 41.1 ± 4.1 vs 37.3 ± 4.2 p= NS Control diet vs Fat n= 5-10). Feeding a high fat diet produced a marked weight gain (60%) in C57BL/6 and 129/Sv animals compared to their control counterparts. Interestingly, the DBA/2 animals did not mirror this trend but showed a 70 % weight increase on the low fat diet compared to C57BL/6 and 129/Sv animals. 129/Sv control animals had significantly lower fasting insulin levels when fed a low fat diet and showed a significant increase in response to high fat. The opposite occurred in the DBA/2 animals in response to high dietary fat. Both C57BL/6 diet groups had significantly higher resting energy expenditure than either DBA/2 or 129/Sv animals. Fat feeding reduced horizontal physical activity in C57BL/6, while having no effect in the DBA animals. 129/Sv animals moved significantly less, irrespective of diet.
Conclusion: These results clearly indicate that high fat feeding produces varied physiological and physical responses in different inbred mouse strains. While the methodology of creating genetically manipulated animals has become surprisingly simple, the science and the interpretation has become more complex than ever. This study clearly shows that inherent metabolic information is important and necessary before embarking on the long and arduous road of producing genetically engineered models of metabolic disorders
Results

	Low Fat Diet (n=10)		High Fat Diet (n=10)			
	C57BL/6	**DBA/2**	**129/Sv**	**C57BL/6**	**DBA/2**	**129/Sv**
Weight Gain (g)	1.26±0.24‡	3.03± 0.49	0.68±0.30‡	3.19±0.43*	3.69±0.30	2.49±0.31*†
Epididymal (g)	0.22±.0.15‡	0.39±.0.40 #	0.26±.0.17‡	0.43± 0.08*†	0.55± 0.04*	0.41±0.05*†
Fasting Glucose (mmol/L)	7.18 ±0.08	6.04 ±0.54	6.63± 0.62	7.83 ±0.43	6.70 ± 0.61	6.63 ±0.77
Fasting Insulin (ng/mL)	0.80 ±0.34	0.65 ±0.16	0.23 ±0.05#‡	1.13 ±0.21	0.41 ±0.07#	0.70 ±0.13*
Food Intake (g)	2.91 ±0.17	2.87 ± 0.17	3.01 ± 0.24	1.79 ± 0.06*	1.98 ± 0.11*	1.89 ±0.14*
Energy Intake (J)	31.98 ±5.9	37.3 ± 5.1	39.13 ± 2.81	40.83 ± 5.25	44.94 ± 6.14	43.10 ± 6.2
Energy Expenditure (kcal/min/kg)	0.232±0.001	0.192±0.001#Φ	0.172±0.001#Φ	0.219±0.012	0.193±0.008#Φ	0.178±0.006#Φ
Spontaneous Horizontal Activity (counts/day)	85481±5330	80855±7961	37353±2912#‡†	62875±12078	81441±9997	44680±9028#‡†

*p<0.05 vs low fat diet, # p<0.05 vs C57BL/6 cont. diet, Φ p<0.05 vs C57BL/6 fat diet, p<0.05 vs DBA/2 fat diet, ‡ p<0.05 vs DBA/2 cont. diet

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Effect of high fat diet on the expression of proteins in muscle, adipose tissues and liver of C57Bl/6 mice.

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Background and Aims: High fat diets are known to induce insulin resistance and obesity in animals. Obesity is a strong risk factor for the development of type 2 diabetes. In a previous study, we had performed a comparative proteomic analysis with genetically obese (lep/lep) and lean mice. In addition, these mice were treated with an insulin sensitizer BRL49653 (Rosiglitazone). In the present study, we wanted to see if insulin resistance associated with high fat feeding produced similar changes in gene expression as those found previously.
Materials and Methods: C57Bl/6 mice were fed on a normal chow diet or a high fat (60% of energy from fat) diet for 3 months. Insulin resistance was established by measurements of blood glucose and plasma insulin during oral glucose tolerance tests. Gastrocnemius muscle, white and brown adipose tissue and liver were taken from the animals and used for proteomic analysis. Expression levels of approximately 10'000 proteins representing the four tissues were assessed by two-dimensional gel electrophoresis (2-DE). For each tissue, 2-DE maps from obese littermates (n=4) were compared to 2-DE maps from lean littermates (n=4). Computer-assisted image analysis allowed the detection of differentially expressed proteins. Their identification was made by tandem mass spectrometric analysis (LC-Q-TOF-MS).
Results: The high fat diet induced moderate obesity and insulin resistance. Altogether, more than fifty proteins were detected to be differentially expressed ($p<0.05$) between obese and lean mice. Interestingly, more than half of these proteins were detected in the brown adipose tissue. Among the proteins identified, mitochondrial proteins were most often observed to be differentially expressed. Mitochondrial enzymes involved in citric acid cycle as well as the fatty acid beta-oxidation were up-regulated in high fat mice. In contrast, a key glycolytic enzyme was found to be down-regulated. Similarly, several stress and redox proteins were down-regulated. A cytosolic factor which was shown to participate in the intra-Golgi protein transport, was up-regulated in high fat mice.
Conclusion: Obesity represents a complex and polygenic metabolic disorder. Therefore, the identification of disease-linked proteins and the discovery of the physiologically relevant biochemical pathways underlying the disease require a global approach. Our study showed that in high fat induced obesity, the expression level of several important proteins in lipid metabolism were up-regulated, whereas a key glycolytic enzyme was down-regulated. In particular, there were significant changes in brown adipose tissue indicating that the high fat diet treated mice were attempting to defend against weight gain by increasing lipid oxidation. There was little overlap between the differential expression of proteins in this study and those found in an earlier study in lep/lep mice.

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Association between oxidative stress and intramuscular lipids in insulin-resistant rat skeletal muscle.

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Background and Aims: Insulin-resistant skeletal muscle frequently displays elevated levels of intramuscular triglyceride (IMTG). The oxidation of this expanded lipid pool may be associated with increased oxidative stress of the myocytes, contributing to insulin resistance. In the present investigation, we tested the hypothesis that alterations in IMTG levels would be associated with parallel changes in local oxidative stress in insulin-resistant skeletal muscle of the obese Zucker (fa/fa) rat.
Materials and Methods: Female obese Zucker rats were treated for 20 days with either vehicle, 76% enriched cis-9,trans-11 conjugated linoleic acid (c9,t11-CLA), 90% enriched trans-10,cis-12-CLA (t10,c12-CLA), or a 50:50 mixture of the two CLA isomers (M-CLA) (by oral gavage at 1.5 g CLA/kg body wt), in order to manipulate insulin-stimulated glucose

transport (assessed in vitro by 2-deoxyglucose uptake), IMTG, and protein carbonyls (an index of local oxidative stress) in soleus muscle.
Results: Insulin-mediated (5 mU/ml) glucose transport was increased ($p<0.05$) by both M-CLA (23%) and t10,c12-CLA (46%), while these same interventions caused significant ($p<0.05$) decreases in IMTG (29% and 34%) and protein carbonyls (48% and 56%), compared to the vehicle-treated control group. The c9,t11-CLA intervention was without effect on these parameters. Linear regression analysis indicated significant negative associations between insulin-mediated glucose transport and either IMTG ($r=-0.631$, $p=0.0028$) or protein carbonyls ($r=-0.616$, $p=0.0038$). Moreover, there was a highly significant, positive relationship between IMTG and protein carbonyls ($r=0.718$, $p=0.0004$) in these soleus muscles of the obese Zucker rat.
Conclusion: These results suggest that the IMTG level may be linked to local oxidative stress, and may be causally associated with the degree of insulin resistance of skeletal muscle glucose transport in the obese Zucker rat. Further investigation is required to more definitively test the cellular mechanisms underlying this provocative concept.

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Amelioration of diet-induced obesity in mice overexpressing uncoupling protein-3 in skeletal muscle.
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Background and Aims: Uncoupling protein 3 (UCP3), which has been hypothesized to uncouple electron transport from ATP synthesis, is expressed abundantly in the skeletal muscle. It is suggested that UCP3 can increase energy expenditure leading to regulation of body weight. Although studies on UCP3 knock-out mice suggest that lack of UCP3 function appears not to be involved in the pathogenesis of obesity or Type 2 diabetes, there still remains the possibility that up-regulation of the UCP3 function is implicated in the improvement of these disorders, or its clinical sequelae. Since 10-20 fold increase of the UCP3 gene expression is achievable through physiological or pharmacological stimuli, we examined the phenotype of transgenic mice of approximately 18-fold overexpression of mouse UCP3 mRNA in the skeletal muscle.
Materials and Methods: We generated transgenic mice of approximately 18-fold overexpression of mouse UCP3 mRNA under control of the skeletal muscle-specific muscle creatine kinase gene promoter. The phenotype of transgenic mice were analyzed either on standard chow diet or on 4-week high fat diet.
Results: On standard chow diet, there were trends towards increased oxygen consumption (202.9 ± 10.1 nmol O_2/mg/min vs. 193.1 ± 7.1 nmol O_2 /mg/min) and decreased mitochondrial proton motive force (196.5 ± 2.2 mV vs. 201.3± 4.1 mV) for the mitochondria of transgenic mice although they did not show any obvious phenotype other than these trends. On 4-week high fat diet transgenic mice exhibited significantly greater oxygen consumption (54.1 ± 4.5 ml/kg/min vs. 43.1 ± 1.6 ml/kg/min, $P<0.05$), markedly less body weight gain (4.4 ± 1.1 g vs. 8.6 ± 0.8 g, $P<0.05$) and less epididymal fat (1693 ± 131 mg vs. 2110 ± 17 mg, $P<0.05$) with amelioration of glucose tolerance than nontransgenic littermates.
Conclusion: The present study demonstrates that 18-fold overexpression of UCP3 mRNA in the skeletal muscle had antiobesity effect on diet-induced obesity. Since 18-fold increase of UCP3 mRNA may be attainable by physiological or pharmacological stimuli, the present study suggests its therapeutic potential in obesity.

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The effects of high fat diet-induced obesity on cardiovascular and autonomic nervous dynamics: a role for hypothalamic endorphins.
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Background and Aims: Obesity and high fat diets are associated with the increased risk of diabetes, cardiovascular disease, and hypertension. The mechanism(s) linking obesity and high fat diet to these diseases are unknown. Obesity is associated with increased leptin production and leptin stimulates the hypothalamic production of proopiomelanocortin (POMC)-derived peptides. Both αMSH and β-endorphins influence cardiovascular and autonomic nervous functions. The aim of this study was to determine if POMC-derived opioids could play an essential role in linking obesity and cardiovascular and sympathetic nervous dynamics.
Materials and Methods: Male Wistar rats were implanted with radio-telemetry transducers to continuously record blood pressure, heart rate and activity. They were fed either a high fat diet or regular diet and maintained for up to 20 weeks. At this time the animals were anesthetized to record additional mean arterial pressure (MAP) responses as well as and renal sympathetic nerve activity (RSNA) responses to opioid agonist or antagonists. Blood samples were collected to measure glucose, insulin, leptin, and beta-endorphins. At the end of the study, the brains were removed and prepared for immunohistochemistry.
Results: The high fat fed rats gained more weight and also had a greater percentage of body fat and a lowered amount of body protein when compared to the aged-matched control. The plasma levels of leptin and insulin were higher and β-endorphins lower in the high fat fed rats. The blood pressures progressively increased over time in the high fat fed rats compared to controls. After 20 weeks the basal MAP, as well as the RSNA, were significantly higher in high fat fed rats compared to controls. The cardiovascular response to the mu agonist (DAMGO) was also increased. Immunohistochemistry demonstrated that the obese high fat fed rats had increased mu opioid receptors in the arcuate nucleus compared to control. This increase in mu receptors was further confirmed by *in situ* hybridization.
Conclusion: These studies suggest that high fat feeding is associated with a progressive increase in blood pressure and sympathetic nerve activity. High fat-induced obesity is associated with an increase in mu receptors and increased sensitivity to mu receptor agonist, and this may contribute to the significantly higher MAP and RSNA observed in obese rats. Supported by NIH GM-058905 and NIH MH-17153.

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Increased insulin sensitivity in prediabetic Goto-Kakizaki young rats precedes the development of insulin resistance in adults.
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Background and Aims: GK rat is a genetic non overweight model of NIDDM in which adult animals are characterised by impaired glucose-induced insulin secretion, decreased β cell mass, decreased insulin sensitivity in the liver and moderate insulin resistance in muscles and adipose tissue. In this model, animals become overtly diabetic at the time of weaning (4 weeks after birth). In a previous study we have shown that one or two weeks old GK rats, are normoglycaemic despite of the severe decrease of β cell number and reduced basal insulinaemia as compared to the age-matched non diabetic Wistar rats. In the work presented here we investigated whether the ability of GK newborns to maintain normoglycaemia despite of the reduced circulating insulin levels reflected an increased sensitivity of target tissues to insulin.
Materials and Methods: We evaluated in 3 weeks old rats, one week before weaning, the insulin secretion in vivo after an intraperitoneal glucose tolerance test. The basal glucose metabolism was determined using [3-^{3}H] labelled glucose and the biological action of exogenous insulin on target tissues was evaluated by euglycaemic hyperinsulinaemic clamp study.
Results: In 3 weeks old animals, basal insulinaemia was significantly lower in GK rats compared to controls. After glucose administration, the mean incremental glucose area was similar in both groups whereas the mean incremental insulin area was significantly ($p<0.01$) lower in GK rats than that found in Wistar rats. We next evaluated basal glucose turn over using [3-^{3}H] labelled glucose. The basal glucose utilisation was significantly higher in GK rats compared to controls ($p<0.01$). To study the effect of exogenous insulin on hepatic glucose production and peripheral glucose

utilisation we performed an euglycaemic hyperinsulinaemic clamp experiment. In both GK and Wistar groups the dose of 1U/h/kg insulin completely blocked hepatic glucose production. The insulin mediated glucose uptake by the whole body mass was significantly ($p<0.05$) greater in GK rats compared to controls as determined by the calculation of the amount of infused glucose necessary to maintain euglycaemia during the experiment.
Conclusion: Altogether our data indicate that in normoglycaemic hypoinsulinaemic 3 weeks old GK rats there is an increased basal glucose metabolism and an enhanced response of peripheral tissues to insulin. This suggests that GK newborns compensate for the primary insulin deficiency by increasing the sensitivity of target tissues to insulin. Such an adaptation is transitory and is followed by insulin resistance which is time-related with weaning nutritional changes and with appearance of basal hyperglycaemia.

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IRS-2 expression, beta cell mass, and insulin resistance in pancreatectomized rats.
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Background and Aims: Some research showed that IRS-2 expression was induced in a cAMP dependent manner in cell culture and with a high fructose diet in experimental animals. In this study, we investigated whether the long-term consumption of cola, 11% sugar or 0.0105% caffeine and exercise increase IRS-2 expression in liver and brain, and they have a beneficial effects on pancreatic beta-cell mass, insulin secretion, and insulin resistance in male 90% pancreatectomized (Px) Sprague Dawley rats weighing 169+35 g.
Material and Methods: In the first study, the rats were freely provided with cola, 11% sugar, 0.0105% caffeine, or water along with 33 energy percent fat diets for 28 weeks. The second experiment investigated exercise and dexamethasone (DEX) effects. Px rats ran on an uphill treadmill at 20 m/min for 30 min every other day for 4 and 8 weeks, and low (0.02mg DXN/kg BW) and high (0.1mg DXN/kg BW) dosages of DEX were treated. In both experiments, at the end of the experimental periods, hyperglycemic clamp and euglycemic hyperinsulinemic clamp were performed in consecutive days to measure in vivo insulin secretion capacity and insulin resistance. IRS-1 and 2 expression of liver and brain was measured by immunoprecipitation followed by western blotting.
Results: Long-term cola intake increased glucose disposal rate and decreased basal hepatic glucose output, and 11% sugar solution and 0.0105% caffeine also showed same results, compared to the control group, but in less degree than cola. In all three groups, IRS-2 expression in liver, but not IRS-1 expression, was increased by 2-3 folds compared to the control. Pancreatic beta cell mass was also higher in cola, sugar and caffeine groups than the control. Low dosage of DEX and exercise synergistically increased insulin sensitivity and pancreatic beta cell mass, and decreased hepatic glucose output. However, high dosage of DEX increased insulin resistance and decreased pancreatic beta cell mass in the 8-week treatment, and exercise could not compensate them. Exercise and low dosage of DEX treatment increased IRS-2 in liver, and their effects were synergistic. In correlation analysis, IRS-2 expression in liver was positively associated with insulin sensitivity ($r=0.45$) and pancreatic beta cell mass ($r=0.36$) and negatively with hepatic glucose output ($r=-0.58$).
Conclusion: Therefore, the improved insulin sensitivity and pancreatic beta cell mass and decreased hepatic glucose output may be related to increased IRS-2 expression in liver, and presumably in islet cells.

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IRS-1 protein degradation induced by oxidative stress is associated with distinct time-course of phosphorylation on Ser307 and Ser632.
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Background and Aims: Continuous exposure of adipocytes and hepatoma-derived cells (FAO) to micromolar H_2O_2 induces the protein degradation of IRS1 and its phosphorylation on Ser/Thr residues. Yet, unlike chronic exposure to insulin, inhibitors of the proteasome-degradation machinery do not inhibit this effect (*Potashnik et al., Diabetologia, In press*). This suggests that unique Ser/Thr residues participate in determining the different fates of IRS1 in response to these inducers of insulin resistance. The aim of the present study was to evaluate the effect of oxidative stress on the phosphorylation state of two distinct Ser residues of IRS1.
Materials and Methods: FAO cells were exposed to an H_2O_2 generating system or to 100 nM insulin for up to 4 h, and phosphorylation on Ser307 or Ser632 was evaluated using residue-specific phospho-antibodies.
Results: Oxidative stress resulted in increased phosphorylation of both Ser307 and Ser632. Interestingly, phosphorylation of the two residues occurred with a distinct time-course. While pSer307 signal displayed a rather acute and short-lived pattern (peaking at 30 min and undetectable at time points over 60 min), phosphorylation on Ser632 was observed beginning at 60 min, peaked at 120 min and was still visible at 180 min. Since Ser632 is within a consensus sequence of the c-Jun N-terminal kinase (SAPK/JNK, P-X-S/T-P), and JNK was phosphorylated by oxidative stress, we next assessed the effect of SP600125 (SP), a specific JNK inhibitor, on IRS1 Ser632 phosphorylation. In both total cell lysates and immunoprecipitates of IRS1, 40 microMolar SP inhibited the pSer632 signal induced by oxidative stress by ~80%. Treating the cells with inhibitors of mTOR, PI 3-kinase, or ERK1/2, had no similar inhibitory effect on the phosphorylation of IRS1 on this site. Inhibition of the proteasome degradation machinery was reported to induce JNK activity. Further consistent with a role of JNK in IRS1 Ser632 phosphorylation, control cells exposed to MG-132, a proteasome inhibitor, exhibited increased pSer632 signal. When used during exposure to oxidative stress, MG-132 did not prevent IRS1 degradation, but resulted in augmented and prolonged duration of its phosphorylation on Ser632. Moreover, these conditions resulted in the appearance of high molecular weight bands, potentially representing accumulation of Ser632 phosphorylated, poly-ubiquitinated IRS1 molecules.
Conclusion: Here we demonstrate that continuous exposure to oxidative stress results in “waves” of residue-specific phosphorylation of IRS1. Furthermore, these results offer a possible link between oxidative stress and IRS1 phosphorylation on Ser632 through activation of JNK.

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Development of diabetes mellitus in the Otsuka Long-Evans Tokushima Fatty (OLETF) rat is related with low activity of skeletal muscle pyruvate dehydrogenase complex.
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Background and Aims: The mitochondrial pyruvate dehydrogenase (PDH) complex catalyzes the irreversible oxidative decarboxylation of pyruvate with the formation of acetyl-CoA and NADH. This reaction links glycolysis and the citric acid cycle in mitochondria. It is hypothesized that the decreased PDH complex activity in peripheral tissues might be related with the development of diabetes. The present study was carried out to verify the regulatory mechanisms of the PDH complex before and after the onset of diabetes mellitus in OLETF rats, a spontaneously type 2 diabetic strain with mild obesity, compared to those in their non-diabetic/lean counterparts Long-Evans Tokushima Otsuka (LETO) rats.
Materials and Methods: Six male OLETF and six male LETO rats aging 8 weeks were randomically selected and killed after 6-hours starvation. After blood sample was taken, the gastrocnemius muscle was excised, freeze clamped at liquid nitrogen temperature, and stored at –80oC until analysis. Remaining six rats of each group were maintained until the diabetic state was confirmed, at 25 weeks of age, and then killed by the same way.
Results: Body weight was significantly ($P<0.005$) higher in OLETF than in LETO rats at 8 weeks of age, and this difference was magnified at 25 weeks of age. The plasma glucose concentration was similar between the two groups at 8 weeks but was significantly higher in OLETF rats at 25 weeks of age ($P<0.001$). The plasma insulin concentration was not significantly different between the two groups of young rats, but was markedly higher in OLETF rats aging 25 weeks ($P<0.001$), indicating a pronounced insulin resistance state. The plasma free fatty acid (FFA) was significantly higher (1.6-fold) in OLETF rats at both 8 and 25 weeks of age. The actual activities of muscle PDH complex in OLETF rats were only 22% and 37% of those in the control LETO rats at 8 and 25 weeks, respectively, suggesting that the glucose oxidation in OLETF rats was depressed even before the onset of diabetes. The activity of the PDH kinase (PDK), which downregulates the PDH complex, was found to be exactly opposite to the activity state of the complex.
Conclusion: These results indicate that the PDH complex plays a decisive role in dictating the rate of carbohydrate oxidation in skeletal muscle. Furthermore, depressed glucose oxidation in young OLETF rats may be related to the development of diabetes mellitus.

PS 37
Hormone-Induced Insulin Resistance

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Dexamethasone prevents activation of the p38 MAP kinase pathway by an upregulation of the dual-specificity phosphatase MKP-1: implications for insulin resistance.

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Background and Aims: A major pathogenic factor in type II diabetes is insulin resistance. Loss of insulin-sensitivity can be induced by a variety of conditions. Steroids of the glucocorticoid family are strong inducers of insulin resistance.We have analysed the effect of dexamethasone on signal transduction pathways in 3T3L1 adipocytes in order to understand the biochemical mechanism that underlies the induction of insulin resistance. Materials and Methods: Fully differentiated 3T3-L1 adipocytes were treated with 100nM dexamethasone and subjected to biochemical analyses.
Results: We observed that incubation of these cells with 100nM dexamethasone for 48 hours induces a 50% reduction in the level of maximally insulin-stimulated glucose uptake. Dexamethasone did not interfere with the PI-3'kinase signalling pathway in such a way that it affected downstream signalling, as demonstrated by the lack of an effect on the insulin-induced phosphorylation of Ser473 of Protein Kinase B (PKB) and the forkhead transcription factor FKHR-L1. The other main signalling pathway of the insulin receptor towards glucose uptake is the tyrosine phosphorylation of Cbl. The insulin-induced activation of this pathway was also unaffected by dexamethasone. Furthermore, dexamethasone-treatment did not affect insulin-induced GLUT4 translocation, which is dependent on both PI-3' kinase activity and Cbl phosphorylation.
The MAP kinase p38 has recently been identified as a modulator of the intrinsic transporter-activity of the GLUT4 transporter in response to insulin. We observed that dexamethasone-treatment induces a strong reduction of p38-phosphorylation in response to insulin. Furthermore, insulin-induced ATF2 phosphorylation (a known *in vivo* target of p38 MAP kinase activity) was also completely abolished by dexamethasone-treatment. Dexamethasone increased MKP-1 (a p38 MAPK phosphatase) 2-fold on mRNA and protein level, with kinetics paralleling the induction of insulin resistance.
Conclusion: Our data suggests that dexamethasone prevents proper activation of the p38 MAP kinase pathway in response to insulin (and the insulin mimeticum Na-arsenite) by increasing the intracellular levels of a MAP Kinase Phosphatase, MKP-1. Consequently, the p38-mediated intrinsic activation of GLUT4 transporters in the plasma membrane is prevented, leading to a reduction in glucose uptake.

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Transcriptional repression of the human insulin receptor gene by 17β-estradiol.

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Background and Aims: We previously demonstrated that 17β-estradiol (E) inhibited human insulin receptor (hIR) gene expression at the RNA level in a dose- and time- dependent manner in U-937 human promonocytic cells. (E)-treatment also caused a decrease in the insulin responsiveness of these cells in terms of glucose oxidation. Based on these findings, the present study was designed to evaluate the possibility that these defects could be primarily regulated at the transcriptional level. We also tried to establish whether the inhibition of insulin action by (E) could be associated with reduced activity of the insulin signalling system at the level of phosphatidylinositol 3-kinase (PI3-·kinase).
Materials and Methods: The –1819 to –271 promoter fragment of the hIR gene cloned at the BglII site of the pCAT3M vector was kindly provided by Drs S.Y. Tsai and G.Elberg. This promoter fragment, considered by these authors as the wild-type promoter, was subcloned at the BglII site of the pGL2-basic vector (Promega) to create the reporter plasmid phIR(-1819)-GL2. The orientation and integrity of the insert was confirmed by restriction analysis. Transient transfections were carried out by electroporation of 20 x 10^6 cells in RPMI 1640 medium with the Bio-Rad gene-pulser II. The cells were electroporated at 250 V, 960 µF, in a volume of approximately 300 µl. After a resting period of 24 h, the transfected cells were left untreated or were treated with 10^{-9} M (E) for 24 or 36 h. The cells were then collected by centrifugation, and luciferase activity quantified. Glucose oxidation was measured at the insulin concentration providing the maximal response, 10^{-7}M, and in the absence/presence of the PI3-kinase inhibitor wortmannin at a concentration of 10-6M, using both untreated cells and cells treated for 24 h with 10^{-9} M (E).
Results: Treatment of the transfected cells with 10^{-9} M (E) for 24 or 36 h, inhibited the promoter activity of the hIR gene by 20% ($p< 0.01$) and 37% ($p< 0.01$) respectively. Wortmannin reduced insulin-stimulated glucose oxidation by 52% ($p< 0.05$) in untreated cells, but failed to reduce the decreased insulin-stimulated glucose oxidation elicited by (E) (69%; $p< 0.01$) in treated cells, suggesting the existence of a reduced PI3-kinase activity in cells treated with (E).
Conclusion: To our knowledge, this is the first demonstration that (E) causes transcriptional repression of the hIR gene. This gene repression seems to provoke an insulin-resistant state with defects, at least in part, in hIR gene expression and insulin action at the level of the PI3-kinase. These defects at more than one site, could have a cumulative effect increasing the degree of insulin resistance.

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Atypical pharmacology and opposing effects of ß-adrenoceptor agonists on glucose uptake and pyruvate oxidation in skeletal muscle.

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Background and Aims: Low concentration (10^{-11} to 10^{-10}M) of the selective ß $_3$-adrenoceptor (AR) agonist BRL-37344 increase 2-deoxyglucose (2-DG) uptake in mouse skeletal muscle. This effect was seen in soleus muscle from ß $_3$-AR knockout mouse and the receptor was tentatively named ß $_{skel}$-adrenoceptor. The present study examines the effects of BRL-37344, clenbuterol and salbutamol (ß $_2$-adrenoceptor agonists) on 2-DG uptake, [2-^{14}C]-pyruvate oxidation and cyclic AMP concentrations in isolated soleus muscle of C57Bl/6J mice.
Materials and Methods: Muscles were preincubated for 60min in KHB buffer and then incubated for 45 min in fresh buffer containing [1-^{14}C] 2-DG or [2-^{14}C]-pyruvate, insulin (0.1mM) and ß -AR agonists (10^{-12} to 10^{-5}M) ± the selective ß $_2$-AR antagonist ICI-118551 (0.1nM) or the selective ß$_1$-adrenoceptor antagonist atenolol (0.1nM).
Results: Concentrations of 10^{-11} and 10^{-8}M BRL-37344 stimulated 2-DG uptake by 17.5% (*P*=0.04) and 74% (*P*=0.001) respectively. The effect of 10^{-11}M BRL-37344 was blocked by both ICI-118551 and atenolol. The effect of 10^{-8}M BRL-37344 was shifted to 10^{-6}M by ICI-118551 but unaffected by atenolol. 10^{-8}M BRL-37344 also stimulated pyruvate oxidation (45%; P=0.006). The increase (18.1%) at 10^{-11}M BRL-37344 was not significant. BRL-37344 (10^{-8}M) had no effect on cyclic AMP levels.
Clenbuterol at 10^{-11}M stimulated 2-DG uptake (29%; *P*=0.017), pyruvate uptake (65%; *P*=0.004) and cyclic AMP levels (186%; *P*=0.001). However, at 10^{-7}M clenbuterol decreased 2-DG (-59%; *P*=0.001) uptake and cyclic AMP levels (-51%; *P*=0.04). Salbutamol similarly stimulated 2-DG uptake at 10^{-11}M but inhibited it at 10^{-7}M.
Conclusion: These findings are consistent with 10^{-8}M BRL-37344 and 10^{-7}M clenbuterol both acting via the ß $_2$-AR but via different second messenger systems to produce opposing effects on glucose metabolism. At 10^{-11}M their similar effects may be mediated via a ß -AR with atypical pharmacology, i.e. blocked with similar potency by atenolol and ICI-118551.

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Increased hepatic fat accumulation independent of obesity is associated with increased metabolism of glucocorticoids by 5β-reductase activity in healthy men.

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Background and Aim: In obesity, cortisol metabolism is altered in association with insulin resistance and activation of the hypothalamic-pituitary-adrenal (HPA) axis. Fatty liver is also associated with insulin resistance, even in the absence of obesity. The underlying mechanisms of fat accumulation in liver are unclear but glucocorticoid excess may contribute. We determined whether liver fat content (LFAT) influences hepatic cortisol metabolism.

Materials and Methods: We measured, in 25 non-diabetic apparently healthy men (age 22-57 years, BMI 21-36 kg/m^2), *in vivo* activities of hepatic 11β-HSD-1 (conversion of oral cortisone to cortisol during suppression of endogenous cortisol with dexamethasone) and 5α- and β-reductases (urinary cortisol and cortisone metabolites), LFAT (proton spectroscopy), intra-abdominal (i.a.) and subcutaneous fat (MRI) and insulin sensitivity of endogenous glucose R_a (EGP) [euglycemic insulin clamp combined with [3-^{3}H]glucose, insulin infusion rate 0.3 mU/kg×min].
Results: Median LFAT was 10 % (range 1-41%). Higher LFAT was not correlated with BMI, i.a. or s.c. fat mass, or 11β-HSD-1 activity, but was associated with insulin resistance (including decreased suppression of EGP (r -0.58, p<0.01) by insulin) and with increased excretion of total and 5β-reduced glucocorticoids (5β-THF r=0.60, p<0.01, 5β-THE r=0.69, p<0.001), independently of obesity. Conversely, obesity was associated with increased 5α-THF, independently of LFAT.
Conclusion: Cortisol metabolising enzymes are differentially affected by accumulation of fat in the liver (increased 5α-reductase) and in peripheral adipose (increased 5β-reductase). Enhanced inactivation of cortisol by 5β-reductase in fatty liver is unlikely to contribute to its pathophysiology but may induce compensatory activation of the HPA axis.

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Evidence that estrogen receptor -α regulates hepatic insulin sensitivity.
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Background and Aim: ER-α but not ERβ play an important role in the regulation of glucose homeostasis. Thus, ER-α knockout mice (ERKO) demonstrate decreased insulin sensitivity. The aim of the present study was to investigate whether insulin resistance in ERKO mice was of hepatic or extra-hepatic origin.
Material and Methods: In anesthetized ERKO and control mice (n=5-7) the right jugular vein and the left carotid artery were catheterised. Thirty minutes later (-100 min) bolus injection of ^{3}H-3-glucose (80 uCi/kg) was given and followed by a continuous infusion of 1.5 uCi/kg/min during 190 min. At t=o minutes, samples for determination of insulin and ^{3}H-glucose were taken and followed by insulin infusion (20 mU/kg/min). Blood glucose was determined every 5 minutes and was kept at 6.5 mmol/l by a variable rate of glucose infusion (40 g/dl). Insulin sensitivity was calculated as the 60-90 min glucose infusion rate divided by the mean of the 60 and 90 min insulin levels. The glucose appearance was calculated at t=0 and t=90 by dividing the rate of infusion of ^{3}H-3-glucose by the plasma glucose specific activity (i.e., dpm per minute divided by dpm per milligram glucose). Endogenous glucose production at 90 min was calculated by subtracting the glucose infusion rate from the glucose appearance. The glucose disposal rate was calculated as glucose appearance rate divided by the glucose concentration.
Results: ERKO mice demonstrate whole body insulin resistance as determined during the clamp, 4.1 ± 0.6 vs 13.8 ± 2.1 (nmol/glucose/kg/min) / (pmol insulin/l) (p<0.012). The basal glucose output (t=0) was not different between the groups, 11.5 ± 1.5 vs 12.4 ± 1.7 mg/kg/min. In contrast, insulin mediated suppression of endogenous glucose production was impaired in ERKO mice (t=90 min), 11.3 ± 1.9 vs 5.9 ± 1.9 mg/kg/min (p<0.014). The peripheral glucose clearence was not different between the groups, 0.14 ± 0.02 vs 0.17 ± 0.02 dl/kg/min.
Conclusion: ERKO mice exhibit whole body insulin resistance which is mainly due to defective suppression of hepatic glucose production by insulin.

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Effect of dehydroepiandrosterone on insulin resistance in Otsuka Long-Evans Tokushima-fatty rats and Japanese male adults.
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Background and Aims: Adrenal androgen, dehydroepiandrosterone (DHEA) provoked a 2-fold increase in [3H]2-deoxyglucose (DOG) uptake for 30 min. In vivo and in vitro effect of DHEA on molecular mechanism of insulin resitance in animal medel of type 2 diabetes and male adults.
Materials and Methods: Otsuka Long-Evans fatty rats(OLETF), animal models of type 2 diabetes, and Long-Evans Tokushima rats (LETO) as control, were treated with 0.4% DHEA for 2 weeks. Metabolic factors of insulin action such as glucose uptake, phosphatidylinositol 3-kinase (PI 3-kinase)-atypical PKCs, and adipocytes differentiation factors such as peroxisome proliferator-activated receptor γ(PPARγ), lipid–binding protein and sterol regulatory element-binding protein were examined. Moreover, 75 g oral glucose tolerance test were performed. before and after treatment with daily oral administration of 25-50 mg DHEA for 2 weeks in 6 male adults.
Results: Insulin-induced [3H]2-DOG uptakes of adipocytes were significantly increased in DHEA-treated OLETF rats when compared with OLETF rats before treatment. Insulin-induced increases in PI 3-kinase activity and membrane-associated PKCepsilon and PKCzeta was enhanced after in vivo treatment with DHEA. These results indicate that “in vivo” DHEA treatment can result in increased insulin-induced glucose uptake with PI 3-kinase–PKCzeta activation in OLETF rat. On the other hand, DHEA is also expected to have a weight reducing effect. epididymal and perirenal adipose tissue in association with decreased plasma leptin levels in OLETF. Adipose tissue from OLETF showed increased expression of PPAR γ protein, which was prevented by DHEA treatment. Further, we examined the effect of DHEA on PPARγin primary cultured adipocytes and monolayer adipocytes differentiated from rat preadipocytes. PPARγ protein level was decreased in a time and concentration dependent manner, and DHEA significantly reduced mRNA levels of PPARγ, lipid–binding protein and sterol regulatory element-binding protein α, but not CCAAT/enhancer binding protein . DHEA-sulfate also reduced the PPAR γ protein, but dexamethasone, testosterone, or androstenedione did not alter its expression. In addition, treatment with DHEA for 5 days reduced the triglyceride content in monolayer adipocytes. Moreover, daily oral treatment with 25-50 mg DHEA for 2 weeks resulted in decreases of glucose levels after 75 g oral glucose tolerance test at 90 and 120 min, but no significant change of insulin levels in non-diabetic male adults.
Conclusion: DHEA treatment provokes glucose uptake through a PI 3-kinase-PKCzeta pathway and downregulates adiposity through the reduction of PPARγ in rat adipocytes, and improves glucose tolerance in male adults.

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Catecholamines attenuate insulin-stimulated glucose uptake in 3T3-L1 adipocytes by inhibition of GLUT-4 translocation.
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Background and Aims: Sympathetic nervous system (SNS) activation inhibits insulin’s ability to stimulate glucose uptake in target tissues. The underlying mechanisms however, are incompletely understood. At the cellular level, adrenergic receptor stimulation may directly affect the insulin signaling pathway. To examine this possibility, we studied the effects of catecholamines in 3T3-L1 adipocytes on insulin-stimulated 2-deoxyglucose uptake and insulin-stimulated translocation of glucose transporters to the

plasma membrane. In addition, we analysed whether catecholamines act through either α- or β-adrenergic receptors to affect proper insulin signaling.
Materials and Methods: Differentiated 3T3-L1 adipocytes were incubated with insulin in the presence or absence of catecholamines. After 10 minutes, 1-[^{3}H]-2-deoxyglucose (2-DOG) was added for 10 minutes. The uptake of labelled 2-DOG in the cell was measured by scintillation spectrometry. To study the contribution of α- and β-adrenoceptors, the α-adrenergic receptor antagonist phentolamine or the β-adrenergic receptor antagonist propranolol were added 10 minutes prior to the experiment. The relative amounts of GLUT-1 and GLUT-4 in the plasma membrane and intracellular vesicles were determined by means of differential centrifugation followed by western blot analysis.
Results: Insulin (10^{-7} M) increased 2-DOG uptake from 1.05 ± 0.07 to 7.22 ± 0.28 nmol 2-DOGxmin^{-1}xwell^{-1} ($P < 0.01$). Both epinephrine (10^{-6} M) and norepinephrine (10^{-6} M) inhibited insulin-stimulated 2-DOG uptake by 38 ± 3 and 35 ± 4 %, respectively ($P < 0.01$). Propranolol ($3x10^{-7}$ M) completely antagonised the inhibitory effects of epinephrine and norepinephrine on insulin-stimulated 2-DOG uptake, whereas pre-incubation with phentolamine (10^{-5} M) had no effect (all values mean ± SEM, results based on at least five experiments). Insulin (10^{-7} M) promoted the translocation of GLUT-1 and GLUT-4 to the plasma membrane. Incubation of cells with insulin in the presence of catecholamines lowered the insulin-stimulated GLUT-4 translocation to the plasma membrane. The effects on GLUT-1 were less pronounced.
Conclusion: Both epinephrine and norepinephrine decreased insulin-stimulated 2-DOG uptake in 3T3-L1 adipocytes by inhibition of GLUT-4 translocation from intracellular vesicles to the plasma membrane. These effects can be explained by stimulation of the β-adrenergic receptor.

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Adrenaline enhances PKB phosphorylation in rat soleus muscle via β-adrenergic receptors, cAMP, and activation of Epac.
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Background and Aims: Interference with insulin signalling in skeletal muscles is of particular importance for regulation of blood glucose concentration since skeletal muscle is the major tissue for insulin-stimulated glucose uptake. The interplay between adrenaline and insulin signalling has not been investigated in skeletal muscles although it may influence insulin sensitivity. The aim in the present study was to investigate the effect of adrenaline on insulin signalling in skeletal muscles.
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 in the insulin pathway was measured with phosphospecific antibodies. Kinase activities were measured after immunoprecipitation with appropriate substrates.
Results: Adrenaline dose dependently increased insulin-stimulated PKB ser473 phosphorylation. In the absence of insulin, adrenaline did not influence PKB phosphorylation. The time-course studies showed the highest insulin-stimulated PKB phosphorylation at 15 min and a gradual decrease. Adrenaline increased insulin-stimulated PKB phosphorylation by 2-3 fold at the different time points. Measurements of PKB activity confirmed that adrenaline increased insulin-stimulated PKB activity. Blockade of PI 3-kinase by wortmannin completely blocked the effect of adrenaline on insulin-stimulated PKB activation, but adrenaline did not increase insulin-stimulated PI 3-kinase activity. The effect of adrenaline on insulin-stimulated PKB phosphorylation was blocked by timolol (β-blocker) whereas blockade of α-receptors was without effect. A cell permeable cAMP analogue mimicked the effect of adrenaline on PKB phosphorylation. Blockade of PKA by H89 decreased adrenaline-stimulated activation of glycogen phosphorylase but did not reduce the effect of adrenaline on PKB activation. In fact, blockade of PKA by H89 increased insulin-stimulated PKB activation with and without adrenaline. Recently, a new cAMP binding protein was cloned. The protein is a GTPase exchange protein and named Epac (Exchange protein activated directly by cAMP). The Epac activator 8-(4-chlorophenylthio)-2`-O-methyl-cAMP increased insulin-stimulated PKB activation but did not activate PKB in the absence of insulin. Glycogen phosphorylase was not activated by Epac activation.
Conclusion: Adrenaline increases insulin-stimulated PKB phosphorylation via β-adrenergic receptors, cAMP and the newly identified Epac signalling pathway. This is the first report of signalling through Epac in skeletal muscles.

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Increased expression of 11beta-hydroxysteroid dehydrogenase Type 1 in subcutaneous adipose tissue in HIV-associated lipodystrophy.
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Background and Aims: Highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of HIV-infection, but is also associated with severe adverse events, such as lipodystrophy and insulin resistance. Patients with HAART-associated lipodystrophy do not have systemic hypercortisolism, although their phenotype shares several similarities with Cushing's syndrome. 11β-Hydroxysteroid dehydrogenase type 1 (11βHSD1) converts cortisone to cortisol. Mice overexpressing 11βHSD1 selectively in adipose tissue are centrally obese, hyperglycemic and dyslipidemic. In obese humans, 11βHSD1 activity is increased in adipose tissue. We determined whether 11βHSD1 expression is increased in adipose tissue of patients with HAART-associated lipodystrophy.
Materials and Methods: A group of HIV-positive patients with HAART-associated lipodystrophy (LD+, n=30) was compared with a group of HIV-positive patients receiving HAART but without lipodystrophy (LD-, n= 13). The mRNA levels of 11βHSD1 and β2-microglobulin (house-keeping gene) in subcutaneous adipose tissue biopsies were measured using real-time PCR. Liver fat (LFAT) was measured using proton spectroscopy, and intra-abdominal (i.a.) and subcutaneous (s.c.) fat by magnetic resonance imaging.
Results: BMIs were comparable (23.6 ± 0.5 vs 22.4 ± 1.1 kg/m^2, LD+ vs LD-, NS), but the LD+ group had significantly more intra-abdominal (1900 ± 200 vs 900 ± 300 cm^3, $p<0.01$) and less subcutaneous (1100 ± 200 vs. 1800 ± 300 cm^3, $p<0.05$) fat than the LD- group. LFAT (8 ± 2 vs. 2 ± 1 %, $p<0.001$) and fasting serum insulin concentrations (11 ± 1 vs. 7 ± 1 mU/l, $p<0.01$) were significantly higher in the LD+ than the LD- group. The mRNA concentration of 11βHSD1 relative to β2-microglobulin was significantly higher in the LD+ than the LD- group (0.29 ± 0.20 vs 0.09 ± 0.07, $p<0.001$). In all HAART-treated patients, 11βHSD1 mRNA levels correlated with features of insulin resistance: fasting serum triglycerides ($r= 0.56$, $p<0.001$), insulin ($r= 0.49$, $p=0.001$) and HDL-cholestrol ($r= -0.48$, $p<0.01$), and intra-abdominal fat ($r= 0.54$, $p<0.001$) and LFAT ($r= 0.45$, $p<0.01$), but not with subcutaneous fat ($r = -0.1$, NS).
Conclusion: Increased expression of 11βHSD1 in adipose may contribute to insulin resistance in patients with HAART-associated lipodystrophy.

PS 38
Insulin Action

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Downregulation of IRS-2 by stable anti-sense transfection impairs action of insulin and metformin in Huh7 human hepatoma cells.
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Background and Aims: Insulin receptor substrate 2 (IRS2) is an important component of the insulin signalling pathway. The IRS2 knockout mouse has significant hepatic insulin resistance, and develops diabetes mellitus within weeks of birth.
We have previously reported that metformin stimulates insulin receptor activity and tyrosine phosphorylation of IRS2, but not IRS1 [1]. The effect of stable antisense IRS2 cDNA tranfection on Huh7 human hepatoma cells was examined.
Materials and Methods: Sense and antisense IRS2 plasmids were generated as reported [2]. Huh7 human hepatoma cells were transfected using lipofectamine. Huh7 cells are insulin sensitive at approximately physiological concentrations.
Recombinant cells were selected with G418, and stably transfected clones were developed. Total IRS2 was assessed by immunoprecipitation and Western immunoblot. Cell growth was assessed by cell counts, performed daily following seeding at 0.5 million cells per well in 6-well plates. Deoxyglucose uptake was assessed following treatment with insulin at 100ng/ml for 10 min or metformin 1µg/ml for 1h.
Results: Three antisense clones demonstrated significant down-regulation of total IRS2 protein (53% $p<0.0001$, 49% $p<0.0001$ and 33% $p=0.0001$ respectively).
Growth curves were performed over 96 hours for 3 Sense clones and 2 Antisense clones. The antisense clones demonstrated retarded growth with the estimated doubling times being 22 hours for the sense clones, and 30 hours for the antisense clones ($p<0.0001$).
Deoxyglucose uptake was examined in the basal state, and following treatment with insulin or metformin. Deoxyglucose uptake increased significantly following insulin treatment (57% increase, $p<0.0001$) and metformin treatment (45% increase $p=0.0002$). In the antisense clones, basal deoxyglucose uptake was decreased by 31% compared to the sense clones ($p=0.0071$). The response to insulin and metformin was no longer statistically significant in the antisense clones.
Conclusion: Downregulation of IRS2 in human hepatoma cells results in impaired cell proliferation. Basal deoxyglucose uptake was significantly decreased in the antisense cells, suggesting that basal function of IRS2 may play a role in glucose uptake. The blunted response in glucose uptake with insulin or metformin treatment in the antisense cells suggests that IRS2 function plays a vital role in glucose regulation in this human liver model.

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mTOR is a key player in the phosphorylation of IRS-1 at serine 307.
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Background and Aims: Insulin receptor substrate (IRS) proteins are important for the transduction of insulin signals to intracellular targets. Serine phosphorylation of IRS-1 has been shown to regulate insulin action in many cell types and phosphorylation of IRS-1 upon serine 307 negatively regulates insulin signaling. We have shown that rapamycin, an inhibitor of mTOR, prevented the development of insulin resistance and the degradation of IRS-1 likely through the inhibition of serine phosphorylation. To gain further understanding of the relationship between mTOR and serine phosphorylation of IRS-1, we examined whether the mechanisms leading to serine 307 phosphorylation were related to mTOR activation in 3T3-L1 adipocytes
Materials and Methods: 3T3-L1 adipocytes were pretreated for 30 min with rapamycin and then incubated for different times in the presence or absence of insulin, anisomycin, TNF α, okadaic acid or different concentrations of aminoacids. IRS-1 was immunoprecipitated and immunoblotted using anti-phospho ser307 antibodies. mTOR in vitro kinase assay was done using mTOR immunoprecipitates obtained from 3T3-L1 adipocytes stimulated with or without insulin
Results: Insulin, anisomycin and TNF α increased serine 307 phosphorylation of IRS-1 through a rapamycin-sensitive pathway, despite disparate activation of upstream signaling pathways. Rapamycin prevented both IRS-1 serine 307 phosphorylation and p70 S6K phosphorylation with an IC50 of 500 pM. In addition, amino acid stimulation activated mTOR and resulted in IRS-1 serine 307 phosphorylation without activating PKB or JNK. Okadaic acid, an inhibitor of the protein phosphatase PP2A, activated mTOR and stimulated the phosphorylation of serine 307 in a rapamycin-sensitive manner. In addition, mTOR immunopurified from insulin-stimulated cells was able to phosphorylate serine 307 in vitro
Conclusion: mTOR is a central player in the phosphorylation of IRS-1 at serine 307 in response to various stimuli and may play a critical role in the development of insulin resistance.

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Role for a novel signaling intermediate, PtdIns 5-P, in acute insulin action in 3T3-L1 adipocytes.
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Background and Aims: Phosphatidylinositol (PtdIns) 5-P is the newest addition to the family of phosphoinositides (PI). While undetectable in many mammalian cells, PtdIns 5-P is highly abundant in 3T3-L1 adipocytes, with basal levels exceeding many fold that of PtdIns 3-P, and reaching as high as ~ 13% of PtdIns 4-P (*Ikonomov et al., 2001, Sbrissa et al., 2002*). However, PtdIns 5-P's role and regulation in 3T3-L1 adipocytes as well as in other mammalian cells is largely unknown. Its intracellular production is associated, at least in part, with the enzymatic activity of PIKfyve, a PI 5-kinase highly enriched in 3T3-L1 adipocytes, that in addition to PtdIns 5-P, produces PtdIns 3,5-P_2. The role of PIKfyve enzymatic activity in several insulin-regulated cellular responses, including GLUT4 vesicle translocation in 3T3-L1 adipocytes, has been recently documented (*Ikonomov et al., 2002*). PtdIns 5-P could also be generated by dephosphorylating the higher 5-PIs but the phosphatases involved in the context of 3T3-L1 adipocytes have not yet been defined. Here we have examined whether PtdIns 5-P production is subject to acute changes in response to insulin stimulation of 3T3-L1 adipocytes.
Materials and Methods: PtdIns 5-P levels were measured by two independent approaches: HPLC-head-group analysis of *in vivo* labeled inositol phospholipids and in *vitro* PI 4-kinase-directed conversion of the endogenous PtdIns 5-P pool to PtdIns 4,5-P_2.
Results: Ten-minute insulin stimulation of [^{32}P]orthophosphate-labeled 3T3-L1 adipocytes resulted in a 40-44% increase in the accumulated radioactive PtdIns 5-P. Likewise, quantitation of the TLC-resolved *in vitro* reaction of extracted cellular PtdIns 5-P, [γ–^{32}P]ATP, and recombinant PI 4-kinase (type II PIP kinase) revealed a 2-fold increase of the produced PtdIns 4,5-P_2 following 10 min adipocyte treatment with insulin, with an effect detectable as early as 2 min after insulin application. However, acute insulin in this cell type did not induce significant changes in the *in vitro* measured PIKfyve-dependent production of PtdIns 5-P implying that if involved, PIKfyve requires a cofactor to increase its kinase activity toward PtdIns in intact cells. Alternatively, increased PtdIns 5-P could be due to a PtdIns 4,5-P_2 breakdown, shown recently to be essential in promoting membrane fission and actin filament remodeling during phagocytosis.
Conclusion: The results demonstrate a substantial increase in PtdIns 5-P levels in response to acute insulin treatment of 3T3-L1 adipocytes and suggest a plausible role of this PI species in the molecular mechanism of insulin-regulated GLUT4 membrane dynamics. Whether PIKfyve enzymatic activity is invoved in the elevated PtdIns 5-P production remains to be identified.

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Phosphorylation of Insulin Receptor Substrate-1 (IRS-1) at Ser318 by Protein kinase C-zeta modulates the interaction with the insulin receptor and the activation of insulin signal transduction.
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Background and Aims: Insulin receptor substrate-1 (IRS-1) was recently identified as a novel upstream substrate for protein kinase C (PKC)-zeta. The resulting serine phosphorylation of IRS-1 down-regulates insulin signal transduction under hyperinsulinemic conditions. To elucidate the molecular mechanism of this feed back loop we aimed to investigate the currently

unknown Ser/Thr sites of IRS-1 phosphorylated by PKC-zeta and characterize their role in insulin signaling.
Materials and Methods: The cell culture experiments were performed in baby hamster kidney cells, stable transfected with the insulin receptor (BHK-IR). The cells were transiently co-transfected with IRS-1 or A318IRS-1, generated by site-directed mutagenesis of Ser 318 to Ala 318, and either a control vector or PKC-zeta.
Results: In baby hamster kidney cells insulin, a strong activator of PKC-zeta, inhibits the insulin-receptor mediated tyrosine phosphorylation of IRS-1 and the interaction of IRS-1 with the activated insulin receptor in a time-dependent manner. We identified by mass spectrometry, one serine residue located adjacent to the phosphotyrosine-binding domain in IRS-1 (serine 318 in rat or serine 323 in human) as a major site of PKC-zeta phosphorylation in IRS-1. Mutation of serine 318 to alanine 318, eliminating the phosphorylation of IRS-1 by PKC-zeta at this site, abrogates the inhibitory effect on the insulin-stimulated tyrosine phosphorylation of IRS-1 by PKC-zeta. Furthermore, preliminary results show that the negative influence of PKC-zeta on the interaction of IRS-1/insulin receptor can also be prevented by this mutation.
Conclusion: These results suggest that phosphorylation of serine 318 might mediate, at least partially, the inhibitory effect of hyperinsulinemia on IRS-1 function, thus introducing new perspectives in the insulin-induced insulin resistance.

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Protein kinase Cs α and δ regulate activation of PKB by insulin in skeletal muscle.
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Background and Aims: Certain members of the Protein Kinase C family of serine-threonine kinases have been found to be involved in upstream insulin signaling. Thus, PKCδ has been shown to interact with insulin receptor to regulate in part its phosphorylation and internalization, while PKCα may regulate IRS activity in response to insulin. One of the major downstream signaling elements is PKB(Akt), which plays an important role in glucose transport and glycogen synthesis. The purpose of this study was to investigate the possibility that PKCs α or δ may play a role in insulin-induced PKB activation.
Materials and Methods: Studies were conducted on primary cultures of rat skeletal muscle age 5-6 days *in vivo*. Primary cultures were freshly prepared for each experiment. PKB activity was determined by (a) translocation from cytosolic to membrane fractions, and (b) by serine phosphorylation as assessed by Western blotting techniques. We used adenovirus constructs of wild type (WT) to overexpress specific PKC isoforms. PKCs were blocked both by expression of dominant negative (DN) isoforms and use of selective PKC antagonists.
Results: PKB activity was increased by insulin stimulation within 5 min and reached a peak by 15-30 min. WTPKCδ increased cytosolic but not membrane expression of PKB. In addition, the effect of insulin and translocation was not altered. In contrast, DNPKCδ increased slightly PKB expression in both cytosolic and membrane fractions, and blocked the translocation induced by insulin. Insulin-induced PKB phosphorylation after 5 min was increased by overexpression of WTPKCδ. DNPKCδ reduced the induction of PKB phosphorylation by insulin. Overexpression of WTPKCα did not alter PKB levels in either cytosolic or membrane fractions in the absence of insulin stimulation, while insulin-induced translocation was unchanged from control. However, translocation of PKB was blocked by expression of DNPKCα. Down regulation of PKCs α and δ by 30 min treatment with PMA blocked both translocation and phosphorylation of PKB by insulin. Finally, insulin the association of both PKCα and PKCδ with PKB.
Conclusions: The results indicate that PKCα and PKCδ act downstream directly with PKB to regulate its activity.

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Long-term expression of insulin in skeletal muscle does not lead to insulin resistance.
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Background and Aims: Transgenic mice expressing insulin in skeletal muscle counteract type 1 diabetic alterations and suggest that muscle cells constitutively secreting low insulin levels may be used in gene therapy for diabetes. However, the long term effects of such a therapy remain unknown. Prolonged local hyperinsulinemia may lead to insulin resistance in insulin expressing skeletal muscle. Here, we examined the effects of chronic insulin expression in skeletal muscle of transgenic mice on whole body glucose metabolism. We also examined the effect of a diabetogenic high-fat diet in these transgenic mice
Materials and Methods: Effects on body weight, glycemia and insulinemia, glucose tolerance and insulin sensitivity were analyzed in old (>12 months) transgenic mice expressing insulin in skeletal muscle and in six months old transgenic mice fed a high-fat diet for 16 weeks
Results: Twelve- and two-month-old transgenic mice expressed similar insulin levels in skeletal muscle. This led to increased intramuscular glucose 6-phosphate and glycogen levels. However, similar blood glucose and insulin levels were found in transgenic and control mice. Likewise, no differences were observed during an intraperitoneal glucose tolerance test. After 16 weeks on a high-fat diet, the body weight increase, glycemia, insulinemia, glucose and insulin tolerance tests were similar in transgenic and control mice
Conclusion: These results indicate that the long-term constitutive insulin secretion by the skeletal muscle did not lead to insulin resistance. Furthermore, when fed a high-fat diet control and transgenic mice responded similarly, indicating that skeletal muscle insulin production did not exacerbated insulin resistance. Thus, these results suggest that long-term insulin expression has no deleterious effects on skeletal muscle insulin sensitivity

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Impaired insulin-induced glycogen synthesis upon chylomicron incubation in L6 skeletal muscle cells.
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Background and Aims: In industrialized countries a considerable part of the population may be up to 70% of the day in a postprandial state which is characterized by marked elevation of triglyceride levels. Several studies have demonstrated that increased postprandial lipemia is an inherent feature of diabetic dyslipidemia. The main lipoprotein fraction mediating the transport of triglycerides in the postprandial state is the chylomicron fraction.
The objective of this study was to determine whether insulin stimulated glycogen synthesis may be altered upon chylomicron incubation. Glycogen synthesis has been demonstrated to be impaired in insulin resistant states.
Materials and Methods: For this purpose, L6 cells were preincubated with purified chylomicrons for 10min, 1hr, 3hrs and overnight. Chylomicrons were isolated by zonal ultracentrifugation and further purified by gel filtration. After the preincubation period, basal and insulin stimulated glycogen content (GC) was determined (in mg/dl glucose following glycogenolysis).
Results: In the basal state, chylomicron preincubation for 10min increased GC slightly compared to incubation without lipoproteins (from 19±1 to 21±1). After 1hr, GC was reduced to 15±2 and after 3hrs GC was further reduced to 8±1. Overnight incubation resulted in a decrease to14±1. Upon chylomicron preincubation for 10min, the insulin stimulated GC did not change compared to incubation without chylomicrons (29±1), whereas after 1hr a significant decrease of GC was observed (20±2). Incubation for 3hrs resulted in a further decrease of GC (7±1) as did the overnight incubation (11±2).
The insulin induced stimulation amplitude of glycogen synthesis upon chylomicron preincubation compared to chylomicron free stimulation got reduced to 90% after 10min and to 50% after 1hr. The 3hrs preincubation with chylomicrons abolished the insulin stimulated glycogen synthesis as did the overnight incubation.
To investigate whether the observed effects may be induced only by the chylomicron fracrtion or whether free fatty acids (FFA) resulting from possible hydrolysis of chylomicron lipoproteins may also be an important factor in inducing the observed effects, we measured FFA-levels in the incubation media at different time points. Compared to chylomicron free media no changes in FFA-levels were observed in the lipoprotein-containing media at all time points.
Conclusion: In summary, our results demonstrate that chylomicrons in a physiological range have marked effects both on basal and insulin stimulated glycogen synthesis. These effects cannot be attributed to FFAs, since FFA-levels in the incubation media did not change upon chylomicron incubation. We conclude that the reduced insulin stimulated glycogen synthesis upon chylomicron incubation may be interpreted as insulin resistance in our cell culture system.

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Monitoring the dynamic of interaction between the insulin receptor and Protein Tyrosine Phosphatase-1B in living cells using Bioluminescence Resonance Energy Transfer.

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Background and Aims: Binding of insulin to its receptor induces autophosphorylation of the receptor on tyrosine residues. This stimulates the tyrosine kinase activity of the receptor, which plays a crucial role in the transmission of the signal. Termination of the signal involves inactivation of the insulin receptor kinase by dephosphorylation of tyrosines located in the activation loop of the receptor.PTP1B is a protein tyrosine-phosphatase predominantly localized on the endoplasmic reticulum. The knockout of PTP1B in mice has demonstrated that this tyrosine-phosphatase plays a major in the regulation of the insulin receptor. In consequence, PTP1B clearly appears as a potential therapeutic target for the treatment of insulin-resistance. Therefore, a better understanding of the dynamic of the interaction between the IR and PTP1B constitutes a major task for the development of compounds that will improve insulin sensitivity.
Materials and Methods: The BRET methodology constitutes a major advance for the study of protein-protein interactions in intact living cells. To study the interaction between two partners, the first partner is fused to Renilla luciferase, whereas the second partner is fused to a fluorescent protein (e.g. YFP). If the two partners do not interact, only one signal, emitted by the luciferase, can be detected after addition of its substrate, cœlenterazine. If the two partners interact, resonance energy transfer occurs between the luciferase and the YFP, and an additional signal, emitted by the YFP, can be detected.In this work, the dynamics of interaction of the insulin receptor fused to Renilla luciferase (IR-Rluc) with a substrate-trapping mutant of PTP1B (PTP1B-D181A) was monitored in living HEK cells using BRET.
Results: Insulin dose-dependently stimulates this interaction, which could be followed in real time for more than 30 minutes. Insulin effect on BRET signal could be detected at very early time-points (30 seconds), indicating that in intact cells, the tyrosine-kinase activity of the insulin receptor is tightly controlled by PTP1B. Interestingly, basal (insulin-independent) interaction of the insulin receptor with PTP1B was much lower with a soluble form than with the endoplasmic reticulum-targeted form of the tyrosine-phosphatase. Inhibition of insulin receptor processing using tunicamycin suggests that basal interaction occurs during insulin receptor biosynthesis in the endoplasmic reticulum.
Conclusion: Our work demonstrate that the BRET methodology can be used to monitor, in real time, in intact living cell, the interaction of the insulin receptor with one of the main tyrosine-phosphatases that control its activity. Moreover, we show that PTP1B located in the endoplasmic reticulum interact with the insulin receptor during its biosynthesis. Localisation of PTP1B to this compartment may be important in order to prevent insulin-independent autonomous activity of the insulin receptor precursor.

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Functional characterization of a PKC-dependent IRS-1 phosphorylation site using mass spectrometry.

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Background and Aims: Insulin receptor substrate-1 (IRS-1), one major intracellular substrate of the insulin receptor kinase, interacts with the insulin receptor at sites adjacent to the N-terminus. Recently it was found that under hyperinsulinemic conditions IRS-1 is phosphorylated by protein kinase C (PKC)-zeta, thereby attenuating the insulin signal transduction. IRS-1 contains 244 serine/threonine residues, which include more than 70 potential phosphorylation consensus sites for protein kinases. The identification of physiologically relevant IRS-1 phosphorylation sites and their functional relevance has progressed slowly, and no PKC-dependent IRS-1 phosphorylation site has been identified yet. Therefore, our aim was to disclose these phosphorylation sites using a mass spectrometric strategy.
Materials and Methods: Using an in vitro kinase assay, the N-terminal part of recombinant IRS-1, expressed as a fusion protein in E. coli, was phosphorylated by recombinant human PKC-zeta. After in gel digestion of the phosphorylated IRS-1 N-terminal protein the resulting phosphopeptides were analyzed by liquid chromatography and electrospray mass spectrometry without radioactive labeling.
Results: Two specific PKC-zeta-dependent serine phosphorylation sites could be identified by mass spectrometric based sequence analysis of the detected phosphopeptides, one of these sites is located at serine 318 (IRS-1 rat, amino acid sequence of the phosphopeptide: TESITATSPASMVGGKPGpSFR). Replacing serine 318 by alanine, by site-directed mutagenesis, confirmed the specificty of the PKC-zeta-mediated phosphorylation. The impact of this new identified PKC-zeta-dependent IRS-1 phosphorylation site on insulin signal transduction is under further investigation.
Conclusion: These results demonstrate the meaningful application of mass spectrometrical methods for functional characterization of phosphoproteins involved in insulin signal transduction.

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Evidence for the rapid stimulation of glucose 6 phosphatase activity by cyclic AMP in enterocyte-like CaCo2 cells.

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Glucose-6 phosphatase (Glc6Pase) is the last enzyme common to gluconeogenesis and glycogenolysis. It is expressed in the liver, the kidney and the small intestine only, and confers on these 3 tissues the capacity to release glucose in blood. Glucose production by these 3 organs is increased in experimental diabetes. We previously suggested that Glc6Pase activity is stimulated by glucagon and catecholamines. We have raised here the question of the role of cAMP in the Glc6Pase activation.
We used CaCo2-cells cultured at postconfluence. Glc6Pase was overexpressed by transduction using adenovirus technology (CMV promoter). Fourty-eight hours after transduction, Glc6Pase activity was overexpressed 10 times in transduced cells: 55.9±2.5 vs 4.9±0.9 nmol/min/mg protein in non transduced-control cells. Glycogen content was not affected in Glc6Pase-transduced cells as compared to control cells : 123.3±2.3 vs 112.3±8.1µg/mg prot. Transduced and control cells were incubated for 1 h at 37°C in the presence of the adenylcyclase activator forskolin (10^{-4}M). After the latter treatment, Glc6Pase activity was increased by 50 % in transduced cells: 85.0±6.0 vs 55.9±2.5 nmol/min/mg prot. in untreated cells. In addition, glycogen stores were more dramatically depleted by forskolin treatment in Glc6Pase-transduced cells (-46±5 %) than in control cells (-26±5 %). Forskolin effects in relation with Glc6Pase activation did not take place at 21°C.
These results strongly suggest the existence of short-term mechanisms of regulation of Glc6Pase activity under the control of cAMP. In addition, these mechanisms play a regulatory role in glycogen storage. Our data also suggest that these mechanisms could be at least partly dependent on membrane fusion events.

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Changes in expression of Na^+, K^+-ATPase in rat skeletal muscle: effects of excercise and high fat diet.

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Background and Aims: Na, K-ATPase has a key role in the regulation of Na^+ and K^+. In skeletal muscle, two α and two β isoforms of Na, K-ATPase are expressed. Na, K-ATPase molecules translocate from an intracellular pool to the cell surface in response to insulin. Decreased content of the pump has been reported in type 2 diabetic patients. The aim of the study was to examine whether high-fat diet (4 and 12 weeks) and short-term moderate exercise training would alter Na, K-ATPase expression and insulin-stimulated translocation of the enzyme in skeletal muscle.
Material and Methods: The expression of Na, K-ATPase isoforms was determined by Western blot analysis in skeletal muscle from Wistar rats after 4 or 12 weeks chow (CD)-or high fat diet (HFD), before and after 5 days swim exercise. Insulin-stimulated translocation of Na, K-ATPase subunits was determined in isolated muscle by Western blotting after a biotinylation and streptavidin-precipitation assay. DNA binding activity of transcription factors MEF2, ZEB, and SP-1 was assessed in muscle nuclear extracts by EMSA.
Results: Insulin resistance in isolated skeletal muscle was observed only after 12 weeks of HFD. Interestingly, after 4 weeks of HFD, α1 subunit protein expression was increased 1.6 fold ($p<0.05$), whereas α2 subunit protein expression decreased 2-fold ($p<0.01$), compared to CD fed rats. Exercise training in HFD group completely restored expression of α subunit isoforms. Na, K-ATPase β1 subunit protein decreased 2.2 fold ($p<0.01$) in HFD vs. CD rats and was restored after exercise training. β2 subunit was not altered by HFD, but decreased 2 fold ($p<0.05$) after 5 days exercise training regardless of diet. The same pattern of Na, K-ATPase subunit expression was persistent after 12 weeks of HFD.
Insulin stimulated translocation of both α1 and α2 subunits of Na, K-ATPase to the cell surface. HFD reduced cell surface abundance of Na, K-ATPase α2 subunit under basal and insulin stimulated condition. This effect was abolished by exercise training. Despite of increase in protein expression, the cell surface abundance and insulin stimulated translocation of Na, K-ATPase α1 subunit were not affected by HFD and exercise training. DNA binding activity of ZEB (AREB6), a transcription factor involved in regulation of Na, K-ATPase α1 subunit, increased ($p<0.05$) after 4 weeks of HFD, with no change after exercise training. DNA binding activity of SP-1, a transcription factor implicated in regulation of α2 and β1 subunit expression decreased after 4 weeks of HFD.
Conclusion: HFD and excercise lead to changes in Na, K-ATPase subunit isoform expression and modulated transcription activity in skeletal muscle. The HFD induced changes of subunits protein expression of Na, K-ATPase prior development of insulin resistance. Short-term exercise training lead to restoration of Na, K-ATPase expression. The findings indicate that altered Na, K-ATPase expression and regulation pattern we have identified plays a role in the development of impaired K^+ fluxes associated with obesity or insulin resistance and we suggest a specific role for α2 subunit isoform of Na, K-ATPase in regulation of skeletal muscle K^+ homeostasis.

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Plasma and muscle lipid parameters in Wistar and ZDF rats during fasting and refeeding.

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Background and Aims: Muscle lipid parameters, e.g. intramyocellular lipids (IMCL) and long-chain acyl CoAs (LCACoAs), are increasingly being thought to be causally involved in insulin resistance. In the presence of an increased IMCL content elevated levels of LCACoAs could negatively interfere with the insulin-signalling cascade and thereby cause insulin resistance.
Materials and Methods: We studied insulin-sensitive Wistar (age 16 wks) and lean ZDF (16 wks) rats and compared them with insulin-resistant (8 wks) and diabetic (16 wks) obese ZDF rats. We measured IMCL and tCr in the M. soleus (oxidative, SOL), M. tibialis ant. (glycolytic, TIB), and M. extensor digitorum longus (intermediary, EDL) by in vivo ^{1}H-MR-spectroscopy (7 T, expressed as IMCL/tCr ratio), and LCACoAs (M. longissimus dorsi) of normal fed rats and after starving for 24 h and 48 h. Additionally metabolic serum parameters were determined.
Results: Insulin-resistant (8 wks), and diabetic (16 wks) obese ZDF rats had elevated blood glucose, insulin and triglyceride levels compared to Wistar and lean ZDF rats. Free fatty acids increased in all 4 rat groups during starvation, while triglycerides, blood glucose and insulin decreased. In Wistar and lean ZDF rats, both insulin-sensitive, IMCL increased during starvation by more than 150 % in both TIB and EDL, while in SOL changes were limited, all IMCL/tCr ratios staying <2. In insulin-resistant (8 wks) and diabetic (16 wks) obese ZDF rats, IMCL/tCr ratios were much higher already in fed conditions and increased dramatically in TIB during starvation (~10 and ~4, respectively). Total LCACoAs were about 20 nmol/g in insulin sensitive Wistar and lean ZDF rats and did not change during starvation. However, in 8 and 16 wks old obese ZDF rats total LCACoAs increased to about 50 and 30 nmol/g, respectively, during starvation. The LCACoA-pattern (16:0, 16:1, 18:0, 18:1, 18:2) was similar in insulin-sensitive Wistar and lean ZDF rats, but differed significantly from that in insulin-resistant 8 wks and 16 wks old obese ZDF rats. In insulin resistant rats levels of palmitoleoyl-CoA (C16:1) were significantly elevated.
Conclusion: We conclude (1) there are significant differences in muscle lipid metabolism (IMCL) with respect to different muscle fibre types, (2) IMCL in TIB and EDL are elevated in insulin-resistant rats compared to insulin-sensitive rats, (3) increased IMCL in TIB in insulin-resistant rats during starvation correlates with increased total LCACoAs, and (4) in insulin-resistant rats there is an obvious dominance of palmitoleoyl-CoA.

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Comparison of lipogenic capacities of human and rat adipose tissues and effects of variation in carbohydrate/lipid diet ratio.

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Background and Aims: *De novo* lipogenesis is considered to be less active in human than in rat adipose tissue. However, This could be explained by differences the nutritional conditions: high carbohydrate diet in rats and high fat diet in humans.
Materials and Methods: To compare the lipogenic capacity of human and rat adipose tissues and to determine the role of difference in proportion carbohydrate/lipid ratio in diet, we studied 1) groups of rats and normal humans receiving either a high carbohydrate (HCHO) or high fat (HF) diet,

2) a control human group (no nutritional control, C), and 3) a human obese group (O). Adipose tissue was sampled in the post-absorptive state for all. We measured 1) the mRNA concentrations (RT-PCR) of fatty acid synthase (FAS), acetyl-CoA carboxylase 1 (ACC1), SREBP-1c and of co-activators (Sp1, Nfy, COUP-TF1) and co-inhibitors (Ids, YY1) of SREBP-1c, 2) the amounts (western blot) of the precursor and mature forms of SREBP-1, and 3) FAS enzymatic activity.
Results:mRNA concentrations of FAS, ACC1 and SREBP 1c were not significantly modified by change in the CHO/fat diet ratio in rats nor in humans. These concentrations were similar in the HCHO, HF and C human groups. FAS and ACC1 mRNA levels were lower ($p<0.05$) in these groups than in rats, and were still lower in O ($p<0.05$). FAS activity was not significantly modified by diet in rats and was higher than in human groups ($p<0.05$). On the contrary, there was no difference for SREBP-1c mRNA concentrations between humans and rats.The mRNA concentrations of SREBP-1c cofactors were lowered in humans ($p<0.05$), except for Id2 and coup-TF1. Both the precursor and mature forms of SREBP-1 protein were less abondant in human than in rat adipose tissue ($p<0.05$), without any significant difference between HCHO and HF diets.
Conclusion: The lipogenic capacity of human adipose tissue is reduced compared to that of rat adipose tissue. These differences does not appear related to difference in CHO/fat diet ratios and is probably explained by the lower amount of precursor and mature forms of SREBP-1c protein in human adipose tissue.

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The effects of insulin and glucose on Aquaporin adipose expression in 3T3-L1 adipocytes.
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Background and Aims: Aquaporin adipose(AQPap) is the physiological glycerol channel specific to adipocytes, and it is expressed predominantly in adipose tissue. The abnormal expression of AQPap in db/db obese mice is linked to insulin resistance. To clarify the regulation of AQPap gene expression, we investigated the effects of hormone such as insulin and nutrients such as glucose on the expression of AQPap in 3T3-L1 adipocytes, in order to know the role of AQPap plays in obesity and diabetes mellitus.
Materials and Methods: 3T3-L1 cells on day 9 after differentiation were incubated with 1000μM,100μM,10μM,1μM insulin for 6 hour respectively , or incubated in DMEM with 10μM insulin for 0, 3, 6h for the experiment on time course, total cellular RNA were extracted and used for RT-PCR. After differentiation on day 9, 3T3-L1 cells were stimulated with different concentrations of glucose(5.6mM, 11.1mM, 16.8mM, 33.3mM) or glycerol(0.17mM, 1.7mM, 17 mM) for 48 hours, then total cellular RNA were extracted respectively, detecting the expression of AQPap mRNA by RT-PCR. After stimulation of insulin in 1000μM to the differentiated 3T3-L1 cells for 48h, or incubated with 16.8mM glucose for 48h, the crude membrane proteins were extracted, then investigated the effects of various factors on AQPap protein expression by Western blotting(15%SDS-PAGE, AQPap antibody concentration is 8μg/ml).
Results: Treatment of differentiated 3T3-L1 adipocytes with 1000μM insulin suppressed AQPap mRNA expression by about 50%, and the pretein level of AQPap by about 64%. The results showed that the suppression of insulin on the expression of AQPap mRNA were dose-dependent and time-dependent in 3T3-L1 cells. After stimulation with high concentration of glucose, AQPap mRNA and protein expression were all augmented in 3T3-L1 cells ($p<0.05$). Incubation with high level of glycerol had no effect on AQPap mRNA expression although with the same osmotic pressure range as glucose stimulation. The expression quantity of AQPap protein was assayed by Gel ultraviolet Transilluminator. Rabbit anti-human AQPap antibody is provided by Chemicon Com.
Conclusion: Insulin is a negative regulator of AQPap expression . While high level of glucose can increase AQPap expression.

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Formation of insulin-responsive vesicles in differentiating 3T3-L1 adipocytes.
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Background and Aims: In adipose and skeletal muscle cells, Glut4 is accumulated in small 60-70S insulin responsive vesicles, IRVs, that are translocated to the cell surface in response to insulin stimulation. Our goal is to determine how these vesicles are formed during adipocyte differentiation.
Materials and Methods: Myc-epitope tagged Glut4 and other chimera molecules were stably expressed in 3T3-L1 cells, and compartmentalization of reporter proteins was studied with the help of confocal immunofluorescence staining, sucrose gradient centrifugation and reconstitution assay in vitro.
Results: In undifferentiated 3T3-L1 cells, myc-Glut4 is localized in heavy intracellular membrane compartment that has been identified as endosomes by confocal immunofluorescence staining. In undifferentiated cells, ectopically expressed Glut4 is not translocated to the plasma membrane and maintains intracellular endosomal localization in both insulin-treated and not treated cells. Between day 2 and day 3 of differentiation, myc-Glut4 is re-distributed from endosomes to small vesicles. Simultaneously with this event, differentiating cells acquire insulin-sensitive glucose uptake. The formation of the IRVs significantly precedes the expression of endogenous Glut4. In order to identify the sequence in the Glut4 molecule that targets the transporter from endosomes to the IRVs we created a chimera molecule consisting of 4 transmembrane protein cellugyrin and the C-terminal cytoplasmic tail of Glut4. Upon stable expression in 3T3-L1 adipocytes, this chimera was targeted to the IRVs. Finally, we show that the formation of small insulin-sensitive vesicles may be reconstituted in vitro using the cytosol from differentiated cells and the donor membranes from undifferentiated cells.
Conclusion: The ability to form small post-endosomal vesicles and not the expression of the Glut4 protein is the key event in the acquisition of insulin sensitivity in differentiating adipocytes. The C-terminal tail of Glut4 contains the IRV targeting information.

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Glucose regulates diacylglycerol intracellular levels by modulating diacylglycerol-kinase activity and subcellular localization.
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Background and Aims: In addition to representing a fundamental energy source for most cells, glucose is an important regulator of signal transduction mechanisms. For instance, acute exposure to glucose activates the glucose transport apparatus independently of insulin, contributing to an increase of glucose uptake in peripheral tissues. This glucose autoregulation is known to involve PKCα cytosolic retrotranslocation. However, the molecular mechanism by which glucose acutely modulates PKCα has not been elucidated yet. We have addressed this issue by analysing glucose regulation of intracellular levels of the PKCα activator_diacylglycerol (DAG) in skeletal muscle cells.
Materials and Methods: L6 cells were labelled with U-^{14}C-glucose to measure DAG levels. DAG has been purified by TLC. Diacylglycerol kinase (DGK) activity was determined by measuring the rate of γ-^{32}P-ATP incorporation into phosphatidic acid by the octyl-β-D-gucoside mixed-micelle assay.
Results: In L6 skeletal muscle cells, exposure to 25 mM glucose for 5 min induced a 2-fold decrease in DAG levels compared with basal. This decrease was paralleled by a similar reduction in PKCα activity. At variance, prolonged exposure of the cells to glucose (30-60 min) induced a progressive increase in DAG levels and PKCα activation. Inhibition of PKCα by bisindolylmaleimide did not modify the acute glucose effect on DAG levels, indicating that glucose regulation of intracellular DAG occurs upstream PKC. A major route for DAG removal is represented by DGK-mediated phosphorylation to form phosphatidic acid. Exposure of L6 cells to 25 mM glucose acutely induced a 5-fold increase in DGK activity. Pharmacological inhibition of DGK with 25μM R59022 or 10μM R59949 reverted glucose-dependent decrease in DAG levels. These same DGK inhibitors also prevented PKCα cytosolic translocation and increased PKCα membrane-associated activity. In addition, subcellular fractionation studies revealed that treatment of L6 cells with 25mM glucose for 5 min induced translocation of the specific isoform DGKζ from cytosol to the plasma membrane.
Conclusion: Thus, glucose regulates PKCα activity by modulating the intracellular DAG concentration. At least in part, this glucose effect is mediated by glucose action on DGK activity and subcellular localization.

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Skeletal muscle ceramide content is inversely related to insulin sensitivity and up-regulated by hyperinsulinemia in humans.

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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. In vitro studies revealed that insulin resistance might be associated with intracellular formation of ceramide, main second messenger in the sphingomyelin-signaling pathway. The aim of the present study was to examine the content and composition of fatty acids (FA) in ceramide and sphingomyelin in human muscle and to evaluate their relationships with insulin sensitivity and regulation by hyperinsulinemia.
Materials and Methods: A total of 24 male subjects with normal glucose tolerance participated in the present study. Insulin sensitivity was determined with euglycemic hyperinsulinemic clamp technique. A biopsy of vastus lateralis muscle was perfomed. Additionally, in 8 subjects second biopsy was taken after 4h clamp. 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.
Results: We identified 11 different ceramides and sphingomyelins according to the FA residues. Ceramide-FA consisted of 42% saturated FA (SFA), 47% monounsaturated FA (MUFA) and 11% polyunsaturated FA (PUFA), while sphingomyelin-FA consisted of 68% SFA, 23% MUFA and 9% PUFA. We found a significant negative correlation between insulin sensitivity and total ceramide content (r=-0.62) and ceramide-SFA (r=-0.64) and MUFA (r=-0.62, all $p<0.05$). Total ceramide and ceramide-SFA correlated also positively with HbA1c, postload glucose and insulin, and triglycerides and negatively with HDL-cholesterol (all $p<0.05$). There were no significant associations between sphingomyelins and insulin sensitivity. Hyperinsulinemia resulted in an increase in total ceramide content of about 36% ($p<0.05$), whereas there was no effect on sphingomyelins. Increase in ceramide was mostly attributable to the increase in ceramide-SFA.
Conclusion: Our data show that sphingomyelin-signalling pathway in muscle might be an important factor determining the development of insulin resistance in humans.

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OMI/HtrA2, a novel interacting partner of PED (Phosphoprotein Enriched in Diabetes) controls PED expression and anti-apoptotic function.

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Background and Aims: PED (Phosphoprotein Enriched in Diabetes) is a 15 kDa death-effector domain (DED) containing protein with anti-apoptotic function. In several cell types, PED overexpression inhibits apoptosis induced by growth factors deprivation and by exposure to stress-inducing agents. To further investigate the molecular mechanisms of PED action, we performed a two-hybrid screening to isolate putative intracellular partners using full length PED as a bait.
Materials and methods: A yeast library of HeLa cells has been screened by using full length PED cDNA cloned in pGBKT7 vector. 45 clones have been isolated and sequenced. Co-immunoprecipitation experiments and GST pull-down assaies were performed in 293T cell lysates. Apoptosis was evaluated by an ELISA procedure, which enables a quantitative analysis of DNA fragmentation.
Results: By using a yeast two hybrid screening procedure, we have found that PED interacts with the mithocondrial pro-apoptotic serine protease OMI/HtrA2, an intracellular binding protein for the caspase-inhibitor XIAP. In 293 cells, OMI/HtrA2 co-immunoprecipitated with PED. In addition, based on pull-down assaies with specific GST-fusion proteins, OMI/HtrA2 binds to the DED, at the N-terminus of PED, but not to the C-terminus of the molecule. Subcellular fractionation followed by Western blotting revealed that OMI/HtrA2 is predominantly mithocondrial. Upon UV treatment, however, OMI/HtrA2 translocated to the cytosol, where PED is mostly localized. Hence, PED-Omi/HtrA2 co-immunoprecipitation was detectable in the cytosolic and not the mitochondrial fraction of these cells. To evaluate the functional role of PED interaction, OMI/HtrA2 cDNA has been transiently transfected in both wild-type 293 and PED over-expressing cells (293_{PED}). Expression of OMI/HtrA2 in wild-type cells determined 4-fold increased basal and UV-induced apoptosis. By contrast, 293_{PED} cells did not undergo apoptosis following UV exposure. Co-transfection of OMI/HtrA2 in 293_{PED} cells reverted PED block of UV-induced apoptosis by >50% ($p < 0{,}005$). Interestingly, PED expression levels were also reduced by about 50% in cells co-expressing OMI/HtrA2 ($p < 0{,}01$).
Conclusion: These data indicate that, following UV exposure, the mitochondrial serine protease OMI/HtrA2 controls PED anti-apoptotic action by migrating into the cytosol, where it binds to PED and regulates its expression levels.

PS 40
Glucose Transport

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Calorie restriction avoids the pinealectomy-induced insulin resistance by improving GLUT4 gene expression and translocation to the plasma membrane.

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Background and Aims: Previous studies have suggested that the pineal gland plays a role in the glycemic homeostasis. The aims of the present study were to investigate the insulin sensitivity and GLUT4 protein in pinealectomized rats, as well as to determine the effects of melatonin and calorie restriction in the pinealectomy-induced modulations.
Material and Method: Wistar rats were pinealectomized (Pinx) or sham operated (C), and studied 30 days later. Melatonin replacement treatment (50 µg/100 g body weight) was performed during 30 days after pinealectomy. Calorie restriction was performed by offering 60% of the standard food intake. Body weight, food intake, pineal gland content of melatonin, and basal plasma glucose, insulin and albumin were measured. In vivo insulin sensitivity was evaluated by the glucose disappearance constant (kITT) during an insulin tolerance test, and GLUT4 mRNA and protein were assessed by Northern and Western blotting, respectively. In vitro effect of melatonin upon GLUT4 protein was investigated in adipocytes isolated from control rats.
Results: Compared to C rats, Pinx rats showed decreased (P<0.05) kITT (40%), GLUT4 mRNA (~70%) and GLUT4 protein (~70%) in white adipose tissue (WAT), and unchanged GLUT4 expression in skeletal muscle (SM). Melatonin-treated Pinx rats restored the kITT and GLUT4 protein to control values. During the 30-min or the 4-hour melatonin (10^{-9}M) incubation, no in vitro effects upon the plasma membrane GLUT4 protein were observed in isolated adipocytes, with or without insulin (10^{-8}M). The calorie restriction imposed to the Pinx rats increased (P<0.05) their kITT value (~40%), which was accompanied in WAT by increased (P<0.05) plasma membrane and microsomal GLUT4 protein content (~240%), as well as increased (P<0.01) GLUT4 translocation to the plasma membrane related to the respective mean of plasma insulinemia (~80%).
Conclusions: The results showed that pinealectomy, for lack of melatonin, decreased insulin sensitivity as well as GLUT4 gene expression in WAT. Calorie restriction improved insulin sensitivity of pinealectomized rats, and that was related to increased GLUT4 gene expression and insulin-induced translocation to the plasma membrane in white adipose tissue.

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Up-regulation of GLUT4 gene expression by thyroid hormone in rat cardiac muscle. Possible involvement of the E-box associated proteins.

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Background and Aims: Triiodothyronine (T3) is known to increase the heart GLUT4 gene transcription rate and probably the synthesis of GLUT4 protein. However, studies about the GLUT4 gene response to T3 are scarce and not well comprehended, since the thyroid response element (TRE) identified on this gene present an unusual low affinity to the hormone. It is also known that the regulatory region of GLUT4 gene presents, besides the TRE, an E-box and AT element. Studies also described that T3 can induce a rapid increase in glucose transport in cardiac muscle cells in culture, which suggest that T3 might induce effects on GLUT4 expression/translocation by other mechanisms. Taking into account these antecedents, the present study was aimed to investigating the acute effect of T3 administration on the GLUT4 mRNA expression, as well as the possible involvement of the phosphorylated MAPK (MAPK-P) and E-box associated proteins in this effect.
Materials and Methods: Male Wistar rats (~250 g) were surgically thyroidectomized under deep anesthesia, and killed, 2 weeks later, after 30 min, 60 min and 24 h of the T3 administration (100 µg/100 g, BW, iv). The ventricles were removed. RNA and protein from cytosol and nuclear fraction were extracted. GLUT4 mRNA expression was determined by Northern blot analysis, using a 32P- cDNA labeled probe. Cyosolic protein was used to determine the content of MAPK-P, and the nuclear protein was used for gel shift assay (nuclear protein extract was incubated with a 32P-γ ATP-labeled 17 bp E-box consensus double stranded oligonucleotide). The product was loaded on a 4% polyacrilamyde gel and the DNA binding activity was assayed.
Results: Tx rats presented an 80% increase on the GLUT4 mRNA expression, after 30 min of the T3 injection. It was followed by a decay and a subsequent increase (~150 %) 24 h after T3 treatment. The MAPK-P content, as well the binding activity of the ventricular nuclear proteins to E-box sequence, was shown to be increased (p<0,01) after 30 min of T3 injection. The binding activity to the E-box was also increased at 1 and 24 h after T3 injection.
Conclusion: T3 enhanced the binding activity of the E-box associated proteins in rat cardiac muscle, which suggests a parallel pathway by which T3 could enhance GLUT4 gene expression. The MAPK-P could be involved in this process, since the phosphorylation of many nuclear proteins seems to be an important step for their binding at specific sequences at the 5' regulatory region of the genes.

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Characterisation of glucose transport in endothelial cells of small contractile arteries.

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Background and Aims: Evidence implicates hyperglycaemia as the precursor of endothelial cell (EC) dysfunction in the onset of diabetes. Several pathways have been identified and suggested to explain the mechanism by which elevated intracellular glucose concentration leads to endothelial dysfunction. How and why glucose tends to accumulate in ECs remains poorly understood.
In this study, we looked at the expression and subcellular distribution of classical GLUT isoforms and SGLT-1 in the ECs of small contractile arteries of the rat. To further elucidate the functional role of glucose transporters in ECs we measured glucose transport in live, pressurized, coronary arteries.
Materials and Methods: Coronary, cerebral, renal and mesenteric arteries were dissected and the presence of GLUT isoforms and SGLT-1 in the EC layer were examined with immunohistochemistry and wide field fluorescence microscopy coupled to deconvolution. Acutely dissected vessels were mounted on glass cannulas in an arteriograph chamber providing both intraluminal flow and superfusion of the adventitial tissue with oxygenated Krebs solution. The chamber was mounted on the stage of a confocal microscope. Phase contrast was used to locate the ECs and smooth muscle cells (SMCs) of the vascular wall, and glucose uptake was monitored using a fluorescently tagged glucose analog 2-NBDG. 2-NBDG is a substrate for both the classical GLUTs and SGLT-1. Images of EC and SMC fluorescence were acquired every 60 seconds for 30 minutes after the addition of 2-NDBG (5mM). To assess the contribution of SGLT-1, glucose uptake by other isoforms was inhibited by cytochalassin B. SGLT-1 was activated by elevated luminal [Na^+](130 mM) and inhibited by phlorizin or low [Na^+](0.5 mM). In addition, the vessel was exposed to a physiological concentration of insulin to determine if glucose uptake is insulin sensitive.
Results: Our results indicate that GLUT-1 to 5 and SGLT-1 are expressed in ECs of all arteries examined. We also found an asymmetric distribution of GLUT-1, GLUT-2 and SGLT-1 in ECs of all four vessels. GLUT-1 and 2 were found mostly on the abluminal side while SGLT-1 was predominantly on the luminal side of the ECs. In addition, we found dense labeling adjacent to the cell-to-cell junctions where the luminal and abluminal membranes are in close proximity and the cytosolic space is minimal. The presence of GLUT-4 and SGLT in ECs suggests a possible regulation of glucose uptake by insulin and Na^+.
Preliminary results in live vessels show cytoplasmic glucose uptake in both cell types, with dense accumulations in the cell periphery, as predicted by the subcellular distribution of the tranporters. The rate of uptake and plateau concentration (t~15mins) was higher in ECs compared with SMCs (dbl exponential fit, λ_{EC}=0.35, λ_{SMC}=0.21).
Conclusion: The specific subcellular organisation of glucose transporters may facilitate transcellular glucose exchange between the blood and the cells of the vascular wall. The differential glucose kinetics of ECs and SMCs may explain the high susceptibility of the endothelium to glucose toxicity.

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Glucose transport regulation by the SUMO-conjugating enzyme Ubc9 during adipocyte differentiation.

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Background and Aims: The SUMO-conjugating enzyme Ubc9 is involved in covalent modification of the GLUT4 glucose transporter with SUMO-1 and modulates glucose transport in insulin-sensitive cells. During the differentiation process, adipocytes develop a glucose transport system that shows high insulin responsiveness. The aim of this study was to define the role of Ubc9 during adipocyte differentiation.
Materials and Methods: 3T3-L1 fibroblasts and adipocytes were transfected with a specific Ubc9 antisense (AS) oligonucleotide to reduce Ubc9 protein levels.
Results: AS-treated fibroblasts and adipocytes showed both a 70% reduction in Ubc9 protein levels ($p<0.05$ vs. untransfected cells or cells transfected with control oligonucleotides). AS-treated fibroblasts showed no changes in 2-deoxy-glucose uptake rates as compared to control fibroblasts, both in the basal state and after insulin stimulation. By contrast, treatment of adipocytes with the Ubc9 AS did not modify basal glucose transport rates, but marked reduced the stimulation of glucose transport by insulin (1.8-fold and 6-fold in AS-treated and control adipocytes, respectively; $p<0.05$). Insulin-induced GLUT4 translocation, detected by immunofluorescence in plasma membrane lawns, was also blunted in AS-treated adipocytes. To investigate the mechanisms responsible for the different effects of Ubc9 during adipocyte differentiation, the intracellular localization of Ubc9 and its substrate SUMO-1 was analyzed in 3T3-L1 fibroblasts and adipocytes. Both immunoblotting studies using subcellular fractions and immunofluorescence analysis using a confocal laser microscope demonstrated that Ubc9 is predominantly localized in the cell nucleus in the fibroblasts whereas the majority of this protein is in cytoplasmic compartments in the adipocytes, in which it appears to co-localize with GLUT4. The localization of Ubc9 in non-nuclear areas was confirmed by expressing tagged Ubc9 constructs, including YFP-Ubc9 and FLAG-Ubc9, in 3T3-L1 adipocytes. By contrast, SUMO-1 was found in nuclear and peri-nuclear compartments.
Conclusion: i.) Ubc9 is key for the full responsiveness of the glucose transport system to insulin in adipocytes, but not in fibroblasts; ii.) the intracellular localization of Ubc9 is regulated during adipocyte differentiation. Thus, Ubc9 may contribute to the development of the highly insulin-responsive glucose transport system in adipocytes through Ubc9 regulation of adipocyte-specific GLUT4 vesicle pools and a change in its intracellular site of action.

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Insulin-like growth factor binding protein-3 (IGFBP-3) shifts the adipocyte towards a less differentiated state and inhibits insulin stimulated glucose uptake.

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Background and Aims: IGFBP-3 is the major serum IGFBP which binds insulin-like growth factors (IGFs). At a cellular level, IGFBP-3 regulates bioactivity of IGFs and it also exerts IGF-independent actions. IGFBP-3 is a binding partner for the retinoid X receptor-α (RXR- α). Peroxisome proliferator activated receptor gamma receptor (PPAR-γ) is a crucial transcription factor in adipocyte differentiation and glucose regulation. As RXR-α is an obligatory dimerization partner for PPAR-γ, we hypothesized that IGFBP-3 may interfere with adipocyte differentiation and glucose uptake through competitive partnering with RXR-α.
We aimed to examine the effects of IGFBP-3 on adipogenesis by studying adipocyte gene expression. PPAR-γ and resistin mRNA were used as markers of the adipocyte phenotype. Plasminogen activator inhibitor-1 (PAI-1) was used as a marker of preadipocyte phenotype. We also aimed to determine the effects of IGFBP-3 on basal and insulin stimulated glucose utilization.
Materials and Methods: Mouse 3T3-L1 preadipocytes were differentiated to adipocytes with IBMX (0.5mM), insulin (0.7 μM) and dexamethasone (0.1μg/mL). Adipocytes were incubated for 24 hrs in either serum-free (SF) media or SF media with glycosylated rhIGFBP-3. RNA was extracted and real-time quantitative RT-PCR was performed. For glucose utilization studies, insulin (10ng/mL) was added for 20minutes. Samples were incubated for 5 minutes with radiolabelled glucose (final concentration 0.2mM 2-[^{14}C] deoxyglucose; 0.1 μCi/mL), radioactivity was determined by liquid scintillation.
Results: Treatment of differentiated adipocytes with IGFBP-3 was found to down-regulate adipocyte specific genes, PPAR–γ and resistin and it up-regulated the preadipocyte enriched gene, PAI-1. IGFBP-3 at 300ng/mL decreased PPAR-γ mRNA expression by 3.1 fold ($p<0.006$) and decreased resistin mRNA expression by 2.3 fold ($p=0.02$). In contrast, IGFBP-3 at 500ng/mL upregulated PAI-1 mRNA by 9.6 fold ($p=0.008$). In all cases, 1 μg/mL of IGFBP-3 had no further effect over the lower doses. When glucose uptake was studied, IGFBP-3 treatment (1μg/mL) for 24 hrs did not affect basal glucose uptake but significantly inhibited insulin stimulated glucose uptake (by 50-64%). A dose response study showed that 50ng/mL reduced insulin stimulated glucose uptake maximally by 40.7% ($p=0.016$).
Using a monoclonal antibody αIR3 which preferentially blocks the type 1 IGF-1 receptor (IGFR1), we showed that the effect of IGFBP-3 on reducing insulin stimulated glucose uptake was independent of the blockade of the IGFR1. This initial data suggests that the action of IGFBP-3 on glucose uptake is independent of IGF-1 acting through the IGFR1.
Conclusion: IGFBP-3 reduced mRNA levels of adipocyte-specific genes, PPAR–γ and resistin and up-regulated the preadipocyte enriched gene PAI-1 indicating a shift of the adipocyte towards a de-differentiated state. IGFBP-3 also inhibited insulin stimulated glucose uptake. Whether IGFBP-3 interferes with adipocyte differentiation and glucose uptake through competitive partnering with RXR-α or by some other mechanism is currently under investigation.

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Cellular signaling in the action of GLP-1 and exendins on glucose transport in human myocytes.

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Background and Aims: GLP-1, as insulin, stimulates glycogen synthesis in rat and human skeletal muscle, and glucose transport in human muscle cells and pieces of mouse skeletal muscle. Exendin-4 (Ex4) and exendin 9-39 (Ex9), structurally GLP-1-related peptides, both have shown to increase glucose transport in myocytes from normal and diabetic patients. Here we have explored, in human myocytes, the role of enzymes proposed to be involved in the insulin action, in the GLP-1 and exendins stimulating effect upon glucose transport (GT).
Materials and Methods: Myotubes were established from satellite cells of dissociated vastus lateralis from 18 normal subjects (15F/3M; age: 80±1 yr; fasting plasma glucose: 106±5 mg/dl), previous informed consent given, undergoing orthopedic surgery; PI3K activation – PIP3 formation – and p44/42 MAPK phosphorylation – Western blot – were measured in cells after 3 min in the absence (control) and presence of 10^{-9} M GLP-1, insulin or exendins; GT – 3H-2-deoxy-D-glucose incorporation – was measured in cells pre-incubated 15 min without (control) and with 10^{-6} M wortmannin (W), $2.5x10^{-5}$ M PD 98059 (PD), 10^{-7} M rapamycin (Rp), or 10^{-4} M H-7 (H-7) or 10^{-7} M Ro 31-820 (Ro), inhibitors of PI3K, MAPKs, p70s6k and PKC, respectively, and then incubated 30 min in the absence or additional presence of 10^{-9} M each peptide.
Results: In cells from 7 subjects, GLP-1 clearly activated ($p<0.001$) PI3K (175±17% of control) as it did insulin (160±7%, $p<0.0001$), Ex4 (136±6%, $p<0.014$) and Ex9 (128±2%, $p<0.06$); the same occurred relative to phosphorylation of p42/44 MAPKs. In cells from 4 subjects, as already known, GLP-1, insulin, Ex4 and Ex9 all stimulated GT (192±9% of control; 141±6%; 146±8%; 147±6%, respectively, $p<0.0001$). In cells from groups of 4 subjects, W abolished the stimulation of GT exerted by insulin (100±3% of control) as well as that by GLP-1 (98±4%) and Ex9 (91±4%), and partially reduced ($p<0.01$) that by Ex4 (119±6%); PD blocked the effect of all peptides; Rp failed to affect the Ex4 action (177±13%), partially reduced that of GLP-1 (120±3%, $p<0.02$; $p<0.0001$ vs GLP-1), and abolished that of insulin. In cells from 3 subjects, H-7 blocked the effect of GLP-1, Ex4 and insulin but not that of Ex9 (138±5% of control, $p<0.0001$), while Ro did not alter the GLP-1 or insulin effect, but inhibited that of Ex4 and Ex9 (93±6% and 100±5%, respectively).
Conclusion: Both MAPKs and PI3K pathways seem to mediate the stimulatory effect of GLP-1, Ex4 and its truncated form Ex9, upon glucose transport in human myocytes, although wortmannin does not completely block that of Ex4; for the GLP-1 action, activation of p70s6k but not that of PKC is required, opposite to what apparently occurs with either exendin.

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Insulin resistance of glucose transport, glycogen synthase, and Akt ser473 phosphorylation in rat skeletal muscle subjected to oxidant stress.

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Background and Aims: Oxidative stress may play a role in the multifactorial etiology of skeletal muscle insulin resistance. To date, no investigation has directly assessed the effect of an in vitro oxidant stress on insulin action in intact mammalian skeletal muscle. Therefore, the purpose of the present study was to determine the in vitro effect of an oxidant stress (hydrogen peroxide) on insulin action in skeletal muscle of the insulin-sensitive lean Zucker rat.
Materials and Methods: Type IIb epitrochlearis and type I soleus muscles isolated from insulin-sensitive lean Zucker rats were incubated in 8 mM glucose for 2 hr in the absence or presence of 100 mU/ml glucose oxidase. Thereafter, basal and maximal insulin-stimulated (5 mU/ml) glucose transport activity (2-deoxyglucose uptake), glycogen synthase activity ratio (± 5 mM glucose-6-phosphate), and Akt1 ser473 phosphorylation were assessed.
Results: The glucose oxidase produced hydrogen peroxide in the incubation medium in the range of 60-90 μM. By itself, hydrogen peroxide significantly ($p<0.05$) activated basal glucose transport activity (48% and 30%) and glycogen synthase activity (18% and 28%) in the epitrochlearis and soleus muscles, respectively. These increases were associated with significant enhancements of Akt1 ser473 phosphorylation (280% and 98%) in these muscles. In contrast, in the presence of insulin, the hydrogen peroxide inhibited the insulin-mediated enhancements in glucose transport (55% and 75%), glycogen synthase (42% and 66%), and to a lesser degree Akt1 ser473 phosphorylation (25% and 19%). There were significant associations between Akt1 ser473 phosphorylation and the glycogen synthase activity ratio in the epitrochlearis ($r=0.524$, $p=0.018$) and soleus ($r=0.453$, $p=0.045$) muscles.
Conclusion: The present results indicate that while an oxidant stress induced by hydrogen peroxide can activate basal glucose transport, glycogen synthase, and Akt1 phosphorylation in insulin-sensitive skeletal muscle, this same oxidant stress will significantly attenuate insulin action on the same variables. The results support a direct role of oxidative stress in the induction of insulin resistance in mammalian skeletal muscle.

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Fat Cell Hormones

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Regulation of adiponectin secretion by endothelin-1.

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Background and Aims: Adiponectin is an adipocyte-derived hormone best known for its insulin sensitizing ability. The expression and circulating concentration of adiponectin is decreased in patients with type 2 diabetes, and increases following treatment with thiazoladinediones. Endothelin-1 (ET-1), a potent vasoconstrictor peptide, has positive inotropic, mitogenic, and metabolic properties and elevated levels have been reported in numerous disease states, including obesity and diabetes. ET-1 has profound effects on adipose tissue metabolism and alters the release of adipose-derived factors such as leptin and resistin. We investigated the role of ET-1 on adiponectin secretion.
Materials and Methods: 3T3-L1 adipocytes were treated with insulin (100nM), ET-1 (100nM), or the appropriate vehicle. Adiponectin secretion into the media was determined by immunoblotting and densitometric analysis.
Results: Adiponectin secretion increased by 84.2% and 109.8% 1hr following insulin or ET-1 treatment, respectively. To investigate whether the stimulatory effects of ET-1 were the result of acute exposure to ET-1, 3T3-L1 adipocytes were treated with or without ET-1 (100nM) for 24 hr then exposed to the conditions described above. Pretreatment with ET-1 inhibited the ability of insulin or ET-1 to acutely stimulate adiponectin secretion. To determine whether the acute effects of ET-1 on adiponectin secretion were mediated by the ET_A receptor, 3T3-L1 adipocytes were pretreated with the specific ET_A receptor antagonist, BQ-610 (1μM), then treated with vehicle or ET-1 (100nM) for 1 hr. BQ-610 inhibited ET-1 stimulated adiponectin secretion by 82.5%.
Conclusions: ET-1 stimulates adiponectin secretion through the ET_A receptor. Chronic exposure to ET-1 dramatically decreases the stimulatory effect of ET-1 on adiponectin secretion. Our findings suggest vascular factors such as ET-1 may play a role in the regulation of whole body energy metabolism.

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Ameliorated hyperglycemia in diabetic animal models by recombinant globular domain of adiponectin.

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Background and Aims: Many studies have shown that adipocyte-derived protein, adiponectin influences insulin-resistance and hyperglycemia. Serum adiponectin levels were decreased in obesity and insulin resistance animal models as well as in human. To verify the hypoglycemic effect by globular domain of adiponectin (gAd), we made the purified gAd protein and gAd producing adenovirus. Purified recombinant adiponectin protein was injected in ob/ob mice, db/db mice, STZ-induced diabetic rats and adenoviral vector was injected in type 1 diabetic animal model (NOD) for efficient adiponectin gene expression.
Materials and Methods: Globular domain of adiponectin gene was cloned in adenovirus shuttle vector including CMV promoter and IgK leader sequence. gAd gene expression and protein secretion *in vitro* were confirmed by western blot using anti-mouse adiponectin antibody. Serum blood glucose levels in rats and mice were measured by One-touch glucose meter.
Results: HEPA cells were transfected by gAd producing adenovirus and the cultured supernatant was injected intraperitorially in STZ-induced diabetic rats and after administration hyperglycemia was reversed to normal blood glucose range but non-infected cell cultured supernatant injection did not influence the blood glucose level in STZ-induced diabetic rats. Also purified protein in HEPA cells stably producing gAd was administrated in ob/ob and db/db mice. The blood glucose levels between 300 - 350 mg/dl were turned to euglycemic range after 4 hour. For an efficient gAd gene delivery and gene expression, gAd producing adenovirus was injected at a

dose of 1 x 10^9 intraveneously in NOD mice. The blood glucose level was lowered to 120 mg/dl comparing that GFP producing adenovirus injection did not change the blood glucose level in NOD mice. Reversed hyperglycemic effect sustained and blood glucose levels were maintained between 110 - 120 mg/dl.
Conclusion: Our data shows that adenovirus mediated globular adiponectin gene delivery has a therapeutic effect on hyperglycemia in various diabetic rodents resulting increased serum adiponectin levels. And yet an immediate hypoglycemic effect of adiponectin in NOD mice and STZ-induced diabetic rats needs further studies that may provide adiponectin has an alternative action to regulate hyperglycemia.

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Reduced adiponectin is related to impaired insulin action in patients with HIV-associated lipodystrophy. Effects of pro-inflammatory cytokines.
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Background and Aims: The HIV associated lipodystrophy syndrome (HALS) is characterized by fat tissue redistribution and insulin resistance (IR). The pathophysiology of HALS-induced IR is still unclear. Adiponectin is an adipose tissue derived protein, which has been implicated in the pathogenesis of IR and atherosclerosis associated with obesity. In the present study, we investigated plasma levels and gene expression of adiponectin and the pro-inflammatory cytokines TNF-α, IL-6 and IL-8 in HALS patients. Associations between adiponectin and insulin sensitivity (IS) were analysed.
Materials and Methods: Eight-teen HIV-positive male patients with HALS were compared with 18 HIV-positive male patients without HALS. Insulin sensitivity was determined by hyperinsulinaemic-euglycemic clamp, fat distribution by DEXA and visceral-AT by CT. At baseline a subcutaneous adipose tissue biopsy was taken from the abdomen. Plasma adiponectin was measured by RIA. ELISA was used for determination of plasma TNF-α, IL-6 and IL-8, while gene expression was measured by real time RT-PCR.
Results: HALS patients had significantly reduced insulin sensitivity compared to non-HALS patients (5.54 vs. 8.24 mg/kg LBM/min^{-1}, $p<0.001$), as well as significantly enhanced visceral-AT ($p<0.001$). Plasma adiponectin was reduced by 41% in HALS patients (7.3 vs. 12.5 µg/ml, $p<0.02$), and subcutaneous-AT adiponectin mRNA was 48% lower in HALS patients ($p<0.03$). TNF-α mRNA levels were significantly higher in HALS patients compared to non-HALS patients (0.032 vs. 0.019, $p<0.05$). The same tendency was found for the gene expression of IL-6 ($p=0.09$) and IL-8 ($p=0.06$). In non-HALS patients positive correlation was found between p-adiponectin and IS ($r=0.48$,$p<0.04$), but in HALS patients the correlation did not reach statistical significance ($r=0.42$, $p<0.09$).
Conclusion: HALS patients are characterized by reduced insulin sensitivity and reduced adiponectin both in plasma and in subcutaneous AT. Gene expression of pro-inflammatory cytokines is increased in HALS patients, suggesting a regulatory role for pro-inflammatory cytokines on the level of adiponectin in patients with HALS. This reduction in adiponectin levels seems to be involved in the reduced insulin action in HALS patients.

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Contrasting effects of resistin on celleular glucose uptake in muscle under experimental conditions reflecting hyper- and hypoinsulinaemia.
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Background and Aims: Resistin is an adipocyte-derived hormone implicated in the pathogenesis of whole-body insulin resistance, but the effects of resistin on glucose uptake in skeletal muscle at different concentrations of insulin have not been clearly established.
Materials and Methods: L6 myoblasts were cultured in 24-well plates and serum starved for 24 hours in 0.5% FCS. They were stimulated for a further 24 hours with varying concentrations of bovine recombinant insulin (10^{-9}M to 10^{-5}M) in the presence and absence of 1µM resistin. Insulin stimulated glucose uptake was measured using a 2-deoxyglucose (2-DOG) uptake assay and the concentration-response curves fitted to a quadratic function to derive $C_{I\ 200}$ values (concentration of insulin required to increase glucose uptake by 2-fold). Resistin concentration-response curves for 2-DOG uptake in L6 myocytes (0.01µM to 5µM resistin) were also characterised in the absence of added insulin in the media.
Results: Coincubation with resistin significantly attenuated insulin-stimulated glucose uptake. Maximum insulin-stimulated glucose uptake was reduced and $C_{I\ 200}$ values (concentration of insulin required to increase glucose uptake by 2-fold) were increased: e.g $C_{I\ 200}$ was $1.45x10^{-7}$M (95% CI: $8.38x10^{-8}$-$2.44x10^{-7}$) with insulin alone (n=12) compared with $2.46x10^{-7}$M (95% CI: $1.16x10^{-7}$-$5.11x10^{-7}$) (n=12) in the presence of resistin 1µM. In contrast, resistin significantly increased insulin-independent glucose uptake at concentrations of 1µM (1.64 fold, $p<0.004$) and 5µM (2.16 fold, $p<0.003$).
Conclusion: The effects of resistin on cellular glucose uptake in muscle depend upon the ambient insulin concentration. Under experimental conditions of hyperinsulinaemia, resistin attenuates insulin-stimulated glucose uptake in L6 myoblasts, but under conditions in which insulin levels are very low there is evidence of a concentration-dependent stimulatory effect of resistin on glucose uptake. Thus, resistin may have opposite effects on basal and insulin-stimulated glucose transport and metabolism in muscle.

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Study on the mechanism of insulin-induced reduction of resistin mRNA in 3T3-L1 adipocytes.
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Background and Aims: Resistin is a peptide secreted by adipocytes and recognized as a hormone that could link obesity to insulin resistance. This study was designed to examine the effect and mechanism(s) of insulin on resistin expression in 3T3-L1 adipocytes.
Materials and Methods: Differentiated 3T3-L1 adipocytes were stimulated with insulin and resistin mRNA expression was examined by Northern blot analysis. In some experiments, the insulin signal was blocked by several chemical inhibitors or overexpression of a dominant negative form (?p85) of the p85 subunit of phosphatidylinositol 3-kinase (PI 3-kinase).
Results: Resistin mRNA expression in pre-adipocytes (differentiation at days 0 and 2) was below the detection limit but was detected from day 4, and reached the maximum level at day 8. Insulin treatment caused a significant reduction of resistin mRNA in time- and dose-dependent manners in 3T3-L1 adipocytes. Insulin-induced decrease of resistin mRNA still occurred under glucose-free conditions. Pre-treatment with PD98059, an inhibitor of extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, or SB203580, an inhibitor of p38 mitogen-activated protein-kinase (p38 MAP-kinase) pathway, did not influence insulin-induced reduction of resistin mRNA. Inhibition of PI 3-kinase by LY294002 or ?p85 also failed to block insulin-induced reduction of resistin mRNA. Cycloheximide, a protein synthesis inhibitor, completely blocked insulin-induced reduction of resistin mRNA. Actinomycin D, a RNA synthesis inhibitor, also blocked insulin-induced reduction of resistin mRNA, and the decreasing rate of resistin mRNA in cells treated with insulin alone was faster than that with actinomycin D.
Conclusion: Insulin downregulates resistin mRNA via PI 3-kinase, ERK or p38 MAP-kinase independent pathway in 3T3-L1 adipocytes. The downregulation mechanism of resistin mRNA by insulin would be an indirect event through the synthesis of novel protein(s) that could accelerate the degradation of resistin mRNA.

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Diabetic *milieu* modulates TGF-β and extracellular matrix (ECM) expression in 3T3-L1 cells.
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Background and Aims: Adipose tissue remodelling is a physio-pathological process responsible for fat mass and depends on cell number and volume, which are modulated by several mediators, including TGF-β and extracellular matrix components. Diabetes causes tissue remodelling dysregulation through several hyperglycemia-induced metabolic alterations,

like non-enzymatic glycation and subsequent modifications of several mediators Based on the frequent association between diabetes and obesity, aim of this study was to evaluate the effect of diabetic *milieu* on parameters modulating adipose tissue remodelling.
Materials and Methods: Murine preadipocytes (3T3-L1) were exposed to high glucose (30 mM, **HG**) vs iso-osmolar mannitol (24.5 + 5.5 mM glucose, **M**) vs normal glucose (5.5 mM, **NG**) for 1-4 weeks, ± 10^{-10}-10^{-6}M insulin, or cultured on glucose-modified BSA (BSA-AGE or BSA-AM in which AGE formation was prevented by co-incubation with aminoguanidine) vs native BSA for 3-10 days. Cell proliferation (^{14}C-Thymidine uptake and crystal-violet binding to nuclei), and TGF-β, Fibronectin (FN), Collagen IV (C-IV), and Laminin (LAM) mRNA expression levels (competitive RT-PCR) were evaluated.
Results: DNA-synthesis and cell number significantly enhance in response to proliferative stimulus (serum and insulin), with no differences between NG, HG and M. Short exposure to HG or M does not alter the mRNA levels of the evaluated modulators. TGF-β expression levels increase in HG (+70,6%, p<0,01) and in M vs NG (+94,9%, p<0,001) at 2nd week, and progressively reduce in subsequent weeks. FN mRNA levels rise at 2nd week in HG (+38,3%, p<0,05) and in M vs NG (+76,2%, p<0,01), with a further increment at the 3rd week (HG vs NG: +67,1%, p<0,05; M vs NG: +93,6%, p<0,005) and a reduction later on. 3 days exposure to HG +10^{-10}M insulin increases C-IV expression (ΔHG/NG+10^{-10}M ins: 0,28±0,19 vs ΔHG/NG: –0,04±0,35). 10 days exposure to HG +10^{-10}M insulin shows a significant increase also in LAM (ΔHG/NG+10^{-10}M ins: 0,35±0,12 vs ΔHG/NG: 0,02±0,37) and C-IV (ΔHG/NG+10^{-10}M ins: 0,37±0,30 vs ΔHG/NG: 0,08±0,22) levels. 10^{-8}M insulin reverses all the HG-induced increment, except for TGF-β in 10 days experiment. No differences in mRNA expression levels have been observed in glycated-BSA experiments.
Conclusion: These findings show that diabetic milieu should modify parameters involved in adipose tissue remodelling. This effect may be influenced by time of exposure to HG and by osmotic mechanisms, but not by AGE formation. These data suggest that hyperglycemia could play a role in determining fat mass.

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Effects of TNF-α and a thiazolidinedione on PAI-1 production in the course of adipocyte differentiation.
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Background and Aims: Obesity and insulin resistance are regarded as a basis for metabolic syndrome. Plasminogen activator inhibitor (PAI)-1, produced from the adipose tissue, liver and vascular cells, is a main inhibitor of fibrinolytic system, and is one of the key molecules to link among obesity, insulin resistance and increased risk for cardiovascular diseases. Adipose tissue is composed not only with mature adipocytes, but also with pre- and immature adipocytes. We previously found that a thizolidinedione, pioglitazone, up-regulates peroxisome proliferator-activated receptery (PPARγ), a master gene for adipocyte differentiation, in preadipocytes, whereas it down-regulates PPARγ in mature adipocytes. To clarify the mechanisms of elevated circulating levels of PAI-1 in patients with insulin resistance, we investigated effects of tumor necrosis factor–α (TNF-α) and a thiazolidinedione on PAI-1 production in the course of adipocytes differentiation.
Materials and Methods: Mouse fibroblast line 3T3-L1 preadipocytes were grown in DMEM. Confluent preadipocytes were induced to differentiate by treatment with an induction medium containing dexamethasone, isobutylmethylxanthine and insulin for 48 h. PAI-1 protein in the incubation medium was measured by an enzyme-linked immunosorbent assay. PAI-1 mRNA levels were quantitated by a real time PCR method, and expressed as relative expression ratios to an endogenous control, 18S ribosomal RNA.
Results: (1) In preadipocytes, 10 ng/ml of TNF-α increased PAI-1 mRNA levels by 34%. (2) Pioglitazone at 1.0 and 10 μM increased PAI-1 mRNA levels by 10% and 40%, respectively. (3) When 3T3-L1 preadipocytes were induced to differentiate, they produced PAI-1 into the incubation medium in a time-dependent manner. (4) Pioglitazone at 1.0 and 10 μM increased PAI-1 production during the early phase of adipocyte differentiation, maximmaly by 4.6 and 11 times of the control, respectively, at day 3. However, after day 6 of differentiation, pioglitazone inhibited PAI-1 production reaching to 25% and 15% of control at the day 12, respectively. (5) Incubation of mature adipocytes with TNF-α for 24 h increased PAI-1 release into the medium to 9.8 times of control. (6) Pioglitazone completely inhibited TNF-α-induced PAI-1 production by 95%. (7) TNF-α induced PAI-1 mRNA expression by 15 times of control. P38/MAP kinase inhibitor, PD98059, and NF- κB inhibitor, emodin, inhibited TNF-α-induced PAI-1 mRNA expression by 57% and 50%, respectively.
Conclusion: TNF-α induces PAI-1 production both in preadipocytes and mature adipocytes via p38/MAP kinase and NF- κB pathways. Pioglitazone up-regulates PAI-1 production in preadipocytes and immature adipocytes, whereas it down-regulates PAI-1 production in mature adipocytes. Pioglitazone also inhibits TNF-α-induced PAI-1 production from mature adipocytes. Thus, a thiazolidinedione regulates PAI-1 production differentially in preadipocytes and mature adipocytes.

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TNF-α promotes mRNA expression and protein production of PAI-1 in hepatocytes.
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Background and Aims: Type 2 diabetes, obesity, and insulin resistance often accompanies fatty liver. In patients with visceral obesity, the liver is exposed with abundant of adipocyte-derived factors such as tumor necrosis factor (TNF) -α. TNF-α has been demonstrated to interfere with insulin signaling, thereby contribute to insulin resistance. We have found that fatty liver is closely associated with insulin resistance and the component of metabolic disease including elevated plasma levels of plasminogen activator inhibitor (PAI)-1. PAI-1, produced from the liver, adipose tissue, and vascular cells, is a main inhibitor of fibrinolytic system. Elevated circulating levels of PAI-1 are regarded to be an increased risk for coronary heart disease in patients with insulin resistance. To clarify the mechanisms of elevated circulating levels of PAI-1 in insulin resistance, effect of TNF-α on PAI-1 production from hepatocytes were investigated.
Materials and Methods: Simian virus 40 large-T (SV40-T) antigen immortalized normal human hepatocyte cell line, THLE-5b cells were incubated with TNF-α in PFMR-4 medium. 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: Exposure of THLE-5b cells to TNF-α resulted in dose- and time-dependent stimulation of PAI-1 release into the culture medium. The maximum effect of TNF-α was found at a concentration of 1.0 ng/ml for 24 hours, increasing PAI-1 release by 238% compared with control (control 393±34 ng/ml, vs TNF-α 936±122 ng/ml, mean±SEM, P<0.05). TNF-α at the concentration of 1.0 ng/ml increased PAI-1 mRNA levels at 3h and 16h to 1.6- and 1.4-folds, respectively, compared with control. We next searched for agents inhibiting TNF-α-induced PAI-1 production. A thiazolidinedione, pioglitazone, at 1.0 and 10μM reduced TNF-α–induced PAI-1 production by 14% and 32%, respectively. A HMG-CoA reductase inhibitor, cerivastatin, at 0.1μM or 1.0μM was also found to completely abolish the TNF-α mediated increase in PAI-1 release by 60% and 76% , respectively.
Conclusion: TNF-α promotes mRNA expression and protein production of PAI-1 by human hepatocytes and may contribute to the impairment of the fibrinolytic system leading to development of atherosclerosis in insulin resistance. Thiazolidinediones and statins are candidates to inhibit TNF-α-induced PAI-1 production.

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Interleukin-6 induces insulin resistance in 3T3-F442A adipocytes.
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Background and Aims: Interleukin-6 (IL-6) is a proinflammatory cytokine that is highly expressed during infection, trauma, or other stress but also in insulin resistant patients with obesity and type 2 diabetes. Circulating IL-6 production might reflect, at least in part, adipose tissue IL-6 production. There is a significant correlation between circulating and adipose tissue IL-6 levels and peripheral insulin sensitivity. Therefore, IL-6 might be involved in insulin resistance. However the mechanisms involved are not clearly elucidated. In vitro studies show that IL-6 exerted antiadipogenic effects. Since little is known about how IL-6 is involved in insulin response, we studied in vitro the effect of IL-6 on adipocyte differentiation, metabolism and insulin response.
Materials and Methods: 3T3-F442A cells were treated with varying concentrations of IL-6 (0-200 ng/ml) for 8 days including the whole differentiation program. The cell response to insulin was tested at several

steps of the signaling pathways: protein expression and phosphorylation of insulin receptor IRβ, its substrate IRS-1, ERK and Akt/PKB. Distal insulin effects were evaluated by activation of lipogenesis and inhibition of lipolysis. Since the cells were chronically treated with IL-6 all along the differentiation program we tested the impact of IL-6 on cell adipogenesis. This was performed by counting the newly formed adipocytes on the basis of their morphology, by the protein and mRNA expression of adipogenic markers: Fatty Acid Synthase, aP2, C/EBPα, PPARγ and by Red-Oil lipid staining. Finally, we tested whether IL-6 deleterious effects could be reversed by rosiglitazone a pharmacological ligand of PPARγ.
Results: Chronic IL-6 treatment induced a dose dependant inhibiting effect on insulin response. IL-6 (100 ng/ml) markedly decreased protein expression of IRS-1 and IR-β which could explain, at least in part, the cellular insulin resistance which is indicated by the altered activation of ERK and Akt/PKB. C/EBPα and PPARγ, two markers of insulin sensibility and differentiation were down regulated at the protein and mRNA levels. Furthermore, IL-6 altered adipose cell function by decreasing lipid accumulation (40%). Low concentration of IL-6 also suppressed lipogenesis and affected FAS and aP2 mRNA expression. Rosiglitazone (1μM) reversed almost all IL-6 effects.
Conclusions: Here we bring new in vitro information that implicates IL-6 in insulin resistance, in accordance with previous clinical studies. We will investigate more precisely the mechanism whereby IL-6 induced a down regulation of insulin signaling.

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Leptin

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Depletion of triglycerides in insulin sensitive tissues after leptin administration is associated with stimulation of fatty acid oxidation.
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Background and Aims: Leptin, a hormone secreted by adipocytes, stimulates fatty acid (FA) metabolism, what may prevent accumulation of lipids in the non-adipose tissues. However, the mechanism of this action has not yet been fully elucidated. We thus investigated whether increased FA oxidation in liver and muscle, as induced by a single injection of leptin is due to activation of the mitochondrial and/or peroxisomal pathways.
Material and Methods: Wistar rats were decapitated in various time intervals (15 min, 1, 3 and 6 hours) after intravenous administration of leptin [1mg/kg BW (Lep)] or saline (C), and blood and tissues were taken for analysis. Triglyceride (Tg), free fatty acids (FFA), glycerol and insulin levels in serum, and tissue lipid content were determined by commercial kits. Hepatic and muscular β-oxidation rate and activities of the key enzymes of mitochondrial (carnitine palmitoyl- transferase-I /CPT-I/and II /CPT-II/) and peroxisomal (/AOX/ acyl-CoA oxidase) FA oxidation were determined radiometrically.
Results: Single injection of leptin led to: a) increased levels of Tg (C: 1.2± 0.1, Lep-3hr: 1.7±0.2 mmol/l, p<0,05), FFA (C: 0.4± 0.08, Lep-1hr: 0.8±0.13 mmol/l, p<0,05) and glycerol (C: 0.3± 0.03, Lep-1hr: 0.5±0.06 mmol/l, p<0.05) in circulation with a maximum response at 3 or 1 hours, respectively, and b) to a decreased liver (C: 4.8±0.6, Lep-6hr: 1.9±0.5 μmol/g, p<0.01) and skeletal muscle Tg (C: 2.3± 0.3, Lep-6hr: 0.8±0.2 μmol/g, p<0.005) content. These effects were associated with an increase of hepatic beta oxidation (medians C: 368, Lep-15min: 709, Lep-6hr: 663 nmol/mg/min) at 15 min and 6 hr time intervals. In skeletal muscle, this increase was significant in all time intervals studied. Measurements of enzyme activities revealed that leptin stimulates peroxisomal (AOX: medians liver: C:0.99, Lep-15min:1.64, Lep-6hr:2.6 nmol/mg/min; medians muscle: C: 0.08, Lep-15min: 0.11, Lep-6hr: 0.18 nmol/mg/min), but not the mitochondrial FA oxidation.
Conclusion: In summary, our data indicate that up-regulation of the β-oxidation pathway both in liver and skeletal muscle after an intravenous leptin injection may prevent the tissue lipid storage. It is likely that the peroxisomal FA oxidation could be one of the principal targets for leptin action.

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The role of leptin receptor-STAT3 signalling in energy homeostasis, neuroendocrine function and glycaemic control.
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Background and Aims: Leptin signals via the long form of the leptin receptor (LRb) to control body energy homeostasis and neuroendocrine function in the central nervous system. Leptin also regulates glucose homeostasis independently of changes in body adiposity. The absence of LRb-signalling in *db/db* mice results in hyperphagia and obesity, infertility, impaired growth and frank diabetes. LRb signals via three independent downstream pathways, including Tyr_{1138} of LRb that recruits the transcription factor STAT3. Here we investigate the role of LRb-STAT3 signalling in the control of glucose homeostasis.
Materials and Methods: In order to understand the mechanisms linking LRb-signalling with glucose homeostasis, we employed a homologous replacement strategy to generate mice in which the leptin receptor gene (*lepr*) is replaced by an allele that contains a substitution mutation of Tyr_{1138} (*lepr*^{s1138}). This strategy specifically disrupts the LRb-STAT3 signal leaving all other LRb mediated signals intact. Parameters of glucose homeostasis were analysed in mice homozygous for the mutation (*s/s*) by longitudinal monitoring of blood glucose and serum insulin. Peripheral insulin sensitivity and insulin production were monitored by glucose and insulin tolerance tests and glucose-stimulated insulin secretion assays.
Results: We have previously reported that disruption of the LRb-STAT3 signal by "knock-in" of a mutant LRb in mice (*s/s*) results in hyperphagia, obesity and neuroendocrine changes attributable to altered hypothalamic melanocortin signalling (similar to *db/db* mice). Here we report that *s/s* mice, like *db/db* mice are hyperinsulinaemic, glucose intolerant and insulin

resistant, however, unlike *db/db* mice they do not become overtly hyperglycaemic.
Conclusions: *s/s* mice, like *db/db* mice have disrupted melanocortin signalling which results in obesity and peripheral insulin resistance. However, obesity and frank diabetes are dissociated in the *s/s* mouse, indicating that LRb-STAT3 independent signals control leptin regulation of glycaemic control.

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Suppressor of cytokine signaling-3 prevents leptin inhibition of human insulin gene transcription.
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Background and Aims: Obesity-induced insulin resistance leads to hyperinsulin secretion as a compensatory response in β cells. Hyperleptinemia is also evident in obese subjects. The leptin receptors have been shown to express human pancreatic islets. Leptin treatment of the islets and the injection into rats resulted in a decreased insulin secretion and mRNA level. However, little is known about signaling pathway(s) from the leptin receptor to insulin gene transcription. We have recently reported that insulin promoter factor-1 (IPF1) stimulated transcriptional activation of human insulin gene. The aim of the study is to identify signaling molecules or pathways involved in the leptin suppression of insulin gene transcription induced by IPF1. Furthermore, to prevent this leptin's suppressive effect, suppressor of cytokine signaling-3 (SOCS3) which has been shown to be an SH-2 containing protein efficiently blocking leptin signaling was employed.
Materials and Methods: (1) 1.0 kb insulin gene promoter was ligated with luciferase plasmid (pINS-LUC). pINS-LUC, IPF1 vector and long form leptin receptor vector were transiently transfected into RINm5F cells. Cells were treated with 50 nM leptin for 48 hrs and subjected to measurement of luciferase activity. (2) Dominant negative form of STAT3 was co-transfected to know contribution of Jak/Stat pathway, and MEK inhibitor PD98059 was used to know contribution of MAP kinase pathway. (3) SOCS3 was co-transfected to see whether or not SOCS3 could block the leptin's suppressive effect.
Results: (1) IPF1 transfection stimulated luciferase activity at 20 fold, which induction was suppressed by 50 to 70% by the leptin treatment. (2) The dominant negative STAT3 transfection resulted in partial rescue of the suppression (20-30% suppression). PD98059 treatment also partially rescued. Both manipulations of dominant negative STAT3 and PD98059 showed complete prevention of leptin's effect. (3) SOCS3 transfection resulted in complete prevention of leptin's suppressive effect to insulin gene.
Conclusion: These data indicate that leptin inhibits IPF1-dependent human insulin gene transcription, which is mediated through both Jak/Stat and MAP kinase pathways. For clinical relevance, hyperleptinemia commonly seen with obese subjects may play as a negative factor to insulin gene expression, suggesting that incomplete compensatory oversecretion of insulin toward insulin resistance. SOCS3 expression in β cells may rescue the leptin inhibition of β cell function in obese condition.

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Regulation of leptin secretion from white adipocytes by norepinephrine and fatty acids.
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Background and Aims: We recently reported that lipolytic agents such as norepinephrine stimulated lipolysis and concurrently counterregulated the stimulatory effects of insulin on leptin secretion from white adipocytes (Am. J. Physiol. 283:C244-C250, 2002). To assess whether there is a cause-effect relationship between stimulation of lipolysis and inhibition of leptin secretion, the effects of fatty acids were investigated in isolated rat adipocytes.
Materials and Methods: Epididymal rat white adipocytes were isolated by a collagenase method (Rodbell 1967) and incubated with several agents for 2 hours at 37 °C in Krebs-Ringer bicarbonate buffers containing various concentrations of albumin, from 0.1 to 4 % (albumin possesses several low and high affinity binding sites for fatty acids).
Results: Palmitic acid (1 mM) mimicked the inhibitory effects of norepinephrine (1 μM) on insulin (10 nM)-stimulated leptin secretion, but only at low albumin concentrations. The 50 % inhibitory concentration (IC_{50}) of the molar ratio [palmitic acid] / [albumin] was equal to 4.5. Therefore, subsequent experiments were carried out at low albumin concentrations (0.1 %). Studies investigating the effects of the chain length of saturated fatty acids [from butyric (C4) to stearic (C18) acids] revealed that only fatty acids with a chain length superior or equal to 8 carbons effectively inhibited insulin-stimulated leptin secretion. Long-chain mono- and poly-unsaturated fatty acids constitutively present in adipocyte triglyceride stores (oleic, linoleic, gamma-linolenic, palmitoleic, eicosapentanoic, docosahexanoic acids) also completely suppressed leptin secretion. Saturated and unsaturated fatty acids inhibited insulin-stimulated leptin secretion with the same potency and without any significant effect on basal secretion. On the other hand, inhibitors of mitochondrial fatty acid oxidation (palmoxirate, 2-bromopalmitate, 2-bromocaproate) obtunded the stimulatory effects of insulin on leptin release without reversing the effects of fatty acids or norepinephrine on leptin secretion.
Conclusion: The results demonstrate that: (1) medium- or long-chain fatty acids mimic the effects of norepinephrine on leptin secretion, (2) the effects of fatty acids are independent from their degree of saturation, and (3) fatty acids do not need to be oxidized by the mitochondria in order to inhibit leptin release. The data strongly suggest that fatty acids play a regulatory role as messengers between stimulation of lipolysis by norepinephrine and inhibition of leptin secretion (Supported by the Institutes of Health Research of Canada).

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Role of phosphatidylinositol 3-kinase (PI3K)signaling in the direct effect of leptin on skeletal muscle thermogenesis.
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Background and Aims: Skeletal muscle is an important site where impairments in metabolism occur in obesity and type 2 diabetes. Leptin is known to be a key hormone in the control of thermogenesis and energy balance. In this worck we have tested the ability of leptin to directly stimulate thermogenesis in skeletal muscle and the role of PI3K signalling.
Materials and Methods: Several strains of mice were studied, all being males and aged 6–8 weeks. BALB/c mice were obtained from CMU, University of Geneva (Switzerland), whereas ob/ob mice (C57BL/6OlaHsd-Lep^{ob}), db/db mice (C57BL/KsOlaHsd-Lepr^{db}) and their respective controls were purchased from Harlan (Horst, The Netherlands). They had free access to tap water, and were maintained on a commercial pelleted laboratory diet (Provimi-Lacta, Cossonay, Switzerland) consisting, by energy, of 24% protein, 66% carbohydrates, and 10% fat. In the study of diet-induced obesity, BALB/c mice were fed for 10–17 days on a high-fat diet providing approximately 50% of energy from lard, 25% from carbohydrates and 25% from protein; the control mice were fed the low-fat chow diet. All diets contained minerals and vitamins at levels recommended by the American Institute of Nutrition.
Each mouse was killed by decapitation between 8.30 and 9.00 h, and their soleus muscles were carefully dissected out intact together with their tendons and freed only of loosely attached connective tissue. These muscles have been used either for ex-vivo calorimetry or PI3K assay.
Results: Using a method involving repeated oxygen uptake determinations in isolated mouse soleus muscle, we recently reported that leptin (a hormone that plays an important role in weight regulation and in substrate metabolism in skeletal muscle) has also direct stimulatory effects on thermogenesis in this tissue – effects which were completely inhibited by either wortmannin or LY294002, two known inhibitors of phosphatidylinositol 3 kinase (PI3K).
Using *in vitro* kinase assays on intact soleus muscles treated *ex vivo* with leptin, insulin or saline solution as control, we report here that in soleus muscle treated with leptin, there is no induction of phospho-tyrosine, IRS1, IRS2 and P85 associated PI3K activity, in contrast to that found in muscles treated with insulin.
Conclusion: Our results suggest that the PI3K requirement for leptin-induced thermogenesis in skeletal muscle is independent of PI3K association to IRS1; IRS2; phospho-tyrosine as well as to an increase of the P85 associated pool.
It is concluded that either leptin induces PI3K associations to molecules other than those investigated or that leptin does not stimulate PI3K activity in muscle but that the direct thermogenic effect of leptin requires basal levels of PI3K activity (that is blocked by either wortmannin or LY294002).

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New approach for obesity by nasal administration of leptin.
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Background and Aims: Exogenously administered leptin inhibits feeding behavior, and reduces body weight. However, „leptin resistance“ disturbs the transport of subcutaneously administered leptin in obese people. Leptin administration in the nasal cavity may access to the brain via an olfactory region. The present studies were undertaken to test the hypotheses that intranasal leptin administration effectively access to the brain, and inhibits appetite.
Materials and Methods: Recombinant mouse leptin (0.5 mg/rat) was administered into bilateral nasal spaces of rats (*i.n.*). Changes in serum immunoreactive leptin (IRL) and cerebrospinal fluid (CSF) IRL concentrations by *i.n.* leptin administration were evaluated and compared with those after intra-peritoneal administration (*i.p.*). The influences of 0.1 % or 0.5 % lysophosphatidylcholine (LPC) as an optimizer of leptin absorption from nasal mucosa was examined. The anorexic effects of *i.n.* leptin administration were compared with *i.p.* leptin in *ad lib.* feeding rats. Furthermore, we observed phosphorylated STAT3-positive cells in arcuate nucleus of the hypothalamus at 6 h after the treatment by using immunohistochemistry.
Results: The *i.n.* administration of 0.5 mg leptin significantly increased CSF-IRL concentrations, but serum IRL concentrations of *i.n.* administered rats were obviously lower than those of *i.p.* administered rats. The addition of 0.1 and 0.5 % LPC dose-dependently increased serum IRL concentrations, but did not affect CSF-IRL concentrations in *i.n.* leptin treated rats. The *i.n.* administration of 0.5 mg leptin significantly inhibited food consumption at 0-1 hour (P=0.002) and 3-6 hour (P=0.007) after the treatment in *ad lib.* fed rats. In contrast, *i.p.* administration of leptin inhibited food intake at 0-1 hour. Phosphorylated-STAT3-immunoreactive cells obviously increased in the arcuate nucleus at 6-h after *i.n.* administration of leptin, but not *i.p.* administration.
Conclusion: Trans-nasal route should be useful for the selective access of leptin to the brain in leptin-resistant obese people.

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Molecular analysis of a human PPARγ mutation associated with lipodystrophy and diabetes.
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Background and Aims: PPARγ is a key regulator of adipocyte differentiation and is the receptor for the antidiabetic thiazolidinedione drugs. Loss of function mutations in the human PPARγ gene are characterized by extreme insulin resistance and type 2 diabetes. Recently we have shown that a novel mutation in the human PPARγ gene (F388L) is characterized by familial partial lipodystrophy, insulin resistance and a reduction in the sensitivity of the receptor to activating ligands. In the present study we further characterize the effect of the F388L mutation on the activity of the receptor.
Materials and Methods: NIH 3T3 fibroblasts were transfected with either WT or F388L mutant PPARγ expression plasmid, an equal amount of RXRα expression plasmid and the PPAR-dependent luciferase reporter pFATP-Luc and treated with varying concentrations of synthetic or natural ligands.
Results: In the absence of ligand the transcriptional activity of the F388L mutant receptor was 3-fold lower than the wild-type receptor. In the presence of saturating amounts of synthetic ligands including rosiglitazone, troglitazone or pioglitazone, or the natural ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (PGJ2) both wild type and mutant receptors showed similar transcriptional activity. However, dose response curves of all thiazolidinediones showed an approximate 3-fold shift to the right in their EC50s. In contrast, the EC50 for PGJ2 was 6-fold higher for the mutant receptor (7.93×10^{-6} M) compared to the wild type receptor (1.46×10^{-6}M). To examine the possibility that the F388L mutation had dominant-negative activity, PPARγ transcription was examined in cells co-transfected with various proportions of both the mutant and wild-type receptors. These experiments demonstrated that the presence of the mutant receptor did not reduce the activity of the co-expressed wild-type receptor regardless of the the ligand concentration.
Conclusion: Our findings suggest that while the F388L mutation in PPARγ reduces the transcriptional activity of the receptor, it does not cause dominant-negative activity.

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AGT-121, a novel hypothalamic gene implicated in the development of obesity.
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Background and Aims: The hypothalamus plays a major role in the regulation of energy balance. We studied hypothalamic gene expression in a unique polygenic animal model of obesity, *Psammomys obesus*, that demonstrates a wide range of body weight, adiposity and metabolic changes when allowed free access to normal laboratory chow, in order to identify novel genes involved in the regulation of energy balance.
Materials and Methods: Differential display PCR was performed on hypothalamic cDNA obtained from lean and obese *P. obesus* to identify differentially expressed genes. Taqman real time PCR and intracerebroventricular antisense oligonucleotides were used to investigate the role of these genes in the development of obesity.
Results: Here we describe a novel gene, AGT-121, initially identified as having increased expression in the hypothalamus of obese compared to lean *P. obesus*. Taqman PCR and Northern blot analysis showed that AGT-121 is expressed exclusively in the brain. Hypothalamic gene expression of an allele of AGT-121 correlated with body weight (p=0.011) and percent body fat (p=0.037) in animals homozygous for the allele. Furthermore, sequence polymorphisms in the 3’ UTR of AGT-121 were associated with obesity in *P. obesus* ($\chi 2 = 6.967$, p=0.031). The entire AGT-121 mRNA sequence is 6,317 nucleotides long and encodes for a 827 amino acid protein, and shares 92% identity with a hypothetical human protein. The function of this protein is unknown. To examine the role of AGT-121 in obesity, an

antisense oligonucleotide designed to the start codon of AGT-121 was injected into the lateral cerebral ventricle of Sprague Dawley rats (24μg/day for 4 days). Preliminary results showed that rats treated with the antisense oligonucleotide had approximately 30% reduction in food intake ($p<0.05$) and weight loss ($p<0.01$) compared with controls (n=4-7).
Conclusion: AGT-121 is a novel, brain-specific gene that may be involved in the central regulation of body weight, and could provide a novel target for the treatment of obesity.

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Gene-environment interactions influence relationships between alcohol and polyunsaturated fat intakes and central abdominal fat in healthy female twins.
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Background and Aims: Moderate alcohol consumption has been suggested to account for the 'French paradox', the paradoxically low rate of cardiovascular disease (CVD) in a population with a high saturated fat intake. Over 50 observational studies have demonstrated that, compared to non-drinkers, moderate alcohol consumers have lower rates of CVD morbidity and mortality. Given the strong association between abdominal fat and CVD, reductions in abdominal fat may mediate this cardioprotection. The reported effect of alcohol intake and other dietary variables, such as dietary fat composition, on body fat and its distribution is controversial in humans. The aims of the current study were, by studying twins: (i) To investigate relationships between alcohol intake and dietary fat composition and body fat and its distribution, independent of genetic, age and other environmental influences; and (ii) To examine the modulating effect of genetic susceptibility on such relationships.
Materials and Methods: We studied 334 healthy, female twins (aged 57.7±6.7 yrs), excluding dietary under-reporters. Total body (TBF) and central abdominal fat (CAF) were measured by dual-energy x-ray absorptiometry. A semiquantitative Food Frequency Questionnaire was used to ascertain dietary and alcohol consumption.
Results: Compared to abstainers, subjects with moderate alcohol intakes (12–17.9 g/d) had less TBF (20.6±5.6 vs 24.8±8.4 kg, P=0.03) and CAF (1.2±0.6 vs 1.6±0.7 kg, P=0.03), remaining significant after controlling for smoking and physical activity. In multivariate analysis, only alcohol consumption and physical activity predicted lower TBF and CAF. In co-twin case-control (monozygotic twin pair) analysis, controlling for genetic, age and other environmental effects, moderate alcohol consumption accounted for 300g less CAF than abstinence and light drinking. Gene-environment interaction analysis indicated that this association was limited to subjects at high genetic risk of abdominal obesity. Despite no relationship between dietary fat composition and TBF or CAF in the overall cohort, we found a gene-environment interaction between polyunsaturated fat intake and genetic risk of abdominal obesity. In women at low genetic risk of CAF, higher polyunsaturated fat intakes were associated with ~50% less CAF than lower intakes (0.9±0.4 vs 1.6±0.4 kg, P=0.0007). No such protective effect was found in those with high genetic risk.
Conclusion: The association between moderate alcohol consumption and lower abdominal adiposity is limited to twins at high genetic risk of abdominal obesity. In contrast, the inverse association between polyunsaturated fat intake and abdominal adiposity is limited to twins at low genetic risk. Lower abdominal fat may mediate the association between moderate alcohol consumption and cardioprotection.

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Prevalence of melanocortin-4 receptor gene mutations in Japanese with morbid obesity and clinical characteristics of probands carrying sequence variation.
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Background and Aims: The melanocortin-4 receptor (MC4R) is a member of the seven membrane spanning G protein-coupled receptor superfamily and singals through the activation of adenylyl cyclase. It has been suggested that *MC4R* mutations constitute the most common monogenic cause of human obesity with up to 4% of all patients with morbid obesity. On the contrary, several reports recently revealed that sequence variation in *MC4R* was not a frequent cause of human obesity. No such mutations have been found in Japanese obese subjects. To elucidate whether *MC4R* mutations are common in Japanese, the prevalence of mutations within the gene encoding MC4R was investigated in severely obese Japanese.
Materials and Methods: Ten unrelated adult subjects (50% females) were included in the study. Genomic DNA was extracted from white blood cells. The coding region of *MC4R* was amplified by the polymerase chain reaction (PCR). PCR pruducts were sequenced directly by dideoxy chain termination method.
Results: We identified a novel homozygous missense mutation of *MC4R* (Gly98Arg) in a 40-yr-old woman, which is located in its second transmembrane domain. The mutation was not found in the control population. Only one homozygous form of *MC4R* mutation (Asn62Ser) has been described from the UK. Her birthweight was 3360g and she gained weight progressively from 10 months of age. At 40 years of age, her weight was 160kg and a BMI of 62kg/m^2. Her parents and niece, who are heterozygous for the mutation, have BMIs of 26, 27, and 37kg/m^2. *In vitro* transient transfection assays revealed no discernable agonist ligand binding and cAMP production in HEK293 cells expressing the mutant receptor. Several features of the phenotype, e.g. hyperphagia, tendency toward tall stature, hyperinsulinemia, and a marked increase in bone mineral density were seen in the proband. Secondary sexual characteristics were apparently normal but her reproductive function has been impaired. This abnormality has not been reported previously in humans with *MC4R* mutation although it is not clear whether this is an associated feature or a coincidence. All there heterozygous carriers are overweight or obese but have not given a history of excessive appetite. A partial reduction in MC4R function can cause obesity with variable penetrance and expressivity. As *MC4R* mutations have been reported to cause a nonsyndromic obesity, all individuals bearing heterozygous mutation have no endocrinological or metabolic problems.
Conclusion: This study represents the first demonstration of a novel pathogenic mutation of *MC4R* in Japan and is the second description of homozygous *MC4R* mutation in human subjects. Though our data show *MC4R* obesity causing mutations in 10% of the patients (i.e. 1 out of 10 patients), the exact frequency of *MC4R* variants in the obese Japanese population remains to be clarified. Further studies are also needed to identify an association between MC4R and reproductive disorders.

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Differential gene expression in the hypothalamus of obese and lean humans: a preliminary report.
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Background and Aims: A large body of evidence indicates that the hypothalamus is central in the regulation of energy homeostasis but little is known about hypothalamic defects that may underlie human obesity. To identify putative common abnormalities, we used cDNA microarray technology to determine hypothalamic gene expression in obese and lean individuals.
Materials and Methods: We studied 5 obese (2M/3F, age at death=51.8±21.3 y, BMI=48.5±12.5 kg/m^2, mean±SD) and 5 lean (2M/3F, 50.8±17.7 y, 23.0±2.6 kg/m^2) brain donors. Based on clinical history and

neuropathology report, donors were free of cancer, diabetes or neurodegenerative diseases and not known to be on any chronic drug treatment at time of death. Five pair-matched (by sex and age at death) assays were conducted using a human neurobiology array (Clontech Laboratories, Palo Alto, CA) consisting of 588 genes. Prior to hybridization, the integrity of the RNA samples was verified using bioanalyzer. Each obese array was normalized to the paired lean array by a normalization coefficient [Σ $(\text{intensity-background})_{\text{all genes Lean}}$/ Σ $(\text{intensity-background})_{\text{all genes Obese}}$]. Only genes with a background-adjusted signal intensity at least 2 fold greater than background and not affected by signal bleed were called present.
Results: According to background-adjusted intensity ratios (obese/lean), the transcript of the 5-aminolevulinic acid synthase mitochondrial precursor gene (ALASH) appeared to be down-regulated in all 5 obese compared to lean donors (mean ratio = 0.36). Two other genes, histidine decarboxylase (HDC) and caspase-10 precursor (CASP10), appeared to be down- (mean ratio = 0.36) and up-regulated (mean ratio = 4.07) respectively, in 4 out of 5 obese donors. Paired t-tests yielded p values of 0.045, 0.075, and 0.064 respectively, for these 3 genes. Other genes were more significant and had high true positive posterior probabilities but did not pass Clontech's quality control indicators for all arrays. Ongoing research is attempting to determine the relative validity of different statistical inference strategies applied to these data.
Conclusion: In conclusion, we report possible common abnormalities in the gene expression profile of the hypothalamus of obese subjects. The altered expression levels of ALASH, HDC, and CASP10 may be cause or consequence of obesity. Further studies are warranted to confirm these preliminary results and decide if these genes should now be considered candidate susceptibility genes for obesity.

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Normalization of BMI during diet treatment is inversely related to gene expression of the beta_2-adrenoceptor in adipose tissue.
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Background and Aims: We have recently shown that the beta2-adrenoceptor protein concentration was markedly increased in adipose tissue from obese subjects (Rasmussen et al. Clin Sci 2003; 104: 93-102). The amount of beta2-adrenoceptor protein was positively correlated to the waist/hip ratio and BMI, and negatively correlated to plasma beta-hydroxybutyrate concentration at the end of 60 hours of fasting. The aim of the present study was to examine gene expression of beta1- and beta2-adrenoceptors in obese subjects in response to weight loss.
Materials and Methods: Eighteen obese subjects were studied during diet induced weight loss for a period of 8 weeks. Receptor mRNA levels were quantified by RT-PCR-HPLC. HPLC was applied for separation of standard and unknown and for quantification.
Results: Subjects lost 12.8±0.8 kg (mean±SEM) during the diet. The mean DNA concentration in adipose tissue was significantly increased after 8 weeks of treatment from 0.079±0.002 mikrog DNA/mg to 0.106±0.005 mikrog DNA/mg adipose tissue ($p< 0.001$) reflecting the decrease in fat cell size. We found a strong correlation between increments in DNA/mg adipose tissue and the corresponding decrements in the waist/hip ratio ($Rs=0.79$, $p<0.005$).
Before weight loss fat free mass (kg) and beta1-adrenoceptor mRNA was negatively correlated ($Rs=-0.80$, $p<0.001$). There was a significant decrease in the beta1-adrenoceptor mRNA level during diet treatment from 0.92±0.09 amol/mikrog DNA to 0.61±0.06 amol/mikrog DNA ($p<0.002$). The beta2-adrenoceptor mRNA level did not decrease significantly. After diet treatment BMI and beta2-addrenoceptor mRNA levels were negatively correlated ($Rs=-0.70$, $p<0.002$). There was a strong correlation between decrements in the glucocorticoid receptor (GCR) alpha mRNA, i.e. from 0.56±0.07 amol/mikrog DNA to 0.41±0.07 amol/mikrog DNA ($p=0.05$), and corresponding changes in the beta1- and beta2-adrenoceptor mRNA levels ($p<0.003$ and 0.002, respectively).
Conclusion: The present study suggests that beta1- and beta2-adrenoceptor mRNA levels are preferentially correlated to the fat free mass and BMI, respectively. The decrease in GCR alpha mRNA may be related to an increase in insulin sensitivity during weight loss (Vestergaard et al. Clin Sci 2001;101: 533-540). It is suggested that the failure to maintain or increase beta2-adrenoceptor mRNA during diet treatment may limit the weight loss

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Age-dependent expression of obesity-related genes in Otsuka Long-Evans Tokushima Fatty (OLETF) rat.
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Background and Aims: Obesity is a well-known risk factor for numerous non-communicable diseases including type 2 diabetes mellitus. Recent research has made a remarkable progress in our understanding of the molecular mechanisms in obesity, and particular attention has been paid in the genes related to the adipocyte differentiation, food intake and energy expenditure. Using OLETF rat, an obese type 2 diabetes animal model which manifest a spontaneous hyperglycemia and hyperinsulinemia along with obesity, we investigated the age-dependent expression of obesity-related genes.
Materials and Methods: We examined the mRNA or protein expression level of several genes in the skeletal muscle of OLETF and its biological control animal (LETO rat) in 8, 10, 25, and 50 weeks of age, respectively. Adipocyte determination differentiation factor 1 (ADD1), peroxisome proliferator activated receptor gamma (PPARγ), uncoupling protein 3 (UCP3), fatty acid binding protein 3 (FABP3), and AMP-activated protein kinase (AMPK) were tested by Northern hybridization, real-time quantitative RT-PCR or Western blotting.
Results: OLETF rats gained weight at a faster rate than LETO rats, and the difference in the rate of weight gain was most prominent around 10 weeks of age. The ADD1 mRNA expression level was 4.7-fold higher in OLETF than LETO rats at 8 weeks of age ($p<0.05$), and the UCP3 mRNA level was 5.5-fold lower in OLETF than LETO rats at 10 weeks of age ($p<0.05$). However, we could not find any significant differences in the mRNA expression of ADD1 and UCP3 at other stage. There were no differences in the mRNA expression of PPARγ and FABP3, and the amount of total and phosphorylated form of AMPK during the study period.
Conclusions: We observed age-dependent changes in the expression of obesity-related genes. Among the genes studied, ADD1 and UCP3 expression in the skeletal muscle may play an important role particularly in the early stage of the development of obesity in OLETF rat.

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Role of hepatic glucokinase (GK) in the stimulatory effect of SREBP-1c on glycolytic and lipogenic gene expression.

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Background and Aims: Hepatic glucokinase (GK) catalyses the phosphorylation of glucose in glucose 6-phosphate (G6P). This step is necessary for glucose metabolism in liver as well as for the induction of key genes of the glycolytic/lipogenic pathway. Hepatic GK is exclusively stimulated by insulin and recently, SREBP-1c has been shown to mediate the effect of the hormone on GK gene transcription. SREBP-1c also stimulates the transcription of lipogenic genes such as Fatty acid synthase (FAS) and Acetyl CoA carboxylase (ACC) but it is not clear whether its effect first requires glucose to be phosphorylated by GK. We first examined the effects of the lack of GK after an acute stimulation by glucose of both glycolytic (L-PK) and lipogenic (FAS, ACC) genes in mice with a liver-specific knock-out of GK (GKKO). High-carbohydrate refeeding (18 h) caused a marked increased in L-PK, FAS, ACC and SREBP-1c gene expression in the liver of control mice. In contrast, none of the glycolytic or lipogenic genes tested were induced in livers of GKKO mice despite a normal induction of SREBP-1c gene expression suggesting that hepatic GK is essential for the acute regulation of hepatic genes by glucose.
Materials and Methods: To test whether the effect of SREBP-1c required glucose to be metabolized we overexpressed the transcriptionally active form of SREBP-1c through the use of a recombinant adenovirus (Ad-SREBP-403c) both in vivo and in primary cultures of hepatocytes.
Results: Forced expression of the mature form of SREBP-1c in liver of 24h-fasted control mice markedly stimulated the expression of GK, L-PK and FAS. Surprisingly, despite the total lack of hepatic GK, overexpression of SREBP-403c also led to an increase in L-PK and FAS expression in liver of GKKO mice. Since overexpression of SREBP-403c also markedly induced the expression of the low-Km hexokinase type II (HKII) in liver, we performed studies in primary cultures of hepatocytes to clearly elucidate the role of glucose phosphorylation in potentiating the SREBP-1c effect. Cultured hepatocytes from control and GKKO mice were infected with 1 pfu of Ad-SREBP-403c in the presence of low glucose (5 mM) or high glucose concentration (25 mM). A high glucose concentration potentiated the effect of SREBP-1c on FAS expression in control hepatocytes and we will determine whether this effect is lost in hepatocytes from GKKO mice.
Conclusion: Our studies will determine whether hepatic GK and/or glucose metabolism is required for the full stimulatory effect of SREBP-1c on glycolytic and lipogenic genes.

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Regulated skeletal muscle gene therapy for Type 1 diabetes: cooperative action of insulin and glucokinase.

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Background and Aims: Type 1 diabetic patients depend upon insulin replacement therapy. However, this therapy is imperfect since proper glycemic control is not always achieved and the resulting chronic hyperglycemia leads to microvascular, macrovascular and neurological complications. Therefore, there is a clear need for new treatment strategies for diabetes mellitus that would permit tight glucose regulation. Transgenic mice expressing insulin in skeletal muscle counteracted type 1 diabetic alterations and suggested that muscle cells constitutively secreting low insulin levels may be used in gene therapy for diabetes. Furthermore, expression of glucokinase in skeletal muscle of transgenic mice reduces diabetic hyperglycemia, without inducing hypoglycemia. However none of these two transgenic mice showed a complete reversion of the diabetic phenotype. Here, we aimed to determine whether co-expression of insulin and glucokinase in skeletal muscle of double transgenic mice may counteract diabetic alterations and maintain normoglycemia
Materials and Methods: Double transgenic mice expressing both insulin and glucokinase in skeletal muscle were obtained. Blood glucose levels, skeletal muscle glucose uptake, glucose tolerance and insulin sensitivity were analyzed in streptozotozin treated double-transgenic mice
Results: After streptozotocin (STZ) treatment, double transgenic mice were able to counteract hyperglycemia and to restore fluid and food intake. In contrast, control mice presented polydipsia, polyphagia and developed severe diabetes. No hypoglycemia was detected in the STZ-treated transgenic mice. In addition, these mice presented normalization in both hepatic and skeletal muscle glucose metabolism and showed increased glucose disposal after an intraperitoneal glucose tolerance test
Conclusion: These results suggest that secretion of basal levels of insulin, in conjunction with increased glucose uptake by the skeletal muscle might permit tight regulation of glycemia. Thus, engineering skeletal muscle to express both insulin and GK may be a feasible gene therapy approach for diabetes mellitus. In vivo gene transfer of insulin and glucokinase genes, using viral and non-viral vectors, to skeletal muscle of type 1 diabetic animal models are now been carried out in our laboratory

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DNA chip analysis of human myotubes from Type 2 diabetic and glucose-tolerant control subjects: kinetics of insulin regulated mRNA expression in vitro.

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Background and Aims: Myotube cultures from patients with type 2 diabetes (T2DM) have been established as an experimental model of T2DM in vitro. Previous mRNA expression studies of skeletal muscle biopsies from diabetic patients suggest that key proteins (glycogen synthase, hexokinase II, protein phosphatase-1, glut4) involved in the metabolic actions of insulin are reduced in T2DM. It is unknown, however, if the observed changes in mRNA expression represents either an adaptive metabolic compensation at the cellular level or a direct expression of a primary genetic trait. Cultures of primary human myotubes offer perfect conditions for performing expression studies under standardised conditions.
Methods: Human primary cells from skeletal muscle biopsies were differentiated into myotubes. Parallel cultures (2 diabetic and 2 normal) in triplicates were incubated with 1mM insulin at 5.5 mM glucose for 0, $^1/_2$, 1, 2, 4, 8, 24h, and mRNA contents were characterised using Affymetrix DNA chip technology (U95Av2).
Results: Approximately-6000 of the "genes" on the U95Av2 chip are expressed in human muscle cells: 102 "genes" change more than ±1.5 fold from base line at two consecutive time points in the time-course for both diabetic and normal myotubes, 50 "genes" changed only in diabetics and 65 "genes" changed only in non-diabetics. The number of changes peaked at 2-4 hrs and 4-8 hrs, and early changes were predominantly up-regulations whereas late changes were predominantly down-regulations.
Conclusion: None of the genes encoding the "classical" metabolic signalling cascade of insulin in this model system of human myotubes appear to be regulated by insulin within 24h when glucose is kept at 5.5mM. These findings suggest that changes previously reported for T2DM in vivo are secondary to the diabetic changes in glucose metabolism and other metabolic homeostases.

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Altered levels of signalling peptides in glucose– and insulin–induced insulin resistance are not mediated by gene expression.

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Background and Aims: Previously we have reported that long-term treatment (24 h) of isolated rat adipocytes with high glucose (≥ 15mM) decreases both basal- and insulin-stimulated glucose uptake capacity by ~20 % and in combination with insulin (10^4 μU/ml) the reduction is 30-50 %. The induced insulin-resistance also leads to alterations in the amount of insulin receptor substrate-1 and 2 (IRS-1/2). Pretreatment with high glucose decreases IRS-1 (20-50%) but increases the amount of IRS-2 (100-400 %) and when high glucose is combined with insulin IRS-1 suppression is amplified and IRS-2 expression almost abolished. Long-term treatment with glucose increased GLUT4 in the cellular membranes (140 %) which was prevented in combination with insulin. The aim of the present study

was to investigate if the observed changes are due to an alteration in the gene expression.
Materials and Methods: Isolated epididymal rat adipocytes were cultured (24 h) under four different conditions: low/high glucose (5mM/15mM glucose) with or without a high insulin concentration (10^4 μU/ml)(n=3). mRNA was extracted and reverse transcription was preformed (superscript II RT). Semi-quantitative PCR was preformed by splitting each cDNA sample in three and running it for three different numbers of cycles in the PCR. β-actin was used as internal control and the amount of mRNA was not affected by the different incubation conditions.
Results: We chose to study the expression of the insulin receptor (IR), IRS-1, PI3-K and PKB and we could find no alteration in the amount of mRNA for any of these signalling peptides upon incubation with high glucose and/or insulin. However GLUT4 mRNA was upregulated when insulin was present in the culture medium and this was independent of the surrounding glucose concentration (5 mM or 15mM). The different culture conditions and the following mRNA preparations were preformed three times and the results were very consistent.
Conclusion: We conclude that gene expression does not seem to be the mechanism for the observed changes in the amount of signalling peptides induced by high glucose and/or high insulin. Insulin is known to upregulate GLUT4 expression so this finding was expected. Another possible mechanism that could explain our findings would be an alteration in the degradation of the signalling proteins and this is currently under investigation.

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Impact of hyperglycemia on steroidogenic gene regulation—- study of the *CYP11A1* gene expression in alloxan-induced diabetic - 2.3kb*CYP11A1/LacZ* transgenic mice.
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Background and Aims: The alteration of steroidogenic gene regulation during diabetes is not clarified. Cytochrome P450scc, encoded by *CYP11A1*, is the key enzyme catalyzing the first and rate-limiting step of steroid biosynthesis. Reports about *CYP11A1* gene expression during diabetes, however, have been discordant. We have generated transgenic mice containing 2.3 kb of the 5'-flanking region of *CYP11A1* driving *LacZ* reporter gene and has demonstrated the characters of tissue-specific and hormonal regulatory expression. To study the hyperglycemic effect, these mice were received alloxan injection to produce a diabetic-transgenic model.
Materials and Methods: One month after hyperglycemia, the adrenal and testes were removed from the freshly killed mice and assayed for β-galactosidase activity. Data were compared between diabetic transgenic mice and their non-diabetic littermates in basal and physiological manipulated states (including ACTH, dexamethasone and β-hCG injection). Three mice were examined in each set. The experiment has been duplicated
Results: The basal *CYP11A1* activity of adrenal and testes in alloxan-treated mice showed more than two-fold higher than those in their non-diabetic littermates (2.2- in adrenal and 2.1- in testes). The injection of ACTH and β-hCG stimulated the increment of transgene expression for 3.1-fold of adrenal and 5.9- fold of testes from basal state in nondiabetic mice. In contrast, the diabetic mice showed less increment after stimulation (1.0- and 2.4- fold of increment of adrenal and testes, respectively). Dexamethasone injection decreased the adrenal *CYP11A1* expression for 70% in diabetes and 20% in nondiabetes.
Conclusion: We found the basal state expression of *CYP11A1* is increased in alloxan-induced diabetic mice not only of adrenal but also of testes. Furthermore, the inadequate response of the steroidogenic gene expression after stimulation might implicate the stress-unbearable of these hyperglycemic mice.

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Microarray analysis of hypothalamic genes regulated by dietary energy restriction in *Psammomys obesus*.
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Background and Aims: *Psammomys obesus* is a unique polygenic animal model of obesity and type 2 diabetes. When fed a standard rodent laboratory diet they exhibit a wide range of body weight and glucose tolerance that closely resembles that observed in cross-sectional studies of human populations. The aim of this study was to use cDNA microarrays to identify differentially expressed genes in the hypothalamus of lean, normal glucose tolerant (nGT) and obese, diabetic *P. obesus* following dietary energy restriction (ER).
Materials and Methods: Daily food intake of nGT (n=8) and obese, diabetic (n=8) animals was measured over 14 days. During the ER study, these animals were restricted to 67% of their normal food intake for a further 2 weeks. Two groups of animals matched for age, weight and blood glucose (nGT n=8, diabetic n=8) were fed ad libitum over the 2 week study period and served as controls. RNA was extracted from the hypothalamus and hybridised to custom-made *P. obesus* cDNA microarrays of 12,288 elements. Microarray data was extracted using Genepix Pro4 (Axon Instruments, CA) and multivariate data analysis was performed using Acuity 3 (Axon Instruments.
Results: Energy restriction in nGT animals resulted in a 7.5% reduction in bodyweight (229±7g to 212±6g, $p<0.05$) but no change in blood glucose levels. In contrast, ER in obese diabetic animals led to significant loss in body weight (260±5g to 237±4g, $p<0.05$) and a significant improvement in glycaemia (13.7±1.9 mmole/L to 4.7±0.5 mmole/L, $p<0.01$). Body weight and blood glucose levels in control animals fed ad libitum did not change significantly over the 2 week study period. Statistical analysis revealed that 309 genes showed evidence of differential expression ($p<0.05$ by t-test not adjusted for multiple testing) between nGT and diabetic animals. Energy restriction lead to altered expression in over 379 genes in nGT animals and 110 genes in obese, diabetic animals.
Conclusion: Hypothalamic genes regulated by dietary energy restriction in nGT and obese, diabetic *P. obesus* included some expected genes such as cytochrome c oxidase and NADH oxidase as well as a number of unknown genes that are currently undergoing further investigation.

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Differential hypothalamic gene expression profile after intracerebroventricular infusion of lipids for 48h in Wistar rats.
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Background and Aims: Dysregulation of non-esterified fatty acids (NEFA) metabolism could be an early event responsible for the etiology of type 2 diabetes. NEFA are likely to be involved in the mechanisms leading to insulin-resistance and exaggerated glucose-induced insulin secretion (GIIS), both features characterising prediabetic state. We previously showed that Wistar rats receiving a 48h intracerebroventricular infusion of triglycerides and heparin displayed an increased GIIS, hepatic insulin resistance, and hypercorticosteronemia. Since this changes occurred without any increase in NEFA concentration in peripheral blood, this situation was likely ascribed to direct effect of NEFA on hypothalamic areas involved in the control of energetic homeostasis. We studied here the differential hypothalamic gene expression profile between rats infused intracerebroventricularly with triglycerides and heparin (icvIL rats) and control rats (C rats) infused with saline and heparin, using a microarray approach in order to identify potential hypothalamic target genes.
Materials and Methods: ARNm were extracted from hypothalamus, labelled with biotinyled nucleotides, and hybridized on rat Affimetrix GeneChip® 34A. After analysis, sequences were ordered according to the importance of the difference of expression between both groups.
Results: 88 genes were found to be differentially expressed in a significant way. Some of them are related to the gamma-aminobutyric acid (GABA) system (GABA receptors types A and B, down-regulated), others are associated with the glutamatergic system (GluR-A, up-regulated, meotropic glutamate receptor 4, down-regulated). Insulin 1 gene is down-regulated, and the gene of vesicular acetylcholine transporter is up-regulated. Many

differentially expressed genes belong to signal transduction pathways (protein kinase C gamma and delta subspecies, down-regulated). Interestingly, several channel and transportation proteins expression are altered; in particular, low voltage-activated, T-type calcium channel alpha subunit gene expression is decreased.
Conclusion: These preliminary results provide new insights into the effect of lipids on nervous activity of hypothalamic nuclei involved in the control of glucose homeostasis. The study of both synthesis and activity of target proteins may allow a better understanding of the molecular mechanisms underlying NEFA action through central nervous system.

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Kidney posttransplant diabetes: autoantibodies and beta-cell function.
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Background and Aim: The etiopathogenesis of posttransplant diabetes mellitus (PTDM) is multifactorial, including genetic and environmental factors with islet and beta-cell toxicity and increase of insulin resistance. Risk factors: age, family history of diabetes, obesity, lifestyle and immunosuppressive regimens (steroids, cyclosporine A and tracolimus-FK-506). The autoantibodies, GAD65 and IA-2, ICAs, IAA are markers of islet autoimmunity in type 1 diabetes (DM1) and C-peptide is related with beta-cell function. At our Center a total of 1140 kidney transplants (Tx) were performed (until November 2002). The aim was to study, at diagnosis, some features of kidney PTDM including autoimmunity and beta-cell function.
Materials and Methods: One hundred and twenty nine (129) patients with PTDM. Family history, BMI (kg/m2), fasting C- peptide and after glucagon test (0 and 6 min). Assays: C-peptide, Immulite 2000 (normal: 1.1-5 ng/mL); ICAs, indirect immunoflurescence; GAD65, RSR's (normal<1U/mL), IA2, RSR's (normal<1U/mL), IAA, DLD (normal<7%). HbA1c (normal: 4-6%). Statistical analysis: mean+/- 2SDS, Student's t test, 95% confidence interval.
Results: One hundred and twenty nine (84M, 44F) PTDM patients with mean age at diagnosis 49.5+/-11.4 y. All patients except one (128) had no autoantibodies to type 1 diabetes with C-peptide at 0 min 4.03+/-2.15 ng/mL, and at 6 min 5.72+/-2.75 ng/mL; pre-Tx BMI 25.05+/-3.75 kg/m2 and at diagnosis 27.25+/-4.38 kg/m2 ($p<0.05$). Only one patient presented, at diagnosis, with GAD65 auto antibodies, a male aged 55 y, fasting C-peptide 0 min 1.3 ng/mL and after glucagon 2.5 ng/mL, a BMI of 18.04 kg/m2, HbA1c 6.8%, on diet and no diabetes in the family. At diagnosis, all patients had triple immunosuppressive regimen (AZA or MMF, CYA or FK-506, steroids); 60% had diabetes in the family. Current treatment consists of glycemic monitoring (100%), diet alone (47.67%), insulin (52.33%) resulting in a HbA1c of 6.5+/-1.2%.
Conclusion: In this study, all patients had normal or high levels of C-peptide and 99.22 % had absence of autoimmune markers of DM1 which strongly suggest the presence of a kind of drug induced DM2. One patient had (GAD65+) associated with a normal beta-cell function, a normal BMI and a good control on diet alone. The possibility of a DM1 LADA shouldn't be excluded. These results suggest that screening PTDM patients for markers of islet autoimmunity probably isn't justified unless on a clinical basis.

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Five suspected cases of fulminant Type 1 diabetes mellitus.
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Background and Aims: A subtype of type 1B diabetes, so-called 'fulminant' type 1 diabetes, has been proposed. This type of type 1 diabetes is characterized by the following criteria: 1) no detectable 'islet-associated' autoantibody; 2) regardless of diabetic ketoacidosis, near-normal HbA1c levels, suggesting extremely acute onset; and 3) high levels of pancreatic exocrine enzymes. We studied five patients suspected of fulminant type 1 diabetes mellitus in our hospital.
Materials and Methods: We studied patients with type 1 diabetes who hospitalized due to diabetic ketoacidosis during the period from 1999 to 2003. We screened fulminant type 1 diabetes using the criteria of the Japanese Diabetes Association Committee for fulminant type 1 diabetes, that is, 1) near-normal HbA1c level, 2) rapid-onset with diabetic ketoacidosis, and 3) rapid loss of insulin secretion. Five patients met the criteria in our hospital.

Results: Of the five patients, 28 to 63 years old, two were men. All five patients did not have GAD65 antibodies and other diabetes-related autoantibodies. The HbA1c levels ranged from 4.8 to 6.7% despite high plasma glucose concentrations, 470 to 1098 mg/d*l*, on admission. Urinary C-peptide excretions were very low in all cases. Elevation of serum pancreatic enzyme concentrations was found in four patients. Two of the three women patients, the onset of fulminant type 1 diabetes mellitus were associated with pregnancy. One woman had Graves' disease at the time of the onset of fulminant type 1 diabetes and another woman fell in Graves' disease after she was diagnosed as having diabetes. Three of the five patients showed symptoms of the cold before they had fulminant type 1 diabetes.
Conclusion: It has been reported that the onset of fulminant type 1 diabetes mellitus was related to pregnancy and occurred during the 2nd and 3rd trimesters. But one of our cases, the women developed typical fulminant type 1 diabetes as early as 7 weeks into gestation. Imagawa et al. proposed that fulminant type 1 diabetes was caused by nonautoimmune process. However Graves' disease, which may be caused by autoimmunological mechanism, was complicated with fulminant type 1 diabetes in our two patients. It seemed that our five cases indicated fulminant type 1 diabetes was not caused by single mechanism.

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Are there clinical differences in patients with Type 2 diabetes with normal and raised serum ferritin concentrations ?
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Background and Aims: Iron overload and therapy trials using phlebotomy in patients with type 2 diabetes have been reported recently (i.e. Fernández-Real et al., Diabetes 2002; 51: 2348-2354). We wanted to clarify, wether a raised serum ferritin level in patients with type 2 diabetes is accompanied by certain patient characteristics indicating a particular diabetes subtype.
Materials and Methods: In 348 consecutive patients with type 2 diabetes (162 female, 186 male, age 62 ± 12 yrs., duration of diabetes 11± 9 yrs., BMI 31.1 ± 5.8 kg/m^2, HbA_{1c} 8.5 ± 1.7 %; treated with insulin in 56 %; with sulfonylurea or analogues in 34%; with metformin in 30%; with acarbose in 6 %; combination possible) serum ferritin was determined. Patients were assigned to two groups with normal (female ≤ 150 ng/ml; male ≤ 400 ng/ml) or raised ferritin levels. Characteristics of patients, therapy and laboratory findings were compared by analysis of variance.
Results: A raised serum ferritin level was frequent among our patients (40 % of tested patients). Among patients with raised ferritin concentrations there were more female subjects (55 vs. 41 %, p = 0.008), serum iron concentration was higher (97 ± 37 vs. 90 ± 34 µg/dl; p = 0.002), serum transferrin was lower (253 ± 43 vs. 273 ± 45; p < 0.0001) resulting in comparable transferrin saturation (p = 0.26). MCV was higher (93 ± 5 vs. 92 ± 5 fl; p = 0.001), haemoglobin concentration was slightly higher (14.09 ± 1.68 vs. 13.97 ± 1.55 g/dl; p = 0.009), just as were elevated liver enzyme parameters (ALAT, p = 0.002; ASAT, p = 0.005; γ-GT, p = 0.02; AP, p = 0.84). Diabetes-related parameters were not significantly different: duration of diabetes 11 ± 9 vs. 11 ± 9 yrs., p = 0.61; BMI 30.9 ± 5.1 vs. 31.2 ± 6.2 kg/m^2, p = 0.50; HbA_{1c} 8.7 ± 1.7 vs. 8.4 ± 1.6 %, p = 0.18, insulin resistance (HOMA-model) 6.1 ± 6.0 vs. 5.1 ± 5.3 fold of a metabolic healthy reference population, p = 0.18. Treatment and blood glucose profiles were not different (p = 0.77). Only one patient(in the group with raised ferritin) had a hemochromatosis gene mutation (homozygous for C282Y). In an additional analysis of 161 patients with type 1diabetes, serum ferritin was elevated in only 14 cases (8.7 %). Otherwise, similar changes were found.
Conclusion: In patients with diabetes type 2 and raised ferritin levels, accompanying changes in iron metabolism , blood count and liver enzyme parameters were found pointing to iron overload. All diabetes-related parameters were not different. Our data do not support a therapeutic benefit of phlebotomy or deferoxamin in patients with diabetes without hemochromatosis or hemosiderosis.

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Does the coincidence of diabetes mellitus affect the prognosis and survival of TIPS patients?
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Background and Aims: Liver cirrhosis is a chronic disease that gradually causes portal hypertension. A method of treating bleeding from oesophageal varices and refractory ascites (caused by portal hypertension) is the implantation of a transjugular intrahepatic portosystemic shunt (TIPS) that decreases the portosystemic gradient. Diabetes mellitus has a greater incidence in patients with liver cirrhosis than the rest of the population. The aim of our project was to determine, on the basis of a retrospective study, whether in diabetics with TIPS over a period of 10years there have been occurrences of complications and higher mortality after TIPS.
Materials and Methods: The group consisted of 430 patients with liver cirrhosis all of which had TIPS. From the total 319 were not diabetics, 111 diabetics of which 59 were on a diabetic diet or per oral antidiabetics and 52 were treated with insulin. We analysed the differences in survival and ; occurrences of early (post procedure) and late complications (hepatic encephalopathy) after TIPS with connection to the presence of diabetes mellitus. For statistical elaboration we have used NCSS 2001 program –Log-rank test, Kaplan-Meierov empirical curve of survival, t-test and chi-square test independence in contingency table.
Results: Diabetes mellitus was more commonly present in patients suffering from liver cirrhosis caused by hepatitis C (p<0.01). We have proved statistically significant lower rates of survival in diabetic patients (p<0.01) than non-diabetic patients (p<0.05) and a greater incidence of encephalopathy(p<0.01). Acute procedures were more commonly performed in non-diabetics than in diabetics (p<0.05). The presence of diabetes did not affect the frequency of acute complications, need for revisions after TIPS and early mortality. The diabetics in our group were statistically significantly older than the non-diabetics (p<0.01) and the age is a factor that most significantly influences the survival. According to Cox regression a one-year rise in age corresponds with an increased risk of death after TIPS by 3.4%. To exclude the effects of age we have only included in our analysis a representative sample population with an age resolution between 40-60 years. In this sample, the presence of diabetes mellitus had no apparent effect on the survival nor on the progression of hepatic encephalopathy. Between the group of diabetics treated with diet or by per-oral anti-diabetic agents; and the group treated with insulin there was no statistically significant difference in survival, incidence of hepatic encefalopathy or occurrence of complications and necessity of revision after TIPS.
Conclusion: Diabetes mellitus was more commonly found in patients with liver cirrhosis caused by hepatitis C. Diabetics were older, yet the presence of diabetes mellitus did not worsen prognosis, occurrence of acute nor late complications after TIPS. Presence of diabetes mellitus is not a contraindication for the implantation of TIPS.

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Anthropometric parameters, metabolic control and thyroid autoimmunity in 127 biopsy-positive patients with Type 1 diabetes and coeliac disease (CD) compared to 18,470 diabetic subjects without CD.
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Background and Aims: CD more commonly occurs in type 1 diabetes (T1D). However, controversy exists on the clinical relevance of CD, as many patients are asymptomatic. Therefore, we analysed data of the German paediatric multi-centre DPV-database comparing clinical parameters in T1D-patients with or without CD.
Materials and Methods: Up to September 2002, data of 19,796 children and adolescents aged <20 years with T1D from 148 paediatric centres were documented in the database. Clinical or laboratory signs for CD were present in 1326 patients (6.7%). The diagnosis was confirmed in 127 patients by small bowel biopsy, while in 1199 patients no histological findings were available; the latter group was, therefore, excluded from

further analysis. In 18,470 T1D patients neither clinical nor laboratory indications for CD were present.
Results: In 13 patients CD was diagnosed before, in 114 patients 4.2±3.8 years after T1D onset. The percentage of females was increased in T1D patients with CD (57% vs. 48%, p<0.05). The onset of diabetes occured significantly earlier in patients who additionally suffered from CD (5.8±4.1 vs. 8.1±4.1 years of age at diabetes onset (mean±standard deviation), p<0.0001). Furthermore, they had lower z-scores for height at onset (-0.11±1.1 vs. +0.15±1.0, p<0.0001) as well as after 5 years of T1D (-0.43±1.0 vs. -0.06±1.0, p<0.0001). In addition, z-scores for body mass index differed between the groups (+0.22±0.8 vs. +0.47±0.9, p<0.005). Daily insulin requirements in units per kg of body-weight were higher in patients with CD (0.88±0.27 vs. 0.82±0.29, p<0.05) while DCCT-standardised HbA1c values were lower (8.2±1.9% vs. 8.8±2.5%, p<0.05). There was no difference concerning the rate of severe hypoglycemia and the number of daily insulin injections between the groups. The prevalence of autoimmune thyroiditis did not differ significantly between the two groups (6.3% vs. 2.3%, n.s.).
Conclusion: Clinical and laboratory findings suggesting CD were present in 1 out of 15 paediatric T1D patients. In 1 out of 156 patients CD was confirmed by small bowel biopsy. Females were more often affected. The majority of these patients developed CD after T1D onset. Both at onset as well as during the course of diabetes, patients with biopsy-confirmed CD differ clearly from T1D patients without CD. The CD positive patients were characterized by earlier onset of diabetes and decreased growth and body weight. These findings emphasize the clinical relevance of coeliac disease in patients with autoimmune diabetes.

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Prevalence and clinical correlates of immune markers for celiac disease in adult subjects with Type 1 diabetes.
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Background and Aims: The ethno-geographical prevalence of celiac disease in type 1 diabetes remains debated, as well as its association with brittleness &/or poor metabolic control. We assessed from consecutive prospective screening the prevalence of circulating anti-gliadin (IgG and IgA) as well as anti-endomysium (IgA) antibodies in adult subjects with type 1 diabetes (out- and inpatients) followed at the Endocrine division of our Academic Hospital, and we determined the prevalence of biopsy-proven celiac disease in subjects concordant for both types of antibodies (i.e. anti-gliadin IgA and anti-endomysium IgA).
Materials and Methods: The cohort included a total of 272 subjects. Mean age was (±1 SD) 47 (17) years, diabetes duration 22 (13), HbA1c 8.2 (1.1) %, sex ratio (M/F) 47/53. Prevalence of thyroid autoantibodies (anti-TG and/or anti-TPO) was 34%. Total serum IgA was 220 (98) mg/dl, with all samples within normal range.
Results: Prevalence rates for anti-gliadin IgG, anti-gliadin IgA and anti-endomysium IgA antibodies were 12% (n=26), 4% (n=11) and 3% (n=8). Neither the prevalences of thyroid autoantibodies nor the degree of metabolic control differed between subjects with or without anti-gliadin and/or anti-endomysium antibodies, although the latter had more brittle diabetes (home blood glucose monitoring SD: 118 (17) *vs.* 86 (23) mg/dl in antibodies-negative subjects, $p<0.01$). Out of 8 subjects (5M/3F) with anti-endomysium IgA, 6 (4M/2F) were also positive for anti-gliadin IgA. Small-bowel endoscopic biopsy was proposed to all subjects with anti-endomysium IgA, irrespective of anti-gliadin positivity, and performed with informed consent on 6 out of these 8 subjects, one female subject being lost to follow-up, and another male subject declining endoscopy. The telltale signs of celiac disease were found in 5 subjects (4M/1F) otherwise positive for both anti-gliadin IgA and anti-endomysium IgA, one female subjects with isolated anti-endomysium IgA titers exhibiting normal jejunal mucosa.
Conclusion: Undetected celiac disease was highly prevalent in subjects concordant for both anti-gliadin and anti-endomysium IgA antibodies, otherwise asymptomatic but for the brittleness of their home blood glucose excursions.

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The influence of diabetes mellitus Type 2 on bone mineral density of premenopausal women.
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Background and Aims: The role of diabetes mellitus type II on bone mineral density still remains unclear in postmenopausal women though most of previous studies have detected a positive effect of the disease on bone mass. The influence of the diabetic state could be different in the premenopausal period. We evaluated the effect of diabetes mellitus type II on bone mineral density (BMD) of premenopausal women.
Material and Methods: Vertebral (L2-L4) and femoral neck (FN) BMD measurements by DEXA were performed in 35 diabetic (DM-PRE) and 205 healthy (H-PRE) premenopausal women aged between 30-49 years. DM-PRE age was 43.4±5.2 years (mean± 1SD), BMI was 28.8±3.8 kg/m^2 while in H-PRE 43.6±4.3 and 27.4±2.7 respectively. In DM-PRE disease duration was 5.2±2.4 years and HbA1c levels 6.6±0.8 %.
Results: L2-L4 BMD values of DM-PRE were significantly higher than H-PRE (1.088±0.142 vs. 1.027±0.125 g/cm^2, p<0.001). FN BMD values in DM-PRE were significantly higher than H-PRE (0.939±0.154 vs. 0.847±0.112 g/cm^2, p<0.01). Proportions of osteopenic-osteoporotic women (WHO criteria) in DM-PRE were significantly lower than in H-PRE (p<0.05). In both groups there was a significant positive correlation between L2-L4 and FN BMD values (p<0.001), but in H-PRE a significant discrepancy occurred between L2-L4 and FN T scores with the former being significantly higher than the latter (-0.4±1 vs. -0.95±1, p<0.01). In neither of the groups was there any significant correlation of BMD values at both anatomic sites with BMI or age. No significant correlation between BMD values and either disease duration or HbA1c levels were observed in DM-PRE.
Conclusions: Diabetes mellitus type II seems to affect positively the bone mineral density of relatively young premenopausal women irrespectively of the disease duration. The disease seems to ameliorate discrepancies between vertebral and femoral neck BMD values observed in healthy individuals. In the premenopausal period aging does not appear to influence significantly the bone mass irrespectively of diabetic state.

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Spectrum of microorganisms causing infection in patients with diabetes.
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Background and Aims: Patients with diabetes are predisposed to infection due to alteration in the host defenses at several levels. These include altered physiology and cell-mediated immunity as well as increased risk due to other coexisting conditions such as malnutrition, vascular insufficiency, cardiovascular and chronic renal disease. Our aim was to isolate the microorganisms associated with a range of infections in patients with diabetes to find out their comparative occurrence and study the spectrum of pathogens with their antibiotic sensitivity pattern at our institution.
Materials and Methods: The study population consisted of **1157** unselected patients of diabetes with foot infection [**n=252**], foot infection/ lower limb amputation [**n=157**], lower respiratory tract infection [**n=202**], urinary tract infection [**n=218**], dental periodontitis [**n=163**], and genital infection [**n=165**]. Samples were collected at surgery or from drainage of the affected tissues, urine, sputum and swabs from the throat and vagina; transported in suitable media and cultured appropriately for aerobic and anaerobic isolation and identification of pathogen. Antibiotic susceptibility testing of all the clinical isolates was carried out using the Kirby-Bauer disk diffusion technique. Multiresistant organisms were subjected to checkerboard synergy studies to ascertain potential synergistic antibiotic combinations.
Results: Patients with foot infections and amputation showed prevalence of gram negative aerobic pathogens [**42.8% and 45.6%**] and anaerobes [**28.9% and 33.5%**] with **9.7**% and **12.7**% pathogens demonstrating resistance to the multiple antibiotics respectively.[**Table**] Lower respiratory infections were mostly due to aerobic gram positive pathogens [**49.2%**] with **16.2** % showing multiple antibiotic resistance. However, the incidence of MRSA was only **4.7%**. In patients with urinary tract infection, gram negative pathogens were predominant [**78.1%**], with **29.68** % demonstrating multiple antibiotic resistance. Dental and genital infections showed higher association of gram positive pathogens [**54.4%** and **69.7%**

respectively]; however these microorganisms were highly susceptible to most of the antibiotics. Incidentally, in these patients, yeast species was isolated in large numbers [**15.9%** and **16.4%** respectively]. The average number of organisms per patient was considerably high in periodontal infections [**3.85**]; followed by amputation [**3.56**], diabetic foot [**3.07**] and genital infections [**2.88**].
Conclusion: The study emphasizes that proper isolation & identification of pathogens with antibiotic susceptibility helps in rationalizing appropriate antibiotic treatment and therefore reduces the duration as well as complications and progress of the illness.

Total cases[1157]	Diabetic Foot [252]	Diabetic with Foot Amputation [157]	Lower respiratory tract infections [202]	Urinary tract infections [218]	Periodontal infections [163]	Genital infections [165]
Total isolates[3034]	775	559	376	219	629	476
Aver.No.Org/case	3.07	3.56	1.86	1.004	3.85	2.88
Aerobic Gm Positive	219	117	185	48	342	332
Aerobic Gm Negative	332	255	144	171	178	36
Anaerobic Gm Positive	175	149	-	-	172	21
Anaerobic Gm Negative	49	38	-	-	76	9
Capnophillic Organisms	-	-	17	-	48	-
Yeast spp.	7	-	30	-	100	78
Multiresistant Organisms	75[9.7%]	71[12.7%]	61[16.2%]	65[29.7%]	-	-

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Clinical criteria to increase the suspicion of underlying pancreatic cancer in patients who are newly diagnosed as having Type 2 diabetes.
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Background and Aims: Although secondary diabetes is rare it is critical to establish the underlying cause as it can directly affect both management and prognosis. As pancreatic adenocarcinoma can solely present with diabetes mellitus (DM), the purpose of this study was to establish sensitive criteria for early diagnosis of this entity in patients with newly diagnosed type 2 DM.
Materials and Methods: During the last ten years we have diagnosed 21 cases of asymptomatic pancreatic cancer in patients with newly diagnosed type 2 DM (15 males, 6 females, aged 45-75) [Group 1]. Screening test was serum CA-19-9. These patients were then compared with a group of age and weight matched patients of the same referral population who had type 2 DM not related to secondary causes [Group 2]. Patients of group 2 were followed for 2-3 years following initial diagnosis to exclude the subsequent development of pancreatic pathology. Comparison between the two groups was based on the following characteristics: 1) presence of family history of type 2 DM, 2) presence of retinopathy and 3) response to antidiabetic drugs.
Results: All patients with pancreatic cancer had CA-19-9 > 300 IU/ml (Normal Value <37IU/ml). Patients of Group 2 had CA-19-9 < 50 IU/ml. Frequencies of aforementioned characteristics between the two groups were as follows: 1) Family history of type 2 diabetes: Group1: 4 patients (19%, 95% CI: 5,4-41,9%), Group 2: 40 patients (80%, 95 CI: 66,3-89,9 p<0.001). 2) Retinopathy: Group 1: 0%, Group 2: 8 patients (19%, 95% CI: 7,2-29,1%, p<0.052). 3) Response to antidiabetic drugs: Group 1: 1 patient (4,8%, 95% CI: 0,1-23,8%), Group2: 48 patients (96%, 95% CI: 86,3-99,5%, p<0,001).
Conclusion: In patients with newly diagnosed type 2 DM, the lack of response to antidiabetic drugs as well as the absence of family history of type 2 DM seem to increase the likelihood of underlying pancreatic cancer. The presence of these findings and the absence of retinopathy should alert the physician for an underlying cause of DM. The absence of retinopathy is statistically significant between the two groups but it does not have strong clinical significance.

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Diabetes Care: Strategies

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Treatment strategies for Type 1 diabetic complications: an audit of practices in France, the DISCO (Type 1 DIabetes Strategies and COmplications) study group.
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Background and Aims: Evidences support that people with type 1 diabetes should be treated aggressively for their blood glucose for primary prevention of diabetic complications, and for their blood pressures with ACE inhibitors for secondary prevention of renal complications. We studied the current practices in this respect in France.
Materiel and Methods: Between September 16 and October 31 2002, a randomly selected sample of 367 diabetologists included each 5 to 10 consecutive type-1 diabetic patients aged 10-45 years. Information was obtained about the demographic characteristics, diabetic complications, treatment modalities, and current HbA1c of 2333 patients with 3-40 years diabetes duration.
Results: Median age was 28 years and median diabetes duration 10 years. 80% of the participants had no retinopathy, 13% background, and 7% proliferative retinopathy; 86% had normoalbuminuria, 12% microalbuminuria, and 2% proteinuria. The severity of complications increased with diabetes duration ($p < 0.0001$). 23.7% of participants had HbA1c ≤ 7%. 74.6% of the participants (58% of those aged < 17 years) were on intensified insulin treatment (≥ 3 daily insulin injections or pump). The mean HbA1c decreased according to the number of daily insulin injections ($p < 0.05$). However, complications were more frequent among participants on intensified insulin treatment than among the others: 24% vs 17% of retinal complications (p: 0.0003) and 17% vs 15% of renal complications (NS), respectively. Conversely, antihypertensive treatment was used by 61.4% of hypertensive (≥ 130/85 mmHg) and 38.6% of normotensive participants. 87.9% of the treated patients used ACE inhibitors.
Conclusions: A large majority of French people with type 1 diabetes, aged 10-45 years, are on intensified insulin treatment. However, this treatment strategy is used more frequently as a secondary, rather than as a primary prevention of diabetic complications. The use of antihypertensive drugs seems adequately focused on secondary prevention of renal complications.

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Intensive glucose control in non-academic intensive care - does the leuven protocol travel?
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Background and Aims: With intensive blood glucose control, Van den Berghe et al. reported a 36% reduction of in-hospital mortality (26.3 to 16.8%) in patients requiring intensive care for ≥ 5d. Since our mortality rate for such patients was 30% we wished to determine whether this approach was practicable and effective in a general non-academic ICU.
Materials and Methods: Epworth Hospital ICU has 15 beds admitting 1000 adult patients annually. We designed an iv insulin infusion protocol aiming to achieve BGL 4.5-6.1mmol/L. All patients admitted from 24/7/2002 were treated with this aim commencing in the operating theatre, on admission to ICU or thereafter if BGL> 6.1. Enteral nutrition was commenced as soon as possible. BGL was monitored 1-4 hourly until discharge or death. At discharge from ICU, BGL control reverted to conventional treatment goals (BGL<11.0). After 6mo, cumulative mortality was assessed by retrospective review of patient charts and extracts from the ICU ward database and compared to a similar cohort of long-stay ICU patients 1y earlier.
Results: During iv insulin infusion in the new intensive glucose control period (2002) 06.00h BGL was 6.5±0.2 mmol/L. Among patients requiring ICU treatment for ≥5d, 12 of 40 (30.0%)(2002) died in hospital equivalent to 11 of 36 (30.6%) in 2001. For each cohort, median (range) APACHE II scores on day 1 in ICU were 25 (17-38)(2002) vs 23(12-44)(2001) and

mean ± S.D. ages were 71.5±12.8y (2002) vs 71.9±9.6y (2001). APACHE II predicted mortality was 47.0±3.6% (2002) vs 42.8±3.7% (2001)(mean±S.E.).
Conclusion: In a non-academic general ICU setting, institution of intensive glucose control did not reduce mortality among patients requiring ≥5d treatment. Hypoglycemia occurred rarely and the protocol was highly accepted by nursing staff. The severity of illness of our patients with mortality 30% was higher than that reported (20% in ICU, 26% in hospital) and our patients may not be directly comparable with other series. Our observed mortality was substantially better than predicted by APACHE II scores. However in our small study, we were unable to reproduce the results of Van den Berghe et al.

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Novel diabetes care credit system: a model for comprehensive and optimal diabetes care.
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Background and Aims: Barriers to improved care for individuals with diabetes and to implementing practice guidelines prompted us to introduce a "Credit System" as a model for optimal diabetes care. Our system empowers the patient to share in the implementation of guidelines with the health care provider and provides specific number of credits for each health parameter considered essential in achieving optimal diabetes care and avoiding chronic complications.
Methods: The credit system provides a specific number of credits to each attribute or health parameter required to achieve optimal care as set forth by current American Diabetes Association and the American Heart Association guidelines.
Each patient is granted a number of credits for each parameter assessing life style attributes such as physical activity, body weight, smoking and alcohol consumption; self-blood glucose monitoring; targeted values of glyco-hemoglobin, blood pressure, and lipids; measurement of urinary albumin; annual physical examination, dilated eye examination, and foot examination; electrocardiogram and, when indicated, complete cardiac evaluation; antiplatelet therapy; and angiotensin receptor blocker (ARB) or angiotensin converting enzyme inhibitor (ACEI) therapy. Finally, the patient's knowledge of diabetes and nutrition, using eight selected questions in each of these two categories is assessed. Maximum number of credits which can be obtained is 30, with the practice goal of at least 25 credits. The plan calls for annual reassessment in each patient, with the expectation of improvement in achieved number of credits.
We enrolled patients with diabetes mellitus during their scheduled office visits. The program was carried out without interfering with the normal flow of the office practice and did not require additional financial support. The body weight and blood pressure were checked at every office visit. HbA1c was measured at least three times per year, and the lipid profile at least once a year. Other parameters were checked as appropriate.
Results: A total of 613 patients with diabetes have been enrolled thus far (310 women, 303 men, mean age 59.91±13.57 years). Although the program is still in progress, interim analysis of the data demonstrates high rate of implementation of guidelines: complete physical examination in 94% participants; foot examination 89%; dilated eye examination 75%; HbA1C < 6% in 26%; < 7% in 58%, < 8% in 82%, serum triglycerides (nonfasting) < 200 mg/dl, 61%; LDL-C < 100 mg/dl, 61%, HDL-C > 35 mg/dl 76%, anti-platelet therapy 60%; quantitative urinary albumin screening 74%, and use of ACEI or ARB in 44%. The mean credit score was 19.5.
Conclusion: The credit system, which has been enthusiastically received by the patients, demonstrates an effective practical means of capturing and implementing necessary parameters that are assumed to lead to optimal diabetes and cardiovascular outcomes. The added advantage is the patients' active participation and their buy-in into continuous improvement.

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Aspirin therapy in patients with diabetes managed in general practice. Are clinical practice guidelines being observed?
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Background and Aims: Patients with diabetes are at increased risk of macrovascular disease and in fact diabetes has recently been designated a coronary heart disease risk equivalent. A number of interventions including management of hypertension and dyslipidaemia, ACE inhibition and aspirin therapy are effective in decreasing this risk and guidelines have been widely published advising appropriate management strategies. In 2002 the Sydney Diabetes Unit of The Royal North Shore Hospital established a Health Assessment Unit (SDHAU) to provide an annual health review of diabetic patients managed by general practitioners. In addition, information was available of the management of diabetic patients in a routine hospital out-patient clinic (OPD). In this report we describe the aspirin use of the first 168 patients (104M, 64F) screened in SDHAU and of 914 (457M, 457F) patients from the diabetes OPD and compare these data to the recently published clinical practice recommendations of the American Diabetes Association.
Results: Of the 168 SDHAU diabetic-patients, 156 (93%) had T2DM, and of the 914 OPD patients 643 (70%) had T2DM. The patients with T2DM form the basis of this report.
Established cardiovascular disease (CVD) was present in 20% (n=31) of the SDHAU patients and in 28% (n=181) of OPD patients. Of these patients 35.5% (n=11) and 54% (n=98) respectively were regular aspirin users. 79% (n=123) of HAU patients and 60% (n=370) OPD patients had at least one cardiovascular risk-factor (obesity, smoking, hypertension, dyslipidaemia or abnormal albumin excretion). Of these, 21% (n=26) and 18% (n=66) respectively were regular aspirin users.
Two-percent (n=3) HAU and 7% (n=42) OPD patients were aged greater than 30 years and did not manifest CVD or any CVD risk factors. 1 of 3 HAU patients and 4.7% (n=4) OPD patients were on regular aspirin therapy.
Conclusion: Despite well publicised clinical practice recommendations and a substantial evidence base, aspirin use is underutilised in patients with established CVD and in those at high-risk of a cardiovascular event, both including patients managed in general practice and those managed in a tertiary referral centre. Reasons for this management gap will be explored. This is the first study to compare aspirin use in patients with T2DM managed in two different out-patient settings in Australia.

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Effects of five-year lifestyle modification on patients with Type 2 diabetes: interim report of the Japan Diabetes Complications Study (JDCS).
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Background and Aims: Short-term lifestyle interventions reportedly contribute to moderate improvement in glycemic controls in patients with established type 2 diabetes. However, their long-term effects remain to be determined in a randomized controlled study. The ultimate goal of JDCS is to determine whether long-term lifestyle intervention can improve glycemic control and prevent complications in patients with type 2 diabetes.
Methods: JDCS is an ongoing randomized, controlled, multi-center, prospective intervention trial with 2205 patients with previously diagnosed type 2 diabetes from 59 Japanese institutes that specialize in diabetes care. The lifestyle intervention program includes intensive lifestyle management at each outpatient clinic visit and telephone counseling. The intervention group receives educational materials about the importance of lifestyle changes, a diary to record the progress of laboratory data, and a pedometer. Parameters related to glycemic control, diabetic complications, dyslipidemia, hypertension and obesity are measured several times a year.
Results: Small but significant differences in HbA1c levels between the intervention (INT) and conventional (CON) therapy groups were seen as early as two years after the start of intervention and were maintained in the fifth year (CON group, 7.69±1.31% vs. INT group, 7.53±1.17%; initial HbA1c levels, 7.80±1.42% and 7.68±1.28%, respectively). Frequencies of CHD and stroke in the diabetic patients (6.1 and 5.3/1000 patients/year, respectively) were three or more times higher than in individuals without diabetes. LDL cholesterol and HbA1c were significant risk factors for CHD and high blood pressure, whereas plasma insulin levels were significant risk factors for stroke in the diabetic patients. Compared with white diabetic patients in the UKPDS, patients in the JDCS had a much lower Body Mass

Index (BMI) (29.4 for UKPDS vs. 23.1 for JDCS), although both patient groups were similarly matched in age, glycohemoglobin A1C level, and disease duration. Moreover, whereas the mean BMI of white diabetic patients was higher than that reported for non-diabetics of the same ethnic origin (BMI 24.1), the mean BMI of Japanese diabetic patients was normal as compared with the Japanese non-diabetic population (BMI 22.7).
Conclusion: The effect of lifestyle modification on improving the glycemic control of patients with established type 2 diabetes is moderate but significant even five years after initiating the intervention program. (This study is conducted by JDCS group consisting of 59 institutes all over Japan.)

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Diabetes and tobacco smoking : the Diabcare France experience.
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Background and Aims: Tobacco smoking and diabetes mellitus (DM) are both major vascular risk factor. However we lack informations concerning the smoking habits of the french diabetic population and their putative clinical and biological consequences .
Materials and Methods: Based on the data collected on the basic information sheet, every year during the one month national campaign of DiabCare-France from 1992 to 2000 (no campaign in 1999), we studied retrospectively 37164 diabetic patients on the basis of their tobacco consumption (TC) and its possible links with metabolic , micro and macrovascular complications.
Results: The study population consisted in 27529 type 2 (14212 males and 13317 females) and 9635 (5014 males and 4621 females) type 1 diabetic patients . They were compared when possible to a french representative data base of non diabetic subject (1992,93, 95, 99) obtained from the French National Institute for Health and Prevention . In this population TC was significantly lower ($p<0.001$) in diabetic patients (21% in 1993, 16% in 1996) compared to the non diabetic subjects (35%), in male as well as female patients.The known raise of TC in female subjects was not observed in diabetic patients till 1998 (8.5%) to 2000 (10%). Type 2 DM smoked significantly less than younger type 1 (16% vs 32% in 1993, 13% vs 25% in 1998, $p<0.01$) ; in both type of DM there were more male smokers . BMI was significantly lower in type 1 and type 2 smokers (S)(mean(SD), 23.2(3.7)vs 24.7(4.4)- 28.6(5.7) vs 29.3(5.8), $p<0.01$), with a higher waist/hip ratio in smokers (0.99(0.10)vs 0.97(0.14)$p<0.01$,systolic blood pressure was lower in S. Glycaemic control was always significantly higher in diabetic S whatever the levels of HbA1c (8%, $p<0.01$). Both type 1 and type 2 DM had higher total cholesterol and triglycerides concentrations and lower HDL-cholesterol ($p<0.01$). Renal function (ie. micro and macroproteinuria, calculated creatinine clearance) was not different between S and non smokers (NS). Among the St Vincent declaration clinical items (orthostatic hypotension, neuropathy , claudication,angina, anerection) and objectives (blindness, myocardial infarction, stroke, renal insufficiency, amputation in the past 12 months), claudication was more frequent in S vs NS ($p<0.001$) . However the collection of these clinical data may have been biased by the overmortality of the diabetic S and that some declared non-S were in fact ex-S. Type 2 DM S required more hospitalizations (8.7(4.6)vs7.6(3.9)days/year $p<0.02$) , and switched quicker from oral antidiabetic agents to insulin ($p<0.001$). Type 1 and 2 DM S less adhered to patients associations, worsely monitored their blood glucose and had a higher alcohol consumption($p<0.01$)..
Conclusion: Tobacco smoking seems to influence intermediate markors (i.e lipid parameters, HbA1c, visceral adiposity)and DM treatment's compliance which is responsible for a worsening of absolute vascular risk and probably overall mortality of this french diabetic population . Smoking cessation is then a key issue in the diabetic patients.

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Clustering of cardiovascular risk factors in middle aged Iranian subjects with impaired glucose tolerance: Tehran lipid and glucose study.
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Background and Aims: Although clustering of cardiovascular risk factors in persons with diabetes is well known, it is not well defined in persons with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). This study has been conducted to gain insight into the clustering of cardiovascular risk factors in persons with IGT.
Materials and Methods: From among 15005 urban individuals, 3-69 years old, chosen by cluster random sampling in the cross-sectional phase of a longitudinal study conducted in the east of Tehran, the database of all 3142 persons aged 30-50 years was used for this study. Known diabetics and smokers were excluded. Fasting blood glucose after 12-14 hours overnight fasting and blood glucose 2 hours after ingestion of 75 gr glucose were measured. Blood pressure, weight, height, and hip and waist circumferences were measured according to standard protocols. BMI and WHR were calculated. We used WHO criteria to define obesity and abdominal obesity. High blood pressure was defined using the sixth report of Joint National Committee (JNC VI) criteria. High lipids were defined using ATP III criteria. Based on WHO criteria, 159 diabetics (group 1), 360 persons with IGT (group 2) and 54 persons with IFG (group 3) were enrolled into the study. Of 2569 healthy subjects, we randomly selected 469 age and sex matched persons for controls (group 4).
Results: The prevalence ratios were as follows: obesity 1.6, 1.6 and 1.2; abdominal obesity 1.4, 1.3 and 1.0; hypertension 2.2, 1.9 and 2.1; hyperchlesterolemia 2.0, 1.9, and 1.5; hypertriglyceridemia 2.2, 1.4 and 1.0; high LDL-C 1.3, 1.4 and 1.3; and low HDL-C was 1.3, 1.0 and 1.2 times higher in groups 1, 2 and 3 than the control group, respectively. Three risk factors were seen in 29.6%, 27.5%, 24.1% and 17.3% and 4 risk factors in 21.4%, 14.2%, 16.7% and 10.4% of groups 1, 2 and 3, respectively.
Conclusion: The prevalence and clustering of cardiovascular risk factors in middle aged persons with IGT and IFG is almost similar to that seen in diabetics.

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Benefit of cooperation in diabetic care teams by using a network computer system.
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Background and Aims: Diabetic population is estimated approximately 7 million in Japan, but the number of the diabetes specialists is limited as to take care of such many patients. We need an efficient tool on corroboration between the practitioners and a diabetes center especially to share sufficient information of patients. We have been using computers at the diabetic outpatient clinic since 1989. Recently, we have developed an electric patient record (EPR) system by a popular database software, FileMaker with personal computers in order to administrate the patient data, and to share the data within diabetes care team to provide more effective education. The system which we have developed is simple and does not require any special computer programmers. However the number of data fields is more than 650 in total with 19 relational files. In order to elucidate the capacity for communications with practitioners who refer patients to our diabetes clinic, for patient education, and protection of privacy of the clients, we examine the function of this system.
Materials and Methods: The data of more than 6,000 patients were collected. Since the decision path of SDMJ (Japanese Version of Staged Diabetes Management) is included in the system, on referring a patient back to his/her practitioners, we attach an appropriate page of the decision path, in which stage the patient stands. We need to show our clinical decision making including algorithm of insulin adjustment to consumers much clearly because along with a practitioners we follow the insulin requiring patients once a month at least for 6 months. For protection of privacy of the clients, FileMaker system does not yet have an encryption technology and its password system is not enough for the complete protection. Therefore, we erased the private data such as address, telephone number against theft. We stopped using stand-alone system and provided a client/server system by FileMaker server5.5 and Windows 2000 server.
Results: It was found that the staff physicians of the diabetes clinic could make a medical letter to a practitioner within ten minutes by our new system because of its commuter auto-function. The level of HbA1c of the patients who were referred decreased 0.9% in average for over the period of more than one year even after the patients returned to their own practitioners without providing the decision path of the SDMJ. It was found that we can share sufficient information for diabetes education without printed matter. Current server technology especially Active-Sync is helpful for protection of the data. In addition, we also added USB chip for mechanical protection. The cost for this system was less than 10,000 US dollars in total.

Conclusion: Diabetes is a unique chronic disease which needs a life long, close alliance between a patient and health care providers. Diabetes is a suitable area to adopt digital record system. Significant potentials of computers for this purpose should be recognized. The capacity of this system is significant especially in communications with practitioners, education of patients, and protection of patients' privacy.

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Clinical Care: Psycho-Social Aspects

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Support and relationship of spouse in persons with diabetes mellitus: a follow up study.

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Background and Aims: Considering that diabetes mellitus makes physical, emotional and financial demands, we examined the support received from the spouse in living with diabetes mellitus
Materials and Methods: At baseline 248 adults (144 men and 104 women; age 48.88+/-11.48 yrs; duration of diabetes 5.83+/-6.43 yrs and fasting plasma glucose 166.67+/- 60.39 mg/dl) with clinical type 2 diabetes mellitus were recruited into a follow up study (visit at baseline, end of 6 mo, 12 mo, and 24 mo), to examine the support and relationship received from the spouse. Among the sample, 182 had come for only one visit (106 men, 76 women), 26 for two visits (13 men, 13 women), 22 for three visits (12 men, 10 women) and 18 for all four visits (13 men, 5 women).
Results: There was no significant difference in the mean age of patient, of spouse, in duration of diabetes or fasting plasma glucose among persons at different visits. We assessed the following aspects at baseline and during each visit: (1) attitude of spouse towards (a) the disease and (b) the spouse with diabetes, (2) support given to person with diabetes in adhering to (a) diet (b) exercise, and (3) the emotional support offered such as (a) conversing about the disease, (b) sharing anxieties (c) offering support in stress during the period preceding the visit. In the first visit greater percent of patients who have visited 4 times report that their spouses feel diabetes may be chronic but is manageable (Chi-square = 38.81,p<.01). The spouses of the patients who have visited once and those who have visited 4 times report that their spouses with diabetes require minimum support. (Chi square = 37.07, p<.01). There were significant differences in emotional and social support provided by conversing about the disease (Chi square = 110.84, p<.01) and sharing fears and anxieties about the disease (Chi-square = 84.45, p<.05). Patients who visited only once receive more of this support. In follow up fewer percent of spouses feel that diabetes is a chronic but manageable condition. This was seen at the second visit for patients who have visited 3 times (CR=6.73, p<.01) and 4 times (CR=5.79, p<.01). Lesser percentage of spouses felt in the second visit that their spouses with diabetes require minimum support among patients who have visited 4 times (CR=2.61, p<.01). However, greater support of their spouses was sought for taking medication at the 2nd visit (CR=2.89, p<.01) and the 4th visit (CR=2.61, p<.01). More patients converse about their disease at the second visit (the differences are significant for all the 4 groups of patients). The support of the spouses was also sought for coping with stressful experiences. We show that in Asian Indians significant social and emotional support is sought and given by the spouse of persons with diabetes mellitus. However patients were more independent in self-care management (i.e. exercising, and taking medicines).
Conclusion: These findings allow us to perceive the interdependence of the person with diabetes and the spouse in living with the disease. Education, support and counseling can be concentrated on this particular unit of the family.

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The relation of family environment with glycemic control and diabetic complications in elderly patients with Type 2 diabetes mellitus.

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Background and Aims: Type 2 diabetes mellitus (DM) in older people is a growing medical problem. Impact of socioeconomic status of older people on diabetes control and complications has not been well studied. We examined whether living alone or with family members affects glycemic control and the development of diabetic complications in elderly patients with type 2 DM.
Materials and Methods: A total of 147 (91F/ 56 M) ambulant patients with type 2 DM aged ≥ 70 yr (age 77.6 ± 0.9 yr, BMI 19.8 ± 0.5 kg/m^2) were studied. The patients were subdivided into the following 2 groups. Group I, 35 patients live alone; group II, 112 patients live with spouse or other family members. Glycemic control was assessed by HbA1c. Diabetic

retinopathy, nephropathy and neuropathy were evaluated by fundoscopic examination by an ophthalmologist, urinary albumin excretion rate, and clinical neurological examinations. Cardiovascular disease was assessed by history of cardiovascular disease and ischemic changes in ECGs. Their diet was assessed by 3-day diet diary. Adherence to medications and exercise, and the level of satisfaction with their diabetes management were assessed by a questionnaire.
Results: There were no significant differences in age and the duration of diabetes between the 2 groups. HbA1C was higher in the group I (7.7 ± 0.6 %) than in the group II (6.5 ± 0.7%). The prevalence of diabetic retinopathy and neuropathy in the group I was higher than those in the group II. However, prevalence of hypertension, nephropathy and cardiovascular disease did not differ between the 2 groups. Compared with the group II, the group I patients revealed poor adherence to diet and medication, and less satisfaction with their diabetes self-management. They perceived more often that their qualitiy of life was impaired by a burden of diabetes management. Even when selected patients with equivalent HbA1c were compared, the group I patients had less satisfaction with self-management and stronger feeling of burden of illness.
Conclusion: Living alone or with family members strongly influences diabetes self-management and diabetes complications in elderly patients with type 2 DM

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Adherence to treatment in patients with Type 2 diabetes mellitus and its relationship with cognitive function.
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Background: Adherence to treatment is a determinant factor to metabolic control, and different factors as social support, stress, depression has been related with no adherence. In addition different studies has suggested that the patients with type 2 diabetes mellitus (DM) have higher risk of poor cognitive function.
Aims: To evaluate the association between adherence to treatment and cognitive function (CF) exploring four domains short-term memory, attention, language, and flexibility.
Materials and Methods: We carried out a cross-sectional study in 96 patients with DM, between 40-60 years old, they were separated in three groups Group 1, men (M); Group 2, premenopausal (PM); and Group 3 women menopausal.(WM). each one with n=32. By mean of questionnaire were obtained psychosocial variables: adherence to treatment, social support, belief in conventional medicine, depressive mood, denial illness. The CF test included: *Wisconsin Card Sorting Test*- flexibility to strategy change, *Sternberg* – memory short-term, *Stroop* - language and reading, *Trial Making* – attention, concentration and vigilance, *Continuous Execution*- visual attention, selective and vigilance , *Verbal Fluency*- language explore. Scores were obtained as corrects, incorrect and reaction time; measured in milliseconds (ms).
Results: The metabolic control was different between the groups, fast glucose and HbA1c respectively M (171±54), (10.3±2.4); PM (190±65), (11±2.3); WM (167±58), (10.5±2.7). Psychosocial variables showed differences between groups to social support p=0.03, depressive mood p=0.02. Adherence to Diet was associated with Stroop reaction time 1 (β=–0.32, p=0.002), corrects 2 (β=-0.34, p=0.001); Continuos Execution, in alarm false (β=-0.38, p=0.03) Trial Making corrects (β=–0.05, p=0.01). Adherence to medication was associated with Stroop corrects 1 (β=0.22 p=0.04), success 2 (β=-0.33 p=0.0004), reaction time 2 (β=–0.31, p=0.01); Sternberg log. Incorrect, (β=–0.48, p=0.03), Cont Exec 1 log corrects (β= 0.39, p=0.004); Trial Making (β=0.45, p=0.01). In addition the Cognitive Function Tests were associated with years since diagnostic and age of the patients.
Conclusion: Adherence to treatment was associated with some aspects of the FC. Adherence to diet were associated with the cognitive tasks Stroop, Continuous Execution and Trial Making tests. Adherence to medication was associated with Stroop, Sternberg, Continuous Execution and Trial Making test. The CF test were associated with year since diagnosis and age of the patients.

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Health related quality of life in elderly Type 2 patients with or without long-term diabetic complications.
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Background and Aims: The greater life expectancy and the increased prevalence of diabetes mellitus (DM), prompted us to study: Health Related Quality of Life (HRQOL) in elderly patients with or without long term diabetic complications and identify any potential correlation with regard to metabolic control, associated conditions (hypertension, cardiovascular disease, hyperlipidemia and obesity) or social parameters.
Materials and Methods: One hundred and five type 2 diabetes patients: 58 males and 47 females; mean age 63.9 ± 8.4 years; mean duration of diabetes 11.8 ± 8 years; 52.4% on oral agents, 15.2% on insulin, 28.6% on combined therapy and 3.8% on diet, volunteered to answer a validated Spanish version of the DQOL, a specific questionnaire for diabetes. Seventy-two out of these 105 answered the EuroQOL, a generic questionnaire, as well. Glucose control was evaluated by means of the last two available HbA_{1C} values with an average of 7.3 ± 1%. Twenty four percent of the included patients, showed long-term diabetic complications. Patients on antidepressants and/or terminal serious diseases non-related to DM were excluded. All data were tabulated for analysis using the SPSS/PC+ statistical package. A "α" type error value ≤0.05 was declared statistically significant.
Results: Social/vocational worry and worry relative to the DM subscales were significantly correlated with DM duration, long-term diabetic complications presence, HbA_{1C}, worse metabolic control and complexity of treatment. Women were seen as more worried than men, and in the EuroQOL, they perceived more pain and anxiety. Likewise, in the analog scale, they felt worse. However, no correlations were shown between satisfaction and impact subscales with specific diabetic complications. Significant differences were not shown in any of the subscales with regard to other clinical associated conditions or working status (employed, unemployed or retired). A cluster analysis showed that 70% of this patient population felt satisfied; the disease had little impact, and they were only moderately worried about the DM.
Conclusion: Although HRQOL in elderly people with diabetes in general terms are good, the complexity of the treatment, the duration of the DM, the presence of specific complications and gender (women), impair HRQOL perception. Therapeutic interventions aimed to correct modifiable parameters can improve the perceived quality of life of these patients.

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The multinational *Men's Attitudes to Life Events and Sexuality* (MALES) study: the influence of diabetes on perceptions of erectile function, attitudes and treatment patterns in men with erectile dysfunction (ED).
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Background and Aims: The objective of the MALES study was to identify prevalence of ED and related health issues in the general population in Europe, North and South America, and to examine the attitudes and behavior of men in relation to these health issues. The present study examined the relationship of self-reported diabetes on mens' perceptions of erectile function (EF), and to patterns of care in relation to ED treatment.
Materials and Methods: From a total of 27,838 men aged 20 -75 years interviewed in 8 countries between February 2001 – April 2001, 3,289 subjects self-reported as having ED and completed a second questionnaire focusing on erectile function and treatment seeking for this condition. Eighty percent of men were recruited through computer-assisted telephone interviewing (CATI) and the remainder by Internet interviews via email

invitation. A standardized questionnaire was used in all cases and the interview lasted approximately 15 minutes. Data were stratified by self-reported history of diabetes and are presented categorically
Results: Of the 3,289 respondents, 544 reported diabetes, 2,624 did not, 121 did not respond. Men with diabetes were almost twice as likely to describe their ED as permanent (68% vs 39%, p<0.0001); were more likely to have discussed their ED with an physician (73% vs 57%, p<0.0001); were more likely to have tried sildenafil more than once (33% vs 21%, p<0.0001), and, of those using sildenafil more than once, more likely to reduce or discontinue it's use (48% vs 38%, p=0.02). Patients with diabetes were more likely to have discontinued or reduced sildenafil as a consequence of reduced efficacy, compared to male non-diabetics (see Table):
Conclusion: Diabetes conferred greater ED severity relative to those without diabetes. These individuals were more likely to seek medical professional assistance and to have tried PDE-5 inhibitor therapy, but they were also more likely to be unsatisfied with sildenafil use, suggesting a need for alternative therapies.

Data expressed as n(%) Based on patients using sildenafil more than once and either reducing or disc

Reason for discontinuing or reducing sildenafil	Diabetics (n=84)	Non-diabetics (n=200)	P-value
It did not work at all	34 (41%)	27 (14%)	<0.003
It only worked occasionally	21 (25%)	34 (17%)	0.1195
My erection was not hard enough	36 (43%)	50 (25%)	0.003
I experienced side effects when I took it	16 (19%)	31 (16%)	0.4628

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The ENTRED study: characteristics of hospitalisations of people treated for diabetes in France, 2001.
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Background and Aims: Hospitalisation-related costs represent almost half of the direct costs of health care received by persons with diabetes in France. We aimed at describing the characteristics of hospitalisations of persons treated with diabetes, and comparing characteristics of those hospitalised to those of others.
Materials and Methods: Based on the administrative database of the national health insurance system that covers all employees in France, 10,000 adults were randomly selected among those reimbursed of a treatment with insulin and/or oral antidiabetic agent in October-December 2001. All hospitalisation-related claims for 2001 were analyzed.
Results: In 2001, hospitalisation claims had been notified for about 2,683 of the 10,000 persons with diabetes (27%), which represented a total of 8,662 hospitalisations and 38,864 days of hospitalisation. Among persons for whom at least 1 hospitalisation claim was notified, an average 3.2 hospitalisations per patient were recorded, corresponding to an average length of 4.4 days per hospitalisation and a total of 14 days per patient per year. Hospitalisation claims were notified by different medical departments: 6% in endocrinology (14% of hospitalised patients), 19% in nephrology (2% of patients), 36% in internal medicine (51% of patients), 22% in surgery (44% of patients), 2% in emergency (4% of patients), 4% in intensive care (7% of patients) and 11% in convalescence (9% of patients). Persons for whom at least 1 hospitalisation claim was notified, compared to others, were somewhat older (65 vs 64 ys, p=0.0003). In a model adjusted for age, they were more often reimbursed for insulin than oral hypoglycaemic treatment (Odds Ratio=2.9 [2.6-3.2]), cardiovascular drug (OR=1.4 [1.2-1.5]) and at least 1 endocrinology visit (OR=1.5 [1.3-1.7]), than people with no hospitalisation claim.
Conclusion: At least 1 in 4 persons with diabetes is hospitalised every year in France. While hospitalisations in endocrinology are not common, about 1 in 5 hospitalisations is notified by nephrology departments, corresponding to few patients but underlying the high burden of dialysis on economic costs. A sub-study of ENTRED is currently being implemented among French public hospitals and private clinics using mailed patient, provider and hospital questionnaires in order to 1) measure a potential claim under-notification from public hospitals, 2) investigate causes of hospitalisations (diabetes- or non-diabetes-related, check-up or complication), 3) compare health status of persons who have been hospitalised to that of others, 4) evaluate hospitalisation-related costs among persons with diabetes.

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Reliability of hemoglobin A1c measurement in patients suffering of sickle cell disease.
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Background and Aims: The use of hemoglobin A1c (HbA1c) for the diagnosis of diabetes mellitus is currently being discussed. Nevertheless,its interpretation can be difficult in populations with high prevalence of hemoglobinopathy (Africa, West Indies). Little is known about that problem.The reliability of HbA1c measurement in these particular populations was assessed in a Parisian hospital where 10% of the patients tested for Hba1c have a hemoglobinopathy. HbA1c and fructosamines were determined in diabetic and non diabetic patients suffering of sickle cell disease and compared with results from patients without hemoglobinopathy.
Material and Methods: HbA1c and fructosamines tests were performed on the same sample. Screening for abnormal hemoglobin and HbA1c measurement were performed at the same time with an HPLC method (A1C2.2 Tosoh bioscience). The percentage of HbA1c was calculated in relation to HbA0 and not in relation to the total hemoglobin. Fructosamines and plasma proteins concentration were determined with Roche reagents. As fructosamines are a marker of circulating glycated proteins, the ratio fructosamines /proteins was calculated to exclude a modified proteins turn-over with high or low concentrations of proteins. Two groups of patients were studied : 41 subjects (26 men and 15 women) homozygote for HbAA, mean age 52 years (range: 31- 76) (group 1) and 41 subjects with the variant HbAS - between 30 and 40% of HbS -(24 men , 17 women) mean age 51years (range : 26 - 70) (group 2).
Results: HbA1c (m ± sd) was 7.3 ± 2.3% in the group 1 and 7.0 ± 1.8% in the group 2 respectively. Fructosamines concentrations were respectively 264 ± 85 µmol/l and 258 ± 59µmol/l. Fructosamines/proteins ratios were 3.35± 0.69 and 3.59 ± 0.71. With the Mann and Whitney test we did not find any significant difference between the two groups for these 3 parameters. A bivariated regression graph exhibited a similar relationship between HbA1c and fructosamines in the two groups.
Conclusion: These results show that HbA1c measured by an HPLC method and calculated in relation with HbA0 is as reliable for patients with sickle cell disease as for people without hemoglobinopathy. If HbA1c measurement was proposed in the future as a tool for screening and diagnosis of diabetes, it could be used in populations where sickle cell disease is frequent as well as in Caucasian populations.

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Glucose monitoring at extended postlunch time: a global assessment for quality and safety of control in Type 2 diabetic patients.
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Background: Glucose self-monitoring in non-insulin-treated type 2 diabetic patients remains questionable. The present study was conducted in order to know whether the monitoring of one particular glucose value can serve as global indicator for both the quality and safety of metabolic control in such patients.
Methods: In 484 non-insulin-using type 2 diabetic patients, plasma glucose (PG) concentrations were determined at fasting (8:00 h) and during postprandial (11:00 h and 14:00h) or postabsorptive (17:00 h) periods. At each time point ROC curves were used to select the optimal cut-off PG value that ensures maximum sensitivity and specificity for predicting HbA1c<7%. Percentages of patients at risk of hypoglycemia, i.e. below a 5.0 mmol/l PG concentration at 17:00 h, were calculated after validation of this threshold from CGMS data.
Results: ROC curves showed that the best sensitivity and specificity and the optimal PG cut-points were obtained at 11:00 h (11 mmol/l), 14:00 h (9 mmol/l) and 17:00 h (7 mmol/l). On the contrary, PG at 8:00 h were less reliable (p<0.005) for predicting HbA1c<7%. The proportions of patients with PG<5.0 mmol/l at 17:00 h were higher in patients with HbA1c<7% than in those with HbA1c>=7% (38.1 vs 3.6%, p<0.0001).
Conclusions: Glucose monitoring at 17:00 h appears as a global marker of control in non-insulin-using type 2 diabetic patients. Striving to maintain this PG value between 5.0 and 7.0 mmol/l seems recommended for minimizing the risk of hypoglycemia while ensuring the quality of the diabetic control.

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Correlation of HbA1c with daily fluctuation of blood glucose in insulin treated patients with Type 2 diabetes mellitus (T2 DM).

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Aim: To evaluate the relative value of plasma glucose (PG) measurements at different daily spots in assessing glucose control of T2 DM.
Materials and Methods: In 40 insulin treated patients, mean age 64±10yr (18 male, 22 female), plasma glucose were obtained at pre-breakfast (8:00AM), 2 h post-breakfast, pre-lunch (13:00PM) and extended post-lunch (18:00PM). The measurements of glucose and HbA1c performed at 2, 4 and 6 months after the initiation of insulin treatment.
Results: Pre-breakfast, 2 h post-breakfast, pre-lunch and extended post lunch plasma glucose values were all significant correlated with HbA1c, but the extended post-lunch plasma glucose had the most significant correlation. The multiple linear regression analysis demonstrated that only the extended post-lunch plasma glucose correlated significantly and independently with HbA1c ($p<0.01$).
The extended post-lunch plasma glucose values were lower than pre-breakfast values (122±49 mg/dl vs 141±55 mg/dl, $p<0.01$) in patients demonstrating good glycemic control (HbA1c < 7%), there was no difference (182±46 mg/dl vs 184±50 mg/dl, p=NS) in patients demonstrating fair glycemic control (7%< HbA1c< 8.5%), whereas the extended post-lunch values were higher than pre-breakfast in patients demonstrating poor glycemic control (HbA1c > 8.5%).
In addition the extended post-lunch values demonstrated better sensitivity (77%), specificity (86%), negative (92.7%) and positive (62.5%) prognostic value for good glycemic control based on HbA1c.
Conclusion: In insulin treated patients with T2 DM the extended post-lunch plasma glucose values are better predictors for good glycemic control than pre-breakfast, post-breakfast, and pre-lunch values.

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The effect of glucose monitoring in insulin requiring subjects with Type 2 DM. Diabetes Outcomes in Veterans Study (DOVES).

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Background and Aims: DOVES is a prospective observational study of factors which alter glucose control in a population with established, insulin requiring, stable Type 2 DM. This study was conducted in three Medical Centers in the US. A substudy of this project involved examination of the effect of frequent glucose monitoring on glucose control.
Materials and Methods: Subjects were asked to monitor their blood glucose (SMBG) 4 times a day using Accu-Check Complete monitors for 8 weeks. The Accu-Check monitors were down-loaded and the values recorded at 4 and 8 weeks. No changes in therapy were done during this study unless values exceeded patient safety standards. Compliance was defined as the number of reading obtained divided by the number specified by the protocol. Statistical analysis included chi-square analysis and unpaired students t-test for group differences, within-subject changes by paired t-test and repeated measures analysis of variance. Multiple linear regression was used to examine associates.
Results: Of 218 subjects entered the study, 17 patients were excluded because of failure to comply appropriately. A total of 35,499 (79%) of the expected glucose measurements were made by 201 subjects included in the final analysis. Although no additional treatments, recommendations, referrals nor feedback were given as a result of participation in this study, SMBG was associated with a significant and sustained decline in HbA1c. The decrease was 0.30 ± 0.68% ($p<0.001$) at 4 weeks and 0.36 ± 0.88% ($p<0.001$) at 8 weeks. The decrease was sustained (0.32 ± 1.17% $p<0.001$) in 152 subjects followed for 52 weeks. Subset analysis showed that these improvements were confined to subjects with entry A1c levels greater than 8% or compliance greater than 75%. Entry HbA1c levels and compliance were strong predictors of 8 week A1c ($r=0.862$ $p<0.001$). Compliance ($p<0.001$), carbohydrate intake ($p=0.005$) and daily insulin dose ($p=0.015$) were the most influential determinants of week 8 HbA1c levels. The age, gender, BMI, level of exercise, or use of oral agents had no effect on the week 8 HbA1c. Not all measurements were required for prediction of week 8 A1c levels. Intensive measurements (4x daily) for one week (either 4,6,or 8 weeks) or rotating once daily glucose measurements provided near equivalent predictive value.
Conclusion: Glucose monitoring in insulin requiring patients with Type 2 DM improved glucose control in spite of no interventions based on results. HbA1c levels decreased with improved compliance and increased with higher carbohydrate intake and daily insulin dose. Glucose control can be improved by glucose monitoring. HbA1c levels can be predicted by selective SMBG.

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Continuous glucose monitoring in Type 2 diabetes and the relationship between post-prandial hyperglycaemia and glycated haemoglobin.

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Background and Aims: Blood glucose is usually measured in the fasting state for home monitoring in type 2 diabetics. Glycated haemaglobin (Hba1c) is thought to relate to the mean blood glucose in the 6 week period prior to sampling. Using the Medtronic Minimed® continuous glucose monitoring system (CGMS) we aimed to investigate in type 2 diabetics the variation in interstitial glucose which is closely related to blood glucose and hence the relationship between blood glucose, fructosamine and Hba1c with special reference to post-prandial hyperglycaemia.
Materials and Methods: 23 type 2 diabetics agreed to undergo continuous glucose monitoring using the Medtronic Minimed® (Medtronic®, Sylmar, CA, USA) system. Data was collected over a minimum of 3 days with a median number of glucose measurements comprising 840 (min-max 635-993). Patients also measured their own finger prick capillary blood glucose at least 4 times every 24 hour period. CGMS measures interstitial glucose concentrations every 5 minutes. A venous blood sample was collected on the first study day to measure Hba1c (DCCT aligned HPLC method) and fructosamine (nitroblue tetrazolium method). Regression coefficients (method of least squares) were calculated for both fructosamine and Hba1c compared with calculated mean blood glucose (MBG), fasting blood glucose (FBG), variation of blood glucose (MAGE using the method of Mirouze et al) and the percentage of blood glucose values over 10 mmol/l (BG >10 mmol/l). Nonparametric statistics using the Spearman Rank Correlation coefficient (SRC) were used to compare Hba1c with calculated values i.e. MBG and BG > 10 mmol/l.
Results: All parameters of blood glucose produced stronger positive correlations with Hba1c than with fructosamine. Hba1c measurements were positively correlated with fructosamine measurements $r= 0.78$ $p<0.0001$. The strongest correlation was between the percentage of blood glucose readings above 10 mmol/l and Hba1c $r= 0.86$ $p<0.00001$. For MBG, FBG and MAGE positive correlations with Hba1c were $r= 0.78$ $p<0.004$, $r= 0.55$ $p<0.002$, $r= 0.39$ $p< 0.0001$ respectively. Using SRC BG >10 mmol/l & MBG correlated with Hba1c; $r_s= 0.65$ $p<0.0005$ & $r_s= 0.53$ $p<0.015$. All patients showed considerable post-prandial glycaemic excursions not suspected from their MBG or FBG values.
Conclusion: All Type 2 diabetics investigated showed considerable post-prandial glycaemic excursions measured by CGMS which would not have been suspected from either individual patients MBG or FBG. Hba1c was most strongly related to the percentage of each patients blood glucose measurements above 10 mmol/l. This suggests that the highest blood glucose concentrations contribute more to the glycosylation process and to the absolute Hba1c than do the MBG as is currently believed. Therefore reducing post-prandial blood glucose values seems a good way of lowering Hba1c and thereby diabetic complications which are clearly related to high Hba1c concentrations.

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A population-based comparison of treatment patterns and glycaemic control in Tayside region in Scotland for 1997 and for 2001.

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Background and Aims: To compare population treatment and glycaemic control patterns of all patients with type 2 diabetes in Tayside, Scotland before and after publication of the United Kingdom Prospective Diabetes Study (UKPDS) in 1998.
Materials and Methods: Cross-sectional study, using linked databases containing demographic, prescriptions, morbidity and biochemical data for all 6,550 Tayside patients with type 2 diabetes in 1997 and 8,686 patients in 2001.
Results: Mean age and diabetes duration (66±13 years and 7.8 years respectively in 1997) were similar for both cohorts. The number of patients

treated with diet alone decreased from 36.2% to 27.7% (p= <0.001). Oral hypoglycaemic therapy (alone or in combination with insulin) increased from 50.0% to 57.7% of patients (p<0.001), and insulin therapy (alone or in combination with oral agents) increased from 14.0% to 16.1% (p<0.001). Metformin use increased from 22.4% to 30.9% (p<0.001). Total sulphonylurea use increased from 34.8% to 37.1% (p=0.00417) but sulphonylurea monotherapy remained unchanged. The mean daily dose prescribed also increased for both metformin (p=0.0063) and sulphonylureas (p=0.0283). Mean HbA_{1c} (DCCT standardised) increased from 7.5(±1.7)% to 7.9(±1.7)% (p<0.001), increasing across all therapeutic groups. Mean BMI increased from 29.1±5.9kg/m^2 to 29.9±5.9kg/m^2 (p= < 0.001), also increasing across all therapeutic groups.
Conclusion: The increased use of oral hypoglycaemic therapy and insulin, in keeping with the benefits of 'intensive therapy' shown in the UKPDS, has failed to deliver improvements in glycaemic control at the population level.

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Blood ketone and leptin levels in diabetic patients with suspected metabolic disorder in the emergency department.
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Background and Aim: The aim of the study is to investigate plasma leptin and ketone levels in diabetic patients with suspected metabolic disorder during their emergency department visit.
Material and Method: Total 139 diabetic patients (mean age: 58 ± 13 years) presenting to the emergency department with any medical complaint, a high glucose level (≥200 mg/dl) associated with any level blood beta-hydroxybutyrate (ß-HBA) were included to the study. Arterial blood gas analysis, urine ketone dip test, point of care testing ß-HBA levels and leptin levels were measured in all patients.
Results: Total 48 of 139 patients were hyperketonemic (ß-HBA ≥0.42 mmol/L) and 18 of these 48 patients were diabetic ketoacidosis. Sixtythree patients were admitted to the hospital and 9 patients died. Body mass index and age were similar in groups of ketoasidosis, hyperketonemic and normoketonemic (p>0.05). Plasma leptin (p=0.03) and ß-HBA level (p=0.00) were statistically significant different between the ketoasidotic(n=18), hyperketonemic (n=30) and normoketonemic (n=91) patient groups. Mean plasma leptin level was lower in hyperketonemic patients than normoketonemic patients (10.4 ± 13.7 vs. 20.5 ± 19.8, p=0.012). Mean plasma leptin level was not different in hyperketonemic patients and diabetic ketoacidosis group (18.9 ± 22.0 vs. 8.4 ± 11.1, p>0.05), admitted (n=63) and non-admitted (n=76) patients (15.6 ± 17.5 vs. 20.2 ± 19.3, p>0.05) and mortal (n=9) and non-mortal (n=130) groups (13.6 ± 11.2 vs. 14.5 ± 12.0, p>0.05). A logistic regression analysis for hospital admission when evaluated with the predictors (initial values of urine ketone, anion gap, corrected pH, bicarbonate, glucose, leptin and point of care testing ß-HBA level) confirmed that point of care testing ß-HBA measurement was an independent predictor for hospital admission (OR 2.57, %95 CI, 1.07 to 6.01, p=0.03).
Conclusion: Our data suggest that hyperketonemia were associated with decreased plasma leptin level, but plasma leptin level was not associated with hospital admission and survival. Point of care testing ß-HBA level was found the only potential predictor for hospital admission in our diabetic patient population.

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The insulinaemia factor in intensive therapy in Type 1 diabetes: intracellular rather than membrane cause of improved glucose oxidation.
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Background and Aims: The aim of the study was to identify the roles of circulating insulin versus circulating glucose in relation to the improvement in insulin (Si) and glucose (Sg) sensitivity found in Type 1 diabetic patients treated with intensive (IIT) rather than standard insulin therapy (SIT).
Materials and Methods: Two studies were carried out on eight well-controlled fasting type 1 diabetic patients. In random order on separate days, euglycaemia (7.5 + 1 mmol/L, mean + SEM) was maintained for 17 hours overnight by employing either (A) a variable insulin infusion alone, adjusted according to 2-hourly blood sugar levels, (average 6 mU/kg/h, standard-insulinaemia), or (B) a constant insulin infusion (12 mU/kg/h, intensive-insulinaemia) plus 10% glucose infusion as required, as models of standard and intensive therapy respectively. Tritiated glucose (15 mcCi/hr) was also infused during the last 2.5 hours of the overnight insulin infusions, in order to assess glycolytic flux. Needle biopsy of skeletal muscle (vastus lateralis) was then performed to measure intracellular substrates, followed by an insulin-supplemented intravenous glucose tolerance test (IVGTT) to estimate Si and Sg, as we have previously described.
Results: Following the 17 hour infusions, intensive-insulinaemia (IIT) compared to standard-insulinaemia (SIT) caused an increase in insulin sensitivity (Si) [10.2 + 5.3 vs 1.7 + 0.6 x 10-4 min-1 per mU/L, p<0.05] and glucose effectiveness (Sg) [3.1 + 0.5 vs 1.7 + 0.4 x 10-2 min-1, p<0.05] plus a significant increase in total body glycolytic flux (GF; predominantly reflecting glucose oxidation) [12.8 + 0.91 vs 9.2 + 0.4 mcmol/kg/min, p<0.02], but caused no significant change in total glucose storage (GS) [3.9 + 1.1 vs 5.8 + 1.6, p=0. 3]. Intensive- versus standard-insulinaemia was associated with lower intracellular glucose (1.7 + 1.3 vs 3.8 + 0.98 mmol/kg dm) and glucose-6-phosphate (G-6-P) (0.37 + 0.09 vs 0.54 + 0.13 mmol/kg dm). The magnitudes of lowering of G-6-P in the intensive- group correlated (r3 = 0.95) with the increases in GF, compared to standard-insulinaemia.
Conclusion: These results indicate that intensive insulin therapy improves basal glucose disposal (Sg) and glucose oxidation (GF) by effects on intracellular pathways rather than on cell membrane glucose transporters. It is possible that this reversal of the observed impairment of glucose oxidation may contribute to the known reduction of tissue damage found in patients maintained on „intensive" insulin therapy regimens.

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Detection of abnormal metabolites in the brain in patients with Type 1 diabetes mellitus in vivi by magnetic resonance spectroscopy.
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Background and Aims: Impairments of cerebral metabolism have been reported in animals with experimental diabetes mellitus and in diabetic patients. Earlier, we found a decrease of brain levels of N-acetyl-aspartate in diabetic patients suggesting the possibility of neurodegeneration. However, the changes of cerebral metabolites composition in patients with diabetes are not fully characterized. Therefore, the aim of this study was to investigate in vivo the composition of brain metabolites in type 1 diabetic patients without overt clinical signs of cerebral dysfunction.
Materials and Methods: We studied 19 patients with type 1 diabetes (age - 29.5±1.3 years, diabetes duration – 11.5±2.8 years, HbA_{1c} – 8.7±1.9%, data are presented as mean±SEM) and 24 healthy control subjects (age – 32.3±7.4 years). The composition of cerebral metabolites was investigated by ^1H nuclear magnetic resonance spectroscopy in vivo in the occipital and frontal brain regions in both hemispheres. We examined specifically the presence of lactate, lipids, myoinositol, and glucose in the brain. Statistical analysis was performed by Student's test.
Results: The traces of lactate, lipids, myoinositol, and glucose were not detected in the brain in the control group. However, we were able to detect the significant quantities of these metabolites in the brain in 9 patients with diabetes (47.4%). The age of patients with or without the presence of pathological cerebral metabolites did not differ between these two groups – 28.2±3.1 vs. 30.1±2.4 years old, p>0.05. The levels of HbA_{1c} were similar in both groups - 9.0±2.4 vs. 8.5±2.3, p>0.05. Patients with abnormal cerebral metabolites tended to have a longer duration of disease (13.0±3.8 vs. 9.6±2.7 years) and higher plasma glucose levels at the time immediately preceding an examination - 15.2±4.1 vs. 10.5±3.2 mmol/L in those with and without abnormal metabolites in the brain, respectively, although this difference did not reach the level of statistical significance, p>0.05.
Conclusion: We speculate that revealed occurrence of abnormal cerebral metabolites could reflect impairment of cerebral metabolism due to vascular or metabolic disturbances and underlie the development of clinically significant cerebral dysfunction in patients with type 1 diabetes mellitus.

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Marked changes in insulin-like growth factor (IGF) system and the phosphorylation of insulin-like growth factor binding protein-1 (IGFBP-1) during recovery from diabetic ketoacidosis (DKA).

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Background and Aims: The IGF system is known to be perturbed in type-1 diabetes, with characteristic decreases in insulin-like growth factor-I (IGF-I) and increased levels of highly phosphorylated IGFBP-1. This is the first systematic study examining how moderate to severe DKA affects the IGF system and how this changes with treatment.
Materials and Methods: Twelve patients (10 male), admitted with DKA were studied. Plasma samples were collected 4 hourly from admission to 24 hours and then at 36 and 48 hours. Patients were commenced on intravenous insulin after collection of the first sample. Immunoassays for IGF-I, total IGFBP-1 (tIGFBP-1), lesser phosphorylated IGFBP-1 (lpIGFBP-1) and binding protein-3 (IGFBP-3) were performed. In addition 15% n-octyl glucopyrasonide (n-og) - polyacrylamide gel electrophoresis (PAGE) of changes in IGFBP-1 phosphoforms were undertaken.
Results: Following the start of insulin therapy there was a dramatic and significant fall in tIGFBP-1 from 0 hours (851ng/ml) to 4 hours (312ng/ml), [mean fall = 538.6 (95% confidence interval-CI = -1076 to 1.3) ng/ml, p=0.05], but no further significant fall until the end of the study, period at 48 hours [mean = -3.85 (95% CI = -14.6 to 6.88) ng/ml/hour, p=0.45]. Similarly levels of lpIGFBP-1 fell significantly over this time period; at 0 hours lpIGFBP-1 = 104.3ng/ml and at 4 hours = 46.9ng/ml [mean fall = 57.33 (95% CI = -119.6 to 4.95) ng/ml, p=0.07]; but no significant change from 4 to 48 hours [mean = -0.49 (95% CI = -1.09 to 0.11) ng/ml/hour, p=0.1]. n-og gel electrophoresis confirmed this decrease in lpIGFBP-1 with time. The ratio of tIGFBP-1 to lpIGFBP-1 decreased, but not significantly, during the first 4 hours [mean = -5.6 (CI = -12.8 to 1.7) ng/ml, p=0.12]. Mean IGF-I concentration at 0 hours was 140.6ng/ml with no change at 4 hours, 134ng/ml [mean = 6.66 (95% CI = -30 to 16.7) ng/ml, p=0.5]. There was a fall in IGFBP-3 from 0 to 4 hours (3.37mg/L to 3.05mg/L), [mean = 0.31 (95% CI = -0.56 to 0.06) mg/L, p=0.01].
Conclusion: Our results suggest that insulin treatment of DKA significantly increases the bioavailability of IGF-I, predominantly by inducing a rapid fall in tIGFBP-1 but also in IGFBP-3. The presence of lpIGFBP-1 may suggest a homeostatic mechanism to increase IGF-I availability at the times of hyperglycemia, previously reported. These data imply synergy between insulin and IGF systems enhancing the tissue availability of IGF-I when insulin becomes available.

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Physical activity and the metabolic health of children: novel findings in six-year-olds (The EarlyBird Diabetes Study).

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Background and Aims: Insulin resistance is closely related to body mass and underlies the development of diabetes and the metabolic syndrome. With the rising prevalence of obesity, markers for these disturbances are likely to present with increasing frequency in childhood. Physical activity improves metabolic health in adults, acting both to reduce fat mass and to lower insulin resistance independently of fat mass. Little is known, however, of the metabolic impact of physical activity in young children. In this study, we examined the impact of physical activity on insulin resistance and fasting lipids in six year-olds.
Methods: EarlyBird is a non-intervention, prospective cohort study of healthy children (recruited at school entry 2000/01). It aims to define the factors responsible for the development of insulin resistance in growing children and to monitor its impact on their metabolic health. This report is based on 237 children (mean age 5.9 years). Main outcome measures were: body mass (BMI), physical activity sampled over seven days (CSA accelerometer), insulin resistance (HOMA-IR), fasting triglycerides and cholesterol/HDL ratio.
Results: 1) The year-on-year correlation of physical activity, measured by accelerometer, was r= 0.49, p< 0.001, of BMI r= 0.89, log insulin resistance r= 0.53, log triglycerides r= 0.45 and cholesterol/ HDL ratio r= 0.62. 2) BMI was not significantly related to physical activity (r= 0.04) 3) Table 1 - There was no correlation between physical activity and insulin resistance in either gender. There was, however, a modest, and significant, inverse correlation between physical activity and triglycerides in both the boys and the girls. In boys, though not girls, there was also a slight but significant inverse correlation between physical activity and the cholesterol/HDL ratio.

Table 1. The relationship between physical activity and metabolic variables.

Partial correlation variable (controlling for BMI)	Boys (n=129) r (p)	Girls (n=108) r (p)
Log insulin resistance	0.06 (0.53)	0.00 (0.98)
Log triglycerides	-0.18 (0.04)	-0.19 (0.04)
Cholesterol / HDL ratio	-0.21 (0.02)	-0.03 (0.78)

Conclusions: The high year-on-year correlation argues for the robustness of the CSA accelerometer as a measure of physical activity in children. Triglycerides are a specific marker for the group of distubances that constitutes the metabolic syndrome. The small but significant correlations between physical activity and lipids are consistent with the findings already reported in adult and obese adolescent populations - that physical activity is an independent determinant of metabolic health. It is also of importance that physical activity shows a degree of tracking. It suggests the habitually inactive child can be identified at a young age. If the lifestyle of such children is amenable to modification, the long term benefits to metabolic health could be substantial.

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Type 2 diabetes mellitus in children, adolescents, and young adults (CAYA-T2DM): a multicentre collaborative study from north India.

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Background and Aim: Asian Indians are highly predisposed to develop type 2 diabetes mellitus. Childhood obesity may be an important contributory factor for recent increase in the prevalence of type 2 diabetes in young individuals (children and adolescents). In this study we evaluated the clinical, anthropometric and biochemical characteristics of patients with early onset of type 2 diabetes (< 25 y) and compared them with age-matched healthy controls.
Methodology: A multi-center collaborative, cross-sectional design. Type 2 diabetic patients (n=28; 19 males, 9 females, age range 10-28 years) and 59 age-matched healthy controls (31 males and 28 females) were studied. Patients with history of ketosis or ketoacidosis and secondary diabetes were carefully excluded. Body mass index (BMI), waist-hip ratio (W-HR), skinfold thickness at 8 sites, percentage of body fat (%BF) and biochemical analysis were performed.
Results: The mean (±SD) age of onset of diabetes was 18.6±4.6 y (range: 1 mo-11 y). History of diabetes in first-degree relatives was obtained in ~90% of patients. Majority of patients (~46%) were on oral hypoglycemic agents (OHAs) alone, 25% were on a combination of OHAs and insulin, and ~27% were on lifestyle measures alone. Acanthosis nigricans was observed in five patients. Mean fasting blood glucose level was 194.5±88.2 mg/dL (range 76-425 mg/dL), HbA1c was 8.8±2.6 gm% (range 5.8-15.1 gm%) and fasting C-peptide was 1.90±1.40 ng/mL (range 0.5-6.3). A significant inverse correlation was observed between fasting blood glucose and C-peptide levels (r=0.49). After adjusting for gender, mean values of BMI (p=0.001), systolic blood pressure (p=0.01), waist circumference (p<0.001), W-HR (p<0.001), sum of four skinfolds (biceps, triceps, subscapular and suprailiac) (p<0.001), % BF (p<0.001), and mean levels of serum triglycerides (p<0.001) were higher in patients than controls. Higher prevalence of obesity (~43%, p<0.001), excess body fat (~59%, p<0.001), high waist circumference (~21%, p=0.01), high W-HR (~46%, p<0.001), hypertension (~21%, p=0.007), hypercholesterolemia (~21%, p=0.01), hypertriglyceridemia (~39%, p<0.001) and low levels of high-density lipoprotein cholesterol (~46%, p=0.03) were observed in patients as compared to controls.
Conclusion: High prevalence of generalized as well as central obesity in young type 2 diabetics patients emphasizes the need of prevention of childhood obesity in children and adolescents in north India.

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Prevalence of diabetes and glucose intolerance in obese children and adolescents.

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Background and Aims: To asses the prevalence of type 2 diabetes mellitus and glucose intolerance (IGT), in an obese population of children and adolescents.
Material and Methods: Two hundred and three Argentine caucasic obese children and adolescents, assisted in the Department of Nutrition and Diabetes of Buenos Aires Children Hospital between November 2001 and November 2002, underwent a two hour oral glucose tolerance test, and glycemia, insulinemia, lipid profile, uricemia, Tanner stage, BMI, blood pressure, were measured. The presence of Acantosis Nigricans, familial antecedents of diabetes mellitus, dislipidemia, hypertension and obesity were documented. Type 2 diabetes mellitus and IGT were defined according to the ADA criteria. Hypertension was defined following the U.S. Task Force guidelines. Obesity was defined according to Cole et al. BMJ, 2000.
Results: Mean age was 11.76±3.30 years (upper quartile, 13.87); BMI: 28.43±4.58 (upper quartile: 31.00). Six of the 203 obese subjects included (2.96%; Fleiss Quadratic 95% confidence interval: 1.21-6.62%) were diagnosed as diabetic, and other 6 showed IGT. Diabetes was more frequent in Tanner stages 4 and 5; IGT was more common in Tanner 3 and 4. Dysglycaemic patients (5.92%) were more obese than normoglycaemics (BMI 31.03±4.93 vs. 28.28±4.53, p=0.053), with higher both systolic and diastolic blood pressure values (SBP: 119.09±9.17 vs. 107.23±14.39 mmHg, p=0.008; DBP: 77.72±10.57 mmHg vs. 68.31±10.45, p=0.004); ApoA levels were lower in subjects with dyglycaemia (p=0.062); the association between dysglycemia and 1st degree familial antecedents of hypertension was close to be significant (r=0.13, p=0.06). In IGT patients, triglyceridemia was elevated in comparison with normoglycaemic subjects (172.40±190.34 vs. 98.41±54.21, p=0.007) and 20% of them were hypertensive. Fasting insulinemia was also higher in IGT group.
Conclusion: The frequency of type 2 diabetes among obese children and adolescents was close to 3%. IGT was present at a similar rate. Lipid abnormalities were found in dysglycaemic patients, as well as higher blood pressure and BMI values.

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Nutritional intervention strategies for children and adolescents with Type 2 diabetes and the metabolic syndrome using staged diabetes management.

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Background and Aims: Childhood obesity has become the most serious and prevalent nutritional disorder in the United States with over 15 percent of children aged 6-19 years old being overweight. Profound health implications are associated with obesity. They include type 2 diabetes, dyslipidemia, hypertension and renal disease, which constitute a metabolic syndrome whose underlying cause is insulin resistance. Impaired glucose tolerance has been detected in 25 percent of obese children (4-10 years of age) and 21 percent of adolescents (11-18 years of age). Overweight adolescents have a 70% risk of becoming overweight or obese adults.
Materials and Methods: Meta-analysis, peer reviewed research, and pediatric endocrinology opinion were the basis of developing clinical pathways. These Staged Diabetes Management (SDM) DecisionPaths were created to address the detection and treatment of obesity in children and adolescents in order to implement systematic nutritional therapies.
Results: SDM Master DecisionPaths that outline major risk areas for weight management were created to help physicians promote specific behavior change goals with children and adolescents regarding food and activity. These major risk areas include: snacks, drinks, sedentary activity, specific meal times, fruit and vegetable intake, and eating outside of the home. After a brief diet and activity history, a physician can prioritize a risk area and focus on the SDM principles of „replacing,“ „reducing,“ or „restricting“ high caloric foods/drinks and sedentary activity within the DecisionPaths. Table 1 reflects the impact of using these SDM principles.

Table 1. Percent Reduction in Kilocalories Using SDM Weight Management DecisonPaths.

	Example: 6 oz. fried potato wedges/day	% daily reduction in kcal	Example: 6 oz. regular cola/day	% daily reduction in kcal
REPLACE	6 oz. baked potato wedges/day	68%	12 oz. regular cola/day	100%
REDUCE	3 oz. fried potato wedges/day	50%	6 oz. regular cola/day	50%
RESTRICT	6 oz. fried potato wedges are restricted to 2xweek/	Weekly reduction : 71%	12 oz. regular cola is restricted to 2x/week	Weekly reduction : 71%

Conclusion: Obesity in children and adolescents has become an emergent risk factor for many chronic diseases associated with insulin resistance including type 2 diabetes, hypertension, and dyslipidemia. Until further research is addressed with this population, physicians have an important role in specific behavior change goals related to food and activity. SDM Weight Management DecisionPaths for children and adolescents can assist physicians in helping their patients work toward positive, healthy behavior changes that will sustain into adulthood.

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A study of insulin and C peptide levels in children with Type 2 diabetes.

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Introduction: Type 2 diabetes among children and adolescents pose a serious challenge to the community as it is being recognized increasingly. The information about its epidemiology is limited. However, there are no definite criteria for diagnosis of this disorder. It is more a disease of exclusion, hence this study was undertaken to see if any clinical or biochemical evaluation can help us to classify Type 2 Diabetes Mellitus in children.This study was conducted in Karnataka, a South Indian state.
Aim: To study Type 2 Diabetes Mellitus in children and differentiate it from Type 1 Diabetes Mellitus based on clinical profile and biochemical parameters.
Materials and Method: Children and adolescents in the age group of 8 to 18 years of either sex, newly diagnosed as Type 2 diabetes and Type 1 diabetes presenting at Endocrine clinic of M.S.Ramaiah Medical Teaching Hospital, Bangalore, India were taken as the study groups. Normal healthy children of the same age group of either sex were taken as control group. A total of 60 children 20 in each of the study groups and control group were selected.
Diabetes was diagnosed as per ADA criteria. Clinical profile like overweight/obesity was determined, based on mean BMI of >24 kg/m^2 and >27 kg/m^2 respectively. Family history and acanthosis nigricans were also recorded in these subjects. Biochemical parameters, mainly serum insulin and C-peptide were estimated. Other parameters like fasting plasma glucose, glycated hemoglobin (HbA_{1c}) and lipid profile were also determined in these children.
Observations: Children with Type 2 DM were overweight with mean BMI of 24.47 kg/m^2 compared to 17.89 kg/m^2 in the control group and 17.80 kg/m^2 in the Type 1 diabetic subjects. Acanthosis Nigricans was seen in 75% cases of Type 2 diabetics but it was not found to be associated with Type 1 cases. Family history of diabetes was present in 90% cases of Type 2 diabetes as compared to 15% in Type 1 DM. Insulin and C-peptide levels were found to be elevated at a mean 0.28 $\pm$ 0.04 and 1.18 $\pm$ 0.09 respectively in Type 2 diabetic children when compared to 0.02 $\pm$ 0.009 and 0.23 $\pm$ 0.08 nmol/L in Type 1 diabetics. There was no significant change in the values for glycated hemoglobin and lipid profile in both the study groups when compared to the normal children. In Type 2 diabetic children the degree of hyperglycemia was found to be lesser when compared to Type 1 diabetics.
Conclusion: Type 2 diabetes in children and adolescents is associated with a strong family history . On physical examination they were overweight with presence of acanthosis nigricans. Raised levels of insulin and C-peptide were observed in Type 2 diabetics in comparison with that of Type 1 diabetics.

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Sex differences in the longitudinal growth of children with Type 1 diabetes.

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Background and Aims: Diabetes is a metabolic disorder which potentially can affect the growth of children. Mild growth impairment in diabetic children has been reported even if control is acceptable. Type 1 diabetes is relatively rare in Chinese. Limited data is available on the longitudinal growth of diabetic children. The aim of this study is to investigate the possible differences in the longitudinal growth in diabetic children by following them from diagnosis to the attainment of final adult height.
Materials and Methods: Seventy eight Chinese children (44 girls, 34 boys) with type 1 diabetes were studied longitudinally from diagnosis until they reached adult height. All patients were measured and weighed at diagnosis and every 3-4 months over the follow-up period. Standing heights were measured by a wall-mount standiometer and weights were measured by digital scale. Their heights and weights were converted to standard deviation scores (SDS) using normal reference standards obtained in local healthy children. Adult height is considered to have attained if the growth velocity is less than 1 cm in the preceding year.
Results: The mean age at diagnosis was 9.1 years.The mean height SDS for boys and girls at diagnosis were +0.62 and -0.12 (p=0.01). The mean final height SDS for boys and girls were +0.15 and -0.37 (p=0.2). At attainment of final height, the mean weight-for-height SDS for boys and girls -0.18 and +0.79 (p=0.005). Their diabetic contol was comparable as indicated by their HbA1C values throughout the follow-up period
Conclusion: This study demostrated that the longitudinal growth patterns of diabetic boys and diabetic girls are different. Boys were tall for age at presentation but achieved normal adult height while girls tended to become obese whey they approached adult height. The differences in the growth pattern are unrelated to their diabetic control. Further studies are necessary to reveal the factors responsible for such differences in their growth patterns.

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Early detection of emotional and behavioural problems in children with diabetes: the validity of the child health questionnaire as a screening instrument.

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Background and Aims: To assess the validity of the Child Health Questionnaire (CHQ) as a screening tool for detecting "at risk" emotional and behavioural maladjustment in children with diabetes, using the Behavior Assessment System for Children (BASC) as a gold standard measure.
Materials and Methods: CHQ and BASC were administered to 103 families with children of type 1 diabetes aged 7 -12 years. Sub-scales of the two measures were compared using Pearson's bivariate correlations. CHQ sensitivity and specificity cut-points were optimised against BASC clinical and borderline categories using receiver operating characteristic (ROC) curves.
Results: BASC Externalising Problems scores correlated strongly with CHQ Behaviour, Global Behaviour, Mental Health, Family Activities and Family Cohesion scales. BASC Internalising Problems scores correlated strongly with CHQ Behaviour, Mental Health and Family Cohesion scales. On ROC curve analysis, the CHQ Mental Health Scale was most effectively identified children classified as borderline on the BASC Internalising Problems Scale, while the CHQ Global Behaviour Scale most effectively identified children classified as borderline on the BASC Externalising Problems Scale.
Conclusions: Significant correlations were seen between the CHQ Global Behaviour and Mental Health scales and the BASC Externalising and Internalising scales respectively. Tandem use of CHQ, as a screening tool, and BASC, as the definitive measure, can be used to identify children with diabetes "at risk" for chronic maladjustment and poor health outcome.

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Predictors of adverse psychological and health outcome in young people with Type 1 diabetes.

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Background and Aims: The incidence of type 1 diabetes is increasing worldwide, imposing enormous public health costs on communities and significantly affecting the health status and quality of life of individual sufferers. If we are to reduce the morbidity associated with this chronic illness, it is critical that risk factors for adverse health and psychological outcomes are identified so that resources can be targeted early to „at risk" individuals. In 1990 the Royal Children's Hospital (RCH) commenced a study to examine prospectively relationships between illness variables, neuropsychological skills and psychological adjustment in children enrolled at diagnosis. Participants have been assessed at diagnosis, one, two, and six years and relationships between these variables and metabolic control examined. The current study examined psychological and illness variables ten years after illness onset. Our aim was to model relationships between demographic variables and psychological adjustment at illness onset and current psychopathology and metabolic control history in order to to identify early risk factors for adverse outcome.
Materials and Methods: A computerised interview, the Diagnostic Interview for Children and Adolescents - Version IV (DICA-IV), was administered to a subset of the cohort (N=41), adolescents then aged

between 11 and 18 years. The DICA generates psychiatric diagnoses based on DSMIV criteria. Lifetime glycosylated haemoglobin levels and history of hypoglycaemic seizures were obtained from the hospital data base.
Results: Fifteen adolescents (37%) received at least one diagnosis, compared to community prevalence estimates of between 14% and 20%. Girls were more likely to receive a diagnosis then boys (X^2=4.98, p<.05) and those with a diagnosis were significantly older (t=2.11, p<.05) than non-symptomatic adolescents. Adolescents with a history of hypoglycaemic seizure were at increased risk for an internalising (anxiety, mood, eating) disorder (odds ratio 3.5). Adolescents with a history of chronic hyperglycaemia exhibited an elevated risk of pathology with an odds ratio of 7.0 for an internalising disorder and 2.8 for an externalising (behaviour) disorder. Discriminant function analyses were used to model relationships between variables at diagnosis and current psychological and health status. Females were at increased risk for an internalising disorder ten years later (F=9.10, p<.001). Children with behaviour problems at illness onset were more likely to have a psychiatric diagnosis ten years later (F=6.90, p=.01) as well as a history of poor metabolic control (F=10.71, p<.001).
Conclusion: These findings confirm clinical perception that psychopathology is elevated in young people with diabetes and is linked with diabetes control. They also suggest that it is possible to identify „at risk“ children very early in the course of the illness, raising the possibility that intensive intervention with these children may reduce both psychiatric morbidity and adverse health outcomes.

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Does functional health of diabetic children deteriorate over time?
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Background and Aims: This study aimed to assess functional health in children with diabetes over a two year period, and to correlate functional health at baseline with change in HbA1c over the same period.
Materials and Methods: Subjects were randomly selected children attending the RCH diabetes clinic. Baseline data (May-August 1998) have previously been reported. 116 subjects were included in the follow-up study (February 2000-May 2001). Functional health was assessed using parent reports on the Child Health Questionnaire (CHQ) for children aged 5-18 years plus adolescent self-reports on the CHQ for those aged 12-18 years. HbA1C was estimated at 3 monthly intervals using a Bayer DCA 2000 machine.
Results: Average parent reported CHQ scale scores and summary scores were not significantly different at baseline and follow up. There was a high correlation (r>0.4) between baseline and follow up scores on all scales and summary scores except the Role/Social Limitations Emotional/Behavioral Scale (r=0.12, p=0.29), and the Role/Social Limitations Physical Scale (r=0.33, p=0.004). Average adolescent self-reported CHQ scale scores indicated a significant improvement in health on the Bodily Pain Scale (t= -2.14, p=0.04), General Health Scale (t= -3.79, p=0.001), and the Family Activities Scale (t= -4.88, p0.4) between baseline and follow up scores on all adolescent self-reported scales except the single item Global Health Scale (r=0.19, p=0.33), the Physical Functioning Scale (r=0.34, p=0.08), the Role/Social Limitations Behavioral Scale (r=-0.01, p=0.97), and the Family Activities Scale (r=0.27, p=0.17). Average HbA1c was significantly lower for the sample at baseline (mean = 7.8) than at follow up (mean = 8.5) (t=-6.42, p<0.001) however, there was a high correlation between baseline & follow up HbA1c (r=0.57). None of the parent reported or adolescent self-reported CHQ scales strongly predicted change in HbA1c between baseline and follow up.
Conclusion: Parents report no significant change in the functional health of their children with diabetes over the course of two years. Adolescents, on the other hand, report an improvement in physical health and family life with a concomitant deterioration in behavioural well-being. Functional health status over time does not appear to be associated with metabolic measures of diabetes control.

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Treatment of Type 1 Diabetes in Childhood

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Continuous subcutaneous insulin infusion (CSII) vs glargine insulin in Type 1 diabetic children: a comparative pilot study.
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Background and Aims: The gold standard of intensive insulin therapy can be obtained in selected cases through continuous subcutaneous insulin infusion (CSII). It has been reported to improve short and long-term glycometabolic control. Insulin glargine is a novel long-acting human insulin analogue that could be used also in diabetic children in order to guarantee the basal insulin supplementation. The aim of this study was to compare the efficacy of two insulin schemes performed as CSII or Multiple Daily Injections (MDI) (glargine once daily at bedtime and human regular insulin at meals) in T1DM children and adolescents for a follow-up period of at least 6 months.
Patients and Methods: We retrospectively collected data from 22 patients that changed their previous insulin scheme (NPH at bedtime and human regular insulin at meals) because of poor glycometabolic control (HbA1c > 8.5%). 11 patients (M:F=2:9; age:13.8±2.8 years, diabetes duration: 4.9±2 years) underwent CSII, while 11 other subjects (M:F=4:7; age:10.1±3.6; diabetes duration=4.5±2.6) continued MDI, but NPH insulin was substituted by glargine insulin. Body Mass Index (BMI), fructosamine (mmol/l), HbA1c (%) and insulin requirement (IU/Kg b.w.) were evaluated at 3 months intervals. All parents signed a consent form.
Results: Both groups showed a decrease in HbA1c values and insulin requirement, that was significant only for CSII group. Main results are reported in the following table. No difference in hypoglycemic episodes has been reported. Patients in the CSII group reported a clear improvement in their quality of life.
Conclusions: It is the first pilot study comparing CSII and glargine insulin in diabetic children. CSII and glargine insulin provide both effective glycemic control in children and adolescent with T1DM. CSII allows to more significantly decrease HbA1c values and insulin requirement and also to reach a better quality of life. We think that CSII remain the gold standard of intensive insulin therapy for all diabetic children with poor glycometabolic control, but glargine insulin is now a valid alternative, especially for those children in whom CSII is considered to be problematic (small age, educational concerns, poor compliance, etc).

CSII	BMI	Fructosamine	HbA1c	Insulin Requirement
0	19.9±2.3	332.3 ± 101.7	8.9 ± 1.4	1 ± 0.2 + 6
months	20.5 ± 2.8	308.3 ± 37.6	7.4 ± 0.8	0.8 ± 0.1
p	NS	NS	0.05	0.002
GLARGINE	BMI	Fructosamine	HbA1c	Insulin Requirement
0	17.2±2.2	348.6 ± 56	8.9 ± 2	1 ± 0.2
+ 6 months	17.5 ± 2.7	342.7 ± 43	2 7.9 ± 1	0.9 ± 0.2
p	NS	NS	NS	NS

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The development of CSII in pediatric Type 1 diabetes patients. Multicenter analysis based on the DPV-science system.
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Background and Aims: In the last years insulin pump therapy has increased in the treatment of pediatric patients with type-1-diabetes. Also in Germany a growing number of children and adolescents use this therapeutic regimen.

Nevertheless only few multicenter studies with large number of pediatric patients have been presented. To describe the development of CSII in pediatric centers from Germany and Austria participating in a multicenter initiative on quality control over the last 7 years.
Materials and Methods: Participating centers in Germany and Austria (Vienna) have provided longitudinal, anonymized data of patients with pump therapy as documented in the DPV system. Patients on CSII (age< 20 years) were documented at 92 centers. The HbA1c was standardized based on the normal range at each center. German reference data for height, weight and BMI were used to calculate SD-scores.
Results: There are 5775 records from 1060 pediatric patients (492 boys and 568 girls) documented in the DPV-system. Mean age at begin of pump therapy was 14.0 +- 3.6 years and hasn´t changed significantly in the last 7 years. The mean age of all pump patients was 14.9 +- 3.7 years, while the mean diabetes duration was 6.9 +- 3.9 years. Regarding different age groups 36 patients were younger than 5 years, 65 between 5 and 10 years, 330 in the range 10 to 15 years, while the majority (629) were older than 15 years.
On average, patients used CSII for 0.9 years with a range from 0-10 years. The average insulin dose was 0.78 Units/ kg and 61% used a rapid-acting insulin in 2002, the use of rapid-acting insulin has remarkably increased in the last 7 years. In the whole group the average HbA1c was 9.07 +- 2.6%, while the rate of severe hypoglycemia was 16.1 per 100 patient-years. The height in pump patients was nearly identical to the reference group (SD-score -0.004 +- 1.02), while the weight and BMI were considerably increased (weight SD-score +0.49 +- 0.95; BMI SD-score +0.59 +- 0.87) and therewith similar to type-1-diabetes patients on multiple injection therapy.
Conclusion: This multicenter analysis showed a remarkable recent interest in insulin pump therapy among pediatric centers and their young patients. The number of patients with CSII is growing constantly, while the age at onset of pump therapy hasn´t changed. Compared to all patients in the DPV-system, patients on CSII seem to have less severe hypoglycemia, while the metabolic control shown as HbA1c is often insufficient. In the future prospective intervention trials should be done to define the outcome of insulin pump therapy in pediatric patients.

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The appreciation of different method therapy with insulin in children and adolescents with diabetes Type 1.
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Background and Aims: Aim of the study: was an estimation of the metabolic control in children and adolescents with diabetes type 1 who received insulin with an intensive or conventional method. The examinations included 367 patients (193 girls, 174 boys) with diabetes type 1 aged3,5 to 19 years, mean 12,6 years. The examinations were performed from march 2002 to september 2002. The patients were divided in 3 groups. Group 1 included 131 patients who received Humalog in multiple daily doses with cartigde, group 2 – 68 patients treated with Humalog in a subcutaneous continuous infusion (SCI) with a personal insulin pump(PIP), group 3-168 patients treated with a conventional method with short acting human insulin 4-5 times a day and basal.
Materials and Methods: Analysed was age at diagnosis and duration of diabetes therapy, HbA1c, BMI, the frequency of mild or severe hypoglycemia and diabetic ketoacidosi.
Results: The mean duration of diabetes therapy was in group 1=5,5±4 ; group 2=5,1 ±3,1, in group 3 = 3,6±3,0 Statistical significant defference (SSD) was shown between gr 1/ 3 and 2 / 3 (p=0,001 vs 0,001). The highest level of HbA1c was ascertained in group 1 = 8,02 ±1,6 %,the lowest in group 2 = 7,43 ±1,16 % in group 3 = 7,9 ± 1,72% SSD was shown between the group 1 and 2 p= 0,007 also 2 and 3 p= 0,055. BMI in group 1 - 21,5 ± 3,0; group 2 = 20,8 ± 3,0 group 3 = 20,0 ± 3,2.SSD was shown between the group 1 and 3 = 0,00001.The frequency a neuroglikopenia in gr 1 was 0,08,in gr 2 – 0,03 in gr 3-0,09 for person/for year. SSD shown between 1 / 2 and 2 / 3 (p=0,002 vs p= 0,004) Mild hypoglycemia in gr 1- 8,8;gr 2 - 8,51; gr 3 - 9,1 for person / month. SSD was shown between gr 2/3 p=0,005. Ketoacidosis in gr 1- 0,13;gr 2- 0,03; gr 3- 0,13/person / year. SSD was shown gr.1 / 2 and 2 /3 (p= 0,002 vs p =0,004).
Conclusion: 1. In patients treated with a personal insulin pump ascertained was a lower HbA1c in comparison with patients treated with other methods. The difference was statistical significant. 2. The frequency of mild and severe hypoglycemia and ketoacidosis was in patients treated with insulin pumps lower than in the remaining groups.

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C-peptide level and clinical remission in children with newly diagnosed Type 1 diabetes.
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Background and Aims: Clinical onset of type 1 diabetes (T1D) is caused by an autoimmune β-cell destruction. Factors associated with a residual insulin secretion, estimated by determination of C-peptide levels and with a clinical remission, are important in the evaluation of modifying the natural history of T1D. The aim of the study was to investigate whether the age at onset, gender, presence of autoantibodies and ketoacidosis at diagnosis and insulin requirement, HbA_{1c} levels could be applied to predict the C-peptide levels and appearance of clinical remission during the first year of T1D in children.
Materials and Methods: 122 type 1 diabetic children, aged: 1.8-18.2 years (average 11.2), 44 female and 78 male were studied. Fasting C-peptide levels were examined by radioimmunoassay at diagnosis and after 10 days and after 1, 2, 3, 6 and 12 months of disease. At diagnosis islet cell antibodies (ICA) were detected by indirect immunofluorescence, antibodies to glutamic acid decarboxylase (GADA) and thyrosine phosphatase antibodies (IA2A) were measured by microradioimmunoprecipitation assay.
Results: Plasma C-peptide levels became decreasing during the first year after clinical diagnosis. Median C-peptide levels peaked at 3 months ($p<0.001$) and declined thereafter. Age at onset was positively correlated to C-peptide levels at each evaluated point of the disease ($r=0.3-0.46$, $p<0.0001$). One year after diagnosis C-peptide levels decreased in ICA(+) ($p<0.04$) and GADA(+) ($p<0.002$) patients but not in ICA(-) or GADA(-) children. There was no significant difference between the IA2A-positive and negative subjects in the C-peptide levels at 12th month of disease. Logistic regression analysis showed that male, younger age, low pH, higher HbA_{1c} and exogenous insulin requirement at onset were associated with lower C-peptide level at diagnosis ($p<0.0001$). The occurrence of clinical partial remission was observed in 44% of children, most often in 2nd and 3rd month of the disease. Higher frequency of remission was associated with higher C-peptide levels at onset of T1D ($p<0.02$). Receiver operating characteristic (ROC) curve method was used for estimation of threshold of C-peptide level for the prediction of clinical remission during the first year of T1D (0.21 pmol/ml, AUC(95%Cl)=0.64(0.54-0.72)).
Conclusion: In conclusion young age, presence of diabetes-related autoantibodies and hyperglycaemia with severe acidosis at the disease onset may be associated with the decreased residual insulin secretion and the different clinical course in T1D.
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Bridging the gap from child to adult: a transition care program which reduces hospital admission rates and improves outcomes in young adults with Type 1 diabetes.
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Background and Aims: "The transition" in patients with type I diabetes is recognised as a difficult period in which to achieve the ideals of the DCCT, good diabetes control and reduced long term complications. During the move from pediatric to adult services many encounter difficulties and become lost to follow up.Glycosylated hemoglobin (HbA1c) is higher and admissions with diabetic ketoacidosis (DKA) peak in the 15-25 years age group.
In July 2001 we began a transition care program to assist young adults aged 15 –25 to remain under a system of care including a diabetes specialist, general practitioner and diabetes educator when moving from pediatric to adult services. The aim was to reduce admissions with DKA and determine if a support program helped to improve diabetes control and compliance with follow up.
Materials and Methods: All patients transferring from pediatric to adult services over an eighteen month period and all patients admitted to hospital with DKA aged between 15 – 25 years have been referred to and followed by the program. Elements of the transition support program include: an appointment reminder system; phone support by the transitional care co-ordinator to resolve problems with follow up, development of acute and chronic self management action plans; case conferencing with care providers for patients with multiple admissions to hospital; carer and young adult education about differences between the adult and pediatric health care systems; and general practitioner education on sick day management.

Results: Of the 82 patients registered with the program, 52 were newly transitioned or newly diagnosed patients aged 15 –25, 10 patients were referred from private practitioners, 5 were admissions with DKA not previously under review, 15 were referred with poor control of which 5 were not under regular review. 80/82 patients have had at least one HbA1c meaurement with mean HbA1c of 9.26 ± 2.09 %. Forty-two patients have had two or more HbA1c measurements with a mean change in HbA1c of –0.55 ± 1.27 % from time of initial transfer to most recent result (P< 0.01). Mean HbA1c of patients admitted with DKA was 11.44±1.94 %(n=16) which was significantly higher than HbA1c of patients registered (mean diff 2.18%, p <0.001). Mean HbA1c of patients referred, who had been lost to follow up, was 11.8% at time of referral which was significantly higher than HbA1c of those under regular review (p<0.01). Admissions with DKA over 18 months of the program have progressively fallen (sustained 40% reduction over each six months of the 18 month program) attributed to reduction in readmission rates with DKA through contact with the program and prevention of loss to follow up after transfer to adult care. All 82 patients registered with the program remain under a system of care.
Conclusion: The program has improved control of diabetes, prevented loss to follow up and improved quality of life for patients registered. A reduction in DKA admissions has resulted in cost savings to the health care system which more than covered the cost of the program.

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Late complications and parameters of mortality among children with Type 1 diabetes mellitus in Uzbekistan.
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Aim: Type 1 diabetes mellitus (DM) progression is associated with high cardio-vascular risk leading to the premature invalidism and mortality. The work aimed at studying late complications, mortality rate, age of diabetes onset and the disease duration at the moment of death as well as mortality causes among children aged from 0 to 14 with I type diabetes mellitus registered at dispensaries in various regions of the Republic of Uzbekistan from 1996 to 1999.
Materials and Methods: Overall screening was used to examine all children registered at dispensaries in Uzbekistan from 1996 to 1999 with HbA1c parameters and microalbuminuria studied. Mortality and lethality rate (the latter is the mortality rate in relation to total number of children with I type DM) as well as death risk were calculated. The data were processed statistically with the Student's criterion used.
Results: There were 5.85, 5.89, 6.82 and 7.03 children with I type DM registered at the dispensaries per 100,000 of the population in Uzbekistan for the period under observation, the disease incidence tending to increase. The prevalence of diabetic complications in children under 15 years was 74.1%. Diabetic retinopathy (53.2%) turned out the most frequent I type DM complication. The frequency decreases as follows: physical development retardation (37%), microalbuminuria (32.5%), chiropathy (27%), diabetic retinopathy (24.5%), diabetic nephropathy (20.4%) and cataract (4.2%). Within the period of 1996-99 there were 0.32, 0.21, 0.16 and 0.24 deaths of children with I type DM per 100,000 of the population, the lethality rate being 5.5%, 3.6%, 2.4% and 3.4%, respectively. No confident reduction in mortality and lethality rate for the period in question was observed (p>0.5). Mean DM duration at the moment of death was 7.55±0.61 years, life duration being 18.9 ± 1.14 years. No sex difference in mortality of I type DM patients was detected (p>0.5). Chronic renal failure with underlying diabetic nephropathy (27.6%), acute pneumonia (23.4%), hyperglycemic ketoacydotic coma (17.0%), tuberculosis (12.7%), acute hepatic failure (6.3%) and other death causes (13%) were found major in children with I type DM. Mortality risk in the cohort confidently reduced within the observed period, 49.7%, 44.5%, 36.1% and 30.3% (p>0.05).
Conclusions: Mortality and lethality rate among children with I type DM are persistently high despite the fact that death risk reduced within the period of study, late complications and concomitant infectious diseases being the major death causes. Tedious work is necessary to prevent late complications and infectious disease as well as thorough monitoring of children with I type DM to preclude mortality in the group of patients.

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Autoimmune thyreoiditis in children and adolescents with Type 1 diabetes: incidence, glycemic control and late complications.
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Background and Aims: In a significant proportion of patients with type 1 diabetes, an autoimmune thyreoiditis (AIT) occurs during the course of their disease. Therefore, we conducted a study to estimate the overall incidence of AIT in children and adolescents with type 1 diabetes, and to evaluate glycemic control and diabetes-specific complications in those patients who had AIT.
Materials and Methods: In 1984, a regular screening program was started at the Charité Children´s Hospital of Berlin, Germany, in order to detect thyroid disorders in patients with type 1 diabetes. Clinical examinations and specific serological and immunological tests were performed in 593 patients. In the presence of thyroid antibodies, the elevation of TSH - with or without ultrasound abnormalities of the thyroid gland - led to a treatment with L-thyroxine. Clinical and metabolic data as well as the incidence of acute and late diabetes complications of patients with AIT were evaluated and compared to controls by matched-pair analysis with sex, age, and diabetes duration as matching criteria.
Results: In total, an AIT was diagnosed and treatment was started in 53 of 593 patients with type 1 diabetes (8.9%) median 4.0 years (0-12 years) after diabetes onset. The cumulative incidence (±SE) of AIT at 10 years of diabetes was 0.14 ± 0.02 in the total group, being significantly higher in female patients (0.19 ± 0.04, p=.019). In 92 subjects (32 boys, 60 girls) available for matched-pair analysis (46 each group), the median diabetes duration was 7.5 years (0.8-17.7 years) and median age at diabetes onset was 7.5 years (0.1-15.3 years). HbA1c values during the first year of diabetes (median 7.8 vs. 7.3%, p=.070) as well as longitudinally measured HbA1c values (8.6 vs. 8.3%, p=.205) were not significantly different between patients with or without AIT. In patients with AIT, there was no significant elevation of the frequency of severe hypoglycemic episodes. Median diabetes duration until the development of incipient retinopathy was 12.7 and 10.9 years in patients with or without AIT, respectively (p=.308).
Conclusion: The development of a frequently subclinical AIT in patients with type 1 diabetes can be detected by regularly performed screening. In case of early diagnosis and treatment of AIT, these patients are not at risk for impaired glycemic control or higher diabetes-specific complication rates.

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Electrogastrography in children with Type 1 diabetes mellitus - predominant role of bradygastria.
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Background and Aims: Electrogastrography (EGG) is a new noninvasive technic suggested for identifying gastric myoelectrical dysrhythmias. Diabetic subjects, who often develop vegatative neuropathy are of special interest in this field. The nature of EGG abnormalities observed in IDDM is not univocal, some authors postulate the percentage of normogastria and dominant frequency (DF) as the most reliable EGG parameters. The aim of the study was to evaluate the gastric myoelectric activity in fasting and post-prandial period in population of children with type 1.diabetes mellitus (DM t.1.).
Materials and Methods: 156 children and adolescents suffering from DM t.1.(aged 5-21yr; duration of DM > 5yr) and 15 healthy controls matched for age were enrolled to the investigation. In all subjects precutaneous elektrogastrography (EGG) was obtained for 30 minutes before, and 90 minutes after a test meal. The following parameters for fasting and postprandial periods were analyzed: normal gastric activity (2-4 cpm [cycles per minute] percentage, bradygastria (less than 2 cpm), tachygastria (over 4 cpm) and DF.
Results: The fasting and postprandial percentage of gastric dysrhythmias were significantly higher in DM subjects comparing with controls (p<0,001). The most distinct difference between DM and controls was marked in the percentage of fasting bradygastria (51,78± 29,13% vs 20 ± 17,81%; p<0.01). There was also lower prevalence of the normal pre-prandial rhythm in diabetics (41,72±31,84 %vs 71,8±13,74%; p<0,01). In the postprandial period in DM children some normalization of gastric myoelectrical rhythm was observed as compared with fasting records (increase of normogastria – 64,92±23,7% vs 41,72±31,84%; decrease of bradygastria – 26,28± 19,85% vs 51,78±29,13%). The increase of

postprandial DF in DM subjects was observed but not significant (1,43±1,19 vs 2,54±1,18).
Conclusions: 1.Our results confirme the derangement of the gastric myoelectrical activity in children with DM t.1.
2. Diabetic children often develop bradygastria (predominantly in the fasting period).

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Gestational Diabetes

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The influence of early diagnosis of gestational diabetes mellitus in maternal and fetal morbidity.
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Background and Aims: According to the Fourth Workshop-Conference on Gestational Diabetes Mellitus (GDM), early screening is recommended only in women with high risk characteristics. However, a recent study has reported a worse outcome for women with early diagnosis. The aim of this study was to analyse the association between gestational age at GDM diagnosis and maternal and neonatal outcomes.
Materials and Methods: 1958 consecutive pregnancies (1891 single, 67 multiple) of women with GDM attended at the Diabetes and Pregnancy clinic were included. GDM screening in our centre includes an O´Sullivan test at the first visit in all non-diabetic pregnant women, that is repeated at 24-28 and 31-34 weeks of pregnancy in women not diagnosed of diabetes. Pregnancies were divided in three groups according to the gestational age at GDM diagnosis: 1st period<24 weeks, 2nd period: 24-30 weeks and the 3rd period: longer or equal to 31 weeks. Intensified metabolic treatment (diet ± exercise ± insulin) was initiated after diagnosis.We examined the association of period of diagnosis with maternal (pregnancy- induced hypertension, insulin treatment and cesarean section) and fetal outcomes (5-minute Apgar<7, macrosomia, perinatal mortality, obstetric trauma, minor and major malformations, hypoglycemia, hypocalcemia, hyperbilirrubinemia, respiratory distress and preterm birth). First, a bivariate analysis was performed to analyse the association between each outcome and period of diagnosis (Chisquare test). Second, a logistic regression analysis was performed using each outcome as the dependent variable and including period of diagnosis, multiple pregnancy and variables measuring GDM severity as a potential predictors. In the case of macrosomia, weight increment during pregnancy, smoking and macrosomia in previous pregnancies were also included as potential predictors. A p value <0.05 was considered significant.
Results: In bivariate analysis, period at diagnosis was found to be associated with 3 out of 3 maternal outcomes and 6 out of 11 fetal outcomes. In multivariate analysis, period was a predictor in 2 out of 3 maternal outcomes (pregnancy-induced hypertension and insulin treatment) and in 4 out of 11 fetal outcomes (preterm birth, 5-minute Apgar<7, perinatal mortality, hyperbilirrubinemia).
Conclusion: Early diagnosis of GDM is a predictor of maternal and fetal adverse outcomes.

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Elective delivery in women with gestational diabetes mellitus.
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Background and Aims: Active induction of labour at term in women with gestational diabetes mellitus (GDM) is performed to reduce the risk of macrosomia and birth trauma. The effect of elective delivery at 39 completed weeks is compared to elective delivery at 38 completed weeks.
Materials and Methods: Retrospective study of two policies of elective delivery in women with singleton gestations and GDM: A) active induction of labour at 38 completed weeks (years: 1988-1989; n=134) versus B) active induction of labour at 39 completed weeks (years: 1999-2000; n=377). Outcome measures were: macrosomia (>4000 g); large for gestational age (LGA), birth trauma and caesarean section.
Results: Mean gestational age at delivery was 38.2 ± 1.8 weeks (median 38) for policy A and 38.9 ± 1.1 weeks (median 39) for policy B (p=0.000). At policy A women were younger (32.0 ± 5.5 vs. 33.1 ± 3.9 years; p=0.036), had higher parity (1.2 ± 1.3 vs. 0.6 ± 0.8; p=0.000) and higher rate of a prior neonate with macrosomia (15.6 vs. 6.1 %; p=0.002). Prepregnancy BMI (A: 25.8 ± 4.7 vs. B: 24.9 ± 4.8 Kg/m2), weight gain during pregnancy (A: 8.7 ± 4.7 vs. B: 9.9 ± 4.5 Kg) and mean glucose levels during pregnancy (A: 92.0 ± 11.3 vs. B: 93.1 ± 10.9 mg/dl) did not differ between policies. No differences were found between policy A and B with respect to macrosomia (3.7 vs. 3.4 %), LGA (9.7 vs. 7.7 %) or birth trauma (1.2 vs. 0.8 %). The rate of caesarean section was reduced during

policy B (30.8 vs. 21.3 %; p=0.033). Comparison of these two policies for women with GDM treated with insulin (policy A: n=51; policy B: n=207) showed no differences for macrosomia (3.9 vs. 5.3 %), LGA (17.6 vs. 9.7 %), birth trauma (2.0 vs. 1.0 %) or caesarean section (37.3 vs. 26.8 %).
Conclusion: Delaying elective delivery to 39 completed weeks in women with GDM did not increase macrosomia or birth trauma and reduced the frequency of caesarean section. However, for insulin requiring women with GDM the rate of caesarean section did not change.

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Antepartum characteristics and birth outcomes of women with previous history of gestational diabetes.
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Background and Aims: Women with previous history of gestational diabetes mellitus (GDM) were found to be at increased risk for type 2 DM during postpartum follow-up period. Various predictors such as obesity, maternal age, parity, family history, previous history of GDM, severity of glucose intolerance and peripartum complications were found to associated with the onset of diabetes. Thus, in this prospective study, we evaluated the antepartum predisposing factors and birth outcomes to predict type 2 DM after GDM.
Materials and Methods: A total 909 of subjects with previous history of GDM were recruited and longitudinal annual examination was made over the 6 years postpartum periods. During 24-26 weeks' gestation, we performed 1 hour 50-g screening and followed by 3 hour oral glucose tolerance test (OGTT) for confirmation of GDM. During OGTT, plasma glucose levels with insulin and c-peptide were measured. Antepartum clinical characteristics and birth outcomes were evaluated using the standardized questionnaire by face to face interview method.
Results: During the 6 years follow-up periods, 116(12.8%) and 120(13.2%) women out of 909 participants were converted to either DM or impaired glucose tolerance (IGT). From the result of antepartum glucose metabolic assessment by 50-, 100-g OGTT, glucose concentrations of all time sequence were significantly high in DM conversion group ($p<0.001$). Insulin level of fasting, 1-, 2-, and 3-hour and 1-hour c-peptide level after glucose loading were significantly high in DM conversion group ($p<0.01$, $p<0.05$, respectively). There was no women with significant peripartum complications of GDM. And APGAR scores of all neonate were over seven points and statistically not significant. Birth weights of neonate from GDM mothers were significantly high in DM conversion group ($p<0.001$). From the questionnaire evaluation, level of education of mother and house income were significantly low in DM conversion group ($p<0.05$). The women without job were also at increased risk for postpartum DM ($p<0.05$).
Conclusion: Antepartum severity of GDM was significantly associated to the onset of type 2 DM during the postpartum period. Life style factors such as socioeconomic status were also found to play as the risk factor in this high risk group for DM. Women with a previous history of GDM continue to revealed as a high risk group for diabetes. Thus, close and routine medical evaluation is necessary for GDM during the postpartum period to prevent diabetes.

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Plasma adiponectin, cytokines and insulin resistance in healthy pregnant women and patients with gestational diabetes.
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Background and Aims: Contribution of adiponectin in correlation with the tumor necrosis factor (TNF)-system and leptin in pregnancy induced insulin resistance was studied. Patients: 24 patients with gestational diabetes (GDM), 40 healthy pregnant women (15 in 1st, 12 in the 2nd and 13 in the 3rd trimester), and 30 matched non-pregnant female with normal glucose tolerance participated in the study.
Materials and Methods: Plasma adiponectin (Linco, USA), serum C-peptide (Biodata, Italy) were measured by RIA, while TNF-α (Sigma, USA), sTNFR-1, -2 (BenderMedSystem, Austria) and leptin (DRG, USA) by ELISA.
Results: Plasma adiponectin levels were significantly ($p<0.01$, Mann-Whitney) lower in GDM patients (7.5± 2.0 μg/ml, X±SD) and in normal pregnants in the 2nd (9.3±2.7) and 3rd (8.0±2.4) trimester as compared to non-pregnant controls (12.5±3.7) and to pregnant women in the 1st trimester (12.3±3.2). Significant negative linear correlations (Searman) were found in patients with GDM among adiponectin and the elevated TNF-α (6.3±0.6 pg/ml, r=-0.77, $p<0.01$), sTNFR-2 (10.0±6.9 ng/ml, r=-0.51, $p=0.01$) and leptin (40.0±24 ng/ml, r=-0.56, $p<0.01$). These correlations were also found but in lesser extent in the healthy pregnant group (TNF-α: r=-0.34, sTNFR-2: r=-0.40, leptin: r=-0.32, $p<0.05$), but with the exception of leptin (r=-0.42, $p=0.03$) were not observed in non-pregnant controls. In all groups a strong negative correlation was found between adiponectin and the BMI values and in GDM patients and healthy pregnants with the fasting C-peptide level (GDM: r=-0.71, normal pregnants: r=-0.49, $p<0.01$) and the C-peptide/Blood glucose ratio (r=-0.56 vs –0.49, $p<0.01$).
Conclusion: Plasma adiponectin levels are decreased in GDM and in the 2nd and 3rd trimesters of normal pregnancy and may contribute to insulin resistance in accordance with the TNF -system and leptin.

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Plasma homocysteine correlates in women with gestational diabetes.
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Background and Aims: Elevated homocysteine concentration during pregnancy is associated with an increased incidence of spontaneous abortion, intrauterine growth retardation, placetal infarction and preeclampsia. There is evidence that elevated homocysteine levels are assosiated with insulin resistance and impaired endothelial function and supplementation with B vitamins improves vascular endothelial function in patients with coronary artery disease. The aim of this study was to examine the potential determinants of serum homocysteine (HC) concentration in a group of women with gestational diabetes (GD).
Materials and Methods: Material included 44 women with gestational diabetes mellitus and 17 healthy pregnant women (control group - CG). Serum HC and C-peptide were determined by ELISA, using Bio-Rad reagents, serum lipids enzymatically, fibrinogen coagulometrically, fasting insulin by immunoradiometric method (IRMA), vitamin B 12 and folates by chemiluminescent immunoassay, free fatty acids (FFA) by enzymatic methods and cystatin C by immunonephelometry.
Results: Mean age in women with GD was 30.5±6.5 yrs, vs. 26.2±4.0 in CG ($p<.02$), mean weight 74.5±15 kg vs. 69±10 kg respectively. Only women with GD treated with diet alone were included in the analysis. Mean values of serum homocysteine (8.0±2.0 vs 7.4±1.1 micromol/l), vitamin B12 (262.0±82.6 vs 287.0±37.5 pg/ml) and folate levels (11.2±6.0 vs 11.1±5.9 ng/ml)did not differ significantly between women with GD and CG. In women with GD in comparison to healthy pregnant women HOMA-IR, a surrogate of insulin resistance (2.7 5±1.67 vs. 1.57±0.9); triglycerides (2.7±0.9 vs. 1.9±0.5 mmol/l); FFA (0.60±0.2 vs. 0.46±0.2 mmol/l); and C-peptide (1.7±1.0 vs. 1.1±0.5 ng/ml) levels were significantly higher ($p < 0.05$). In women with GD serum vit B12 correlated negatively with homocysteine (r=-0.44, $p<0.01$), with serum triglyceride (r=-0.42, $p<0.01$) and with C-peptide (r=-0.43, $p<0.01$), while folates correlated with homocysteine (r=-0.54, $p<0.001$). In multiple regression analysis with serum HC as dependent variable and vitamin B12, folate, cystatin C and HOMA-IR as independent variables, folate and vit B12 entered the analysis in women with GD (beta=-0.42 and-0.34, respectively, $R^2=0.33$, $p<0.01$), while in healthy pregnant women cystatin C (beta=0.42),and HOMA-IR (beta=-0.71), $R^2=0.54$,$p<0.01$.
Conclusion: The results of the study indicate, that: 1) women with GD are characterized by higher insulin resistance, measured by HOMA-IR, higher triglyceride, FFA and C-peptide levels, the metabolic syndrome components, in comparison to control group and 2) in women with GD serum homocysteine is significantly related to vit B12 and folate levels, while in healthy pregnant women to HOMA-IR and cystatin C levels, what may suggests the importance of the B-group vitamins supplementation in women with gestational diabetes.

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Insulin resistance and beta cell function in women with gestational diabetes: studies in the post partum.

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Background and Aims: A pregnancy complicated by gestational diabetes (GD) identifies women at increased risk of developing type 2 diabetes post partum. We have previously shown that women with GD are more insulin resistant and have lower beta cell function than appropriate controls. The aim of this study was to determine if women with GD and a persistent abnormality in glucose tolerance post-partum had more severe insulin resistance and/or less efficient beta cell function during pregnancy and/or postpartum than women with GD who had a normal glucose tolerance post partum.
Materials and Methods: Results are reported on 102 consecutive women who had gestational diabetes and a 75g GTT with a basal insulin measurement during pregnancy and post partum. The women (aged 33.0 ± 4.6 years, mean ± SD) were studied at 28 ± 4 weeks of gestation and 14 ± 4 weeks post-partum. Delivery was at 39.0 ± 1.6 weeks. Insulin resistance (IR) and beta cell function (BCF) were calculated from HOMA.
Results: In the group as a whole, women with GD had better glucose tolerance and were less insulin resistant post-partum. IR (HOMA) fell from 2.6 ± 1.8 to 1.6 ± 0.9 ($p < 0.001$). Women with abnormal glucose tolerance post-partum (n = 17: group 1) were more insulin resistant than women with normal glucose tolerance post-partum (n = 85: group 2): IR 2.2 ± 1.3 vs. 1.5 ± 0.8 ($p = 0.026$) when studied post-partum. The fall in IR in Group 1 from pregnancy to the post partum was not significant whereas the fall was significant in Group 2. Group 1 had significantly lower BCF during pregnancy than Group 2 calculated at similar IR. This was associated with more marked glucose intolerance in pregnancy (2hr glucose 10.4 v 8.9 mmol/L; $p = 0.047$) compared with Group 2. Women in Group 1 were older (35.4 ± 4.5 vs. 32.5 ± 4.5 years, $p = 0.017$) and of greater parity (1.1 ± 1.1 vs. 0.5 ± 0.8 $p = 0.014$) than women in Group 2 but had similar weight, height and waist measurements. A stepwise logistic regression on the whole group revealed the following predictors of an abnormal GTT post partum: the 2 hour glucose in the pregnancy GTT, OR (95% CI) 1.82 (1.12, 2.95), $p = 0.016$; parity (not influenced by BMI) OR (95% CI) 2.48(1.29, 4.78), $p = 0.007$. Ethnicity (Asian) just failed to reach significance OR (95% CI) 3.37 (0.83, 13.7),$p = 0.089$.
Conclusion: In women with abnormal glucose tolerance at follow up: 1. Insulin resistance did not fall from pregnancy levels; and 2. Beta cell function was impaired in pregnancy and remained low compared with women with normal glucose tolerance. Both more marked insulin resistance and lower beta cell function were associated with abnormal glucose tolerance post partum. In this study parity rather than BMI predicted an early post partum abnormality in glucose tolerance. Ethnicity may also be important in predicting an early abnormality.

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Relationship between variables related to insulin resistance and mild gestational hyperglycemia or gestational diabetes.

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Background and Aims: Purpose of this study was to identify the relationships between mild gestational hyperglycemia (MGH) or gestational diabetes (GDM) and other variables associated to insulin resistance syndrome.
Materials and Methods: After 1-h-50g-glucose-challenge-test (GCT) performed between the 24th and the 28th gestational week in 542 consecutive women, those with 1-h-plasma glucose<7.8mmol/l were classified normotolerant (NGT, n=436; 80%), those with 1-h-plasma glucose≥7.8mmol were further categorized by a 100g-3-hr-OGTT as diabetic (GDM, n=49; 9%) and, if OGTT was negative, as MGH (n=57; 11%).
Results: As compared to NGT or MGH, GDM women were older (33±4(SD)y vs. 31±5y and 32±4y; $p<0.05$ by ANOVA), and with a higher pregestational BMI (25±4kg/m^2 vs. 23±4kg/m^2 and 23±4kg/m^2; $p<0.05$). Either 1-h-GCT-plasma-glucose (6.47±1.72,NGT, 9±0.94,MGH and 9.39±1.06mmol/l,GDM) and the median value of insulin sensitivity index (ISI) measured according to Matsuda and DeFronzo (5.26,NGT, 4.33,MGH and 2.77mg*dl^{-1}*min^{-1},GDM) were each other different in the 3 groups ($p<0.05$). Mean blood pressure, plasma triglycerides, uric acid and total cholesterol were similar in all groups, while basal GCT-plasma-glucose (4.3±0.69,NGT, 4.41±0.58,MGH and 4.83±0.67mmol/l,GDM), basal C-peptide (7.1±3.4,NGT, 7.9±4.7,MGH, and 11.1±16.3nmol/l,GDM) and HDL-cholesterol (1.49±0.46,NGT, 1.39±0.46,MGH and 1.55±0.33mmol/l,GDM) were higher in GDM ($p<0.05$). After adjusting for all confounders (including basal, 1-h-GCT-plasma-glucose, parity and family history of diabetes), GDM was characterized by an older age ($p=0.03$), a higher basal GCT-plasma-glucose and a higher HDL-cholesterol, while ISI progressively decreased from NGT through MGH to GDM ($p<0.05$).
Conclusion: In conclusion, even in presence of a progressive decrease in insulin sensitivity GDM and MGH rank equally as to main determinants of the insulin resistance syndrome, being GDM significantly associated with an older age, a more elevated basal GCT-hyperglycemia and, unexpectedly, a raised level of HDL-cholesterol.

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Prevalence and risk factors for postpartum carbohydrate intolerance in women with gestational diabetes mellitus.

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Background and Aims: Controversy still surrounds the prevalence of and predictive factors, screening and pregnancy outcome in gestational diabetes mellitus (GDM). We evaluated the risk factors and pregnancy outcome in GDM, and the incidence of postpartum carbohydrate intolerance.
Materials and Methods: 2416 pregnant women were screened by a two-step approach: Step One: Universal screening with a 50-gram 1-hour glucose challenge test (GCT); Step Two: 100-gram oral glucose tolerance test (OGTT) in women with a GCT plasma glucose of ≥130mg/dl. Women with two or more abnormal OGTT readings were formally diagnosed with GDM, based Carpenter and Coustan's criteria. All pregnancies were followed up until delivery. Available GDM patients underwent a 75-gram 2-hour OGTT 6 to 12 weeks after delivery. Postpartum diabetes mellitus was diagnosed according to ADA criteria. Univariate and multivariate analyses were used to estimate the contribution of different risk factors.
Results: The prevalence of GDM in our sample was 4.7% (95% CI: 3.91-5.64). There was a significant difference between GDM women and those with a normal GCT in terms of age (29.09±6.13 vs. 24.92±5.31 years; $p<0.001$), BMI (27.43±4.33 vs. 24.78±2.09; $p<0.001$) and parity (1.79±2.09 vs. 0.90±1.34; $p<0.001$). The prevalence of pospartum carbohydrate intolerance was 23%, with 6.3% having type 2 diabetes. The prevalence of fetal macrosomia, hyperbilirubinaemia, hypoglycaemia and hypocalcaemia was 18.2%, 16%, 9%, and 6%, respectively. A positive family history of diabetes or poor obstetric outcome was more common in women with GDM than in those with a normal GCT. Multivariate analysis identified fasting blood glucose at diagnosis, obesity (BMI>27), GDM onset early in pregnancy, and requirement for insulin during pregnancy as independent predictors of postpartum carbohydrate intolerance.
Conclusion: Compared with figures reported elsewhere, the prevalence of GDM in our sample was a moderate one. Important risk factors for GDM are advancing age, grand multiparity, obesity, and family history of diabetes and/or poor obstetric outcome. Nearly 25% of GDM patients developed carbohydrate intolerance, including overt diabetes, in the postpartum period. Fasting hyperglycaemia, maternal obesity, early-onset GDM, and insulin requirement during pregnancy are independent predictors of postpartum carbohydrate intolerance. We recommend postpartum monitoring of women with GDM because of the high proportion of them who go on to develop permanent carbohydrate intolerance.

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Parameters predicting future development of DM2 in women with gestational diabetes.

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Background and Aims: Gestational diabetes mellitus (GDM) is considered as a serious risk factor for the future development of type 2 diabetes mellitus (DM2). The aim of this study is to evaluate laboratory

parameters during GDM pregnancy that may predict the candidates for DM2 among these women.
Materials and Methods: 597 women with a history of GDM, based on ADA 2000 criteria, underwent an OGTT-75g after delivery (median interval 7 months). Of these, 480 (80.4%) were normal (N); 84 (14%) developed glucose intolerance (fasting and/or 2h post load) (GI); and 33 (5.6%) had DM2. In all women during pregnancy the following parameters were reported: Age, pre-pregnancy BMI, blood pressure (BP), family history (FH) for DM, gestational age (GA) when the diagnostic OGTT-100g was performed, glucose (G) and insulin plasma levels, and HbA1c. For statistical analysis ANOVA, x^2 test, receiver operator characteristic (ROC) analysis, and stepwise logistic regression were used and relative risk was calculated.
Results: DM2 were heavier compared to N (BMI 30±6 versus 26±5) and had more frequent FH (mother DM2 42% vs N 16% and father DM2 36% vs N 17%). GDM occurred earlier in pregnancy in DM2 compared to N (GA17±9 vs 27±6 weeks respectively). DM2 had significantly higher (p<0.001) HbA1c and G levels during diagnostic OGTT-100g (5±1.4% vs 4±1, G0' 125±34 vs 93±13, G60' 252±55 vs 202±29, G120' 231±62 vs 174±34, G180' 177±57 vs 136±34 mg/dl). There was no difference in age, BP, and insulin levels between the two groups. The discrimination ability of various parameters for DM2 prediction (ROC) was G0' 100, 60' 213, G120'186, και G180'145 mg/dl, HbA1c 4.3%, and the corresponding RR was 15.8, 7.5, 8.3, 3.7 and 7.7. In stepwise logistic regression the combination of G0'>100mg/dl, G120' >186mg/dl, and HbA1c> 4.3% predicted correctly the future DM2 in 87% of cases
Conclusion: GDM women with fasting G0'>100mg/dl and G120'>186mg/dl and HbA1c> 4.3% are the more vulnerable candidates for DM2 development in the near future. Therefore this subgroup of prior GDM women have to be followed closely after delivery for early preventive and/or therapeutic intervention.

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Prevalence of metabolic syndrome in women with previous gestational diabetes.
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Background and Aims: Women with previous Gestational Diabetes Mellitus (pGDM) may be considered at risk for metabolic syndrome (MS) later in life, but its prevalence in pGDM women is still undefined. Moreover, definition of MS and various cutoffs for its components have varied widely. This study was performed to evaluate MS prevalence according to different diagnostic criteria in pGDM women.
Matherials and Methods: We studied 116 pGDM women and 27 controls (CON) 2 years after delivery. In all women plasma glucose, insulin, lipid profile, fibrinogen, C-reactive protein (CRP), blood pressure and anthropometric parameters were determined. MS was diagnosed using WHO criteria (1998) as well as NECP-ATP III recommendations (2001)
Results: The two groups were comparable for age (35.4±4.5 vs 34.2±4.4 yrs) and use of oral contraceptive (23.2% vs 29.6%). pGDM had higher waist circumference (85.6±13.5 vs 79.3±10 cm; p<0.01) and BMI (24.9±5.1 vs 23.4±kg/m2; p<0.07). In pGDM, fasting plasma glucose (91±11 vs 82±10 mg/dl; p<0.002), insulin (9.7±6.5 vs 6.8±3.44 _UI/ml; p<0.05) and HOMA-R (2.22±1.56 vs 1.25±0.76; p<0.001) were higher. On the contrary, HDL cholesterol (HDL-C) was lower (54.1±14.1 vs 58.6±11.8 mg/dl; p0.06). When normal weight (BMI<25 kg/m2) pGDM (n=60) and CON (n=20) were compared, fasting plasma glucose, insulin and HOMA-R remained significantly higher in pGDM (all p<0.01). MS was diagnosed using WHO criteria as well as NECP recommendations (ATP III). No CON met the diagnostic criteria for MS. In pGDM, MS prevalence was 3.5% and 12% according to WHO and ATP III respectively. Only one woman was identified by both criteria. Women with MS showed a significantly (p<0.001) higher values of CRP than women without MS (5.7±4.9 vs 1.7±2.6mg/l). After multivariate analysis, CRP (r=0.35; F test 17.5) and LDL-C (r =0.48; F test 18.6) resulted independently associated with MS.
Conclusions: The study shows that MS can be found in pGDM women in a sizable proportion early after delivery. The prevalence of MS is significantly influenced by different diagnostic criteria. In addition to the traditional components, women with MS show high level of others cardiovascular risk factors.

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Six-year results of the implementation of Israeli-Georgian program diabetes in pregnancy.
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Background and Aims: The Purpose of the work was to evaluate the six-year results (1997-2002) of the program Diabetes in Pregnancy, aimed at approximating pregnancy outcomes (PO) in diabetic woman to that of the non-diabetic ones.
Materials and Methods: The study included in total 103 women with pre-gestational DM (pre-GDM) separated into 3 groups: Gr.1 - 44 women, who received preconception care. Gr.2 – 20 women enrolled in the study at 10.8± 2.3 week of gestation, these women were previously supervised at the Children's Endocrinology Center. Gr.3 – 39 women enrolled in the study at 13.5±2.9 week of gestation, previously supervised by general practitioners. Strict metabolic control and fetal surveillance were performed throughout the pregnancy. In Control group (CGr) -57 women with pre-GDM who had 82 prior pregnancies without proper perinatal care (1990-1996).
Results: At conception Gr.1 women were well-controlled: HbA1c – 6.1±0.4%. While in Gr.2 and 3 HbA_{1c} at entry was statistically higher (Gr.2 – P<0.01, P<0.001). By the term HbA_{1c} levels decreased to 5.7±0.2 % (Gr.1), 6.1±0.3% (Gr.2), 6.7±0.3% (Gr.3). In Gr.1 one spontaneous abortion (SA) (2.3%) was registered. Remaining 43 women delivered. In Gr.2 one (5%) induced abortion (IA) was performed; infants were born to 19 mothers. In Gr.3 we observed – SA- (7.7%), IA- (5.1%), intrauterine deaths (ID) (7.7%), infants were born in 79.5% of cases. In CGr pregnancy outcomes were significantly worse: SA-(40.2%), IA-(23.1%), ID-(23.1%), only 13.4% of women gave birth to living infants.
Conclusion: Planned pregnancy and strict diabetes control throughout the pregnancy increases the level of good outcomes from 13.4% to 97.7%. PO for the participants depended on when care during pregnancy was initiated and whether preconception care was carried out. The results stress the necessity to implement preconceptional management for all diabetic women in the Public Health System of Georgia.

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HbA1c and fructosamine by trimester and birth weight in pregnancy in T1DM.
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Background and Aims: Macrosomia rates remain high in T1DM pregnancies. Relative importance of HbA1c compared to fructosamine as a risk marker for macrosomia remains uncertain. The impact of glycaemic control in early versus late gestation on birth weight is unclear.
Materials and Methods: We report results on 97 singleton infants born at term to mothers with T1DM. All had HbA1c measured at booking and on a monthly basis and fructosamine levels at booking and on a two to four weekly basis for the duration of pregnancy. For the purposes of this study HbA1c (normal range 3.9-6.0%) and corrected fructosamine (cF) (normal range < 250 mmol/l) at 13 weeks, 26 weeks and 37 weeks are used as markers for glycaemic control at end of the 1st trimester, 2nd trimester and term respectively. Macrosomia for the purpose of this study is defined as birth weight at term of > 4.0 kg. 16% of deliveries in our non diabetic population have birth weight > 4.0 kg.
Results: 32/97 (33%) of the infants in this study had birth weight of > 4.0kg. Those infants with birth weight < 4.0kg (n = 65) has lower HbA1c (6.4 ± 0.8% vs. 6.9 ± 0.7%, p = 0.03) and cF (292 ± 30 vs. 314 ± 32 mmol/l, p = 0.03) at 13 weeks gestation than those with birth weight > 4.0 kg. HbA1c and cF did not differ significantly at booking, 26 weeks and 37 weeks. Birth weight in those pregnancies with a cF < 250 mmol/l at 13weeks (n = 9) was lower than those with cF > 250 mmol/l (3.22 ± 0.4 kg vs. 3.85 ± 0.47 kg, p < 0.001). Macrosomia rates in those with cF < 250

mmol/l at 13 weeks was 0%. Birth weight in those pregnancies with mean HbA1c < 6% at 13 weeks did not differ from those with HbA1c > 6% (3.64 ± 0.38 vs. 3.84 ± 0.47kg, p = 0.17). Those with HbA1c < 6% at 26 weeks had a lower birth weight than those with HbA1 > 6% (3.71 ± 0.4 kg vs. 3.97 ± 0.48 kg, p < 0.05). Macrosomia rates in these groups were 25% vs. 50%.
Conclusions: Pregnancies of normal birth weight infants had lower HbA1c and fructosamine by 13 weeks gestation than those of macrosomic infants. Pregnancies with cF levels < 250 mmol/l by 13 weeks are associated with birth weights below the mean for our non-diabetic population. This association is not seen with HbA1c at 13 weeks. Optimal glycaemic control at 26 weeks as assessed by both HbA1c (< 6.0%)and cF (< 250 mmol/l) is associated with lower birth weights and reduced macrosomia rates but not to background level. In T1DM in pregnancy, glycaemic control as measured by HbA1c and fructosamine must be optimised prior to the end of the second trimester to reduce macrosomia rates to those in non diabetic pregnancy.

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Long-term breast-feeding in women with Type 1 diabetes.
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Background and Aims: Breast-feeding may be more difficult and of shorter duration in women with type 1 diabetes due to neonatal morbidity and fluctuating blood glucose values. The aim was to evaluate the frequency of breast-feeding for more than 4 months in mothers with type 1 diabetes. The possible causes for unsuccessful breast-feeding are sought identified.
Material and Methods: Eighty out of all 85 consecutive women with type 1 diabetes giving birth to a single living baby in the period may 2001 to september 2002, were interviewed about breast-feeding using a structured questionnaire about 5 days and 4 months after delivery. HaemoglobinA1c during pregnancy,White class, mode of delivery and gestational age were identified from the medical records. The neonates were routinely with their mother for the first 1-2 hours to facilitate bonding and breast-feeding, whereafter they were admitted to the NICU for the first 24 h. Early feeding was established. Neonatal hypoglycaemia was defined as at least one blood glucose < 2.5 mmol/l within the first 2-24h. Neonatal morbidity was defined as CPAP for > 1 hour, need for antibiotic treatment, or phototherapy.
Results: Five days after delivery 55 women were exclusively breast-feeding, 17 women were partly breast- feeding, 8 were bottle-feeding with their own milk or formula-milk. Four months after delivery the figures were 42 women (52%); , 11 (14%) and 27 (34%), comparable to the background population: 51%; 25% and 24% (NS).
Mothers who were breast-feeding after 4 months (66%) were characterised by a higher prevalence of infants born at term without significant neonatal morbidity (57%) compared to mothers who stopped breast-feeding (22%) (p<0.01). Haemoglobin A1c during pregnancy, duration of diabetes, White class, mode of delivery and neonatal hypoglycaemia were similar in the two groups. Four months after delivery exclusively breast-feeding mothers received 90 % of their prepregnancy insulin dose, formula-feeding women received 105% (p<0.05), the number of mild hypoglycaemia per week was comparable. Thirty (83%) out of 36 mothers delivering a healthy infant at term were still breast-feeding 4 months later while only 23 (52%) out of 44 women delivering an infant preterm and/or with neonatal morbidity were breast-feeding after 4 months (p<0.05).
Conclusion: Women with type 1 diabetes are breast-feeding for 4 months as in the background population. Premature delivery and neonatal morbidity, but not fluctuations of blood glucose were the main causes for unsuccessful breast-feeding.

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Changes in apolipoproteins during pregnancy in women with Type 1 diabetes.
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Background and Aims: The aim of the study was to evaluate the changes in ApoA and ApoB apolipoprotein levels and their equivalents: lipoproteins in pregnant women with type 1 diabetes.
Materials and Methods: The investigation was conducted in 46 pregnant women with Type 1 diabetes belonging to classes B, C and D, according to White. In the following pregnancy terms apolipoprotein was determined by ApoA and B, total cholesterol (TC), LDL and HDL. The Control Group (CG) was 27 non-pregnant women with Type 1 diabetes. All the patients exhibited very good glycemia compensation (HbA1c average=6,0% in the pregnant women, 6,2% for the non-pregnant women).
Results: The pregnant women with Type 1 diabetes in the first term of pregnancy do not differ from the non-pregnant women with type diabetes as far as TC, LDL and HDL levels are concerned, whereas in the pregnant women the apolipoprotein Apo A level is higher and apolipoprotein B level is lower, which may be the result of raising the insulin treatment and fast glycemia control at the beginning of pregnancy. Later in the period of pregnancy in patients with Type 1 diabetes the TC, Apo B and LDL values increase significantly in the T-II and T-III. The increase in Apo B concentration as compared with LDL was considerably higher statistically in the T-II (40.9% vs 21.1 %, p<0.05) and T-III (92.2% vs 42.3%, p< 0.01), which may be the reflection of an unfavourable change of the LDL particle structure into a more atheromatous. However, no significant differences in Apo A and HDL concentration increase were observed (in T-II: 20.7% vs 21.2%, NS; in T-III: 25.0% vs 14.0%, NS).

Table 1. Lipoprotein and apolipoprotein levels in pregnant women with successive pregnancy terms in Type 1 diabetes and in the control group (Mean±SD).

Parameters	Term I	Term II	Term III	CG
ApoB	55.9 ± 13.7	78.8 ± 27.5	107.4 ± 27.3	65.9 ± 21.7
LDL	93.2 ± 29.2	113.0 ± 31.0	132.7 ± 35.5	102.2 ± 30.8
ApoA	113.4 ± 27.5	136.9 ± 27.7	141.8 ± 35.8	98.1 ± 16.9
HDL	65,1 ± 19,0	78.8 ± 17.7	74.1 ± 20.1	58.8 ± 16.4
TC	175.3 ± 38.7	216.6 ± 40.6	251.5 ± 44.3	175.9 ± 30.5

Parameters	I vs II I	vs III	II vs III	CG vs I	CG vs II	CG vs III
ApoB	p<0.001	p<0.001	p<0.001	p<0.05	p<0.05	p<0.001
LDL	p<0.001	p<0.001	p<0.01	NS	NS	p<0.001
ApoA	p<0.001	p<0.001	NS	p<0.05	p<0.001	p<0.001
HDL	p<0.001	p<0.01	NS	NS	p<0.001	p<0.01
TC	p<0.001	p<0.001	p<0.001	NS	p<0.001	p<0.001

Conclusion: The changes of apolipoprotein B level in the pregnant women with Type 1 diabetes are not equal to the level of its equivalent LDL lipoprotein, which indicates the appearance of quality changes of lipoproteins particles during pregnancy.

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Differing causes of perinatal mortality in Type 1 and Type 2 diabetic pregnancies.
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Background and Aims The increasing incidence of Type 2 diabetes (T2D) means that in many parts of the world there are now more pregnancies in women with T2D than there are in women with Type 1 diabetes (T1D). Auckland, New Zealand, has a high prevalence of T2D in the indigenous Maori, and in migrants from the Pacific Islands and southern Asia. We describe the incidence and causes of fetal loss in our clinic over a 17 yr period, 1985-2002.
Materials and Methods There were 258 pregnancies in women with T1D, 388 in women with established T2D and 257 in women with newly recognized diabetes (13% of women identified with gestational diabetes in our clinic prove to have diabetes [almost always T2D] on early postpartum testing). All pregnancy losses, other than spontaneous miscarriage at <20 weeks' gestation, were included. The losses were classified as either elective terminations (for congenital anomaly), intermediate fetal death (20-28 weeks), late fetal death (28 weeks to term) or early neonatal death (1 day to 1 month postpartum).
Results The total number of pregnancy losses in T1D was 9/266 (3.4%), with the majority (78%) attributable to either prematurity or congenital malformation. There were no late fetal deaths, and no intermediate fetal deaths attributed to chorioamnionitis. The total number of pregnancy losses was higher in women with T2D, 19/393 (4.8%), and in those with newly recognized diabetes, 12/258 (4.7%). In these groups, 11 of 31 losses (35%) were late fetal deaths, and 6 of 31 (19%) were intermediate fetal deaths

resulting from chorioamnionitis. Only 8 of 31 (26%) were attributable to prematurity or congenital malformation. The etiology of fetal losses differed according to the type of diabetes (X^2=9.08, p=0.01).
Conclusions Fetal losses now occur at a higher rate in T2D than in T1D, and that there are significant differences in the causes of fetal loss. Late fetal death and chorioamnionitis are both significant causes of loss in T2D, but are very uncommon in T1D.

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Placental growth hormone and insulin-like growth factor I in pregnancies in Type 1 diabetic subjects.
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Background and Aims: Human placental growth hormone (hPGH) gradually replaces pituitary GH during pregnancy. hPGH may influence fetal growth and has been found to correlate with insulin-like growth factor I (IGF-I) in normal pregnancy and in pregnancies in women with non-insulin dependent diabetes mellitus. hPGH also correlates to birthweight in normal pregnancies. The present study investigated the relationship between hPGH, IGF-I and birth weight and placental weight in pregnancies in type 1 diabetics.
Materials and Methods: A cohort of 51 type 1 diabetic pregnant women were followed during pregnancy with repeated blood sampling. Median 14 blood samples were obtained per subject, and analyses of hPGH, IGF-I and -II were performed. Results were compared to clinical characteristics. hPGH was determined with a radioimmunometric assay (Biocode, Liege, Belgium). Total IGF-I was determined with an immunofluorometric assay. Non-parametric tests were used for data analysis. Data are given as median (range).
Results: Characteristics of the cohort are given in Table 1 below. hPGH was detected as early as gestational week 6, where after a gradual rise was observed to a maximum in week 34 – 35 with levels at 23.1 microg/l (6.7 – 157). IGF-I values decreased from first to second trimester ($p = 0.005$), reaching a plateau from where increasing levels was observed during the third trimester. Correlation between hPGH and IGF-I was observed from week 24 to week 35 ($0.35 < r < 0.6$; $0.001 < p < 0.03$). Hereafter a trend was observed, but decreasing numbers of blood samples limited analyses. hPGH values correlated to birth weight and placental weight independent of choice of hPGH value (maximum value, mean value in the last four weeks of pregnancy, last recorded value or Area Under the Curve-estimates) in both the entire cohort and in the subgroup delivering at term.
Conclusion: The present study indicates a potential role for hPGH in the growth of the foetus. In type 1 diabetic pregnancies, hPGH appears to be associated with IGF-I and birth weight and placental weight.

Table 1	Median	Range
Age	28 years	20 - 37
Parity	1	0 - 2
Duration of diabetes	16 years	0 - 29
Pre-pregnancy BMI	23.2 kg/m^2 [a]	18.8 - 31.1
Creatinine in first trimester	56 micromol/l [c]	41 - 74
Gestational age at delivery	258.5 days (36+6 weeks)	227 - 282 (32+3 - 40+2)
Birth weight	3870 g.	1640 - 5300
Placental weight	752.5 g. [b]	300 - 1150

Characteristics for the 51 type 1 diabetic subjects. a) n = 50, b) n = 48, c) n = 45.

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Effect of pregnancy on diabetic retinopathy progression; changes in systemic levels of angiopoietic factors.
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Background and Aims: To study the levels of angiopoietic factors in pregnant women with and without Type 1 diabetes and to relate these levels to progression of diabetic retinopathy during pregnancy and postpartum.
Materials and Methods: In prospective follow-up study of 63 pregnant women with Type 1 diabetes and 11 non-diabetic pregnant women, diabetic retinopathy was graded from colour fundus photographs. Levels of angiopoietic cytokines and receptor (angiopoietin-1 and -2 and hVEGF-A and sVEGF receptor-1), circulating insulin-like growth factor-binding protein 3 (IGFBP-3), and glycodelin, were measured during the first and third trimester of pregnancy and 3 months postpartum.
Results: Levels of angiopoietin-2 and IGFBP-3 were lower in the diabetic women (p=0.015 and p=0.001, respectively) during pregnancy, and hVEGF-A lower at 3 months postpartum than in non-diabetic controls (p=0.002). At baseline, levels of angiopoietic factors showed no correlation with severity of retinopathy. During pregnancy and postpartum, glycodelin and hVEGF-A levels were lower in diabetic women with progression of retinopathy, whereas the other factors were similar to those without progression. In linear regression analysis, only sVEGFR-1 during the third trimester was associated with retinopathy severity (R^2=0.16, P=0.04). Other factors were dropped from the model.
Conclusions: In diabetic women, IGFBP-3, angiopoietin-2 and hVEGF-A are downregulated, whereas levels of angiopoietin-1 and glycodelin are unchanged compared to levels in non-diabetic controls during pregnancy and/or postpartum. The systemic growth milieu of pregnancy does not seem to favour the progression of diabetic retinopathy.

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Is caffeine a risk factor for adverse pregnancy outcome in women with Type 1 diabetes mellitus?
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Background and Aims: The effect of maternal caffeine consumption during pregnancy on perinatal outcome is limited and controversial. The current study was undertaken to test the hypothesis that caffeine consumption during pregnancy in women with type 1 diabetes mellitus (IDDM) is associated with increased risk of adverse maternal and perinatal outcome.
Materials and Methods: This is secondary analysis of pregnant women with IDDM who participated in a controlled clinical trial. These women were interviewed monthly by a trained non-medical member of the research team regarding typical daily consumption of caffeine, smoking habits and alcohol intake. Information about typical daily consumption of caffeine including amount and type (coffee, tea and soft drinks) was obtained. This design was used to minimize bias that may be introduced by the caregiver obtaining this information.
Results: Data on caffeine consumption was collected on 193 consecutive pregnancies over a seven-year period. Fifty-four percent of women reported consuming at least one 8-ounce drink (range:1-3) containing caffeine daily during the first trimester of pregnancy. Sixty-five percent of women reported consuming at least one 8-ounce drink (range:1-3) containing caffeine daily after 20 weeks' gestation. The association of caffeine consumption during pregnancy with maternal and perinatal outcome is depicted in the tables below. Covariates and confounders considered were; age, years since diagnosis of diabetes, previous spontaneous abortion, presence of diabetic retinopathy and nephropathy, glycemic control as measured by glycohemoglobin A_1 concentration and cigarette smoking.
Conclusion: Caffeine consumption during pregnancy is independently associated with increased risk of preterm birth and related complications. An intriguing finding is that caffeine consumption is independently associated with significant reduction in risk of preeclampsia in this population.

Table 1	Caffeine 1st Trimester) (%		Odds Ratio for Caffeine (95%CI)	
Outcome	Yes (N=104)	No (N=89)	Unadjusted	Adjusted
Spontaneous Abortion	18 (17.3%)	5(5.6%)	3.5 (1.2, 9.9)	1.9 (0.6, 6.5)
Major Congenital Malformation	4 (4.6%)	2(2.4%)	2.0 (0.4, 11.2)	—-

Table 2	Caffeine after 20 weeks' n (%)		Odds Ratio for Caffeine (95%CI)	
Outcome	Yes (N=110)	No (N=59)	Unadjusted	Adjusted
Preeclampsia	14 (12.7%)	15 (25.4%)	0.4 (0.2, 1.0)	0.3 (0.1, 1.0)
Delivery <34 weeks'	14 (12.7%)	4 (6.8%)	2.0 (0.6, 6.4)	2.4 (0.7, 8.4)
Delivery <37 weeks'	40 (36.4%)	15 (25.4%)	1.7 (0.8, 3.4)	2.2 (0.9, 5.5)
Respiratory Distress Syndrome	17 (16.0%)	4 (6.9%)	2.6 (0.8, 8.1)	3.3 (1.0, 11.3)
Infant LOS >7 days	28 (25.9%)	10 (17.5%)	1.6 (0.7, 3.7)	2.2 (0.8, 6.2)
	Unadjusted mean ± std		Adjusted mean ± std	
	Yes (N=110)	No (N=59)	Yes (N=110)	No (N=59)
Birth weight (grams)	3228 ± 700	3436 ± 741	3243 ± 562	3325 ± 638
Gestation at delivery (weeks)	36.7 ± 2.5	37.4 ± 2.4	36.7 ± 2.5	37.8 ± 2.8

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Early microalbuminuria is an important predictor of development of preeclampsia in women with Type 1 diabetes – results from a nation-wide Danish study.

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Background and Aims: A few reports from Rigshospitalet, Copenhagen have found that microalbuminuria predicts preeclampsia. The aim was to study the relationship between preconceptional and/or 1st trimester microalbuminuria and development of preeclampsia in a large group of Danish women with type 1 diabetes.
Materials and Methods: During 1993-99 all type-1 diabetic pregnancies were reported to a central registry in the Danish Diabetes Association. Information of maternal demography, glycemic control, diabetes-related complications and pregnancy complications was prospectively collected by 1-3 caregivers in each centre. We included 898 women without diabetic nephropathy (albuminuria > 300 mg/24h) and without medically treated hypertension at the time of the first visit. Preeclampsia was defined as blood pressure > 140/90 and proteinuria (0.3 g protein/24h).
Results: The patients were 28 (25-32) years (median and interquartile range) and had a body mass index (BMI) of 23 (21-25) kg/m2. Diabetes duration was 11 (5-17) years and 3rd trimester HbA1c 6.6 (6.1-7.4) % (reference interval 4.4-6.4 %). Sixty-one percent were nulliparous and 4% had proliferative retinopathy. Early microalbuminuria was detected in 10% of the women and among these 42% developed preeclampsia. The frequency of preeclampsia in the normoalbuminuric group was 13%. After adjustments for a number of other factors, significant predictors (odds ratios with 95% confidence intervals) for development of preeclampsia were: Microalbuminuria 4.5 (2.6-7.9), nulliparity 3.2 (1.9-5.4), 3rd trimester HbA1c 1.3 (1.1-1.5) per 1 % increase and BMI 1.1 (1.0-1.1) per 1 kg/m2 increase.
Conclusion: Women with early microalbuminuria have a nearly 5-fold increased risk of developing preeclampsia. Accordingly, this risk group should be subjected to intensified surveillance. Optimising glycemic control might reduce the risk of preeclampsia.

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Carbohydrate Metabolism in Pregnancy

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Normal range of haemoglobin$_{A1c}$ in pregnant women is lower than in non-pregnant women.

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Background and Aims: Prior to pregnancy the target for metabolic control is haemoglobin A1c values near the normal range. Whether the normal range of haemoglobin A1c is changing during pregnancy is not well established. Thus the aim was to evaluate the normal range of haemoglobin A1c in early and late pregnancy as well as in non-pregnant women matched for age and BMI.
Materials and Methods: Healthy non-pregnant women aged 30 years were investigated as a part of a population survey ("Inter 99", N=145). One hundred randomly selected normal pregnant women from our obstetric clinic with random capillary blood glucose below 7 mmol/l were investigated in week 14 (range 8-17). Ninety-eight randomly selected normal pregnant women with a normal 75 g oral glucose tolerance test in week 33 (30-37) were investigated the same day as they had the test. All had Haemoglobin A1c measured with the HPLC method (variant, Bio-Rad LABS, Steno Diabetes Center). For calculation of BMI, pre-conception height and weight was used in the pregnant women.
Results: Haemoglobin A1c in healthy non-pregnant, early pregnant and late pregnant women were as follows (mean (SD)): 5.46 (0.38); 5.14 (0.32); 5.02 (0.32) % (non-pregnant vs. early pregnancy, p<0.0001, early vs. late pregnancy p<0.009, non-pregnant vs. late pregnancy p< 0.0001), BMI: 24.5 (4.6); 23.0 (3.6); 22.3 (2.8) kg/m^2 (p<0.001, trend test) and age: 30; 30.8 (5); 29.2 (3) years, respectively. After excluding the overweight women (BMI>25 kg/m^2) from the groups, haemoglobin A1c was still significantly different: 5.43 (0.4); 5.13 (0.3); 5.03 (0.3) % (p<0.001 trend test) while BMI was comparable: 21.7 (2.0); 21.6 (1.7); 21.5 (1.9) kg/m^2 (NS). Normal range of haemoglobin A1c in non-pregnant 30 years old women was 4.7-6.2 % and 4.5-5.8 % in early pregnancy and 4.4-5.7 % in late pregnancy.
Conclusion: Normal range of Haemoglobin A1c is significantly lower in pregnant women compared to non-pregnant women.

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Glucose uptake and insulin-signaling in human adipose tissue in pregnancy.

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Background and Aims: Pregnancy is a state of insulin resistance. Insulin stimulates glucose uptake via PI3K-dependent (IRS-1, PI3K, Akt, PKC, GLUT4) and PI3K-independent signaling pathways (Cbl, CAP, CkII, C3G and TC10, GLUT4). In contrast, hyperosmolarity stimulates glucose uptake via a PI3K-independent pathway.We hypothesized that adipose tissue in pregnancy and gestational diabetes mellitus (GDM) is insulin resistant due to defects in the subcellular localization of key molecules involved in insulin-stimulated glucose uptake. An assessment of hyperosmolarity-stimulated glucose uptake would help differentiate if the PI3K-independent pathway was involved.
Materials and Methods: Paired subcutaneous (SC) and omental (OM) adipose tissue biopsies were obtained from 16 normal glucose tolerant (NGT) pregnant women, 14 GDM and 19 nonpregnant controls (NPC). Insulin- and hyperosmolarity-stimulated glucose uptake was measured and correlations made with clinical variables and the subcellular localization of the insulin receptor (IR), GLUT4, p85 (the regulatory subunit of PI3K), and CAP. The subcellular fractions were obtained by differential centrifugation of adipose tissue lysates yielding plasma membrane (PM), high density microsomes (HDM), low density microsomes (LDM) and cytosol. Densitometry of Western blots was performed to quantify the expression of the proteins in question.

Results: Basal and insulin-stimulated glucose uptake was not impaired in SC or OM adipose tissue in either pregnant group compared with nonpregnant controls. In the NPC, insulin-stimulated glucose uptake inversely correlated with BMI (r=0.684, P=0.001). Hyperosmolarity stimulates glucose uptake in SC adipose tissue of NPC. This was impaired in NGT pregnant women and GDM (1.52±0.19 vs. 0.73±0.12 vs. 0.88±0.07; NPC vs. NGT vs. GDM, P=0.02). The subcellular distribution of the IR, p85, and GLUT4 was similar in the three groups of women. In the SC depot, insulin-stimulated glucose uptake correlated with the expression of GLUT4 at the PM (r=0.51, p=0.05). The expression of CAP was significantly reduced in the OM depot in NGT pregnant women compared with NPC (p=0.05). The expression of CAP was reduced in both the SC and OM depots in women with GDM compared with NGT pregnant women (p<0.02).
Conclusion: These results demonstrate that adipose tissue in NGT pregnancy and GDM is not insulin resistant compared with NPC. The resistance of SC adipose tissue in pregnancy to hyperosmolarity, associated with a reduction in the expression of CAP, particularly in GDM, alludes to a defect in the PI3K independent pathway and/or an abnormality in a specific pool of GLUT4. The PI3K independent pathway requires further investigation in states of insulin resistance.

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Serum concentrations of soluble tumor necrosis factor receptor 2 (sTNFR2), glycated hemoglobin at delivery and birthweight.
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Background and Aims: Serum concentrations of acute phase proteins and several other inflammatory parameters have been found to be associated with insulin resistance. Both low and increased birthweight have been related with gestational diabetes mellitus and with increased risk for developing insulin resistance in adulthood. We aimed to study the serum concentrations of sTNFR1 and sTNFR2 (which reflect the activation of the TNF-α system) in association with glucose tolerance during pregnancy, as determined by maternal glycated hemoglobin at delivery, and birthweight.
Materials and Methods: Fifty-four healthy (except for 4 cases of gestational diabetes) pregnant women (age: 31.4±4.4y; pregestational BMI: 24.0±4.0kg/m2) were studied for serum levels of glucose, HbA1c, sTNFR1 (ELISA) and sTNFR2 (ELISA) at delivery. Clinical variables included: age, height, pregestational BMI and BMI at delivery (pre-BMI and BMI), weight increment during gestation, cigarette smoking, parity and gestational age- and sex-adjusted birthweight (in SD score).
Results: sTNFR1 was 2.16±0.84 mg/L; sTNFR2: 5.17±1.37; glucose at delivery: 81.9±22.9 mg/dl; HbA1c: 4.27±0.47. Gestational age was: median 39 (37-41); birthweight: 3,311±385 grs; BW SDS: 0.05±-0.026). Univariate analysis is shown in table. Predictors of HbA1c at delivery were: sTNFR2, explaining 11% of HbA1c variance (excluded variables: age, serum glucose and BMI). Predictors of BW SDS were: sTNFR2, explaining 8% of BW SDS variance (excluded variables: maternal height, either pre-BMI or BMI, serum glucose, smoking and parity).
Conclusion: Circulating sTNFR2 could be a marker of glucose intolerance during gestation and a determinant of birth weight.

	sTNFR1	sTNFR2	Pre-BMI	ΔWeight	BMI	Glucose	HbA1c
BW SDS	r=0.03 p=0.81	r=-0.34* p=0.026	r=0.037 p=0.80	r=0.063 p=0.67	r=0.052 p=0.73	r=-0.34* p=0.020	r=-0.12 p=0.46
HbA1c	r=0.34* p=0.029	r=0.39* p=0.012	r=0.36* p=0.018	r=-0.36* p=0.017	r=0.30 p=0.055	r=0.17 p=0.29	
Glucose	r=-0.05 p=0.73	r=-0.07 p=0.63	r=0.03 p=0.88	r=-0.012 p=0.94	r=0.013 p=0.93		
BMI	r=0.039 p=0.80	r=0.30* p=0.044	r=0.93* p<0.000	r=-0.30* p=0.037			
ΔWeight	r=0.005 p=0.97	r=-0.111 p=0.47	r=-0.60* p<0.000				
Pre-BMI	r=0.033 p=0.83	r=0.24 p=0.112					
sTNFR2	r=0.48* p<0.000						

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Role of insulin resistance in the pathogenesis of pregnancy induced hypertension and preeclampsia.
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Background and Aims: The association of pregnancy induced hypertension (PIH) and preeclampmsia (PE) with insulin secretory capacity and insulin resistance is still not fully clear. Both PE and diabetes are claimed to be insulin resistant conditions. Insulin secretory defect (which is the primary pathology in many diabetic patients) may, however, also be conceived to be responsible for creating insulin resistance by secondary mechanism. Parallel investigation of insulin secretory capacity and insulin sensitivity in PIH and PE patients with and without diabetes, and in nondiabetic Controls, has been undertaken to understand the role of these factors in the pathophysiology of these disorders.
Materials and Methods: Nineteen nondiabetic-PIH (PIH), 17 nondiabetic-PE (PE), 15 diabetic-PIH (DPIH) and 12 diabetic-PE (DPE) subjects were studied along with 27 diabetic pregnant (DP) and 32 nondiabetic, non-PE pregnant (NDP) Controls. All subjects were aged between 20 to 35 years and they were at 3rd trimester of gestation. Fasting and 2 hour serum glucose and fasting serum insulin (fluorescence-based ELISA) and serum C-peptide (chemiluminiscence-based ELISA) were measured. Insulin secretory capacity (HOMA B) and insulin sensitivity (HOMA S) were estimated by homeostasis model assesment (HOMA) method.
Results: Fasting and postprandial blood glucose levels of PIH and PE groups werenot different from those of Control. The PIH and PE groups, however, showed considerable degree of hyperinsulinemia as evident from serum C-peptide (p<0.009 and 0.026) as compared to Control levels. The DP and DPE groups showed serum C-peptide level almost equivalent to those of the Control Group but the DPIH group showed significant difference as compared to Control group [mmol/l, {Mean (Range)}: {0.54 (0.20-1.78)} in Control vs {0.92 (0.17-2.31)} in DPIH, p<0.003]. B-cell secretory capacity [HOMA B (%)] in PE and PIH groups showed significantly higher values as compared to Control group [mmol/l, {Mean (Range)}: {139.6 (59.9-724.9)} in Control vs {217.3 (53.1-552.8)} in PE, p<0.037 and {193.3 (104.4-401.9)} in PIH, p<0.035]. But the diabetic groups (DPE and DPIH) showed no significance difference as compared to Control. In contrast to the DP group the PE and PIH (p<0.002 and p<0.001) groups showed increased B-cell secretory capacity [HOMA B(%)]. Although the PE and diabetic groups showed opposite trends regarding B-cell function the PE, PIH and DPIH groups showed significantly low insulin sensitivity [HOMA S (%)] as compared to Control [mmol/l, {Mean (Range)}: {85.85 (24.8-227.30) in Control} vs {48.9 (17.4-264.2) in PE, p<0.012}; {57.5 (0.90-130.6) in PIH, p<0.020} and {46.1 (17.7-299.5) in DPIH, p<0.003}].
Conclusion: : PE and PIH are insulin resistant conditions in which a hypersecretory response from the pancreatic B-cells occurs as a compensatory measures. In contrast, coexistence of both B-cell secretory failure and insulin resistance is associated with diabetes with or without the presence of PE or PIH.

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The impact of pregnancy weight and glucose on the metabolic health of mother and child (The EarlyBird Diabetes Study).
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Background and Aims: The association between obesity and insulin resistance is well established. The long-term effects of the gestational environment on the metabolic status of mother and child, however, remain unclear. It is possible that the increasing prevalence of type 2 diabetes in today's children is linked to increasing weight of their mothers during pregnancy. The aim was to examine the relationships between pre-pregnant weight, glucose during pregnancy, infant's birth weight, and weight, glucose and insulin resistance five years later in both mother and child.
Materials and Methods: Baseline data were examined from a prospective, non-intervention cohort study of 300 healthy children (mean age 4.9 years) and their mothers (mean age 33.4 years). Outcome measures: Mother: pre-pregnant weight, random third trimester glucose (n= 221) and fasting glucose (n= 26), weight, glucose and insulin resistance five years later (n= 273). Child: birth weight, current weight, glucose and insulin resistance five

years later (n= 283). Six mothers with diabetes or gestational diabetes were excluded from this analysis.
Results: 1) 36% of mothers were overweight or obese at conception, rising to over 50% five years later, when her insulin resistance correlated with her weight (r= 0.58, p< 0.001). 2) Pre-pregnant weight predicted fasting glucose in pregnancy (r= 0.49, p= 0.02), mother's insulin resistance five years later (r= 0.49, p<0.001), offspring's birth weight (r= 0.16, p= 0.007), and its weight at 5y (r= 0.28, p<0.001). 3) Random glucose (range 3.7-7.6 mmol/l) predicted maternal glucose five years later (r= 0.20, p= 0.005). 4) Fasting glucose in pregnancy (range 3.8-5.8 mmol/l) predicted birth weight (r= 0.51, p= 0.014), glucose (r= 0.69, p<0.001), and insulin resistance (r= 0.54, p= 0.01) five years later. 5) Girls were 0.52 standard deviations heavier than average at five years, and their weight correlated with their insulin resistance (r= 0.33, p<0.001). 6) No maternal measure predicted insulin resistance in the child at five years.
Conclusions: Maternal weight during pregnancy has an important influence on the gestational environment, the effects of which are still evident some five years later. Fasting pregnancy glucose is a function of the mother's pre-pregnant weight, and is a substantially better predictor than random glucose of infant's birth weight and her future insulin resistance, even for a normal range of fasting glucose. Antenatal weight management and routine screening during pregnancy with fasting glucose are recommended.

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Optimised management of diabetic pregnancy reduces fetal loss.
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Background and Aims: The outcome of pregnancy in diabetic women remains worse than that of their non-diabetic peers even in specialist units. We set out to optimise outcome in our established combined diabetes-antenatal clinic.
Materials and Methods: Following an audit in 1995, services were re-organised, targeting more intensive diabetes management and increased use of fetal monitoring to determine timing of delivery. Fetal outcome was monitored throughout 1996-2002.
Results: Between 1992-95, 197 pregnancies occurred in 99 gestational (GDM) and 98 established (EDM) diabetic women; compared with 441 (297 GDM and 144 EDM) between 1996-2002. Fetal loss rate (still births and lethal congenital abnormalities) in the first cohort was 60.9 per 1000, similar to published rates from specialist services elsewhere, and fell to 13.6 per 1000 in the second. In the first cohort, there were 7 still births (2 GDM; 1 Type 2 and 4 Type 1 DM) and 5 lethal congenital abnormalities (2 Type 2; 3 Type 1); in the 2nd there were 5 still births (3 GDM; 1 Type 2, 1 Type 1) and 1 lethal congenital abnormality (1 GDM). This equals a fall in fetal loss in the GDM from 20.2 to 13.5 per 1000 and in EDM from 102 per 1000 to 13.9, against a background rate for our local population of ~10.8 per 1000. Intervention rates and gestational age at delivery remained stable throughout both periods but in the second, early delivery in a small number of cases was indicated by adverse fetal monitoring.
Conclusion: We conclude that in diabetes, both gestational and established, better fetal outcome can be achieved by intensive management regimens.

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Umbilical cord insulin in small for gestational age babies of nondiabetic and diabetic mothers in Bangladeshi population.
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Background and Aims: Insulin levels in the umbilical cord blood may provide some idea about the inheritance of the basic defect (insulin secretory dysfunction vs insulin resistance) in the babies of diabetic mothers have a great chance of developing type 2 diabetes mellitus in later life. The interpretation, however, is still complicated by the possible contribution by the intrauterine environment of the mother. In the perspective of the reported ethnic variations on this issue, which is also known to be strongly related to the nutritional status of the mother and baby, we have studied cord insulin in low and normal birth weight babies from nondiabetic, type 2 and GDM mothers in a Bangladeshi population.
Materials and Methods: Umbilical cord blood was obtained (from venous side after double clamping of the cord) from small for gestational age babies of nondiabetic (NDSGA group), type 2 diabetic (DSGA), and GDM (GDSGA) mothers as well as from average for gestational age babies of nondiabetic (NDAGA), diabetic (DAGA) and GDM (GDAGA) mothers. The number of babies in each group was 30. Weight of the babies was measured by weighing balance and Plasma insulin was estimated by chemiluminiscence- based ELISA.
Results: The NDSGA group showed significantly lower levels of insulin [cord blood insulin, µIU/ml, Median (range), 3.8 (2.0-7.0), p<0.0001] as compared to NDAGA group [7.3 (4.0-12.0)]. The DAGA group and GDAGA showed significantly higher cord insulin level [DAGA, 29.0(15.0-43.0); GDAGA, 25.5(12.0-43.0), p<0.001 with both groups] compared to the NDAGA group [7.3 (4.00 -12.00)]. GDSGA and DSGA showed significantly higher insulin [14.00 (5.00-25.00) and [17.00(6.00-32.00), p<0.001 with both groups] compared to the NDSGA group [3.8(2.0-7.0)]. The findings in the GDAGA and GDSGA groups were almost similar to the DAGA and DSGA groups respectively with slightly lower insulin levels in babies from GDM mothers.
Conclusion: Low fetal growth is paralleled by lower insulin in babies from nondiabetic mothers probably as a compensatory response. The response remains proportionately the same in babies from diabetic mothers although the level of insulin is set at a much higher level in response to hyperglycemia. Lack of disproportionate hyperinsulinemia in SGA babies seems to contradict the notion that fetal undernutrition creates programmed defect in insulin sensitivity leading to overt diabetes in adult life. GDM, although genetically a different disorder than type 2 DM, produces exactly the same kind of response implying that reduced insulin secretion is probably a basic inherited defect although hyperglycemia and intrauterine factors in the mother may determine the final status of b cell function and insulin sensitivity in the baby.

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Metformin reduces maternal weight gain in pregnant PCOS women without adverse effects on fetal outcome. Results of a randomised pilot study.
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Background and Aims: The majority of women with polycystic ovary syndrome (PCOS) are overweight. When they become pregnant women with polycystic ovary syndrome (PCOS) have an increased incidence of pregnancy complications and poor pregnancy outcome. Furthermore, overweight itself is associated with pregnancy complications and poor pregnancy outcome. Metformin is, more or less, established as the first line drug in the treatment of PCOS. Recently the use of metformin in pregnant women with PCOS has been reported. However, these studies have been retrospective or non-randomised. In a pilot study we investigated the possible effect of metformin on maternal weight gain during pregnancy.
Material and Methods: 23 pregnant women with PCOS were included in a randomised double blind placebo controlled pilot study before gestational week 9. All the women had received a diagnosis of PCOS before the present pregnancy. They were treated with metformin 850 mg bid or identical placebo capsules until delivery. Results are given as means and standard deviations (SD). Mann-Whitney statistics were used to compare groups.
Results:

Maternal bodyweight (kg) and weight change during pregnancy:

	Week 8		Change to week 16		Change to week 28		Change to week 36	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Placebo	11	82.1 ± 21.4	11	1.7 ± 3.0	10	6.2 ± 3.6	9	9.4 ± 4.9
Metformin	12	91.4 ± 20.2	12	-1.9 ± 3.0	12	2.5 ± 3.7	11	5.4 ± 4.0
P-value		ns		0.006		0.017		0.034

Fetal outcome:

	Placebo (n=11) Mean ± SD	Metformin (n=12) Mean ± SD	P-value
Weight at birth (g)	3111 ± 723	3555 ± 389	.091
Fetal length (cm)	46.3 ± 8.7	50.0 ± 2.3	.17
Gestational age (days)	261 ± 47	282 ± 9	.26

Discussion: To our knowledge this is the first prospective randomised double blind study on the effect of metformin in pregnant women with PCOS. In accordance with our hypothesis metformin reduced pregnancy induced weight gain in women with PCOS. This effect was seen without

adverse effects in the mother or fetus. To the contrary, this small pilot study indicates that there might be a positive effect on fetal outcome in terms of birth weight. The effect on fetal outcome should be investigated in larger randomised studies.

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WITHDRAWN

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Is altered protein kinase C-activity involved in diabetic embryopathy?
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Background and Aims: Diabetic pregnancy is associated with an increased risk for congenital malformations and growth retardation. The estimated risk for malformations in a diabetic pregnancy is 3-5 fold higher compared to a non-diabetic pregnancy. The mechanisms causing these disturbances are not completely clarified. It has been suggested that Protein Kinase C is involved in the induction of diabetic complications. Protein Kinase C may also be involved in the induction of embryonic dysmorphogenesis in diabetic pregnancy. The aim of this work was to investigate the role of different isoforms of Protein Kinase C for the induction of developmental malformations in the embryos of diabetic rats.
Material and Methods: Rat embryos were collected on gestational day 10,5 and 11,5 from non-diabetic and diabetic rats. The embryos were homogenized and centrifuged in order to separate the membrane fraction from the cytosolic fraction. Protein Kinase C bound to the membrane is considered to be active while Protein Kinase C in the cytosol is considered to be inactive. Membrane and cytosolic fractions of the α, β I, β II, ε, δ, γ, ζ isoforms of Protein Kinase C were used for Western Blot to estimate enzyme activity. Total RNA of embryos was used for cDNA preparation. One µl of cDNA was used for real-time PCR to estimate gene expression.
Results: We found that malformed embryos from diabetic rats had increased activity of most Protein Kinase C isoforms compared to normalformed embryos from diabetic rats and embryos from non-diabetic rats. There was no clear difference in enzyme activity between normalformed embryos of diabetic rats and embryos from the non-diabetic group. When we measured the gene expression of each Protein Kinase C-isoform we did not find any difference between the groups.
Conclusions: We suggest that the induction of malformations in embryos of diabetic rats is associated with increased Protein Kinase C- activity.

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What proportion of birthweight is attributable to maternal glucose among infants of diabetic women?
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Background and Aims: It is unclear whether maternal demographic factors or glucose concentrations have greater impact on birth weights of infants of diabetic mothers. If maternal glucose is most influential, then maintaining normoglycemia should be a primary therapeutic goal. If, however, potentially alterable demographics (e.g. weight gain, smoking) have a greater effect on birth weight than does glucose, then the focus of care should include normalization of these clinical factors. The primary purpose of this study was to establish the proportion of birth weight that is attributable to maternal demographics and glucose concentrations in insulin-treated diabetic women.
Materials and Methods: Maternal demographic and self-monitored memory-based glucose data were analyzed. The data of only women who had at least 12 weeks of treatment during pregnancy, who had checked their glucose at least two of the requisite four times daily (fasting and 1 hour post-prandial) and who delivered live born singletons having no anomalies were evaluated. Demographic variables analyzed were maternal age, ethnicity, parity, pre-pregnancy BMI, weekly weight gain, hypertension, and smoking status. Birth weight was expressed as the population-specific percentile birth weight for gestational age and gender. Maternal glucose concentrations were analyzed collectively, by trimester, and by time of day. The primary analytical approach used was multiple regression. This method allowed us to measure the proportion of variance in babies' birth weight percentile that was attributable to demographic and clinical characteristics in addition to glucose values.

Results: Data of 101 diabetic women (7 type 1, 48 type 2, and 46 gestational) qualified for analysis. Among the three groups there were no significant differences in ethnicity, maternal age, parity, weight gain, birth weight, or gestational age at delivery. The three groups did differ with regard to mean (± SD) maternal BMI (respectively 25.9±2.5, 34.6±7.5, and 34.9±8.6 kg/m^2, p=0.005), and weeks gestation at initiation of glycemic control (respectively 9, 12, and 20 weeks, p=0.0001). Mean maternal glucose was 5.9±1.8 mmol/l. Significant differences were found among the three groups for maternal glucose concentrations across all trimesters (p=0.0001). However, no trend in maternal glucose concentrations was found over the three trimesters. None of the maternal demographics was selected as independently associated with birth weight in the multivariate regression analysis. Only maternal glucose during third trimester was found to be independently correlated with birth weight (adjusted r^2=0.14; p=0.006). Maternal glucose at no one time of day was more closely associated with birth weight than at other times of day.
Conclusion: Among insulin-requiring diabetic women maternal glucose in third trimester explains 14% of the variance in birth weight, and appears to be more influential on this outcome than any other maternal factor. The primary focus of care of the pregnant diabetic woman should be optimization of maternal glycemic control.

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Maternal and neonatal lipid profiles and their relation to fetal growth in pregnancies with gestational diabetes.
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Background and Aims: Management solely focussed on maternal glucose control does not lead to a normalization of the macrosomia rate in gestational diabetes mellitus (GDM), especially in obese women. Therefore, we investigated the correlation between maternal and cord blood lipid profils and their relation to fetal and neonatal anthropometry in GDM pregnancies.
Materials and Methods: In 173 GDM pregnancies measurements of cholesterol (Chol), and trigylcerides (TG) were performed in maternal serum at diagnosis and delivery and in cord blood (CB). Lipid values were correlated with growth parameters in a continuous fashion by bivariate regression and as categories by chi square test.
Results: Maternal Chol and TG at diagnosis were weakly correlated with the abdominal circumference (p= 0.001, r=0.19 and 0.25). No association was found between maternal lipids at study entry or delivery and neonatal birth weight (BW), body mass index (BMI) or fat mass. All maternal lipids at delivery were associated with CB lipids (p= 0.038 - <0.001, r=0.32-0.38). There were thresholds levels for Chol and TG of 240 mg/dl for an increased rate of elevated CB Chol and TG (56.4 vs 83.6%, p=0.01 for Chol; 12.5 vs 35.0%, p=0.01 for TG). CB TG were negatively related to BW, BMI and fat mass (p= 0.02- 0.001, r= 0.23-0.34). CB TG from neonates with BW <10th percentile was elevated in 75% vs 25% for neonates with growth ≥10th percentile (p<0.001)
Conclusion: Maternal lipids are not predictive for abnormal growth while maternal lipids at delivery correspond to neonatal lipid profiles. Neonatal lipids are conversely related to neonatal growth which might indicate a higher lipolytic activity due to a catabolic metabolism in growth-retarded fetuses.

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Maternal diet influences postnatal growth and glucose tolerance in adult offspring.
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Aims: We investigated the effect of maternal diet on postnatal growth in delivered rats and adult offspring glucose tolerance.
Materials and Methods: Female wistar rats at mating day 7 were randomly assigned to high fructose diet (Fru), high fat diet (Fat) or control diet(C). Pregnant rats were fed the experimental diet for 2 weeks and fed for the same or the control diet for 3 weeks after delivery, thus they were divided into 7 groups;(a)Fru-Fru, (b)Fru-C, (c)Fat-Fat, (d)Fat-C, (e)C-Fru, (f)C-Fat, and (g)C-C. The control diet was contained (as percent of calories) 59% vegetable starch, 11% fat, and 30% protein, the high fat diet contained around 20% fat, and the high fructose diet contained 68% fructose. Body weight and food intake were monitored weekly. The intravenous glucose tolerance test (IVGTT; 0.5g /kg body weight) was performed in mother rats after lactation and in the offspring rats at the age 5weeks. Blood samples were collected at 0,15, and 30 min and blood glucose and plasma insulin concentrations were measured.
Results: Maternal body weight and food intake were not different in 7 groups. The dams in (a)+(b)+(e) had elevated levels of blood glucose and plasma insulin concentrations. Body weight in (a)+(e) were significantly lower than that in (e)+(f)+(g), and that in (c)+(d) were significantly higher than that in (e)+(f)+(g). The offspring in (c)+(d)+(f) had gained their weight more than the others during the lactation, however after then they had gained their weight less than the control group. The increase in body weight of offspring was remarkably inhibited for 5 weeks by Fru feeding. The offspring in (a)+(b) had significantly elevated blood glucose levels and lower insulin levels in IVGTT.
Glucose levels and insulin levels in (c)+(d) were significantly higher than those in others, respectively.
Conclusion: Exposure to fru during the pregnancy and lactation inhibited the growth and insulin secretion in the offspring. Exposure to fat during pregnancy and lactation caused insulin resistance in the offspring.
Results

group	a	b	c	d	e	f	g
diet during pregnancy	Fru	Fru	Fat	Fat	C	C	C
diet during lactation	Fru	C	Fat	C	Fru	Fat	C
Glucose in dams	U				U		
IRI in dams	U				U	U	
body weight at birth in offspring	D	D	U	U			
at age of 3weeks(lactation) in offspring	D	D	U	U	D	U	
at age of 5weeks(weaning) in offspring	D	D			D		
Glucose in offspring	U	U					
IRI in offspring			U	U		U	

U was up; significantly higher than the control group.
D was down; significantcy lower than the control group.
Fru was high fructose diet. Fat was high fat diet. C was control diet.

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The cardiovascular risk factors in offspring of mothers with previous history of gestational diabetes mellitus.
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Background and Aims: The objective of this study was to investigate the long term adverse effects of maternal gestational diabetes mellitus(GDM) on the glucose metabolism and cardiovascular disease(CVD) risk factors in offspring of diabetic mothers.
Materials and Methods: In this multi-centered prospective study, a total of 919 subjects were recruited but 218 offsprings were followed prospectively. During six years of postpartum period, 85 women with a previous history of GDM were converted to either diabetes mellitus(DM) or impaired glucose tolerance(IGT)(AGT group), and 123 stayed as normal glucose tolerance(NGT group). In mother, 75 gram 2 hour oral glucose tolerance test(OGTT), obesity measurements (ie., height, weight, waist and hip circumferences, skinfold thickness, and total body fat), vital signs, and lipid profiles were evaluated. In offsprings, same evaluation as mother was made, but 2 hour OGTT was modified using 1.75g of dextrose/kg of children's body weight. Furthermore, relative obesity was assessed on the basis of the symmetric index. The mtDNA content was also measured by a real-time polymerase chain reaction method.
Results: The following cardiovascular risk factors were more prominent in offspring of AGT group when compared to NGT group: waist and hip circumference(p<0.001), fasting glucose, 2 hour postprandial glucose, fasting insulin level(p<0.01), symmetric index, percent body fat, and 2 hour postprandial insulin level(p<0.05). The slope of the regression line predicting symmetric index in offspring of AGT group was slightly higher than the NGT group (p=0.055, β_A=0.0143, β_N=-0.0131). The slope of the regression line predicting 2 hour postprandial and C-peptide for offspring of

AGT group was significantly higher than that of NGT group($p<0.01$, $\beta_A=0.598$, $\beta_N=0.101$). This study showed that offspring of women who had a history of GDM and developed IGT or DM in 6 years postpartum had significantly adverse results on glucose metabolism, as well as CVD risk factors.
Conclusion: This prospective study identified early manifestation of diabetes and CVD risk factors in offspring of GDM mothers. Thus, implementation of early medical evaluation of diabetes, obesity and cardiovascular disease in offspring of diabetic mother merits potential prevent of chronic diseases in later life.

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Pregnancy outcome in women with cystic fibrosis related diabetes.
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Background and Aims: The survival of patients with cystic fibrosis (CF) has improved remarkably over the last decades. In an adult population, issues such as family planning and pregnancy have become important. Pulmonary function impairment has been suggested as the most important predictor of maternal and foetal outcome, but cystic fibrosis related diabetes (CFRD), more frequent in adult population, is another one. The aim of this retrospective study is to describe pregnancy outcome in CFRD women.
Materials and Methods: Between 1997 and 2001, 22 CF women consulted for consideration of pregnancy. They were take in charge by a multidisciplinary team (obstetrician, geneticit, pneumologist, endocrinologist,pediatrician) . Medical and ethical decisions were based on the short and medium term prognosis for the woman. Pre-conceptional assessment included genotype and phenotype features of woman's pulmonary function, pancreatic sufficiency, nutritional status, diabetes mellitus and liver disease. During pregnancy, management included nutritional supplementation, intensification of chest physiotherapy and intravenous antibiotic treatment of pulmonary infections. Screening for diabetes was done before and during pregnancy. Prenatal care included monthly visits with the obstetrician, the pneumologist, and the endocrinologist.
Results: Five women had pre-pregnancy diabetes mellitus, and one developed a gestational diabetes early in the course of the pregnancy. Between the 5 CFRD women, 2 were discouraged for having a pregnancy: one had a rapid worsening of her nutritional and pulmonary status, the other one had undergo an hepatic and pulmonary transplantation 7 years ago, and had a renal failure. For the 3 remaining women, insulin was initiated before pregnancy in 2, and at the begin of the pregnancy in 1. The last woman developed gestational diabetes, and need insulin treatment at 10 weeks gestation. Insulin treatment included rapid onset insulin 3 to 4 times daily, before main meals; 1 patient also needed NPH insulin at bedtime. One patient had an early pregnancy loss at 12 weeks, it is the only one who had a bad glucose control (HbA1c 8.8 %; normal range: 4.3 – 5.7 %). The median HbA1c during pregnancy were 4.8 %, 5.2% and 5.6 % in the other women. A cesarian section was done in the 3 other patients, the median gestational age was 38,3 weeks, and the birth weights were normal (median: 3200 g). No perinatal complications were observed either in infants or mothers. The median weight gain was 8,3 kg. During pregnancy all the patients were hospitalized for pulmonary infection, one for poor weight gain, and one for severe hypoglycemia. After delivery the pulmonary status were stable. All 3 children are healthy.
Conclusion: Screening for diabetes is mandatory in CF women before and during pregnancy. A good diabetic control seems to be important for the pregnancy outcome, and insulin treatment is usually required.

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Congenital anomaly rate in offspring of women with diabetes treated with Humalog®
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Background and Aims: Pregnancies complicated by maternal diabetes mellitus (DM) are at increased risk of fetal complications such as congenital anomalies (CA). A direct correlation exists between improved glycemic control and reduction of CA. To date, a limited number of case reports and clinical studies with small sample sizes assessing Humalog use during pregnancy have been published. The aim of this study was to determine the rate of major CA in offspring from a large cohort of women with pregestational DM treated with Humalog.
Materials and Methods: To be included in this global, multi-center, retrospective medical chart review, all mothers had to have pregestational DM and treatment with Humalog for at least 1 month before conception and during at least the first trimester of pregnancy. Information on the pregnancy outcome as well as other parameters known to affect neonatal outcome were collected by medical personnel independent of the sponsor. Data collected on all suspected cases of CA were assessed independently by two dysmorphologists (Frías and Hoyme).
Results: Data was collected from 55 centers in 8 countries in Africa, Asia, Europe, and the Americas. The charts of 496 women were reviewed for 533 pregnancies (518 Type 1 and 15 Type 2 DM) resulting in 542 offspring. The average age of the mothers was 29.9 years (±5.2) (mean [±standard deviation]) and 86% of the mothers were Caucasian. Only 29% (154) mothers had received pre-pregnancy counseling. Humalog continued to be the main pre-meal insulin for over 96% women during the 2nd and 3rd trimester. Of the 542 offspring, 500 were live births, 38 abortions (31 spontaneous and 7 elective), and 4 stillbirths. Twenty-seven (5.4%) infants were born with major anomalies and 2 (0.4%) with minor anomalies according to the final assessment of the 2 dysmorphologists. Average infant birth weight was 3463±765g and gestational age 36.7±2.2 weeks. Mother's average, standardized HbA1c declined during the pregnancy: 8.9%±4.2, 7.7±3.2, 6.0±2.6, and 6.2±2.4 for the first prenatal visit, 1st, 2nd, and 3rd trimesters respectively. Rate of severe hypoglycemic episodes was 0.3±2.0, 0.2±0.7, and 0.0±0.7 per trimester for the 1st, 2nd, and 3rd trimesters respectively.
Conclusion: The current background rate of major CA in the general population is usually quoted as 2-4%. A recently published study of 145,196 pregnancies (1991-2000) found a 6.1% rate of major anomalies in infants from mothers with pregestational DM (n=410). In our study the rate of major CA was 5.2% for the infants of mothers with pregestational DM treated with Humalog before and during their pregnancy.

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Evaluation of metabolic effectiveness of exercise habit using respiratory gas analyses and a newly developed cookie test.

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Background and Aims: To uncover the metabolic effectiveness and mechanism of chronic exercise habit using respiratory gas analyses and a newly developed cookie test
Materials and Methods: Male students with or without exercise habit(at least 1 hour , more than 3 days per week) performs treadmill for the measurement of AT(anerobic threthold) and VO_2 Max. Resting energy expenditure was also measured**.** They ingest a newly developed cookie(75g flour starch and 24g fat) and gas analyses as well as blood measurement for glucose intolerance, postprandial dyslipidemia, hyperinsulinemia and insulin resistance have been carried out.
Result: Resting energy expenditure in subjects with exercise habit was 800-1100 kcal/day/m^2 and 20-40% lower than non-exercise habit, which was attributable to the lowered fatty acid oxidation. However, after cookie ingestion, the exogenous fat is preferentially oxidized. At AT exercise group showed 8.4 Mets, while in non-exercise 6.5 and at VO_2 max O_2 uptake was 3389 ml/min and 2462 respectively. Basal and post cookie glycemic response was significantly less together with lowered insulin response in exercise group, indicating improved insulin sensitivity. AUC IRI and AUCGlucoseXAUCIRI, which indicate insulin resistance were significantly lower, further supporting the enhanced insulin sensitivity. Basal TG was less in exercise group and by PAGE analyses, exaggerated post coolie hyperlipidemia observed in non-exercise group was not observed. HDL was higher in exercise group. Serum level of leptin was significantly less in exercise group, which may explain the lower energy expenditure and activated appetite.
Conclusion: In the exercise group, lowered resting energy expenditure which was attributable to the low fatty acid oxidation was clearly demonstrated. Oxygen uptake at AT and VO_2max increased over non-exercise group, indication the more efficient energy use and higher physical ability. After cookie ingestion, the exogenous fat is easily oxidized indicating the increased mitochondrial activity. Less glycemic response together with low insulin level demonstrates enhanced insulin sensitivity. Basal TG and its post cookie rise was blunted in the exercise group together with lowered VLDL response indicate the activated LPL through increased insulin sensitivity for lipid metabolism. The newly developed cookie test can evaluate glucose intolerance using the same criteria as liquid glucose in subjects without exocrine pancreatic diseases, postprandial dyslipidemia, hyperinsulinemia and insulin resistance and be more widely used in not only suspected diabetes, but also obesity, hypertension, dyslipidemia and life style related disorders.

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Remaining endogenous insulin secretion predicts the long-term hypoglycaemic effect of a very low calorie diet in obese Type 2 diabetic patients.

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Background and Aims: A very low calorie diet (VLCD, 500 kcal/day) improves glycaemic control in some but not all obese patients with type 2 diabetes (DM2). It is unknown whether a patient reacts favourably or not. We aimed to identify specific endocrine and metabolic markers that predict a favourable response to a VLCD in obese DM2 patients. A favourable response was a priori defined as a fasting plasma glucose concentration (FPG) < 10 mmol/l after 30 days of dietary restraint.
Materials and Methods: 14 obese patients (BMI 31.3-48.6 kg/m^2; duration DM2: 0.5-21 yrs) were given a VLCD for 30 days. Patients had moderate to poor glycaemic control (FPG 12.8 ± 2.96 mmol/l {mean ± SD, HbA_{1C} 8.5 ± 1.6 %) despite oral blood glucose lowering medication and/or insulin (66-340 units/day). Before the start of the VLCD (day -1) all hypoglycaemic agents (including insulin) were discontinued, at least for the duration of the study. On day 2 and 30 of the VLCD an intravenous glucose tolerance test (IVGTT, 25 gram glucose) was performed and body weight was measured.
Results: Eight patients could be qualified as responder. Responders and non-responders could already be distinguished at day 2, when weight loss was still minimal (-0.9 to -3.2 kg), reflecting salt and fluid loss. Despite the cessation of all hypoglycaemic agents responders had a minimal increase or even a decrease in FPG (0.64 ± 2.29 mmol/l) at day 2 whereas non-responders exhibited an increase in FPG (4.15 ± 3.25 mmol/l), p=0.035. After 30 days, FPG improved further in the responder group (-4.33 ± 2.43 mmol/l) whereas FPG deteriorated in the non-responders (+3.92 ± 5.17 mmol/l), p=0.002. Both responders and non-responders showed substantial and equal weight loss at day 30 (12.23 ± 3.6 and 12.15 ± 2.5 kg respectively,NS). Responders displayed a more prominent second-phase insulin response to an intravenous glucose load on day 2 (AUCinsulin 2014 ± 978 mU/l/10-60 min) and day 30 (AUCinsulin 1494 ± 906 mU/l/10-60 min) whereas non-responders showed almost no extra insulin release at any time (AUCinsulin 722 ± 223 and 388 ± 115 mU/l/10-60 min on day 2 and day 30, p=0.004 and p=0.022 as compared to responders on day 2 and day 30). A first phase insulin response was absent in all patients. K-values of plasma glucose concentration profiles (as a measure of peripheral glucose uptake) were higher in responders than in non-responders (baseline k-values 0.50 ± 0.08 resp. 0.35 ± 0.13, p=0.016). K-values were not predictive for the response to a VLCD however, and neither responders nor non-responders showed any improvement of k-values after weight loss. Fasting insulin and fasting C-peptide levels on day 2 were significantly higher in responders than in non-responders (p=0.023 and p=0.009 respectively).
Conclusion: Responders had significantly higher fasting insulin and C-peptide levels and a higher AUCinsulin on day 2 than non-responders. So, preservation of the capacity of β-cells to secrete insulin predicts a favourable metabolic response to a VLCD in obese DM2 patients.

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Low plasma total homocysteine in Japanese Type 2 diabetic patients.

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Background and Aims: A high plasma total homocysteine (tHcy) concentration is considered to be an independent risk factor for atherosclerotic diseases. Elevated tHcy is associated with vitamin status, genetic factors, male gender, aging, smoking, hypertension, and several other diseases. Recently, many studies have that hyper-homocysteinemia to be common among subjects with diabetes mellitus. Therefore, we studied the relationship between homocysteine and glycemic control, and investigated the factors to predicting homocysteine concentrations including nutoritional background in Japanese Type 2 diabetic patients.
Materials and Methods: One hundred fifty Japanese patients (76/74: male/female), 40-69 years old, were studied. Plasma tHcy, serum folate, vitamin B2, B6, and B12 concentration were determined. A 677C-T methylenetetrahydrofolate reductase (MTHFR) mutation was detected by the PCR-RFLP method. Pulse wave velocity (PWV) was measured using an automatic device (form PWV/ABI, Nihon Colin, Aichi) as an indicator of atherosclerosis. Dietary assessments were made using a food-frequency questionnaire.
Results: The duration of diabetes was 14±7 years (Mean±SD). The numbers of patients treated by diet therapy, oral hypoglycemic agents and insulin were 27 (18 %), 67(45 %) and 56 (37 %), respectively. HbA1c was 7.4±1.5 %, serum creatinine was 0.9±1.8 mg/dl, serum blood urea nitrogen was 15.3±5.5 mg/dl, the urinary albumin-creatinine ratio was 185±649 mg/gCre. In all subjects, tHcy was low at 7.5±3.6 nmol/mL, but females had lower tHcy than males of ($p<0.001$). The hbPWV value correlated with tHcy ($r=0.372$, $p=0.0001$). The serum folate concentration was high at 14.5±7.4 ng/mL. Moderate hyper-homocysteinemia (>12 nmol/mL) was present in only 9 patients (6 %). Those of four subjects were associated with homozygousity for a 677C-T MTHFR mutation, and their folate concentrations were low. Those of five subjects were associated with other MTHFR genotypes complicated by neuropathy. The frequencies of food consumption of vegetables, seaweed, and soybeans products were high in our subjects. Females consumed vegetables more frequently than males ($p<0.05$). By stepwise regression analysis to predict the log tHcy concentration, habitual supplement intake and the frequency of consumption of other vegetables were negative predictors of the log tHcy

concentration in males (p<0.001), and habitual frequencies of consumption of other vegetables and low fat milk were negative predictors in females (p<0.0001).
Conclusion: Plasma tHcy was low and serum folate was high in Japanese Type 2 diabetic patients. The frequencies of consumption of certain foods by our Type 2 diabetic patients appear to be benefical for maintaining high serum vitamin concentrations and a low plasma tHcy concentration.

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Influence of a modified Atkins diet on glucose metabolism and weight loss in obese Type 2 diabetic patients.
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Background and Aims: Examine the influence of a low-carbohydrate, high-protein diet, on glucose metabolism and weight loss in obese type 2 diabetic patients, and possible untoward effects of this ketogenic diet. The study diet, modified from the typical Israeli diet, consisted of increased intake of dairy products, vegetable oils, eggs, chicken and fish.
Materials and Methods: 56 obese type 2 diabetic patients not treated with insulin, aged 35-75, BMI 30-40 Kg/m2 (mean 33.2+10.9 Kgm2) , HbA1C>7% (mean 9.0± 1.6%) were placed on the recommended American Diabetes Association (ADA) diet for one month. They were then randomly assigned to either the ketogenic diet - modified Atkins (initially 25 grams carbohydrates and subsequently 40 grams) or to a standard ADA calorie-restricted diet for a period of 3 months. Patients were evaluated for change in weight, HbA1C, blood pressure, blood glucose levels, ketones, blood pressure, HbA1C and plasma lipids profile.
Results: Preliminary results [on the first 31 patients who have completed the study], showed equal weight loss in both groups (Atkins: -2.5+2.9 kg, ADA: -2.5+3.6kg, P=0.74). However, the decline in HbA1C was greater on the Atkins (-2.29+1.55%) than the ADA diet (1.09+1.07%), p=0.019. Findings for fasting glucose were consistent. No deleterious effects of the Atkins diet on plasma lipids or blood pressure was evident.
Conclusions: In uncontrolled obese type 2 diabetic patients, the low carbohydrate diet was better able to regulate glucose levels. Based on our initial results, the modified-Atkins diet appears to be more efficacious than the ADA diet in Type 2 diabetics and is apparently safe for short periods.

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Effects of chromium treatment in patients with poorly controlled, insulin-treated Type 2 diabetes mellitus. A randomised, double-blind, placebo-controlled trial.
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Background and Aims: Chromium is an essential micronutrient involved in carbohydrate and lipid metabolism. Different studies have shown that chromium deficiency can cause impaired glucose tolerance and diabetes mellitus in humans. Studies in which high quantities of supplemental chromium were used in non-Western populations have demonstrated major beneficial improvements in glycaemic control. The aim of this study was to determine the effect of chromium treatment on glycaemic control and on clinical parameters of the insulin resistance syndrome in patients with insulin-treated, but poor controlled, type 2 diabetes mellitus in a Western population.
Materials and Methods: In this 6-month, double-blind, placebo-controlled study, 52 patients with type 2 diabetes mellitus and HbA1c > 8% despite insulin requirements > 50 U per day, were randomly assigned to receive treatment with placebo, 500 µg chromium picolinate or 1000 µg chromium picolinate per day. The primary endpoint was a change in HbA1c from baseline after 6 months of treatment. Secondary endpoints were changes in serum lipids, BMI, blood pressure and insulin requirements.
Results: Mean HbA1c in the 1000 µg treated group decreased from 9.5 (SD 0.8) to 9.0 (SD 0.8) after 6 months (p=0.032). Cholesterol/HDL ratio decreased with 0.46 (SD 0.47) in the 500 µg treated group (p=0.019) and with 0.32 (SD 0.50) in the 1000 µg treated group (p=0.048). Trends for improvements were found for triglycerides levels in both chromium groups and for blood pressure in all groups. Diastolic blood pressure was significantly lower in the placebo group. BMI and insulin requirements showed no changes. Between the treatment groups, all endpoints were not significantly different at the end of the study.
Conclusion: Chromium treatment lowered HbA1c and improved lipid profile in patients with type 2 diabetes in poor metabolic control in a Western society. Larger designed studies are necessary to confirm these improvements of chromium versus placebo, and whether it is possible to select subgroups of patients with certain phenotypes that may or may not benefit from chromium therapy.

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The effect of Mediterranean diet on blood pressure and lipid profile of DM2 patients.
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Background and Aims: The effect of a 28-day consumption of a Greek mediterranean diet (rich in fiber,mono- and polyunsaturated fatty acids and complex carbohydrates) was studied in 58 patients with DM2.
Materials and Methods: Anthropometric and biochemical parameters were evaluated in 35 men aged (M±SEM) 61,6±1,57 years and 23 women aged 58,8±1,97 years with DM2,on treatment with diet or/and oral hypoglycaemic agents.The food,rich in olive oil,vegetable and fruit,was prepared and provided daily by the commercial firms Goodies and Olympic catering in the context of a larger research project supported by the Greek secretariat for research and technology.The diet was isocaloric to the current so that patients could not reduce their weight during the test period.The BMI and HbA1c of patients were less than 28 and 7%.The anthropometric and biochemical parameters were assessd before and after the end of the 28 day-period on the diet.The Wilkoxon matched paired test was applied for the statistical analysis.All values are expressed as means±SEM.
Results: It was found that in diabetic men,following the diet,BMI did not change significantly but the waist perimeter and waist to hip ratio were significantly reduced (p< 0,02). The systolic blood pressure dropped from 137,5±1,96 to 129,3±1,7 mmHg,(p=0,0001) and the diastolic from 83,2 to 80±1,2 mmHg (p=0,006).In diabetic women there was a significant reduction of the hip perimeter (111,2±1,8 versus 109,2±2,0 cm,p=0,02) but no change in BMI,waist perimeter and waist to hip ratio.A significant drop only in systolic blood pressure was also observed (138,6± 3,1 versus 128,4± 2,0 mmHg,p=0,001).In both men and women there was a significant reduction of apolipoprotein –B levels (men 114,1± 4,1 vv 98,9 ±4,1 mg/dl,p=0,0003,women 109,2±7,7 vv 99,8±7,1 mg/dl,p=0,01) whereas the plasma cholesterol,LDL,HDL and triglycerides did not change.
Conclusions: It is concluded that even a short term consumption of meditarranean diet can have profound effects on blood pressure and apolipoprotein B levels which are accompanied by changes in anthropometric parameters considered risk factors for cardiovascular disease.

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Effect of glycine on insulin secretion, fasting and postprandial glucose levels in patients with Type 2 diabetes mellitus.
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Background and Aims: Glycine is a nonessential amino acid. Low plasma glycine levels have been found in lean non-diabetic insulin-resistant offspring of diabetic parents. A dose of glycine (5 g) has increased the insulin secretion in healthy first-degree relatives of type 2 diabetes mellitus patients. The aim of this study was to evaluate the effect of glycine on insulin secretion, fasting and postprandial levels in patients with type 2 diabetes mellitus.
Materials and Methods: A randomized, double-blind, placebo-controlled clinical trial was performed in 20 type 2 diabetes mellitus patients without pharmacological treatment. 10 volunteers received a dose of glycine at 1 g orally 20 min before the breakfast during a week; after that the dose was forced-tritated every week up to 5 g of glycine to complete the study in 5 weeks. The other 10 patients received placebo in the same way. Basal and at the end of the study an hepatic and a metabolic profiles, including hemoglobin A1C (A1C), insulin, fasting and posprandial glucose levels were performed. HOMA formula was used to calculate the insulin secretion. Every week was measured fasting and postprandial glucose

levels. Mann-Whitney U, Friedman and Wilcoxon tests were used to perform the statistical analyses. The hospital-based Ethics Committee approved the study protocol.
Results: The basal clinical characteristics and the laboratory measurements were similar between groups. There was a significant reduction ($p = 0.028$) of the glucose postprandial levels in glycine group. There was a significant reduction ($p = 0.017$) of the A1C levels in glycine group (8.1 ± 1.2 vs. $6.9 \pm 0.4\%$) after 5 weeks. Fasting glucose and insulin secretion were not modify with the pharmacological intervention. There were not adverse effects with the administration of glycine.
Conclusion: Administration in forced-tritated dose of up to 5 g of glycine decreased the postprandial glucose and the A1C levels after 5 weeks of treatment in type 2 diabetes mellitus patients.

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Who profits most from weight reduction? Weight reduction decreases IL-6 levels dependent on the C–174G polymorphism within the IL-6 promotor.
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Background and Aims: IL-6 has been described to independently predict risk of type 2 diabetes. IL-6 C–174G polymorphism influences IL-6 transcription in vitro. Therefore, it is the aim of this study to investigate the effect of weight reduction on changes in IL-6 serum levels dependent on the patient´s C-174G polymorphism within the IL-6 promotor.
Materials and Methods: The investigation was performed in 39 morbidly obese patients (36 female, 3 male) undergoing vertical banded gastroplastic operation (VBG). Blood samples were drawn after a 10 h overnight fast before VBG operation and 14 month thereafter, respectively. IL-6 was measured by ELISA. IL-6 C-174G polymorphism was determined by the primer elongation method using the SNuPE kit (Amersham). Patients were dichotomised by carrying the C allele or not carrying the C allele. Differences in IL-6 between before and after VBG were tested for significance by Student´s T-Test for paired analysis. IL-6 values were used ln transformed to achieve normal distribution. Data presented are mean ± SEM.
Results: We observed the following distribution of genotypes: GC 51.3 %, GG 43.6 %, CC 5.1 %. Mean Body mass index (BMI) before operation was 48.1 ± 1.1 kg/m^2. After VBG patients carrying GG genotype reduced BMI in average by 15.7 ± 2.4 kg/m^2. The group with GC or CC genotype reduced BMI by 14.8 ± 1.2 kg/m^2. Weight reduction as a consequence of VBG operation in patients with GG genotype led to a reduction of IL-6 by 1.2 ± 1.5 pg/ml in average. This decrease was not statistically significant ($p= 0.31$). In contrast, weight reduction yielded a significant decrease of IL-6 by 2.3 ± 1.2 pg/ml in average for patients carrying GC or CC genotype ($p= 0.028$).
Conclusion: Weight reduction as a consequence of VBG operation was associated with different effects in respect to serum IL-6 values dependent on the C-174G polymorphism within the IL-6 promotor. Only patients carrying the C allele showed a significant reduction of serum IL-6. Since IL-6 was shown to be an independent predictor of type 2 diabetes weight reduction seems to have an additional protective effect in individuals carrying the C allele at this polymorphism.

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White bean amylase inhibitor administered orally reduces glycaemia, water and food intake, and enterocyte disaccharidase activity in Type-2 diabetic rats.
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Background and Aims: Post-prandial hyperglycaemia can be controlled by delaying digestion and complex carbohydrate absorption. The objectives of the present work were to isolate and purify a pancreatic alpha-amylase inhibitor (AI) from white beans (*Phaseolus vulgaris*), and to study the effect on metabolic control of administering the AI orally for 22 days to non-diabetic (ND) and type-2 diabetic (neonatal diabetes models n0-STZ and n5-STZ) male Wistar rats at 3 months old.
Materials and Methods: The AI was purified by ion exchange chromatography. Doses of 100 mg/kg b.w. dissolved in 0.9% NaCl were administered orally for 22 days. At 09.00 every day, the glycaemia was measured and the ingestion of food and water was recorded. At the beginning, halfway through, and at the end of treatment the plasma insulin levels were measured by radioimmunoassay. At the end of treatment, the sucrase and maltase enzyme activities were determined in isolated enterocytes. Values correspond to mean±SEM for each untreated (NaCl) vs treated groups (AI).

Results: The glycaemia (mmol/L) declined from day 4 of the AI administration in ND (5.48±0.08 vs 4.39±0.13; p<0.05), n0-STZ diabetic (7.94±0.42 vs 5.56±0.32; p<0.01), and n5-STZ diabetic (17.34±2.58 vs 11.93±1.96) rats, and this decline was maintained until the end of the treatment: ND (5.22±0.21 vs 3.97; p<0.01); n0-STZ (8.10±0.19 vs 5.21; p<0.01); and n5-STZ (16.36±2.14 vs 7.69±1.34; p<0.01). No significant changes were observed in insulin levels. While there was no reduction in water intake (ml/day) in the ND rats (31±1 vs 32±0.01), there was a decrease in the AI-treated diabetic rats: n0-STZ (30±0.1 vs 22±1.5; p<0.01) and n5-STZ (76±5 vs 57±5; p<0.01). There was decreased food intake (g/day) in all three groups: ND (23±0.31 vs 20±0.03; p<0.05); n0-STZ (22±0.55 vs 16±0.98; p<0.01); and n5-STZ (31±0.58 vs 23±0.2; p<0.01). The enterocyte sucrase and maltase activities (U/g proteins) were high (p<0.01) in the untreated diabetic rats, n0-STZ (45±4 and 152±10, respectively) and n5-STZ (67±12 and 151±10, respectively) with respect to the ND rats (24±2 and 74±16, respectively). After AI treatment, the enzyme activities declined in both diabetic rats, n0-STZ (21±2 and 85±11; p<0.01) and n5-STZ (28±7 and 75±19; p<0.05), to values close to those in the ND rats.
Conclusion: AI purified from white beans and administered orally for 22 days *in vivo* to Wistar rats significantly reduced glycaemia levels in both the ND and diabetic (n0-STZ and n5-STZ) animals. It also reduced the intake of food and water, and normalized the elevated sucrase and maltase levels of the diabetic rats.

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Chronic salacia extract administration promotes a decrease in postprandial glucose and insulin concentrations in Zucker fatty rats.
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Background and Aims: Salacia oblonga is a plant that has been used in the treatment of diabetes in the Indian traditional medicine. It has been described that this antidiabetic effect is mediated through the inhibition of the α-glucosidases activities from the brush border of the intestinal mucosa. The main aim of this study was to evaluate the effect of chronic administration of salacia extract on postprandial glycemic and insulinemic responses in an animal model of insulin resistance, the obese Zucker fatty rat.
Material and Methods: Twenty Zucker fatty rats 15 week old were divided into two groups. One group (n=10) was fed with a standard semipurified diet (AIN 93-M) for 8 weeks. The other group (n=10) was fed with the same diet supplemented with 0.13% salacia extract. After 7 weeks of feeding the animals underwent to a meal tolerance test using maltodextrin (1g/kg body weight). Blood samples were obtained at baseline and 15, 30, 60, 90, 120 and 180 minutes postprandial time for measurement of glucose and insulin. At the end of the study period, the rats were sacrificed and the jejunum mucosa was removed for the determination of sucrase, maltase and isomaltase activities. In addition, leptin and β-hydroxybutyrate were determined in plasma as markers of diabetes progression.
Results: Chronic administration of salacia extract promoted a decreased of 25% in the glycemic response (p<0.05) respect to control animals. Salacia also induced a decrease of 34% (p<0.05) in insulin basal levels. Maltase and isomaltase activities in the jejunal segment were inhibited to 30% and 80% (p<0.05) respectively, whereas sucrase activity was not affected. Salacia treatment also promoted a decrease of 20% (p<0.05) and of 25% (p<0.05) in plasma leptin and β-hydroxybutyrate concentrations respectively.
Conclusions: Chronic salacia treatment improved postprandial glycemic response that follows a meal tolerance test in an animal model of insulin resistance. The hypoglycaemic effect was due to inhibition of α-glucosidases activities in jejunum. In addition, insulin sensitivity might be modulated by salacia through the reduction in insulin and leptin concentrations in plasma.

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Gene expression analysis of the effect of chromium picolinate in human skeletal muscle culture.
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Background and Aims: Recent data suggest that chromium enhances insulin action, but the cellular mechanism is unknown. To identify candidate genes involved, we used a human skeletal muscle culture and with microarray analysis, sought to determine whether gene expression profiles were altered in the presence of chromium.
Materials and Methods: Gene expression analysis was assessed for human skeletal muscle cells incubated with chromium picolinate (CrPic, 10 ng/ml), insulin (200 nm), CrPic/insulin (10 ng/ml / 200nm), or control. After incubation, microarray analysis was performed using Affymetrix U95A chips, and genes were identified that increased or decreased in response to the treatments. Genes exhibiting >1.2-fold change in comparison to control were considered altered using the Affymetrix absolute call algorithm. Gene expression data were normalized against control, and fold changes determined using GeneSpring software (Silicon Genetics, Redwood, CA).
Results: Of the 22,283 genes and ESTs present in the chip, 3176 (14%) were down-regulated and 1111 (5%) up-regulated with CrPic only; 1263 (6%) were down-regulated and 2481 (11%) up-regulated in insulin-treated cells; and 3174 (14%) down-regulated and 2202 (10%) up-regulated in the combined treatment. To identify genes involved in Cr-enhanced insulin action, gene expression of the combination was compared to insulin only, resulting in 32 selected down-regulated genes, including 2 postulated to play a role in insulin action: tumor necrosis factor alpha-induced protein 6 (TNFAIP6) and F-box only protein 5 (ubiquitin-protein ligase and ubiquitin-conjugating enzyme). Real-time quantitative PCR confirmed that gene expression of TNFAIP6 in CrPic- (0.84±0.034) and CrPic/insulin-treated cells (0.66±0.04) was significantly down-regulated (p<0.01) vs the control (1.0±0.01) or the significantly up-regulated insulin-treated cells (1.09±0.03). Similar data were shown with F-box 5 protein as gene expression was higher (p<0.01) in insulin-treated cells (1.18±0.03) compared to control (1.0±0.01), CrPic (0.96±0.01), or CrPic/insulin-treated cells (1.0±0.04). The gene expressions of ubiquitin-conjugating enzyme E2 variant, ubiquitin-carrier protein E2-EPF, and ubiquitin-specific protein 20 (1.1, 1.0, and 0.3, respectively) in CrPic-treated cells were significantly lower than those of insulin-treated (1.5, 1.76, and 1.2, respectively) or CrPic/insulin-treated cells (1.3, 1.6, and 0.9, respectively) (p<0.01 for CrPic-treated cells vs insulin- or CrPic/insulin-treated cells).
Conclusion: Gene expression analysis suggests that CrPic may down-regulate genes in human skeletal muscle that are potentially involved in cellular insulin action (e.g., TNFAIP6 and ubiquitin-associated proteins).

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Determination of the glycaemic index in diet products: how to overcome the limitations of the classical approach.
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Background and Aims: The glycaemic index (GI) is defined as the incremental area under the blood glucose response curve of a 50 g carbohydrate (CH) portion of a test food in relation to the response to a 50 g glucose intake. However, many modern dietary food products contain little amounts of carbohydrate so that the intake of a 50 g carbohydrate portion would require a huge serving size (whereas usually patients will just exchange their „normal" food product (FP) with a similar dietary product (DP). Nevertheless, as low-GI diets have been shown to reduce coronary risk factors the determination of GI in dietary products is of high interest. In order to overcome the above mentioned limitations of the GI, we determined the GI of 7 FP and compared the incremental areas under the blood glucose response curves to those obtained with 7 comparable DP provided in the same serving size as their FP-counterparts.
Materials and Methods: Ten healthy volunteers ((4 male, 35±7 years (mean±SD), BMI 23±2 kg/m^2) participated in this open, randomised study with 16 experimental days. At each study day, the subjects ingested either a 50 g carbohydrate portion from one of the FP (serving size 57-106g) or the same serving size of the respective DP. Changes in blood glucose and satiety (assessed by a rating scale) were determined over 120 min after food intake. The results were set into relation to those obtained with an oral 50 g glucose load (measured twice in each individual) and expressed as GI.
Results: All DP provided low GI (< 50%) and showed a clear reduction in GI compared to their FP counterparts (despite a higher CH content in some

of the DP), table. SI was comparable (data not shown) between FP and DP indicating that patients exchanging normal FP with DP will use about the same serving size.

Food (serving size)	Food Products (Non-Diet) CH (%)	GI*	Dietary Food Products CH (%)	GI*
Chocolate cream (87.2 g)	57	42±10	49	21±7
Coconut bar (98.8 g)	51	56±14	47	37±13
Waffles (79.0 g)	63	43±10	62	42±9
Chocolates (106.2 g)	47	32±10	52	25±8
Cookies (99.0 g)	51	42±12	58	28±9
Candies (56.9 g)	88	62±7	88	5±2
Jam (81.2 g)	62	59±14	43	35±8

* Data are given as mean±SEM

Conclusion: This first determination of the GI of dietary products offered in equal serving sizes as normal food product-counterparts provided reasonable results. Future studies will further evaluate this variation of the GI-determination which offers an easy and logical approach to evaluate the glycaemic index of dietary products even with a very low carbohydrate content.

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The Glycemic Index (GI) of standard and diabetes-specific clinical nutrition products.

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Background and Aims: Low-GI foods are more and more applied as a tool in the dietetic treatment of diabetic patients, as is also recommended by official diabetes organisations. For diabetic patients who are not willing or capable to eat and drink sufficiently, special diabetic feeds for nutritional support are available. However, the GI of many of these diabetes-specific products has never been assessed nor compared with the GI of standard products in a controlled manner. Therefore, in this study the GI scores of 6 standard clinical products and 6 products specially designed for diabetic patients were tested.
Materials and Methods: In a randomised, double blind, cross-over trial, the GI of 12 different clinical nutrition products was assessed. Ten healthy volunteers received a portion of product containing 25 grams of available carbohydrate on different occasions after an overnight fast. As some of the tested products contain relatively little available carbohydrates, portions containing 25 grams of carbohydrate were used in order to keep the amount of product acceptable. The reference food was 25 grams of glucose dissolved in 200 ml water. Venous blood samples were taken at set time points for up to two hours. Plasma glucose concentrations were measured in whole blood (EML-105, Radiometer, Copenhagen, Denmark). There was a wash out period of at least 4 days between tests. The positive incremental area under glucose response curve (AUC) was calculated for each product according to the trapezoidal rule. The GI was calculated as: (AUC of the test product / AUC of the reference food) x 100%.
Results: Each product was tested by 5 male and 5 female volunteers (age 23±2, BMI 22±2 (mean ± SD)). The GI scores of the tested products are shown in table 1 (mean ± SEM). The average GI of the standard products was 42.1 ± 5.9. The average GI of the diabetes-specific products was significantly lower (19.4 ± 1.8; $p<0.01$ Mann-Whitney U test).
Conclusion: Foods with a low GI are recommended for the nutritional treatment of diabetic patients. Our study showed that clinical nutrition products that are specifically designed for diabetic patients have a lower GI than standard products and thus have less glucose-raising potential. Therefore the use of diabetes-specific products seems to be superior to standard feeds and should be advised to diabetic or hyperglycaemic patients in need of nutritional support.

Table 1: GI for each product.

Standard products	GI (glucose = 100)	**Diabetes-specific products**	GI (glucose = 100)
Nutridrink MF[1]	53 ± 19	Diasip[1]	12 ± 3
Nutridrink[1]	61 ± 19	Nutrison Diabetes[1]	17 ± 4
Fortimel[1]	25 ± 8	Glucerna[2]	15 ± 3
Nutrison Standard[1]	34 ± 8	Glucerna SR[2]	23 ± 5
Biosorb Drink[1]	50 ± 16	Fresubin Diaben[3]	22 ± 7
Nutrison MF[1]	28 ± 10	Novasource Diabet[4]	26 ± 5
Mean ± SEM	42.1 ± 5.9	Mean ± SEM	19.4 ± 1.8

MF: multi fibre. SR: Slow Release.
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Physical Exercise

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Synergistic effect of insulin and prior moderate exercise on muscle glycogen synthesis is associated with muscle glyconeogenesis.
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Background and Aims: Exercise is an important adjunct in diabetes treatment partly since it may enhance insulin sensitivity. This is manifested by increases in insulin-stimulated post-exercise muscle glycogen resynthesis. Exercise also enhances muscle glyconeogenesis. Possible relationships between the two are examined.
Materials and Methods: 4 groups of rats underwent: (i) 4h period of rest +3h recovery (ii) 4h rest +3h insulin infusion (iii) 4h swim + 3h recovery and (iv) 4h swim + 3h insulin. [^{3}H-6]- and [^{14}C-6]-glucose were infused concurrently. Insulin sensitivity was calculated as the ratio of tracer-determined metabolic clearance rate, and insulin conc. Glycogen was measured in soleus, red (rg) and white (wg) gastrocnemii, together with its direct formation from circulating glucose (^{3}H-glucose incorporation) and glyconeogenesis (local [^{14}C-1]glucose formation).
Results: Recovery from swimming demonstrated a recovery of glycogen concentrations to those in (i): 3.36± 0.50 (rg) & 4.27± 0.74 (wg) vs 3.79± 0.59 (rg) & 4.43± 0.83 (wg) mg/g tissue. This was accomplished by an increase in the direct formation of glycogen (1.57± 0.28 (rg) & 1.02± 0.21 (wg) vs 0.54± 0.10 (rg) & 0.11±0.02 (wg) mg/g tissue, $p<0.05$) and accompanied by an increase in glyconeogenesis (0.37±0.11 (rg) & 0.28±0.05 (wg) vs 0.11±0.02 (rg) & 0.027±0.005 (wg) mg/g tissue, $p<0.05$). For swim+insulin (iv), (plasma insulin: 81±15 vs 17±1µU/ml), the exercise-related increase in glyconeogenesis (0.57±0.18 (rg) & 0.43±0.13 (wg) vs 0.23±0.06 (rg) & 0.080±0.012 (wg) mg/g tissue for rest + insulin (ii)) predicted an increase in total glycogen formation from circulating glucose (9.0±0.8 (rg) & 3.9±0.7 (wg) vs 2.8±0.4 (rg) and 0.53±0.10 9 (wg) mg/g tissue for rest + insulin, $p<0.05$) , which exceeded the additive effects of recovery from prior exercise alone (iii) and insulin alone (ii) ($p<0.05$). Total glycogen concentrations increased above resting (i), only with swim+insulin (iv): 8.0±1.2 (rg) and 5.3±1.1 (wg) vs rest + insulin (ii): 4.1±0.1 & 3.0±0.2 mg/g tissue ($p<0.05$). Insulin sensitivity tended to increase after exercise: 0.70±0.20 vs 0.54±0.20 ($p=0.08$). The attenuated increase in sensitivity can be explained by differential effects of exercise on muscle glycogen: the soleus demonstrated neither the increase in glyconeogenesis after exercise (iii), nor in total glycogen synthesis following swim + insulin (iv).
Conclusions: The synergistic enhancement of glycogen synthesis by insulin and prior exercise parallels the exercise-stimulated new glycogen in unsupplemented recovery, when viewed across muscle groups. Although the effects on glyconeogenesis were quantitatively not large, increases in glycogen recycled within the muscle were only seen consistently under the same circumstances as increases in direct glycogen synthesis. It therefore appears that the exercise effect on new glycogen synthesis is strongly associated with an increase in glyconeogenesis. The synergistic effect of prior exercise and insulin on glycogen synthesis is expressed differentially in different tissues and even in different muscle types (eg slow-twitch oxidative vs fast-twitch glycolytic). This may help to explain the variability of results which is seen in the *systemic* effects of exercise on insulin sensitivity.

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Effects of diet and exercise therapy on insulin sensitivity and intramyocellular and intrahepatic lipid contents in Type 2 diabetic patients.
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Background and Aims: Insulin resistance is associated with increased lipid contents in muscle and liver. Our aim was to examine the effects of diet and exercise therapy (D+E therapy) on insulin sensitivity and intracellular lipid contents in type 2 diabetic patients.
Materials and Methods: Type 2 diabetic subjects (DM,n=4,BMI: 24.2±2.31kg/m^2, glycated albumin (GA): 24.4± 2.83%) were admitted to our hospital for 2 weeks and were continued diet (25~30kcal/kg) and exercise (300~400kcal/day) therapy. We performed hyperinsulinemic-euglycemic clamp (insulin infusion rate; 100mU/m^2/min, target plasma glucose; ~95mg/dl) and measured glucose infusion rate (GIR) to examine insulin sensitivity. Intramyocellular lipid (IMCL) of tibialis anterior muscle and intrahepatic lipid (IHL) were evaluated by ^{1}H magnetic resonance spectroscopy. IMCL was quantified as the area under the curve of the methylene signals of lipids peak at ~1.25ppm (AUC-IMCL) and was divided by internal reference (AUC-creatine). IHL was calculated from the AUC of the methylene signals of intrahepatic lipids (peak at ~1.25ppm) and expressed as ratio of AUC-IHL/(AUC-IHL+AUC-H_2O). We also measured IMCL, IHL and insulin sensitivity of non-diabetic healthy subjects (C, n=6, BMI :22.8± 1.38 kg/m^2).
Results: At baseline, both IMCL and IHL were higher in DM group than in C group (IMCL; 3.63± 1.01 vs. 1.82± 0.95, $P<0.03$, IHL; 7.94± 5.81 vs. 2.13± 1.25%, $P<0.05$). GIR, a marker of insulin sensitivity, was lower in DM group than in C group (5.54± 1.08 vs. 10.7± 1.80 mg/kg/min, $P<0.003$). After 2-week D+E therapy on D group, GA was decreased from 24.4± 2.83 to 20.4± 1.27% ($P<0.03$). IMCL was also decreased by 18% (3.63± 1.01 to 3.00± 0.89, $P<0.01$), while GIR was increased by 41% (from 5.54± 1.08 to 7.83± 1.46 mg/kg/min, $P<0.03$). Further more, IHL was decreased by 21% (from 7.47± 5.62 to 5.76± 5.35, $P<0.03$).
Conclusion: These results indicate that IMCL and IHL may regulate insulin sensitivity and glucose metabolism in type 2 diabetic patients.

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Exercise training improves cardiorespiratory fitness and endothelial function in women with Type 2 diabetes (NIDDM).
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Background and Aims: NIDDM is associated with impaired left ventricular (LV) diastolic filling, reduced arterial compliance (AC) and abnormal peripheral vascular endothelial function. These maladaptations may contribute to the increased cardiovascular (CV) mortality and morbidity associated with the diabetic state. Previous investigations have found that exercise training can attenuate the age-related decline in LV filling, AC and endothelial function. However, the role that exercise training plays on altering LV diastolic filling, AC and endothelial function in individuals with NIDDM has not been well studied. The aim of this investigation was to determine the effects of exercise training on CV function in women with NIDDM.
Methods and Materials: Thirty women with NIDDM were screened for this investigation, and assigned to training (ET) or control (CT) groups. Twenty-four women (mean age: 57 ± 5 yrs; BMI: 34 ± 6kg/m^{-2}) completed the investigation (ET = 17, CT = 7). Cardiorespiratory fitness was assessed using a graded exercise test on a cycle ergometer. Doppler and M-mode echocardiophic imaging were used to measure LV filling and morphology. Large and small AC were derived from arterial waveforms of the radial pulse, using applanation tonometry. Brachial artery endothelial function was assessed from the vascular response to reactive hyperemia (flow-mediated dilatation, FMD) and sublingual glyceryl trinitrate in a subset of 7 subjects in each group. Hematological measurements included: fasting lipid profile, glucose, insulin and C-reactive protein. Insulin sensitivity was estimated from the HOMA index. The ET group performed a minimum of 60 minutes of aerobic and strength training, 3 days per week for 10 weeks. The CT group maintained habitual physical activity levels.
Results: Cardiorespiratory fitness increased significantly in the ET group (19.5 ± 3.5 mL/kg^{-1}/min^{-1} to 22.0 ± 5.6 mL/kg^{-1}/min^{-1}) ($p<0.05$), despite no change in LV diastolic filling or LV morphology. Exercise training did not alter large (pre: 9.5±3.8; post: 10.6 ± 3.9; $p = 0.58$) or small (pre: 4.5 ± 4.1; post: 3.1 ± 1.2; $p = 0.42$) AC. FMD increased significantly ($p < 0.05$) from 1.7 ± 4.4 to 9.1 ± 3.3% in the ET group. No changes were observed in any hematological values following 10 weeks exercise training. All measured variables remained unchanged following 10 weeks in CT.
Conclusions: Exercise training improves cardiorespiratory fitness and endothelial function without altering LV diastolic filling, small or large artery compliance in women with NIDDM. Enhanced FMD following Exercise training cannot be explained by changes in lipid profile, insulin sensitivity or C-reactive protein. These results suggest that exercise training may serve as an adjunctive therapy for CV dysfunction associated with NIDDM.

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Hyperglycaemia does not impair acute maximal exercise performance in non-diabetic men.

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Background and Aims: The effects of variations of blood glucose concentration (BGC) on exercise performance are poorly investigated. This information could be of great importance for the pre-competition preparation of both non diabetic and diabetic athletes, particularly as the prevalence of this last group in elite competitions is increasing. This study was undertaken to examine the impact of two BGC levels (euglycaemic and hyperglycaemic) on exercise performance and metabolism during a maximal 30 second cycling test (Wingate test). It was hypothesised that hyperglycaemia would impair maximal exercise performance.
Materials and Methods: Five non-diabetic male subjects (age = 22.2 ± 3.5 years; body mass index = 22.6 ± 0.4 kg•m^2) attended the laboratory on two occasions in a fasted state, having abstained from heavy exercise, alcohol and caffeine for at least 24 hours. They underwent hyperinsulinaemic clamping at either euglycaemia (5mmol•l^{-1}) or hyperglycaemia (8mmol•l^{-1}) in a randomised, single-blind, crossover design. After steady-state conditions were achieved, they performed a maximum effort sprint on a mechanically braked cycle ergometer (Monark 864, Varburg, Sweden) for thirty-seconds against a calculated frictional resistance equivalent to 7.5% of the subject's own body mass. Measurement of both performance and metabolic variables were recorded.
Results: Results are shown in table 1. No significant differences were found in any of the considered parameters between the two conditions (two tailed, Student's paired t-test; P<0.05).
Conclusion: Hyperglycaemia does not impair maximal exercise performance in non-diabetic subjects, and the metabolic responses to this type of exercise do not differ significantly between the two considered conditions. However, further studies are required at higher and lower BGCs, adopting differing types of exercise, and in people with diabetes in order to improve further our understanding of the relationship between BGC and maximal exercise performance.

Table 1. Performance and metabolic variables measured in the two conditions. Data are mean ± SD.

Variable	Euglycaemia	Hyperglycaemia
Peak Power (Watts)	1093.6 ± 84.4	1093.5 ± 66.5
Mean Power(Watts)	709.7 ± 50.1	710.9 ± 52.8
Peak pedal rate (rpm)	167.4 ± 7.2	166.5 ± 8.6
Mean pedal rate (rpm)	126.7 ± 5.9	125.9 ± 8.8
Peak lactate (mmol/L)	13.1 ± 0.75	13.6 ± 1.2
Resting Free fatty acids (mmol/L)	0.22 ± 0.7	0.48 ± 1.7
Post exercise Free fatty acids (mmol/L)	0.3 ± 0.1	0.5 ± 0.5
Rate of perceived exertion	18.3 ± 0.43	18.0 ± 0.71

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Effects of aerobic plus resistance exercise (ART) on Type 2 diabetes mellitus: a 2-year follow-up.

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Background and Aims: Aerobic training improves glycemic control in T2DM patients. Resistance training has also been shown to improve glycemic control in addition to increasing muscle mass. However, limited information is available on the long-term effects of ART in this patient population. Thus, we conducted a prescribed and supervised trial to determine the safety and efficacy of ART on cardiovascular risk factors, glycemic control and body composition in T2DM patients.
Materials and Methods: A diet and an algorhythm of ART together with their own pharmacological therapy was prescribed to 120 sedentary T2DM (60 M/60 F) age 60.9 ±7.3 yrs duration of diabetes 9.8±7.3 yrs.
ART algorhytm was composed by Stretching + Aerobic Exercise (30-45 min of non-weight bearing exercise / brisker walking treadmill / running with increasing intensity) + Resistance Exercise (30-40 min of neuromuscolar electrical stimulation / circuit training of strenght exercise with low weight)
Sixty-two patients (30M/32F) accepted to do ART for 60-70 minutes 3 times x week x 2 years, while 58 (30M/28F) did not , and represented the control group (CON). There were no differences between the groups at baseline . Over the two years of research, before and after each ART setting, both blood pressure and blood glucose were evaluated, while the Hba1c, BMI, waist, glycemic and lipidic profile were tested every 3 months. Body composition was evaluated at T0 by means of (DXA) and after two years.
Results: The reduction in HbA1c (-1.23 ±0.9 vs. -0.05±0.3%, P<0.01), SBP (-5±1.9 vs. -1.3±1.3%, P<0.05) and DBP (-6.9±0.9 vs. -1.1±1.5%, P<0.05) was greater in the ART group compared to the CON group. No between-groups differences were observed, for the change in BMI, and TC. FFM increased in the ART group (+ 2.5 1,6 %) and decreased in the CON group (-1.4 ±1.06%, P<0.05). In contrast Body Fat (-19.1±1 vs. +2.7±0.4%, P<0.01), Waist (-7.8±2 vs. +0.45±1%, P<0.01), LDL (-3.9±2 vs. +5.9±2.6%, P<0.05) and TG (-27.6±8 vs.+6.1±4%, P<0.05) decreased in the ART group and increased in the CON group.
Conclusion: These findings show that ART is safe and effective as it improves glycemic control and body composition and reduces cardiovascular risk factors.

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Improved self-perceived physical functioning following supervised resistance training in older persons with Type 2 diabetes.

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Background and Aims: Recent studies have demonstrated that progressive resistance training (RT) can improve glycaemic control and lean muscle mass in older persons with type 2 diabetes. However, the effects of resistance training on the physical and mental health status of patients with type 2 diabetes have not been reported. We investigated the impact of high-intensity RT performed in a supervised setting on self-perceived health status in older persons with type 2 diabetes.
Materials and Methods: We studied 36 overweight (BMI ≥ 27 kg/m^2), sedentary men and women (mean age ± SD; 67.4 ± 5.6 yrs) with established (>6 mo) type 2 diabetes. All participants followed a healthy eating plan (fat <30% total energy intake) designed to elicit moderate weight loss and were randomised to either: 1) RT [3/wk, 3 sets/8reps ~ 75-85% of one-repetition maximum strength (1RM)] (RT, n=19), or 2) light exercise training [stretching, 3/wk] (LT, n=17) for 6 months. The short-form SF-36 questionnaire was used to assess eight health concepts measuring both the physical and mental dimensions of health. Self-reported questionnaire data was collected at baseline and after 6 months. No between-group differences were observed at baseline.
Results: Subject retention after 6 months supervised training was 81% (RT 84%; LT 76%), and exercise compliance did not differ between groups (RT 88%; LT 85%). Baseline and 6 month self-reported health status data at both baseline and 6 months testing points was available for 28 participants (RT, n=16, LT, n=12). At the end of 6 months supervised training, a significant improvement in self-reported physical functioning was observed in the RT group compared to the LT group (RT 11.6 ± 12.5% vs LT -0.2 ± 8.0%, P<0.05). No significant between-group differences were detected for the change in: role limitation due to physical problems (RT 9.4 ± 40.7% vs LT -18.8 ± 38.6%); role limitation due to emotional problems (RT 2.1 ± 25.7% vs LT -13.9 ± 26.4%); social functioning (RT -4.2 ± 22.2% vs LT -10.2 ± 19.8%); mental health (RT -0.5 ± 13.4% vs LT 0.0 ± 8.5%); vitality (RT 5.0 ± 8.9% vs LT -1.3 ± 11.7%); bodily pain (RT -6.9 ± 26.9% vs LT -6.5 ± 30.9%) or general health perception (RT 7.2 ± 10.8% vs LT -2.1 ± 20.5%).
Conclusion: These findings show that supervised high-intensity RT can lead to improved self-perceived physical functioning in older persons with type 2 diabetes. Although RT did not alter other self-perceived health concepts, the findings suggest that, when combined with healthy eating, RT may offer an effective lifestyle management strategy.

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Weight, adiposity and physical activity as determinants of insulin sensitivity in Pima Indian children.

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Background and Aims: Obesity, which is strongly associated with insulin resistance, may be caused by low levels of physical activity (PA).We questioned whether habitual PA (measured by questionnaire and doubly labeled water) has an independent effect on levels and changes in an insulin sensitivity index (ISI = 10^4/fasting insulin x glucose) in 90 (39M/51F) Pima Indian children at 5 and 10y of age.
Materials and Methods: Adiposity was determined by dual energy x-ray absorptiometry; behavioral measures of PA were determined by questionnaire (ACT= #/wk, TIME= hr/wk) and metabolic measures of PA were determined by the doubly labeled water method (AEE= activity energy expenditure, PAL = physical activity level).
Results: In cross-sectional analyses, ACT was correlated with ISI at 5 y (r= 0.24, p= 0.02) and 10 y of age (r= 0.21, p= 0.05) but these relationships were not independent of weight (partial r= 0.16, p= 0.14 and r= 0.08, p=0.64 for ACT at 5 and 10 y, respectively). ACT was associated with ISI independent of adiposity at 5 y (partial r= 0.22, p=0.04) but not at 10 y (partial r= 0.08, p=0.64). PAL was correlated with ISI at 10 y of age (r= 0.39, p= 0.03) but not independent of weight (partial r= 0.29, p=0.11) or adiposity (partial r= 0.33, p=0.07). Longitudinally, ISI decreased from 5 to 10y of age and increases in weight and adiposity were associated with decreases in ISI (r= -0.51, r= -0.41, respectively; both p< 0.0001). ACT decreased from 5 to 10 y of age but children who had smaller decreases in ACT had smaller decreases in ISI, independent of increases in weight or adiposity (partial r= 0.22, p= 0.04 adjusted for either weight or adiposity).
Conclusion: These data suggest that early establishment and maintenance of an active lifestyle has a beneficial effect on insulin resistance, regardless of changes in weight or adiposity. This is particularly relevant given the current epidemics of both obesity and type 2 diabetes in children.

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Insulin Therapy: Short-Acting Analogues

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Subcutaneous lyspro and intravenous regular insulin treatments are equally effective for the treatment of diabetic ketoacidosis.

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Diabetic ketoacidosis (DKA) is a serious and potentially life threatening complication. Patients with DKA must be treated basically with intravenous (i.v) hydration and regular insulin therapy under close monitoring in intensive care units (ICU).
Background & Aims: We wanted to show that treatment of DKA with rapid acting insulin (insulin lyspro) via subcutaneous (s.c) route would be equally effective and safe as i.v regular insulin therapy.
Materials and Methods: Twenty patients (Mean age; 43,75±19 years, 11 women and 9 men) were enrolled into the study. The patients were randomly divided into two groups, Group L (Mean age 38,70±19,66 years, 5 women and 5 men) and R (Mean age 48,8±17,86 years, 6 women and 4 men). Following a bolus injection of 0.15U/kg i.v regular insulin, group L received half of this dose as insulin lyspro while group R treated conventionally with standard i.v regular insulin infusion. Doses of insulin therapy titrated according to serum glucose, ketone, and pH levels. Both treatments were continued until all parameters became into normal limits. During treatment all patients were carefully monitorized for serum glucose, pH, β-hydroxybutyrate, electrolyte, urine ketone levels and urine output. Also demographic features, serum lipid and HbA1c were recorded. We compared the time period of normalization of monitorized parameters in both groups. And also means of each patients' serum glucose and pH levels' standard deviations were compared to assess variation of serum glucose and pH during the treatment in both groups.
Results: On admission, patients' serum glucose, pH, β-hydroxybutyrate, electrolyte, lipid and urine ketone levels did not differ between groups L and R. During the treatment there was no need for additional therapies in both groups. At the end of the treatment period, time that needed for normalization of serum glucose (12,7±7,45 and 9,4±8,85 hours; p:N.S), capillary β-hydroxybutyrate (15,3±8,69 and 11,2±4,93 hours; p:N.S), blood pH (6,8±5,67 and 8,2±5,61 hours; p:N.S), and urine ketone levels (22,3±10,93 and 17,2±7,02 hours; p:N.S) were not different in group R and L. During the treatment, 24-hours variation of serum glucose (45,62±22,25 and 46,44±20,84 mg/dL; group L and R respectively; p: N.S) and pH (0,036±0,025 and 0,051±0,025 group L and R respectively; p: N.S) levels were similar in both groups.
Conclusions: At the end of our study, we showed that treatment of DKA with s.c lyspro insulin and i.v regular insulin were found to be equally effective and safe.

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Post-challenge glucose responses to fixed mixtures of insulin lispro, human insulin 30/70, and insulin glargine in patients with Type 2 diabetes.

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Background and Aims: Control of postprandial glucose (PPG), an important part of diabetes management, can be achieved by the use of rapid-acting insulin analogs. However, the ability of basal insulin such as insulin glargine and NPH to control PPG remains unclear. This study compared the ability of fixed-mixtures of insulin lispro 25/75 (25% lispro/75% NPL) and 50/50 (50% lispro/50% NPL), human insulin 30/70, and insulin glargine to control PPG elevations in patients with type 2 diabetes.
Materials and Methods: This two-part, randomized, double-blind, three-way crossover study involving 23 insulin-treated patients (male/female) with type 2 diabetes was carried out at a single site. Patient characteristics at baseline included the following (mean +/- sd): age (yrs) 61.3 +/- 10.0; BMI (kg/m^2) 33.0 +/- 3.8; HbA1c (%) 8.1 +/- 1.6. A single dose (approximately 2/3 the daily insulin dose) of insulin lispro 50/50, insulin lispro 25/75, or insulin 30/70 was administered before a standard breakfast

test meal and serial glucose levels were measured. Twenty-two subjects received all premixed insulin treatments. The glucose responses were compared to those of healthy, untreated subjects (n=10) given the same meal but receiving no insulin. Thirteen subjects who completed the first part of the study were subsequently administered insulin glargine at bedtime for at least 5 days in the absence of other insulin before returning for the same breakfast test meal and serial glucose measurements. The insulin glargine dose was determined at the discretion of the investigator and adjusted according to patient self-monitored fasting blood glucose values.
Results: The fasting blood glucose levels before the test meal did not differ among insulin treatment groups. The doses of pre-mixed insulins (the average of the three periods of the cross-over) did not differ significantly: 50/50, 43.8 ± 17.8 U; 25/75, 44.1 ± 18.2 U; 30/70, 44.1 ± 18.2 U. The mean dose of insulin glargine was 37.3 ± 10.5 U. Following the test meal, the 2-hr PPG, T_{max}, C_{max} , and incremental AUC values decreased as the proportion of rapid-acting or regular insulin components of the fixed-mixture preparations increased:

Post-Test Meal Glucose Responses (mean ± SD)

Pre-Test Meal Therapy	Fasting Blood Glucose (mmol/L)	2-hr PPG (mmol/L)	T_{max} (min)	C_{max} (mmol/L)	Incremental AUC (mmol/L X min)
Glargine	9.2 +/- 1.7	14.5 +/- 2.6[a]	118.5 +/- 38.0	15.2 +/- 2.4	947 +/- 344[a]
30/70	8.9 +/- 1.9	11.8 +/- 2.6[a]	99.1 +/- 55.9	12.8 +/- 2.3[b]	548 +/- 281[a]
25/75	9.0 +/- 2.2	11.0 +/- 3.7[a]	80.5 +/- 47.8	12.3 +/- 2.9[b]	388 +/- 261[a]
50/50	8.3 +/- 1.8	8.8 +/- 2.9[a]	65.9 +/- 39.5[b]	10.8 +/- 2.0[b]	270 +/- 248[a]

[a] pair-wise comparisons among all therapies ($p<0.05$). [b] significantly different from glargine ($p<0.05$)

Conclusion: The rise in PPG after a test meal is attenuated by premeal administration of a fixed mixture of insulin and is directly related to the amount of rapid-acting insulin contained in the insulin formulation. In patients with type 2 diabetes, basal insulin treatment in the absence of a rapid-acting insulin component insufficiently controls postprandial glucose levels.

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No increase in the duration of action with rising doses of insulin aspart.
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Background and Aims: Regular human insulin (HI) when injected in high doses shows a considerable prolongation in its duration of action putting patients into a risk of late postprandial hypoglycemia. This double-blind, randomized, 6-way cross-over trial investigated if this undesirable effect is less pronounced with the fast-acting analogue insulin aspart (IA).
Materials and Methods: The pharmacokinetic (PK) and pharmacodynamic (PD) properties of HI and IA were compared in doses of 6, 12, and 24 IU s.c. in 14 healthy subjects (4 female, 28±4 years (mean±SD), BMI 24±2 kg/m^2) using the euglycemic glucose clamp technique (clamp level 5.0 mmol/l, continuous insulin infusion 0.15 mU/kg/min).
Results: In comparison to HI, IA showed higher maximal glucose infusion rates (GIR_{max}, p=0.006), an earlier onset of action (lower values for the time to the maximal effect t- GIR_{max}, $p<0.001$)), and a shorter duration of action (lower GIR-$AUC_{6\text{-}12h}$, p=0.0001). Whereas HI showed a significant increase in the duration of action with higher doses (indicated by a significant increase in GIR-$AUC_{6\text{-}12h}$), there was only a moderate, non-significant rise in GIR-$AUC_{6\text{-}12h}$ for IA with higher doses (table). Accordingly, the PK-results (obtained with specific ELISAs for serum insulin (INS) or IA-concentrations) showed a non-significant rise in INS-$AUC_{6\text{-}12h}$ for IA with higher doses in contrast to a significant increase for HI.
Conclusion: Insulin aspart does not show a significant prolongation in its duration of action with higher doses. This indicates that insulin aspart, in contrast to regular human insulin, can be safely applied even with high doses without increasing the risk of late postprandial hypoglycemia.

	Human Insulin			Insulin Aspart		
	6 IU	12 IU	24 IU	6 IU	12 IU	24 IU
GIR_{max} (mg/kg/min)#	8.7±2.7*	10.3±2.4*	13.6±3.0	9.4±3.0+*	14.1±4.5	15.7±4.2
t-GIR_{max} (min)#	180±48	190±24	185±37	118±32	130±33	144±18
$AUC_{GIR\ 6\text{-}12\ h}$ (mg/kg)#	1371±394*	1424±346*	1698±215	1166±281	1289±284	1299±251
$AUC_{INS\ 6\text{-}12\ h}$ (nmol/L)#	40.4±12.8+*	51.8±17.3	60.9±17.9	1.5±4.5	1.8±3.5	4.9±5.4

Characters denote significant differences: #HI vs. IA, +vs. 12 IU, *vs. 24 IU

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Evaluation of the pharmacodynamic and pharmacokinetic profiles of insulin glulisine – a novel, rapid-acting, human insulin analogue.
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Background and Aims: Insulin glulisine is a new rapid-acting, human insulin analogue. This study was designed to compare the pharmacodynamic (PD) and pharmacokinetic (PK) time–concentration and time–action profiles of insulin glulisine, with those of insulin lispro and regular human insulin (RHI).
Materials and Methods: In this single-dose, randomized, double-blind, three-way crossover, single-centre study, a total of 16 healthy male subjects (aged 19–30 years, body mass index 21–26 kg/m^2) received subcutaneous injections of 0.3 IU/kg body weight insulin glulisine, insulin lispro or RHI as part of the euglycaemic clamp procedure. Serum insulin concentration and glucose infusion rate (GIR) time profiles were measured in terms of maximum observed concentration (C_{max}), maximum GIR (GIR_{max}), time to maximum concentration (T_{max}) and time to maximum GIR (t_{max}). In addition, the area under the serum insulin concentration and GIR-time curves up to 2 hours and clamp end ($AUC_{0\text{–}2\ h}$, $AUC_{0\text{–clamp end}}$), mean residence time (MRT) and duration of action (t_d) were also measured, as shown in the table.
Results: The absorptions of insulin glulisine and insulin lispro were twice as rapid as RHI, and both insulins had an MRT half that of RHI. In addition, T_{max} and t_{max} were twice as rapid for both insulin glulisine and insulin lispro compared with those for RHI. Total glucose disposal was similar for all insulin preparations.
Conclusion: In this study, insulin glulisine demonstrated PK and PD profiles of a rapid-acting insulin analogue.

Variable	Insulin glulisine 0.3 IU/kg	Insulin lispro 0.3 IU/kg	RHI 0.3 IU/kg
Pharmacodynamics:			
Maximum GIR* (mg/min/kg)	12.5	13.1	11.7
t_{max}* (min)	90.0†	87.6†	195.3†
$AUC_{(0\text{–}2\ h)}$ (mg/kg)	1026.0	976.3	674.8
$AUC_{(0\text{–clamp end})}$ (mg/kg)	2839.6	2942.6	3234.7
t_d (min)	318†	329†	385†
Pharmacokinetics:			
C_{max} (µ IU/mL)	196	156	84
T_{max} (min)	56†	50†	99†
$AUC_{(0\text{–clamp end})}$ (µ IU/min/mL^{-1})	29302	22116	21673
MRT (min)	105	117	182

Values are means unless otherwise indicated; * Derived from 'smoothed' individual GIR profiles; Median value

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Pharmacokinetic and glucodynamic profiles of insulin glulisine: an evaluation following subcutaneous administration at various injection sites.
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Background and Aims: Insulin glulisine, a new rapid-acting human insulin analogue, was developed to have a more rapid onset of activity and shorter duration of action than regular human insulin. The effect of different injection sites on the pharmacokinetics (PK), pharmacodynamics and absolute bioavailability of insulin glulisine following single 0.1 IU/kg body weight subcutaneous (s.c.) administration into femoral, deltoid or abdominal areas, as well as after bolus intravenous (i.v.) injection of 0.1 IU/kg body weight insulin glulisine, was evaluated in this study.
Materials and Methods: This single-centre, randomized, open-label, four-way crossover trial, enrolled 16 male volunteers (age: 19–28 years, body mass index: 21–26 kg/m^2) who underwent the euglycaemic clamp procedure. Serum concentration (C) and glucose infusion rate (GIR) time curves were analyzed for maximum C (C_{max}), time to C_{max} (T_{max}), maximum GIR (GIR_{max}), time to GIR_{max} (t_{max}), and area under the C and GIR time curves to clamp end ($AUC_{0\text{–clamp end}}$), as well as mean residence time (MRT) and duration of action (t_d) (see table).
Results: Similar results for absorption, absolute bioavailability and glucose disposal were obtained regardless of s.c. injection site, although more rapid insulin delivery was noted for the abdominal route.
Conclusions: Insulin glulisine can be considered a rapid-acting insulin analogue because it has similar rapid PK, absolute bioavailability and

glucodynamics after s.c. injection into the femoral, deltoid or abdominal sites.

Variable	Femoral s.c.	Deltoid s.c.	Abdominal s.c.	Forearm i.v.
Pharmacodynamics:				
GIR_{max} † (mg.min^{-1}kg^{-1})	7.9	7.3	8.0	14.3
t_{max} † (min)	157*	135*	127*	24*
$AUC_{(0-clamp\ end)}$ [mg/kg]	1520	1464	1498	1228
t_d (min)	268*	270*	251*	160*
Pharmacokinetics:				
C_{max} (µIU/mL)	57	68	84	3014
T_{max} (min)	66*	58*	44*	NA
AUC $_{(0-clamp\ end)}$ [µIU.min.mL^{-1}]	9625	10288	10551	14755
MRT (min)	114	103	89	2
Absolute BA (%)	68	71	73	NA

Values are geometric/arithmetic means unless otherwise indicated; * Median values; † Determined from 'smoothed' GIR profiles; NA = not applicable; BA = bioavailability

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Investigating the drug accumulation of two premixed insulins.
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Background and Aims: Premixed insulin preparations might not exhibit their full metabolic potential after the first injection, as the effect may persist into the next dose. This double-blind, randomised, two-period crossover study investigated both the pharmacokinetics (PK) and pharmacodynamics (PD) of two premixed insulins at the onset (Day 1) and after one week (Day 8) of thrice daily treatment
Materials and Methods: Twenty-seven patients with type 1 diabetes (18 male, age 27±8 years) were included. The patients started treatment for one week with BIAsp 30 or BIAsp 70 (30% and 70% soluble insulin aspart, respectively, the remainder protaminated). Then followed a 2-6 week washout period, before shifting to one week of treatment with the alternative insulin. On Day 1 and Day 8 of the two treatment periods, the PK (based on plasma insulin aspart profiles) and PD (based on glucose infusion rate (GIR) profiles) properties of the two insulin preparations were assessed in a 12h euglycaemic clamp (target blood glucose (BG) level: 5mM; BG stabilized during a 3-6h baseline period; basal insulin infusion rate: 0.15mU/(kg•min)).
Results: As expected, BIAsp 30 and BIAsp 70 showed clearly different time-action profiles (Table 1). A slight upward shift in the insulin aspart (IAsp) profiles was seen after one week of treatment, but for PD no difference was seen between Day 1 and Day 8.

Table 1: PK and PD Results Summarized

Pharmacokinetics	Day 1 BIAsp 30	Day 1 BIAsp 70	*p*-value	Day 8 BIAsp 30	Day 8 BIAsp 70	*p*-value
Cmax,IAsp (pM)	327	607	0.000	358	698	0.000
AUC,0-6h,IAsp (pM×h)	987 **(a)**	1663 **(a)**	0.000	1178	1862	0.000
AUC,6-12h,IAsp (pM×h)	211 **(a)**	187	0.118	343	213	0.000
Pharmacodynamics						
GIR,max (mg/(kg×min))	4.75	6.05	0.002	5.16	5.84	0.137
AUC,0-6h,GIR (mg/kg)	1264	1455	0.018	1262	1474	0.016
AUC,6-12h,GIR (mg/kg)	567	347	0.025	646	340	0.000

(a) p<0.05 when comparing Day 1 and Day 8.

Conclusion: This study proves that neither BIAsp 30 nor BIAsp 70 show any clinically relevant accumulation in the metabolic effect. Future studies investigating accumulation of intermediate-acting or long-acting insulin preparations should investigate both PK and PD properties under 'multiple dose' conditions.

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Safety and efficacy of long term exposure to biphasic insulin aspart compared with biphasic human insulin in people with Type 2 diabetes.
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Background and Aims: Biphasic human insulin 30 (BHI 30), a mixture of free (30%) and protamine-bound (70%) human insulin is the most widely used premixed insulin. It offers good glycaemic control in patients with diabetes both at meals and between meals. The present trial evaluates the safety and efficacy of a corresponding recent biphasic formulation of the rapid acting insulin analogue BIAsp 30 versus BHI 30 over a 4-year period.
Materials and Methods: The trial began as a 3-months efficacy comparison with twice daily treatments in both groups (called *BIAsp* and *BHI*, respectively), but was extended several times for long-term safety and efficacy comparison of the treatments. 294 people started at the efficacy trial and 204 (79 with type 1 and 125 with type 2 diabetes) continued in the first 21 month safety extension period. In a further 2-year extension 60 people (41 with type 2 diabetes) started in the *BHI* group and 55 (32) in the *BIAsp* group. Data for efficacy (HbA_{1c}) and safety (hypoglycaemic episodes and adverse events (AEs)) were collected for the group of people (115) who continued beyond the first 2 year period. Most of these were in treatment for at least $3^1/_2$ years, but many withdrew just before 4 years of treatment (At 47 months 88 people were still in treatment but at 48 months the number had dropped to 43).
Results: All data presented here relates to the 73 people with type 2 diabetes who continued beyond the first 2 years. 2-year data have previously (EASD 2001) been presented for people with type 2 diabetes who completed the first 21-month extension trial.
Efficacy: HbA_{1c} measurements at baseline, at 24 months and at last visit were 8.03, 8.06 and 7.98 in the *BIAsp* and 7.88; 7.98 and 8.26 in the *BHI* group, respectively. Standard deviations ranged between 0.95 and 1.2, irrespective of the treatment. No significant between-group differences were found at last visit (p=0.23).
Safety: Number of people with at least one major hypoglycaemic episode were: during the first 2 years 1 in the *BIAsp* group and 7 in the *BHI* group (p=0.04), during entire trial period 4 in the *BIAsp* group and 11 in the BHI group (p=0.08), during 4 years of treatment no people in the BIAsp group experienced major nocturnal episodes (between 24:00 and 6:00), whereas 6 people in the *BHI* group experienced such episodes (p=0.02). Minor nocturnal episodes showed a similar trend. The corresponding numbers for the 4-year period were 5 in the BIAsp group and 11 in the BHI group (p= 0.15).
The frequency and severity of AEs was similar in the two treatment groups.
Conclusion: In people with type 2 diabetes, overall glycaemic control and frequency of adverse events was similar during twice daily treatment in four-years with either BHI30 or BIAsp 30. The number of people experiencing major hypoglycaemic episodes was lower in the BIAsp group during the entire treatment period, and was significantly lower during the first 2 years of treatment. The number of nocturnal major hypoglycaemic episodes was significantly lower in the BIAsp group.

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Pharmacokinetics and glucodynamics of Biphasic Insulin Aspart 30 and 50 in Japanese Type 2 diabetes.
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Background and Aims: Biphasic Insulin Aspart 30 and 50 (BIAsp30 and BIAsp50, respectively) are biphasic insulin analogues containing soluble rapid-acting insulin aspart (IAsp) and protamine-crystallized protracted IAsp with a ratio of 30%:70% and 50%:50%, respectively. We investigated the pharmacokinetics and glucodynamics of BIAsp50 and BIAsp30 derived from serum IAsp profile and glucose infusion rate (GIR) profiles obtained from the glucose clamp method.
Materials and Methods: Single subcutaneous doses of BIAsp30 and BIAsp50 were administered at 0.3 U/kg to 10 Japanese Type 2 diabetic patients under a randomized, double-blind, two-period crossover fashion. Under the euglycaemic clamp technique, the pharmacokinetic and glucodynamic parameters were determined over 8 hours following injection.
Results: The mean C_{max} was significantly higher for BIAsp50. The ratio (BIAsp50/BIAsp30) for C_{max} was 1.63. Other endpoints, such as AUC_{0-}

$_{120min}$, $AUC_{0-240min}$ and $AUC_{0-480min}$ were estimated to be approximately 68, 47 and 32% higher, respectively, in BIAsp50. The amount of insulin aspart absorbed during the early period of the AUC tended to be higher with BIAsp50 due to its higher percentage of soluble IAsp than BIAsp30. The GIR_{max} did not differ between the treatments in spite of a statistical difference seen in the pharmacokinetic parameter C_{max}. The $AUC_{GIR,\ 0-120min}$ was estimated to be 31% higher for BIAsp50. The GIR during the first 2 hours tended to be higher with BIAsp50 due to its higher percentage of soluble IAsp than BIAsp30. No serious adverse events were reported during the trial. All adverse events were mild and were judged unlikely related to the trial product.
Conclusion: This study demonstrated that C_{max} was statistically higher for BIAsp50 when compared to BIAsp30. $AUC_{GIR,\ 0-120min}$ was estimated to be higher for BIAsp50. The difference of C_{max} between the products was not reflected in the GIR_{max}. No safety concerns were raised in this trial.

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A randomised, multicentre trial of biphasic insulin aspart versus biphasic human insulin in Type 2 diabetes.
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Background and Aims: Biphasic insulin aspart (BIAsp) is a mixture of soluble (rapid-acting) and protamine crystallized (intermediate-acting) insulin aspart (IAsp). BIAsp30 contains 30% soluble IAsp. The aim of this trial was to compare BIAsp30 with biphasic human insulin 30 (BHI30) in Japanese Type 2 diabetes.
Materials and Methods: In this multicentre, open-labelled, randomised, parallel group study, 437 patients were asymmetrically randomised and 428 patients were treated with either BIAsp30 twice daily immediately before meals (321) or BHI30 twice daily 30 minutes before meals (107). Baseline demographics (gender, age and BMI) and diabetic characteristics (duration of insulin treatment, HbA_{1C}, total daily insulin dose) were comparable between the treatment groups. Efficacy was assessed by HbA_{1C} at 24 weeks, and at 48 weeks and blood glucose (BG) before and after breakfast at 24 weeks.
Results: The trial endpoint, the least-squares means of HbA_{1C} at 24 weeks (adjusted for baseline values) was 7.31 ± 0.04% and 7.20 ± 0.06% (mean ± SE) for BIAsp30 and BHI30, respectively. The mean treatment difference in HbA_{1C} at 24 weeks adjusted for baseline HbA_{1C} was 0.10 (95% C.I.: -0.04; 0.25) %. Thus, the planned non-inferiority criterion (the upper limit of 95% C.I. < 0.6%) was fulfilled and BIAsp30 was as effective as BHI30. The least-squares means of HbA_{1C} at 48 weeks (adjusted for baseline value) was 7.37 ± 0.04% and 7.35 ± 0.07% for BIAsp30 and BHI30, respectively. The mean treatment difference in HbA_{1C} at 48 weeks adjusted for baseline HbA_{1C} was 0.02 (95% C.I.: -0.15; 0.18) %. The BIAsp30 group had a statistically significant lower BG level at 90 minutes after breakfast compared to the BHI30 group (234.1 ± 3.4 vs. 247.9 ± 5.8 mg/dl, p=0.0414), and a statistically significant higher BG level before breakfast compared to the BHI30 group (160.2 ± 2.3 vs. 145.3 ± 3.9 mg/dl, p=0.0010), when adjusting for baseline values. However, after 24 weeks of treatment, BG level before breakfast decreased slightly in both treatment groups. The mean postprandial BG increment after breakfast was statistically significantly lower in the BIAsp30 group than in the BHI30 group (73.8 ± 2.9 vs. 103.3 ± 5.0 mg/dl, p<0.0001). The frequency of subjects having at least one hypoglycaemic episode was similar in both treatment groups, 56.1% in the BIAsp30 group and 57.0% in the BHI30 group. Major hypoglycaemic episodes (required third-party help) were rare in this trial. The estimated relative risk (BIAsp30/BHI30) of having a minor hypoglycaemic episode was 0.69 (95% C.I.: 0.46; 1.04). This result represented approximately 30% lower risk in the BIAsp30 group relative to the BHI30 group, however the difference of the risk was not statistically significant.
Conclusion: Overall glycaemic control with BIAsp30 twice daily immediately before meals regimen was just as effective as with BHI30 twice daily 30 minutes before meals regimen in Type 2 diabetes. The frequency of hypoglycaemic episodes was similar in both treatment groups.

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Long-Acting Insulin Analogues

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Type 1 diabetes patients can temporarily switch from continuous subcutaneous insulin infusion with insulin aspart to basal bolus therapy with insulin aspart and insulin glargine.
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Background and Aims: The safety and efficacy of multiple daily injection (MDI) therapy of insulin aspart (IAsp) and insulin glargine were compared to continuous subcutaneous insulin infusion (CSII) with IAsp.
Materials and Methods: This study was a multicenter, open-label, randomized, cross-over study of adult type 1 subjects previously treated with CSII (37 men/63 women; BMI (±SD), 26.9 ± 4.0 kg/m^2; age, 43 ± 11 yrs). Before randomization, all subjects were treated for 1 week with IAsp using CSII, switching on a unit-by-unit basis from their prestudy CSII insulin. Thereafter, 50 subjects were randomized to MDI therapy (IAsp immediately before each meal and glargine at bedtime) and 50 subjects continued on CSII therapy. For MDI–treated subjects, the glargine dose was equal to the total dose of the 24-hour CSII basal rate. After 5 weeks, subjects were switched to the alternative treatment for 5 weeks. During the last week of each treatment period, subjects wore a continuous subcutaneous glucose monitoring system (CGMS) for 48 to 72 hours.
Results: The CGMS showed that glucose exposure was significantly lower with CSII than with MDI therapy, based on AUC glucose ≥80 mg/dL over a 48-hour glycemic profile (CSII: 2059 ± 1310 mg·hr/dL; MDI: 2687 ± 1734 mg·hr/dL; P<0.01). Fructosamine values at the end of each treatment period were significantly less with CSII than with MDI therapy (343 ± 47 umol/L vs 355 ± 50 umol/L, respectively, P<0.001). Incidence of hypoglycemia was similar for both treatment therapies. A similar percentage of subjects reported hypoglycemic episodes (CSII: 92%, MDI: 94%) and nocturnal (midnight to 8 am) hypoglycemic episodes (CSII: 73%, MDI: 72%). Major hypoglycemia (with CNS dysfunction requiring assistance of another individual) had low occurrence in both groups (CSII: 2 episodes, MDI: 3 episodes).
Conclusion: CSII therapy with IAsp results in lower glycemic exposure without increased risk of hypoglycemia as compared to MDI therapy with IAsp and insulin glargine in type 1 diabetes patients. Type 1 patients can safely switch from CSII therapy to MDI therapy for temporary situations.

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Reduced hypoglycemic episodes and improved glycemic control in children with Type 1 diabetes using insulin glargine therapy.
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Background and Aims: The purpose of this study was to determine if the addition of insulin glargine (Lantus®) could improve glycemic control in children with type 1 diabetes without increasing hypoglycemic episodes.
Materials and Methods: 114 subjects (54 boys, 60 girls) ages 2 to 18 years (mean age 12.6±4.2, duration of diabetes 5.4±4.0) were studied. All subjects had diabetes for at least one year prior to initiating insulin glargine, had two A1C levels above 8.0% during the prior 9 months, and had at least 9 months of follow-up care. Subjects received 3 insulins; insulin lispro and two long acting insulins (morning NPH and evening insulin glargine).
Results: The frequency of low blood glucose levels (<60 mg/dl per meter printouts and patient reports, ± 1SEM) per week, decreased from 2.0±0.1 in the 9 months prior to treatment to 1.3±0.1 during the 9 months on insulin glargine (p<0.001). Severe hypoglycemic events (seizure or loss of consciousness) decreased from 22 (in 14 subjects) in the 9 months prior to insulin glargine treatment to 9 (in 8 subjects) in the 9 months during insulin glargine. The reduction in severe events was primarily due to the decline in nocturnal episodes (14 pretreatment events to 4 events on insulin glargine, table).
The mean A1C level and the daily insulin dose (units/kg) were significantly lower during the 9 months of insulin glargine treatment compared to the prior 9 months (table).

Conclusion: The addition of evening insulin glargine resulted in a reduction in non-severe and severe hypoglycemic events as well as a significant reduction in A1C levels.

RESULTS SUMMARY

Outcome Measures	*Baseline*	*Treatment*	*p-value*
Non-severe hypoglycemia/wk	2.0±0.1	1.3±0.1	<0.001
Total severe hypoglycemia/9 mos	22	9	*
Nocturnal severe hypoglycemia	14	4	*
A1C(%)	9.6±0.1	9.3±0.1	0.01
Total units/kg	1.02±0.03	0.96±0.03	0.01

* Severe hypoglycemia did not occur with sufficient frequency to perform statistical analysis for this number of patients.

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Fluctuation of serum insulin levels after single and multiple dosing of insulin glargine.

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Background and Aims: Large fluctuation of serum insulin concentration is of clinical concern because it increases the risk of unpredictable hyper- and hypoglycaemia. The aim of this study was to establish the degree of fluctuation from mean serum insulin levels following administration of insulin glargine (LANTUS®).
Materials and Methods: Subjects from three Phase I studies were included in the analysis: 1) a single-dose, randomized study of healthy subjects (n=36) receiving insulin glargine, NPH insulin or ultralente (0.4 IU/kg); 2) a single-dose, randomized study of patients with Type 1 diabetes (n=20) receiving insulin glargine or NPH insulin (0.3 IU/kg); 3) a multiple-dose study of patients with Type 1 diabetes (n=15) receiving insulin glargine (patient-tailored doses) plus insulin lispro over 11 days. Fluctuation, defined as the percentage deviation around the average serum concentration over 24 hours (PF24), was evaluated for each patient and a mean PF24 value for each treatment group was calculated; lower PF24 indicates a more physiological profile.
Results: In study 1, mean PF24 in patients receiving insulin glargine (20%, 1.8 µIU/mL) was significantly lower than in those receiving NPH insulin (32%, 3.8 µIU/mL; both p <0.0001 vs insulin glargine) or ultralente (47%, p <0.0001 vs insulin glargine; 3.7 µIU/mL, p=0.0034 vs insulin glargine). This equates to more than 50% less variability with insulin glargine versus NPH insulin or ultralente. In study 2, insulin glargine was also associated with a significantly lower mean PF24 (14%, 1.7 µIU/mL) versus NPH insulin (26%, p <0.0001 vs insulin glargine; 4.8 µIU/mL; p=0.0003 vs insulin glargine), again a demonstration of over 50% less variability with insulin glargine versus NPH insulin. Fluctuation levels estimated from the single-dose studies were comparable to estimates in the steady state by analysis of patients in study 3 who received multiple insulin glargine doses. Mean PF24 values were not significantly different on days 2 (20%, 4.3 µIU/mL), 5 (20%, 4.0 µIU/mL) or 12 (20%, 5.0 µIU/mL).
Conclusion: These results show that single doses of insulin glargine provide less fluctuation in serum insulin levels versus NPH insulin or ultralente. Furthermore, these minimal fluctuations with repeated dosing make insulin glargine more closely approach a physiological basal insulin profile and should increase the predictability of insulin action, thereby reducing unpredictable hyper- and hypoglycaemia.

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Improvement in overall blood glucose control with insulin glargine plus insulin lispro in comparison with NPH insulin plus unmodified human insulin in people with Type 1 diabetes.

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Background and Aims: Studies with the long-acting insulin analogue glargine (LANTUS®) have shown lower pre-breakfast blood glucose levels and reduced nocturnal hypoglycaemia compared with NPH insulin, but inconsistent improvement in HbA_{1c}. Insulin lispro (Humalog) also reduces nocturnal hypoglycaemia but does not reduce HbA_{1c} without intensification of basal insulin therapy. This trial investigated whether HbA_{1c} could be improved with insulin glargine plus insulin lispro combined, compared with NPH plus unmodified human insulin.
Materials and Methods: This was a 32-week, open, randomized, multicentre, 2-way cross-over clinical trial in people with Type 1 diabetes on a multiple insulin injection regimen. People with diabetes (n=56, baseline HbA_{1c} 8.0 ± 0.8 [± SD] %) were randomized to evening insulin glargine plus meal-time insulin lispro (G+L), or to NPH insulin (once or twice daily) plus meal-time unmodified human insulin (NPH+HI). Blood glucose control was evaluated by HbA_{1c}, patient self-monitoring, in-patient 24-h profiles and hypoglycaemic events.
Results: Fifty-one patients completed the study. Basal insulin dose was higher with G+L *vs* NPH+HI (24.5 *vs* 22.0 U/day, p=0.005), but meal-time dose was lower (29.7 *vs* 34.1 U/day, p=0.004); total dose was similar (54.2 *vs* 56.1 U/day, NS). HbA_{1c} was lower with G+L than with NPH+HI (7.5 *vs* 8.0 %, difference -0.5 [95% CI -0.7, -0.3] %, p<0.001). This was confirmed by a 10 % lower 24-h plasma glucose AUC (187 *vs* 208 mmol/l.h, p=0.032) with a 25 % reduction in plasma glucose excursions above 7.0 mmol/l (AUC 47 *vs* 63 mmol/l.h, p=0.014), but with no reduction in night-time plasma glucose AUC or increase in plasma glucose AUC below 3.5 mmol/l. Post-prandial AUC was also lower (75 *vs* 88 mmol/l.h, p=0.002). Mean 24-h self-monitored blood glucose (SMBG) level was lower with G+L (7.8 *vs* 9.7 mmol/l, -1.9 [-3.1, -0.8] mmol/l, p=0.001), with statistically significant effects on pre- and post-breakfast SMBG levels (p=0.005 and p<0.001, respectively). Frequency of nocturnal hypoglycaemia was lower with G+L during months 2 to 4 (33 *vs* 42 patients, p=0.013), and during month 1 (21 *vs* 32 patients, p=0.001); other hypoglycaemia was similar between regimens.
Conclusion: The combination of insulin glargine and a rapid acting insulin analogue *vs* NPH+HI in a multiple injection regimen in Type 1 diabetes improves overall glycaemic control together with a reduction in nocturnal hypoglycaemia to a clinically significant degree.

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Insulin detemir reaches steady-state after the first day of treatment and shows a peakless time-action profile with twice daily-applications.

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Background: This euglycemic glucose clamp-study compared the effect of the basal insulin analogue insulin detemir (IDet) in single-dose and under steady-state (SS) conditions with that of NPH insulin (NPH) at SS.
Material and Methods: 25 Type 1 diabetic patients (7 women and 18 men, 39±12 yrs (mean±SD), BMI 24±3 kg/m^2) were included. Patients participated in three 24-h glucose clamps (clamp level 5.5 mmol/l) with insulin injections at 0 and 12 h (in fixed, individualised doses). The first clamp assessed the metabolic effect of NPH at SS. The second clamp investigated the effect of two single injections of IDet (NPH treatment was stopped app. 24 h before the clamp). Patients continued IDet treatment at 12-hour intervals for 7-14 days, after which the third clamp was performed with IDet at SS.
Results: The overall metabolic effect of IDet at SS was similar to that of NPH (total area under the glucose infusion rate (GIR) profile, $GIR\text{-}AUC_{0\text{-}24h}$: 5697±1861 vs. 5929±1965 mg/kg) whereas a significantly lower effect (5187±1784 mg/kg, p=0.01 vs. SS) was observed following the first two single IDet injections. The metabolic effect after the second IDet injection was similar to that at SS. At SS IDet provided a flat and peakless profile over 24 hours while a clear peak in the metabolic effect after each injection was observed with NPH. The more constant metabolic effect of IDet under SS was reflected in lower Delta-GIR values (difference between maximum and minimum of the GIR-values divided by the mean: 0.69±0.30 (IDet-SS) vs. 0.80±0.44 (NPH)) and by almost identical values for the area under the GIR-curves for 0-12 h (2962±1042 mg/kg) and 12-24 h (2967±1005 mg/kg). The pharmocokinetic data were in accordance with the GIR-profiles.
Conclusion: Insulin detemir reaches steady-state after 2-3 days, depending on dose and dose frequency. The peakless time-action profile of insulin detemir may have advantages over NPH insulin for the treatment of people with diabetes.

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Insulin glargine versus NPH insulin: a prospective 18 months comparison in Type 2 diabetic patients with a multiple injection regimen.

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Background and Aims: The UKPDS has established that long term tight metabolic control delays the onset and progression of diabetic micro- and macrovascular complications. To meet glycemic goals multiple injection regimens are one therapeutic option. Up to date only marginal long term data is available about the long-acting insulin analog Glargine (G).
Materials and Methods: We chose a prospective long term approach to determine the safety and efficacy of G compared to NPH insulin (NPH) in type 2 diabetic patients. We analysed a subgroup of patients with a multiple injection regimen and compared G once daily with NPH twice daily for 18 months in a prospective, single centre, open-label study. Up to date 56 (G) respectively 53 (NPH) patients completed the trial. All patients used a short acting insulin analog as pre-prandial insulin.
Results: Mean age was 60.8 years (G) and 62.0 years (NPH), duration of diabetes was 14.6 years (G) and 15.1 years (NPH), mean fasting C-peptide was 0.78 ng/ml (G) and 0.81 ng/ml (NPH). After 18 months the average daily dose was 0.29 IU/kg (G) (start dose : 0.28 IU/kg) compared to 0.38 IU/kg (NPH) (start dose: 0.35 IU/kg) with no significant differences in preprandial insulin doses. In the G group HbA1c levels improved significantly from 7.4 to 7.0% (-0.42%, SD 0.77, $p<0.003$). In the NPH group the decrease of HbA1C from 7.4 to 7.2% was not significant (-0.19, SD 0.8, $p<0.06$). Both groups did not show a significant increase of mean body-mass-index. Compared to the NPH group a lower number of symptomatic hypoglycaemias were documented in the G group. In both groups no severe hypoglycemias were detected.
Conclusion: In patients with type 2 diabetes treated with a multiple injection regime, G once daily resulted in a significant improvement of metabolic control without change in body weight in a 18 months clinical trial. G appears to provide a clinical advantage over NPH also with respect to the incidence of mild hypoglycaemia.

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Latin American clinical trial project on insulin glargine versus NPH insulin plus glimepiride in Type 2 diabetes.

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Background and Aims: This multicentre, open-label, 24-week randomized trial compared efficacy (non-inferiority based on baseline to endpoint change in HbA_{1C} and safety of once-daily bedtime insulin glargine (LANTUS®) or NPH insulin plus once-daily morning glimepiride ($_{AMARYL®}$) $_{fixed}$ dose in Latin American patients with type 2 diabetes.
Materials and Methods: Patients poorly controlled by oral agents received insulin dose individually titrated to fasting blood glucose (FBG) target ≤ 100 mg/dL.
Results: Patients (n=449 and 480 for efficacy per-protocol [PP] and safety [ITT] populations respectively) had an average age 56.6 ± 9.7 years and an average body mass index (BMI) of 27.3 ± 3.8 kg/m^2; baseline HbA_{1C} was 9.1 ± 0.9%. Baseline to endpoint change in HbA_{1C} was similar with insulin glargine (-1.4%) and NPH insulin (-1.5%). At endpoint, similar patients percentages in insulin glargine and NPH insulin groups (PP set) achieved FBG ≤ 120 mg/dL (66.7% and 65.3%, respectively) and $HbA_{1C} \leq 7.5\%$ (51.2% and 48.3%), respectively). However, confirmed nocturnal symptomatic hypoglycemia (BG ≤ 72 mg/dL) occurred significantly less frequently with insulin glargine versus NPH insulin (15.6% vs 27.3%, respectively; $p < 0.01$). Further preliminary analysis showed that more patients on insulin glargine achieved $HbA_{1C} \leq 7.0\%$ without confirmed nocturnal symptomatic hypoglycaemia versus NPH insulin treatment (27.2% vs 18.6%, respectively; $p = 0.036$).
Conclusion: These results show that a single combination of once-daily basal insulin plus once-daily glimepiride using an insulin titration schedule can restore glycaemic control in majority of type 2 diabetes patients who are poorly controlled on oral agents. This occurs more frequently without confirmed nocturnal hypoglycaemia when insulin glargine is used compared with NPH insulin. These results support those of European and North American studies, despite cultural differences in dietary habits.

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Quality of glycaemic control and well-being of patients with Type 2 diabetes mellitus after switching from conventional insulin therapy to once-daily insulin glargine in combination with oral antidiabetic drugs – SWITCH-pilot, a prospective, randomized trial.

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Background and Aims: Patients with Type 2 diabetes on multiple daily insulin injections often have comparable glycaemic control, but poorer well-being, versus patients on conventional insulin therapy (CIT; twice-daily premixed insulin). The addition of the long-acting insulin analogue glargine (Lantus®) to inadequate dietetic or oral therapy has been evaluated in previous studies; however, it is unclear whether glargine in combination with oral therapy can be used in poorly controlled patients with Type 2 diabetes previously treated with CIT.
Materials and Methods: In a randomized, 16-week trial, 52 patients with Type 2 diabetes, HbA_{1c} >7.8% and CIT were randomized to either glargine (once daily in the morning) with glimepiride (group A, n=17), glargine with glimepiride and metformin (group B, n=18) or continued CIT (group C, n=17) (Table 1). Glycaemic control, incidence of hypoglycaemia and patients' well-being and treatment satisfaction were evaluated.
Results: HbA_{1c} decreased in all treatment groups and there were no between-treatment differences at endpoint in fasting blood glucose and the incidence of hypoglycaemia (Table 2). In 3 patients (in groups A and B), treatment with glargine and oral agents was stopped before study end due to gastrointestinal discomfort and diarrhoea. Although there were no significant differences in well-being and treatment satisfaction at endpoint, 15/17 patients (88%) in group A and 12/18 (67%) in group B opted to continue therapy with glargine and oral agents. In group C, 1 patient (6%) switched to multiple daily insulin injections.
Conclusion: Compared with CIT, glargine plus oral therapy shows at least equal efficacy and high acceptance. Therefore, as for patients with Type 2 diabetes on previous oral therapy, switching to glargine seems to be a real alternative for patients with long-term CIT.

Table 1. Baseline characteristics of patients randomized (excluding 3 patients who stopped therapy before study closure)

	Group A	Group B	Group C	A vs B	A vs C	B vs C
Patients (n)	17	15	17			
Age (years)	61.53±10.61	64.57±8.32	69.66±6.43	0.379	0.011	0.061
Duration of diabetes (years)	15.17±8.42	14.77±7.91	16.24±6.66	0.890	0.686	0.573
Duration of insulin therapy (years)	3.77±2.00	4.50±1.59	4.33±1.41	0.269	0.355	0.752
Body mass index (kg/m²)	32.23±3.15	31.37±2.89	30.98±3.25	0.420	0.260	0.717
C-peptide (nmol/L)	2.71±1.84	2.90±1.44	2.98±2.09	0.757	0.692	0.902
Insulin dosage (IU/d)	77.59±32.12	77.59±32.12	65.18±34.48	0.255	0.286	0.936
HbA_{1c} (%)	8.22±0.69	7.99±0.70	8.08±0.84	0.343	0.596	0.730

Table 2. Quality of diabetes control, frequency of hypoglycaemia and treatment satisfaction at endpoint (excluding 3 patients who stopped therapy before study closure)

	Group A	Group B	Group C	A vs B	A vs C	B vs C
Patients (n)	17	15	17			
Body mass index (kg/m²)	32.58±2.87	31.25±2.85	31.06±3.49	0.201	0.177	0.870
Insulin dosage (IU/d)	75.06±44.06	48.13±33.47*	70.59±35.04*	0.064	0.745	0.075
HbA_{1c} (%)	7.81±0.55*	7.41±0.94*	7.83±1.13*	0.147	0.939	0.263
Fasting blood glucose (mmol/L)	6.95±2.25	7.16±1.74	7.38±2.39	0.744	0.566	0.774
Symptomatic hypoglycaemia (number of events/patients)	3/3	7/4	3/3	0.166	1	0.166
Treatment satisfaction (points)	31.59±7.55	32.67±5.15	30.88±6.05	0.645	0.766	0.380

*p <0.05 vs baseline

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Impact of different insulin glargine regimens on glycemic parameters in intensively treated adults with Type 1 diabetes.

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Background and Aims: The new 24 hr insulin analogue, insulin glargine (Lantus®) is approved and recommended for use as a single injection at bedtime. In some patients and caregivers, compliance to therapy may be better if a larger dose of insulin is taken in the morning.
Materials and Methods: A questionnaire (locally generated and approved by the IRB) and electronic database were used to assess various glycemic parameters for 104 consecutive type 1 diabetic subjects on insulin glargine (as their only long acting insulin) throughout the study period for at least six months. All subjects were on intensive diabetes management (≥4 injections/day) and received a short acting insulin preprandially. Twenty-two subjects were taking glargine in the morning, thirty-seven subjects were taking glargine in the evening and forty-five subjects were splitting the glargine dose to both morning and evening injections.
Results: The mean (± SD) age was 36.3 ± 11.4 yrs, 30.8 ± 8.3 yrs, and 31.5 ± 8.4 yrs for the morning, evening, and split groups respectively (p>0.05). The mean (± SD) duration of diabetes was 21.2 ± 11.6 yrs, 15.7 ± 9.2 yrs, 17.1 ± 9.1 yrs for the morning, evening and split groups respectively, (p>0.05, table). The mean (± SEM) duration of treatment on glargine was 13.5 ± 1.1 months, 13.1 ± 0.8 months, and 14.2 ± 0.7 months for the morning, evening, and split groups respectively (p>0.05). Mean (±SEM) baseline, end of the study (7.4% ± 0.2, 7.5% ± 0.2, 7.5% ± 0.1) and change in HbA1C values were not significantly different for the morning, evening and split groups respectively, p>0.05, table).

Glargine Group	HbA1C ± SEM Baseline	HbA1C ± SEM End of Study	Severe Hypoglycemic Episodes/yr	Glargine Insulin dose (units)	Short Acting Insulin Dose (units)	Total Insulin Dose (units)	Mean Weight (kg)
Morning	7.7% ± 0.3	7.4% ± 0.2	1.4 ± 0.4	29.9 ± 1.7	19.7 ± 1.7	49.5 ± 2.6	74.7 ± 2.5
Evening	7.8% ± 0.3	7.5% ± 0.2	0.6 ± 0.2	28.8 ± 2.0	22.6 ± 2.4	51.3 ± 4.0	76.1 ± 2.5
Split morning and evening	7.9% ± 0.2	7.5% ± 0.1	0.4 ± 0.2	40.9 ± 2.5	20.8 ± 1.8	61.6 ± 3.7	80.0 ± 2.7
P-value	p > 0.05	p > 0.05 p	> 0.05	p < 0.01 p	> 0.05	p = 0.05	p > 0.05

The mean (±SEM) glargine insulin dose was significantly greater in the split group as compared to the morning or evening groups (p<0.01, table). There were no differences between the groups in severe hypoglycemic episodes per patient years, weight, or short acting insulin dose (p>0.05). Dividing the glargine insulin dose did not provide any benefit and morning single injection is as good as evening single injection. Since glycemic parameters were similar with morning and evening injections, the morning single dose is likely to be better accepted in clinical practice by patients and caregivers.
Conclusion: Similar glycemic control can be achieved by administering glargine in the morning, evening or using a split dose. Therefore, splitting the dose does not offer any advantages.

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Treatment with insulin detemir is associated with predictable fasting blood glucose levels and favourable weight development in subjects with Type 2 diabetes.

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Background and Aims: Insulin detemir, a soluble, basal insulin analogue, maintains glycaemic control effectively with predictable glucose levels in subjects with type 1 diabetes on a basal/bolus regimen. The aim of this trial was to compare the effect of a basal/bolus regimen with insulin detemir or NPH insulin (NPH) in combination with insulin aspart (before meals) in subjects with type 2 diabetes.
Materials and Methods: This was a 6-month, multinational, open, parallel group comparison in subjects randomised 2:1 to insulin detemir or NPH and dosed once or twice daily according to previous treatment. A total of 505 subjects with type 2 diabetes (insulin detemir: 341, NPH: 164) with (mean age: 60 yrs, duration of diabetes 13 yrs, BMI: 30.4 kg/m^2, HbA_{1c}: 7.85%) received trial products.
Results: After 6 months, HbA_{1c} had decreased by 0.26% point (insulin detemir) and 0.36% point (NPH). Mean difference (insulin detemir-NPH) in HbA_{1c} was 0.16% point, (95% CI [0.003; 0.312]). HbA_{1c} values were similar in the groups using a once (40% of subjects in both groups) or twice daily basal regimen (p=0.73). Fasting plasma glucose did not differ between the two groups: 9.7 mmol/l (insulin detemir) and 9.6 mmol/l (NPH), (p=0.66). Treatment with insulin detemir resulted in a lower within-subject variation in self-measured fasting blood glucose compared to NPH (SD=1.3 *vs* 1.4 mmol/l, p=0.02). Self-measured 9-point blood glucose profiles were similar in the two groups (p=0.58). The risk of hypoglycaemia was similar with the two treatments (p=0.48) as was the incidence of adverse events. Body weight (adjusted for baseline and change in HbA_{1c}) was significantly lower in the insulin detemir group than in the NPH group after 6 months (p=0.020), with a weight increase of 0.9 kg *vs* 1.6 kg in the NPH group.
Conclusion: Similar glycaemic control was found with insulin detemir and NPH when used in combination with insulin aspart in subjects with type 2 diabetes. Furthermore, treatment with insulin detemir resulted in less increase in body weight and more predictable levels of fasting blood glucose. The safety profile was comparable between the two treatments.

PS 60
Insulin Therapy (Miscellaneous)

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Unmasking sequence effect from carryover effect in insulin crossover studies.

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Background and Aims: Crossover designs are efficient alternatives to parallel designs. The efficiency of these designs comes from patients serving as their own control. Between-patient variation is eliminated from the treatment comparison, which results in sample size reduction. Despite the benefits of the crossover design, handling potential carryover effects still remains a challenge. This abstract presents results from six insulin clinical trials with the goal of justifying the use of crossover designs in insulin clinical research.
Materials and Methods: A total of 690 patients with type 1 or 2 diabetes were enrolled in six multicentre randomized comparative crossover studies of insulin analogs and human insulins for 6 to 8 months. Efficacy parameters included HbA1c at endpoint of each treatment period and self-blood glucose monitoring before and after meals at approximately monthly intervals during the study. Koch's sum and difference approaches were used to test for carryover and treatment effects, respectively. Because sequence and carryover effects are confounded in the crossover models, we used the change from baseline measurements to eliminate the sequence effects due to baseline imbalance, which occurs frequently in crossover trials that have a small sample size. This approach reduced the false detection of carryover effects.
Results: Table 1 displays the continuous variable results. After elimination of sequence effects, only one significant carryover effect was detected out of forty tests. Even so, some carryover effect estimates, indicated in bold, appeared to be relatively larger than their treatment effect counterparts. By averaging the effects across six studies, the carryover effect estimates were very close to 0. This illustrates that even though the true carryover effect may be 0 or negligible, the estimator has a large variance. These treatment effect estimates are also consistent with what has been observed from parallel studies using similar comparators.
Conclusion: The crossover design is often used in diabetes studies where the disease state is chronic and the study drugs have a short duration of action. By unmasking the likely sequence effect from carryover effect, we can justify the use of crossover designs in insulin clinical research.

Variable		Study A	Study B	Study C	Study D	Study E	Study F	Mean	SD
HbA1C	τ	-0.01	0.13	-0.20	0.19	-0.08	-0.41	-0.06	0.22
	λ	0.22	**-0.84**	0.28	-0.14	0.34	-0.02	-0.03	0.44
Fasting BG	τ	-0.50	0.20	-0.20	1.01	0.05	0.76	0.22	0.57
	λ	0.22	**-1.25**	**1.89**	-0.54	**-1.47**	0.13	-0.17	1.22
Morning 2-hr	τ	-1.54	-1.94	-1.08	-0.83	-1.13	-0.80	-1.22	0.44
PPBG	λ	**1.53**	-0.36	0.79	**-1.90**	-0.38	0.66	0.06	1.21
Noon BG	τ	0.22	-0.81	-0.04	0.02	-0.15	NA	-0.15	0.39
	λ	0.32	-0.70	-1.27	-1.98	0.11	NA	-0.70	0.96
Noon 2-hr	τ	-0.70	0.46	0.36	0.68	-0.48	NA	0.06	0.61
PPBG	λ	0.90	0.65	1.82	-0.09	0.68	NA	0.79	0.69
Evening BG	τ	0.90	0.76	0.05	0.78	0.24	0.12	0.48	0.38
	λ	0.16	-0.62	**1.55**	**-1.54**	0.66	**1.50**	0.29	1.22
Evening 2-hr	τ	-0.76	-0.21	-0.97	-0.57	-1.94	-1.30	-0.96	0.61
PPBG	λ	0.85	**-1.03**	**-1.22**	**-2.20**	**1.37**	-0.48	-0.45	1.34

Abbreviations: BG = blood glucose; PPBG = postprandial blood glucose; τ = treatment effect; λ = carryover effect

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Prevalence and incidence of autoimmunity in Type 1 diabetic patients treated by implantable or external pumps: a prospective study.

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Background and Aims: Autoimmune (AI) diseases are increased in type 1 diabetic patients. A possible initiation of autoimmunity in type 1 diabetic patients by treatment with continuous intraperitoneal insulin infusion (CIPII) has been evaluated by the EVADIAC group through a national multicentric prospective study.
Materials and Methods: Prevalence of clinical AI diseases (Hashimoto's, hyperthyroidism, pernicious anaemia and vitiligo) and subclinical diseases (anti-thyroperoxydase (anti-TPO) antibodies(ab), anti-intrinsic factor (antiIF) ab, TSH levels), was estimated by comparing two groups of type 1 diabetic patients matched for age, sex and duration of disease : 121 type 1 patients treated with CSII (SC) since 6.1 ± 4.5 years (men/women, 58/63; mean age ± SD, 46.4 ± 11.1 years; mean duration of diabetes ± SD, 23.7 ± 8.9) and 153 treated with CIPII (IP) since 6.1 ± 3.5 years (men/women, 74/81; mean age ± SD,47± 10.3 years; mean duration of diabetes ± SD, 25 ± 9.9). The incidence of AI diseases was appreciated by repeating this cross-sectional study one year later (14 ± 2 months). TSH and antiTPO ab estimation was carried out using luminescence immune assay kits from BRAHMS (Berlin, Germany), antiIF ab level by a semi quantitative radioimmunoassay (DPC, Los Angeles, USA). Anti-insulin ab level was also studied in all patients and expressed as % of total radioactivity.
Results: No significant difference was observed for the prevalence of Hashimoto's disease, hyperthyroïdism, pernicious anemia, and vitiligo : 7.4 %, 2.4 %, 0.8 %, 4.1 %, in SC group, and 8.4%, 1.3 %, 1.9 %, 1.3 % in IP group respectively. Moreover, no significant difference was observed for antiTPO ab , TSH abnormal and anti-IF ab levels respectively : 30.6 %, 12 %, 4.1% in SC group, and 25.9%, 8%, 3.9% in IP group (ns). As already known anti-insulin ab level were increased in IP group (32.9% IP vs 20.2 % SC p<0.0001), but no correlation was observed with either clinical or subclinical AI disease. The incidence study performed in 58 SC and 103 IP treated patients) showed no significant difference for clinical AI diseases and antiTPO ab, TSH abnormal, antiIF ab levels respectively : 5.5 %, 2.2 %, 0 % in IP group and 5.5%, 0%, 1.7% in SC group.
Conclusion: This large scale study eliminates the risk of increased autoimmunity by treatment with implantable pumps.

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Physician resistance to prescribing insulin: an international study.

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Background and Aims: This study examined resistance to prescribing insulin. Our aim was to determine whether resistance to prescribing insulin was associated with a variety of factors, including physician and practice characteristics (age, gender, generalist/specialist, country, practice location and size), characteristics of their patients (% minority and perceived levels of self-care compliance and psychological problems), provider treatment philosophy, and attitudes toward insulin.
Materials and Methods: We surveyed 2,681 physicians who treat type 2 patients with diabetes. Data are from an international study of Diabetes Attitudes, Wishes and Needs (DAWN) in 13 countries from 11 regions (Australia, France, Germany, India, Japan, Netherlands, Poland, Scandinavia, Spain, UK, USA). Approximately 250 physicians were interviewed in each region.
Results: All reported findings are p<.05 using hierarchical multivariate regression. Physicians differed substantially by country in attitudes about prescribing insulin, even after controlling for provider characteristics. Almost half of physicians (42%) were reluctant to start their patients on insulin until it was absolutely essential (and another 16% were undecided/ambivalent). Resistance was highest in India, Spain, Germany and Japan, and lowest in Scandinavia and Netherlands.
Physicians less resistant to prescribing insulin were younger, more likely to be specialists, and located in more multidisciplinary settings. Years of treating diabetes patients and number of diabetes patients treated were not related to insulin prescribing resistance. Those who speak to and write for audiences of lay persons and providers were less resistant to prescribing insulin.
Physicians who were more resistant to prescribing insulin saw their own patients as more compliant with medication. Patient characteristics, including their attitudes toward insulin treatment (as perceived by their physicians), were not related to resistance to prescribing insulin. The two strongest predictors of resistance to prescribing insulin were general reluctance to use medications to control blood glucose (associated with

higher resistance) and belief that earlier use of insulin would reduce the cost of diabetes care (associated with lower resistance).
Conclusions: Physicians report substantial resistance to prescribing insulin. This resistance may be an important element in the delay of insulin use when it would be helpful, and may keep patients from being able to manage their diabetes effectively. Understanding the determinants of this resistance may facilitate efforts to increase physician willingness to prescribe insulin as a means of improving diabetes management.

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Predictors for a successful add-on therapy with basal insulin in patients with Type 2 diabetes and secondary failure of oral antidiabetic drugs.
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Background and Aims: The addition of a basal insulin, such as insulin glargine (LANTUS®), is a common treatment strategy for patients with Type 2 diabetes inadequately controlled on oral antidiabetic drugs (OADs).
Materials and Methods: This open, multinational, multicentre, randomized, 6-month study, which initially examined the efficacy and safety of glimepiride (3 mg) plus bedtime NPH insulin (n=232) or bedtime (n=227) or morning (n=236) insulin glargine, identified potential factors that could predict the treatment success of add-on insulin using multivariate, stepwise linear respectively logistic regression. Dependent variables were change in HbA_{1c} and frequency of symptomatic hypoglycaemia. Independent variables were baseline insulin type, age, sex, diabetes duration, study country, type of pre-existing OAD and HbA_{1c}, body mass index (BMI) and fasting C-peptide levels.
Results: Baseline characteristics were: age, 61 ± 9 years; diabetes duration, 10 ± 7 years; BMI, 28.7 ± 4.1 kg/m^2; OAD treatment duration, 8 ± 6 years. HbA_{1c} decreased from 9.1 ± 1.0% (baseline) to 8.1 ± 1.3% (endpoint; $p < 0.001$). There was correlation (R^2=0.26) between the type of insulin therapy, HbA_{1c} and BMI at baseline, type of pre-existing OAD treatment, sex, country, fasting C-peptide level and reduction in HbA_{1c}: HbA_{1c} reduction was greater in patients taking morning insulin glargine, with highly increased HbA_{1c} and low BMI at baseline, pre-existing monotherapy with a sulphonylurea, low C-peptide levels, and male. Episodes of symptomatic hypoglycaemia were correlated (concordance of prediction 74%) with type of insulin therapy, BMI, country, sex, fasting C-peptide level and pre-existing OAD with metformin: episodes were lower in patients taking insulin glargine at bedtime, with a high BMI, high fasting C-peptide and male. Cessation of metformin increased symptomatic hypoglycaemia risk.
Conclusion: Morning insulin glargine plus OAD therapy was associated with the greatest decrease in HbA_{1c}, notably in non-obese patients with increased baseline HbA_{1c}. Bedtime insulin glargine was associated with the fewest symptomatic hypoglycaemia episodes. Diabetes duration and age were not crucial predictors for successful add-on insulin therapy.

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Self-reported compliance with insulin injection therapy in subjects with Type 1 and 2 diabetes.
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Background and Aims: Recent studies have indicated that many people with diabetes do not follow their prescribed insulin treatment regimens. Insufficient compliance may partially explain the poor clinical outcomes in diabetes despite the availability of efficacious therapies. This preliminary study examined the prevalence of self-reported poor compliance with insulin injections among people with either type 1 or type 2 diabetes, and the association of poor compliance with important clinical and psychosocial endpoints
Materials and Methods: In the absence of validated electronic systems for registering insulin treatment compliance, patient compliance was assessed by a nuanced self-report questionnaire. The questionnaire was administered as part of a larger psychosocial test battery to a sample of 170 insulin treated people with type 1 and 2 diabetes at three diabetes clinics in the US. Most recent Hba1c values were obtained from the medical records.
Results: Mean subject age was 49 years (range 19-82), 64% were women, 59% had type 2 diabetes; Most subjects used a regimen of two injections per day (44%), 71% used syringe and vial, 29% used pen or pump. The table below shows the percentage of subjects admitting to skipping injections.
Think about skipping an injection 61%
Skip injections on purpose: 33 %
Forget to take injections: 53 %
Skip because of forgetting insulin supplies: 40 %
Postpone injections to more convenient time: 58%
Subjects reporting full compliance (34%) had significantly lower A1c values than subjects admitting to skipping injections. (Mean A1c=7.93 versus A1c=8.54, $p<0.05$, Mann-Whitney), and had impaired treatment satisfaction and diabetes related quality of life as assessed with the Insulin Treatment Satisfaction Questionnaire (ITSQ) and the Problem Areas in Diabetes Questionnaire (PAID) ($p<0.05$).
Conclusion: This preliminary study found that a large proportion of people taking insulin report that they do not always comply with their insulin regimen as prescribed by their doctor. Self-reported partial compliance was moderately associated with impaired blood glucose control, treatment satisfaction and quality of life. Larger studies are required to adequately determine the prevalence of and clinical consequences of poor compliance in different populations of people with diabetes. Such studies require the development of reliable methods for assessing compliance with insulin therapy.

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Deleterious effects of increased body weight associated with intensive insulin therapy for Type 1 diabetes: increased blood pressure and worsened lipid profile partially negate improvements in life expectancy.
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Background and Aims: Weight gain is an unwanted side effect of improved glycemic control in type 1 diabetes, associated with increased blood pressure (BP) and worsening lipid profiles, leading to higher risk of macrovascular disease. While improved glycemic control *per se* should improve long-term patient outcomes, it is possible that increases in BP and worsening lipid profiles may limit these benefits.
Materials and Methods: To test this hypothesis, a validated diabetes model was used to project life expectancies in type 1 diabetes cohorts defined to fit the characteristics of Diabetes Control and Complications Trial (DCCT) patients catagorized by increase in body weight under either conventional or intensive therapy. These were: A) conventional glycemic control in the subgroup who were in the lowest quartile of weight gain after 6.5 years (but HbA1c increased by 0.9% points); B) conventional control in the highest quartile of weight gain (but no change in HbA1c from baseline after 6.5 years); C) intensive glycemic control in the lowest quartile of weight gain (leading to a 1.4% point decrease in HbA1c, but no increase in weight or associated BP and improved in lipid profile); and D) intensive glycemic control in the highest quartile of weight gain (leading to a 1.9% point decrease in HbA1c, an increase of 6 mmHg in systolic BP, and worsened lipid profile). Data were derived from DCCT and other published sources. Markov modeling techniques were used to describe the long-term incidence and progression of diabetes-related complications (angina, myocardial infarct, stroke, heart failure, peripheral vascular disease, neuropathy, foot ulcer, amputation, renal disease, and eye disease). Probabilities of developing complications and HbA1c- and BP-dependent adjustments were derived from published clinical trials and population studies, including the DCCT, and Framingham Heart Study.

Results: Generally, intensive therapy improved life expectancy compared to conventional therapy. Increased incidence of cardiovascular diseases due to worsened lipid profile and BP reduced the improvements in life expectancy anticipated with improved glycemic control by 16%.

Group	Description	Projected Life Expectancy from Age 26 (years)
A	Conventional, no weight gain	29.13
B	Conventional, BMI increase 13% from baseline	29.81
C	Intensive, no weight gain	32.65
D	Intensive, BMI increase 29% from baseline	32.08

Conclusion: Concerns about weight gain should not deter intensive insulin therapy. However the importance of improving glycemic control without increasing body weight and associated increased BP has been confirmed. New forms of therapy/intervention that improve glycemic control without increasing body weight/BP will represent an important improvement in the management of type 1 diabetes patients.

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Insulin initiation and follow up in Type 2 diabetic patients hospitalized in France.

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Background and Aims: To describe the characteristics and 6-month outcome of Type 2 Diabetic patients after insulin treatment initiated at hospital.
Materials and Methods: This was an observational prospective survey proposed to all French hospitals/clinics specialized in diabetes. The investigators had to recruit all type 2 diabetic patients hospitalized in June 2001 and for whom long-term insulin treatment was indicated. Patients' characteristics, insulin regimen, blood glucose control and body weight were analyzed initially (M0) and after 6 months (M6) of insulin treatment. Occurrence of episodes of hypoglycemia was fully documented during the follow up.
Results: 902 patients were recruited by 232 centers and 797 patients met the inclusion criteria. When insulin was initiated, patients characteristics were: a balanced sex ratio (51 % females), age: 64 ± 12 years, body weight: 79 ± 17 Kg, BMI: 29 ± 6 kg/m^2, median duration of diabetes 11 years. Treatment prior to insulin initiation included 2 OHA (oral hypoglycemic agents) or more in 71 % of cases, 1 OHA in 21 % and no oral treatment in 8 % of cases. Baseline blood glucose control was poor: fasting plasma glucose (FPG): 13,0 ± 6 mmol/l and HbA1c 10,3 ± 2,2%. After 6 months 638 out of the 797 analyzed patients continued insulin, 37 stopped it, 109 were not reexamined and 13 patients died. At M6, the 638 patients on insulin had a much better blood glucose control (mean decrease in HbA1c: 2.2%, p<0.0001 and mean decrease in FPG: 4 mmol/l, p<0.0001) and a slightly increased body weight . The insulin regimen was unchanged at M6 in the vast majority of patients and was as follow: 39 % had 1 injection/day (basal insulin at bed-time: 2/3 of cases), 48 % had 2 injections/day and 13 % had 3 injections/day or more. In addition, 33% of patients still received 2 OHA, 27% had 1 OHA and 40% had no oral treatment. The number of injections/day at M6 was related to HbA1c decrease: 1 inj bedtime/day: -1.7 %; 2 inj./day: - 2.5% and 3 inj/day: -3%, but also to body weight increase (1inj/day: + 1.2 kg ±4; 2 inj/day: +2.4 kg ±5; 3 inj/day: +3.7 kg ±5) as well as to the frequency of hypoglycemia: only 14 % of patients experienced at least 1 episode with inj/day versus 20 % with 2 inj/day and 32 % with 3 inj/day. The mean insulin dose at M6 was 30 IU/day ± 19 (+ 3 IU/day vs M0, p<0,0001). Most of the patients used a pen and did injections themselves (73 % at M0, 81% at M6).
Conclusion: In France, insulin initiation in type 2 diabetes occurred after 11 years of diabetes in patients with very poorly controlled blood glucose level. In daily practice multiple daily injections regimens were associated with the largest HbA1c decrease but a single daily injection exhibited a more favorable efficacy/safety ratio profile with a limited weight gain and a low frequency of hypoglycemia.

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Different effects of withdrawal of insulin or glibenclamide treatment on beta cell function in recently diagnosed Type 2 diabetic patients.

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Background and Aims: We investigate the effects of insulin vs. glibenclamide treatment in recently diagnosed Type 2 diabetic patients. Recently we showed (EASD 2001) that early insulin treatment prolonged beta cell function after one year; furthermore we found better metabolic control (HbA1c) after two years of insulin compared to glibenclamide treatment. We now demonstrate that the time-dependency of effects of treatment withdrawal differs between treatment groups.
Material and Methods: 39 patients with ICA-negative Type 2 diabetes diagnosed 0-2 years before inclusion entered this Swedish multicenter trial and were randomised 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. Other measurements of beta cell function: Islet Amyloid Polypeptide (IAPP) and fasting insulin and proinsulin.
Results: Treatment withdrawal for 2 vs. 3 days after one year of treatment did not significantly affect fasting blood glucose in either group. However, the C-peptide response to glucagon increased between 2 and 3 days of withdrawal in the insulin group, whereas it stayed the same in the glibenclamide group, resulting in a significantly increased response in the insulin vs. glibenclamide group (p<0.05). The concomitant IAPP response to glucagon tended to be reduced in the insulin vs. the glibenclamide group (p=0.13). Metabolic control after 2 years of treatment was better in the insulin vs. the glibenclamide group (delta HbA1c -1.26 vs. -0.47 %, p<0.05). Treatment withdrawal for 2 vs. 3 days after 2 years of treatment resulted in no change in fasting blood glucose in the glibenclamide group (8.83 vs. 8.88 mM) but an increase in the insulin group (8.59 after 2 days vs. 9.28 mM after 3 days withdrawal, p<0.05). This increase was accompanied by a rise in fasting insulin (p<0.05), a tendency for a rise in proinsulin but no significant increase in C-peptide glucagon response day 2 vs. day 3.
Conclusions: The results can be interpreted to show that insulin treatment has a transient beneficial effect on beta cell function caused by beta cell rest, whereas a later beneficial effect on metabolic control is due to exogenous insulin acting mainly as a supplement for correction of insulin deficiency. Both these effects are beneficial and constitute strong arguments for early insulin treatment in Type 2 diabetes.

PS 61
Oral and Inhaled Insulin

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Inhalation of insulin in dogs: assessment of insulin levels.
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Background and Aims: Pulmonary insulin delivery is being developed as an alternative to conventional subcutaneous (sc) insulin. In 15 healthy Beagle dogs, we determined whether pulmonary delivery is associated with different distribution of insulin levels in the arterial, deep venous, and portal circulation, compared to sc insulin.
Materials and Methods: Continuous vascular access for measuring blood glucose, plasma C-peptide, and insulin was established by indwelling Silastic® catheters into the left femoral artery, the portal vein, and inferior vena cava. Catheters were also placed into cephalic or saphenous veins for iv delivery of somatostatin to suppress endogenous insulin secretion and glucose to maintain normoglycaemia. Six dogs received sc human regular insulin 0.36 U/kg, while dry powder human insulin 1 and 2 mg was given to another 3 and 6 dogs, respectively, by endotracheal delivery. Glucose was continually infused to maintain euglycaemia.
Results: Post inhalation of 1 and 2 mg, arterial insulin levels quickly rose to a maximum (mean ± SEM) of 55 ± 6 and 92 ± 9 µU/mL, respectively, before declining to fasting levels by 3 h. Portal levels were lower than arterial levels at both doses, peaking at 43 ± 9 and 74 ± 11 µU/mL, respectively. Deep venous peaks were between arterial and portal levels at 51 ± 9 and 80 ± 16 µU/mL, respectively. By contrast, sc insulin was associated with a delayed and lower peak arterial concentration of 55 ± 8 µU/mL at 64 min, returning to baseline after 6 h. Peak portal insulin levels were comparable to those achieved by 1 mg, but significantly less than 2 mg, of inhaled insulin. However, the AUC in the portal circulation was comparable for sc and 2 mg groups. With sc insulin, the highest plasma levels were seen in the deep venous circulation, peaking (78 ± 10 µU/mL) at 64 min. Interestingly, more glucose was required to maintain euglycaemia with 2 mg inhaled insulin (3.1 ± 0.5 g/kg) than with sc (2.6 ± 0.5 g/kg) or 1 mg inhaled insulin (1.4 ± 0.6 g/kg).
Conclusions: Post insulin inhalation, arterial and venous levels are not significantly different, presumably due to high intrapulmonary blood flow.

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Increase in serum insulin levels is correlated with lung distribution after pulmonary delivery of Technosphere/Insulin.
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Background and Aims: Thechnophere/Insulin (TI) is a new formulation for effective pulmonary delivery of human insulin into the systemic circulation. In order to investigate the biological activity of TI, the lung distribution was assessed by radiolabelling the particles and gammma scintigraphy after pulmonary delivery in healthy human volunteers,
Materials and Methods: The study was performed with 5 healthy subjects (3 men, 2 women, mean(STD): age: 27(1) years, BMI: 23(3) kg/m^2). Radiolabelling with ^{99}Tc was performed by means of a passive, mass adhesion technique using a nebulizer chamber. The respirable fraction after the labelling was 58.9 % as assesssed by Andersen cascade impactor analysis.
Results: It could be shown by gamma camera imaging that 58.9 % of the total exposed dose was deposited into the lungs with an equal distribution between the two lung wings. No activity could be observed in the trachea or the larger bronchioli. The distribution into the entire lung tissue was accompanied by an almost immediate serum increase of insulin that reached peak serum concentrations of 46(21) µU/ml within 15 min after inhalation and went back to baseline within 2 hours. There were no changes in the pulmonary function tests before and after the inhalation and no adverse event was reported.
Conclusion: In conclusion, it has been demonstrated that equal distribution of Technosphere/Insulin into the lung tissue is accompanied by a consecutive increase in serum insulin concentrations. The fast systemic absorption of Technosphere/Insulin may be substantially supported by the utilization of the entire exchange surface of the lung alveoli.

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Efficacy and safety of inhaled insulin (Exubera®) compared with rosiglitazone in Type 2 diabetes patients not optimally controlled on diet and exercise: results of a 3-month, randomised, comparative trial.
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Background and Aims: This study investigates whether pre-meal, rapid-acting, dry powder inhaled insulin (Exubera®: INH) can provide acceptable glycaemic control to significantly more subjects than rosiglitazone in type 2 diabetes patients not optimally controlled on diet and exercise.
Materials and Methods: In this Phase III, multicentre study, patients (ages 35–80) were randomised to receive 3 months' treatment with either INH prior to meals (n=76), or rosiglitazone 4 mg BID (ROS; n=69), both in conjunction with a diet/exercise regimen. INH was delivered as 1–2 inhalations of 1 or 3 mg.
Results: The INH and ROS groups had similar baseline HbA_{1c} values (mean 9.5% vs 9.4%, respectively). The primary efficacy endpoint was the percentage of subjects attaining HbA_{1c} <8.0% at study end; significantly more patients achieved this in the INH group (82.7%) compared with the ROS group (58.2%) (adjusted odds ratio: 7.14; 95% CI [2.48, 20.58]). Further improvement in glycaemic control (HbA_{1c} <7%) was achieved by 44.0% and 17.9%, respectively (adjusted odds ratio: 4.43; 95% CI [1.94, 10.12]). Absolute HbA_{1c} decrease was significantly greater in the INH (-2.3%) vs the ROS (-1.4%) group (adjusted difference: -0.98%; 95% CI [-1.23, -0.55]). These metabolic improvements were accompanied by changes in body weight (1.9 kg for INH vs 0.8 kg for ROS [NS]). The rate of overall hypoglycaemia (events/subject-month) was higher in the INH group compared with the ROS group (0.7 vs 0.05; risk ratio: 14.72; 95% CI [7.51, 28.83]), but no severe hypoglycaemic events were reported. INH-treated patients developed increased insulin antibody serum binding compared with ROS-treated patients, but without attributable clinical manifestations. Changes in pulmonary function were small and comparable between groups. Overall, no serious adverse events were reported throughout the study.
Conclusions: This study suggests that inhaled insulin (Exubera®) may be a valuable therapy for subjects with type 2 diabetes who do not achieve adequate glycaemic control on diet and exercise alone.

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Within-subject variability of inhaled insulin (Exubera®) versus subcutaneous regular insulin in elderly obese patients with Type 2 diabetes mellitus.
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Background and Aims: Within-patient reproducibility is an important component of insulin delivery. This study aimed to determine the within-subject variability (CVw) of inhaled insulin (Exubera®; INH) absorption in elderly, obese type 2 diabetes patients compared with that of subcutaneous insulin (SC).
Materials and Methods: Twenty subjects (10 male, 10 female; mean age 72 years, BMI 33 kg/m^2) were studied 4 times (2 INH/2 SC) in a randomised sequence crossover. For each dosing, after an overnight fast, subjects either inhaled 4 mg INH or received 12 IU SC (abdominal injection). Serum free insulin and fasting plasma glucose levels were measured for 6 h after each administration. CVw values were computed by two methods: an ANOVA method (CVw[ANOVA]) and an average of ratios method (CVw[AR]). The ANOVA approach is similar to the method for statistical analysis of bioequivalence, whereas the AR approach has often been used in studies of other insulin products. This was done as CVw[AR] produces estimates that are statistically biased downwards.
Results: Compared to SC, peak insulin concentration (C_{max}) with INH was 70% higher and occurred earlier. Systemic insulin exposure over 2 h ($AUC_{0–2h}$) with INH was 87% higher than with SC, whereas $AUC_{0–6h}$ was similar. CVw% with INH (C_{max}, $AUC_{0–6h}$) was comparable to, and ($AUC_{0–2h}$) lower than, that with SC for either CVw method. Post-dose glucose pharmacodynamics were consistent with the insulin pharmacokinetics.

Conclusions: At doses producing comparable systemic insulin exposure over 6 h, inhaled insulin (Exubera®) is absorbed more rapidly than SC insulin, and has a similar or better CVw of insulin delivery, in elderly obese patients with type 2 diabetes.

* Adjusted geometric mean

		INH	SC	INH/SC Ratio (%)	90% CI
AUC_{0-2h} (µU·min/mL)	Mean*	3472	1852	187	138, 255
	CVw% (ANOVA)	23	47	49	34, 71
	CVw% (AR)	21	40		
AUC_{0-6h} (µU·min/mL)	Mean*	4696	4823	97	68, 139
	CVw% (ANOVA)	31	43	71	49, 104
	CVw% (AR)	25	28		
C_{max} (µU/mL)	Mean*	48.62	28.57	170	131, 221
	CVw% (ANOVA)	27	36	74	51, 108
	CVw% (AR)	20	28		

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The effect of smoking cessation and subsequent resumption on absorption of inhaled insulin (Exubera®).

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Background and Aims: We compared absorption of inhaled insulin (Exubera®; INH) in active smokers (Sm) at baseline, after smoking cessation, and after smoking resumption.
Materials and Methods: Baseline-adjusted pharmacokinetics and glucodynamics of subcutaneous insulin (SC) and INH were measured in 20 healthy nondiabetic male Sm (mean BMI 22 kg/m^2, age 28 years, 16 cigarettes/day) and 10 matched nonsmokers (NSm) after receiving INH (1 mg; INH1) or the approximate SC equivalent (3 U) in a randomised crossover method after an overnight fast. Sm then received INH 12 h (INH2), 3 days (INH3) and 7 days (INH4) into a smoking cessation period. They then resumed smoking (20/day) for 2–3 days before again receiving INH one h after the last cigarette (INH5). Serum insulin, C-peptide and blood glucose were measured for 6 h after inhalation.
Results: Prior to smoking cessation, C_{max} and AUC_{0-6h} of INH were 2–3 times higher, and T_{max} earlier, in Sm than NSm, while systemic exposure was equivalent for SC insulin. Persistent smoking cessation decreased AUC_{0-6h} up to 50% within 1 week, close to that in NSm, although C_{max} and T_{max} indicated still faster absorption. Smoking resumption completely reversed the effect of smoking cessation. Glucodynamics corroborated the findings in insulin absorption without overt hypoglycaemia.
Conclusion: Cessation and resumption of smoking greatly alters absorption of inhaled insulin. As rapid changes in systemic insulin exposure increase variability in glycaemic control, patients with diabetes who smoke should abstain before and during treatment with Exubera®. This is consistent with uniform recommendations that people with diabetes refrain from smoking altogether.

	AUC Ratio, % (90% CI)	C_{max} Ratio, % (90% CI)	ΔT_{max}, min (90% CI)
INH1(NSm)/INH1(Sm)	64 (44, 92)	36 (24, 54)	33 (17, 50)
INH2/INH1(Sm)	122 (100, 149)	123 (102, 152)	1 (-5, 6)
INH3/INH1(Sm)	90 (71, 112)	69 (56, 85)	16 (7, 25)
INH4/INH1(Sm)	73 (58, 92)	59 (49, 72)	20 (10, 30)
INH5/INH1(Sm)	122 (99, 149)	109 (88, 135)	9 (3, 14)
INH1(NSm)/INH4	87 (58, 131)	61 (44, 85)	13 (-8, 34)
SC(NSm)/SC(Sm)	98 (80, 120)	85 (66, 111)	30 (14, 74)

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Oral delivery of insulin using polymere nanoparticles in the rat.

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Background and Aims: The most physiological and convenient route for the administration of insulin is the oral route. However, insulin is less absorbed by the intestinal mucosa and is destroyed by proteolytic enzymes in the gastro-intestinal tract. Thus, we have associated insulin to a new nanoparticles system made of an uncharged polymer (polycaprolactone, PCL) and a polycationic polymer (Eudragit®RS). The aim of this work was to analyse the biological effects of these nanoparticles (NP) given by gavage to diabetic rats and to understand the interaction of the positively charged NP with the electronegative intestinal mucosa in rats.
Materials and Methods: NP were prepared according to the double emulsion technique. They were characterized according to their diameter, encapsulation efficiency and release of insulin. Diabetic rats were obtained by an i.v. administration of streptozotocin. Glucose and insulin levels were evaluated in blood for 24 hours after gavage. Oral glucose tolerance tests were performed 4 and 8 hours after gavage. At various time intervals after administration of fluorescein isothiocyanate (FITC) labelled insulin-NP in the intestinal lumen of an isolated intestinal loop, samples of intestinal mucosa were processed for fluorescence microscopy; FITC-insulin levels were measured by fluorometry in the mesenteric blood, the intestinal mucosa and lumen.
Results: (1)The mean diameter of NP was around 350 nm and the encapsulation efficiency was superior to 95%. (2) Insulin NP (100 U/kg b.w.) administered by gavage to fasted diabetic rats, significantly reduced blood glucose levels by 36% ($p<0.05$), 57 ($p<0.01$), 57 % ($p<0.01$) and 14% ($p<0.05$) respectively after 4, 6, 8 and 24 h after gavage when compared to rats treated with empty NP. A reduction in glycemia was also observed with 50 and 25 U/kg insulin NP, still significant with 50 U/kg but not with 25 U/kg. Plasma insulin levels increased as well. Peroral insulin NP lowered the glycemic response to an oral glucose challenge (2g glucose/kg) performed 4 or 8 hours later by 60 % ($p<0.01$) and 38 % ($p<0.05$) respectively. (3) Observed by fluorescence microscopy, insulin NP appeared in the follicular mucosa 30 min after intra-luminal administration but not in the non follicular mucosa. The concentration of FITC-insulin decreased in the intestinal lumen by 78% ($p<0.001$) from 30 min to 4 h after intra-luminal injection. In the intestinal mucosa, it increased up to 30 min, then decreased by 72% ($p<0.001$) from 30 min to 4 h. In mesenteric blood, insulin concentration increased by 88% ($p<0.001$) at 30 min and by 252% ($p<0.001$) at 4 h.
Conclusion: Insulin-loaded nanoparticles prepared with PCL and Eudragit®RS exert an antidiabetogenic effect when administered perorally in diabetic rats. These results may be explained by the mucoadhesive properties of the polycationic polymer allowing the intestinal uptake of insulin NP followed by the release of insulin in the mesenteric blood.

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Dose response effect of a single dose of orally administered hexyl-insulin monoconjugate (HIM2) in healthy nondiabetic subjects.

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Background and Aims: Treatment of diabetics with multiple insulin injections remains a barrier for patient compliance and achievement of optimal glycemic control. HIM2 is an orally active modified recombinant insulin. The objectives of this randomized, open-label study were: (i) to determine the dose response effect of a single dose of HIM2 on endogenous (primarily hepatic) glucose production (EGP) and the rate of glucose disposal (Rd) by peripheral tissues using the euglycemic clamp technique in healthy nondiabetic subjects; (ii) to examine the reproducibility of HIM2 administered to the same individual on a separate day; (iii) to compare the results with HIM2 to those obtained with subcutaneous lispro insulin.
Materials and Methods: After an overnight fast, 6 subjects (2 males/4 females; age = 31.3± 5.5 y; BMI = 23.1± 3.9 kg/m^2) received, in random order, three oral doses of HIM2 (0.125, 0.5, 0.75 mg/kg) on separate days. Each subject also received a repeat oral dose of HIM2 (0.5 mg/kg) and two subcutaneous doses of lispro insulin (0.1 U/kg) on separate days. Following each insulin dose, plasma glucose concentration was maintained constant at the fasting level with a variable 20% glucose infusion for 240 min. 3-^{3}H-glucose was infused for 120 min before and throughout the 240 min glucose clamp. Plasma 3-^{3}H-glucose specific activity and insulin concentration were obtained every 15 min.
Results: Fasting plasma insulin (18±2 uU/ml) increased to a maximum of 102, 321, and 561 uU/ml at 60 min in the 0.125, 0.5, and 0.75 mg/kg of HIM2, respectively. Glucose kinetics (mean ± SEM) during the clamp are shown below in table 1 (EGP) and table 2 (Rd) (*ANOVA: $p<0.01$ between doses).
Conclusion: Oral HIM2 effectively inhibits EGP and augments Rd in a dose dependent fashion in nondiabetic subjects and its biologic effectiveness is sustained for > 240 min. Oral HIM2, at dose of 0.5 mg/kg, is equivalent to 0.1 U/kg of lispro given subcutaneously. The variability in the effect of HIM2 (25±7%) and lispro (27±1%) on Rd was similar. If confirmed in diabetic patients, oral HIM2 can provide an effective and reproducible means of controlling postprandial plasma glucose excursions.

Table 1

EGP (mg/kg.min)	0.125 mg/kg	0.5 mg/kg	0.75 mg/kg	Lispro
Basal	2.36±0.12	2.24±0.16	2.18±0.12	2.31±0.07
0-60 min	0.69±0.18	0.98±0.20	0.75±0.12	1.40±0.39
60-120 min	0.93±0.14	0.48±0.18*	0.25±0.10*	0.69±0.57
120-180 min	1.32±0.20	0.75±0.20*	0.52±0.16*	1.05±0.57
180-240 min	1.58±0.22	0.86±0.26*	0.96±0.18	1.47±0.51

Table 2

Rd (mg/kg.min)	0.125 mg/kg	0.5 mg/kg	0.75 mg/kg	Lispro
Basal	2.36±0.12	2.24±0.16	2.18±0.12	2.31±0.07
0-60 min	3.72±0.30	5.22±0.56*	5.52±0.46	3.16±0.45
60-120 min	3.41±0.24	4.75±0.58*	6.28±0.60*	4.91±0.50
120-180 min	2.70±0.28	3.95±0.74*	4.64±0.64*	4.83±0.45
180-240 min	2.49±0.20	3.19±0.64*	3.45±0.38	3.94±0.24

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Treatment with Oralin in comparison with regular and rapid insulins in Type 1 diabetic patients evaluated by euglycemic clamp technique.

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Background and Aims: The improvement in glucose control in diabetes mellitus is a major challenge for those on multiple daily injections. The goal of this study was to investigate the time-action profile of Oralin in comparison with s.c. injected regular insulin or Rapid insulin in 15 Type 1 diabetic patients using the euglycemic clamp technique. The RapidMist, a novel Diabetes Management System, allows insulin to be orally absorbed and can provide significantly improved needle-free postprandial glucose control. This system is based on a unique liquid aerosol formulation (Oralin), which allows a precise insulin dose delivery by mouth.
Materials and Methods: In this study 15 Type-1 diabetic subjects were clamped under euglycemic condition using glucose clamp (clamp level 120 mg/dl with continuous i.v. insulin infusion 0.1 mU/kg/min). After a stable baseline establishment at 40 min period, patients received either 10 puffs of Oralin or 0.1 u/kg s.c. regular insulin or rapid insulin injection (7-9 u) in a randomized, 3-way, crossover study on three separate occasions. The glucose infusion rates were registered for the next 4 h period.
Results: Oralin showed faster onset of action - within the first 10 mins of the dose administration and reached peak levels within 50-70 mins (Tmax=50 mins, $p<0.0001$) as compared to S.C. regular insulin – which began at approx 45 min and reached peak levels within 90-100 min. The onset of action of Oralin was comparable to rapid insulin. The duration of the action was shorter for the Oralin treated group than the s.c. insulin group (120 min vs 230 min). The mean peak of tissue glucose uptake for Oralin, regular and rapid insulin were 0.186, 0.225 and 0.475 ml/min ($p<0.05$), respectively. The peak serum insulin levels of Oralin were comparable to the s.c. regular insulin and significantly lower than the rapid insulin. No drug related adverse events were observed in this study.
Conclusion: Oralin showed faster onset of action with similar peak activity when compared to s.c. regular injected insulin with time to peak (Tmax) resembling that of rapid insulin. These characteristics are well suited for the treatment of diabetes and control of meal-related glucose levels safely and effectively.

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Reproducibility of Oralin absorption in Type 1 diabetics on 3 different occasions.

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Background and Aims: The improvement in glucose control in diabetes mellitus is a major challenge for those on multiple daily injections. The RapidMist, a novel Diabetes Management System, that allows insulin to be orally absorbed, can provide significantly improved needle-free post-prandial glucose control. This system is based on a unique liquid aerosol formulation (Oralin), which allows a precise insulin dose delivery directed in the mouth. The goal of this proof of concept study was to evaluate the efficacy and the reproducibility of the absorption of the Oralin spray (15 puffs, no NPH) in Type-1 diabetic patients after a standard meal challenge at breakfast-time on 3 different occasions.
Materials and Methods: In a randomized crossover study, 12 Type-1 diabetic patients received Oralin spray via the RapidMist device on 3 different occasions 3 to 7 days apart or s.c injection (8-9 units) with a 360 cals Ensure liquid meal 15 minutes after each treatment.
Results: The table shows serum insulin levels after each dose of Oralin or s.c. injection.
Conclusion: The onset action of Oralin was much faster and reached its peak level (Tmax) at 20 mins when compared to s.c. injection (Tmax= 60 mins). There were no statically significant differences on 3 different occasions in Oralin absorption ($p<0.857$). The rise in serum insulin concentrations in the Oralin treated group was comparable to s.c. injection. There was no inter and intra-subjects variability observed in the Oralin treated group.

Time	Insulin (uU/ml) s.c. Injection	Insulin (uU/ml) Oralin Day-1	Insulin (uU/ml) Oralin Day-2	Insulin (uU/ml) Oralin Day-3
0 min	15.7	16.7	12.6	14.4
20 min	33.3	88.4	89.6	92.8
60 min	76.7	38.3	49.8	46.6
120 min	34.9	24.2	27.5	32.9
240 min	15.2	12.6	13.3	20.8

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The sulphonylurea binding site is located at the β-cell surface.
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Background and Aims: Hypoglycemic sulphonylureas, a class of drug widely used in the management of Type II diabetes, stimulate insulin secretion *via* interaction with specific receptors located in the ß-cell plasma membrane. These high affinity receptors (SUR1 for 'sulfonylurea receptor-1' protein) associate, inside the β cell plasma membrane, with the Kir6.2 protein (which forms the ionic pore) to constitute the K_{ATP} channels, a major piece in the regulatory mechanisms induced by sulfonylureas, but also by glucose *via* the channel sensitivity to the intracellular ATP/ADP ratio. While it is widely accepted that sulfonylureas trigger insulin secretion by interacting directly with the plasma membrane SUR1 protein, a polemic exists concerning the location of the sulfonylurea binding sites, seen as present either at the intracellular or at the extracellular cell surface. We have addressed this issue by direct measurement of the qualitative and quantitative binding characteristics of sulfonylureas on three *in vitro* models, including intact β cells.
Methods: Using the MIN6 ß-cell line, we compared the characteristics of ^{3}H-glibenclamide binding on 1) intact cells freshly detached from the culture flask using PBS-EDTA; 2) the same cell preparation with additional permeabilization by saponin; 3) membranes prepared from intact cells prepared as in 1). The integrity of plasma membrane of intact cells and the efficiency of the permeabilization process were monitored by measuring the ATP content of the cells at the end of the binding experiments.
Results: ^{3}H-glibenclamide binds in a reversible manner to intact MIN6 cells very similarly to that of permeabilized cells or membranes. Affinity constant and number of sites, calculated from Scatchard plots, were very similar in each model: Kd values were 1.71±0.28 nmol/l for intact cells, 0.85±0.18 nmol/l for permeabilized cells and 0.7±0.12 nmol/l for membranes, while the calculated number of sites were 22,243±3,100; 23,584±3,700, 21,233±3,100, respectively. The significant ($p<0.01$) two-fold difference observed for Kd values in intact cells as compared to permeabilized cells or membranes could be explained by modifications in the cytosolic environment in the latter conditions, ions and nucleotides playing a role in the regulation of K_{ATP} channels and their sulfonylurea binding properties. Dissociation experiments, conducted either with an excess of glibenclamide or of tolbutamide, showed the same time-course for intact cells, permeabilized cells and membranes : dissociation was rapid and almost complete, 20% of the specific binding remaining after a 40 minute-dissociation experiment. Pharmacology of the binding site as determined by use of a variety of unlabelled sulfonylureas are very similar in all experiments.
Conclusion: We conclude that sulfonylureas have free and direct access to their binding sites from the extracellular space, do not need to enter the cell to bind their receptor for triggering insulin secretion and, accordingly, that the sulfonylurea receptor is located at the cell surface of the ß-cell.

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Synthesis and characterisation of ß-cell specific positron emitting radiolabeled sulfonylureas for the non-invasive visualisation of the ß-cell mass in vivo using positron emission tomography (PET).
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Background and Aims: The non-invasive imaging and quantification of the ß-cell-mass in-vivo remains a challenge. The reason for this inability, is the lack of a specific radiolabeled ß-cell-tracer. Therefore, the aim of the present study was the synthesis and characterisation of potentential, high-affinity positron emitting radiolabeled sulfonylureas.
Materials and Methods: In a first step, 20 different fluoralkylated and benzylated derivates of glibenclamid (GI 1-20) were synthesized. Afterwards, the potential influence of this systematic derivatisation on the binding-affinity to the human SUR1 receptor, the lipophilic behaviour and the insulin-secreting capacity was analysed and compared with the original glibenclamid (GI 0).
Results: The systematic derivatisation leads only to marginal changes concerning the binding affinity to the human SUR1 receptor compared to the original glibenclamid [e.g. (GI 0) Kd 0.28 ± 0.02 nmol vs. (GI 4) Kd 0.24 ± 0.01 nmol vs. (GI 15) Kd 1.37 ± 0.25 nmol]. But, there is a strong correlation between the binding-affinity and the lipophilic behaviour of the derivates (with increasing lipophily the binding- affinity to the human SUR1 receptor increases also and vice versa. By none of the 20 derivates these observations had an influence on the insulin secreting capacity compared to the original glibenclamid [e.g. (GI 15) 280±40% vs. (GI 0) 270±30%, n.s.).
Conclusion: On the basis of these preliminary data we expect, that the synthesized derivates could be possible candidates, for the non-invasive visualisation of the ß-cell-mass in-vivo.

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Influence of treatment compliance on blood glucose control: results of 2 controlled studies comparing glimepiride and glibenclamide.
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Background and Aims: Translating efficacy results from controlled clinical trials to general practice raises the problem of patients' treatment compliance in daily life. The objective of the present work was to compare the efficacy of glimepiride (GMP) and glibenclamide (GBC) across 2 studies that used different methodologies.
Materials and Methods: 2 separate comparative studies of similar design were performed. In both studies, diabetic patients poorly controlled by either diet alone or one oral antidiabetic drug were randomly allocated to GMP or to GBC after a 2-week observational period. In the AGATE study, patients were aged 65-85 and received the study treatment according to a double blind double dummy technique during 17 weeks: GMP doses ranged 1-4 mg once daily and GBC doses ranged 1.25 mg once daily-5 mg twice daily. In the OBADE study, patients were aged 35-65 and received either GMP once daily or GBC 2- to 3- times daily in an open fashion during 26 weeks: GMP doses ranged 1-6 mg once daily and GBC doses ranged 1.25 mg twice daily-5 mg 3 times daily. HbA1c was measured centrally at baseline and at the end of the treatment period. Efficacy was assessed by the adjusted between-group difference in final minus baseline HbA1c. The delta of equivalence was fixed at ± 0.40%.
Results: 224 patients and 233 patients were respectively randomized and treated in the AGATE study and in the OBADE study. Groups were comparable at baseline in both studies. In the AGATE study, final HbA1c was 7.3±1.1% and there was a final adjusted difference in HbA1c in favor of GBC: 0.32% (90%CI: +0.10 to +0.54%). In the OBADE study, final HbA1c was 7.0±1.2% and there was equivalence between the 2 drugs: the final adjusted difference in HbA1c was –0.0017% (90%CI: -0.25 to +0.24%). Compliance to study drug was supposed equivalent between the 2 treatment groups in the AGATE study as the number of drug intake was similar in both arms due to the double dummy technique. Conversely, in the OBADE open study where treatment was taken once daily in the GMP group and 2- to 3- times daily in the GBC group a difference in compliance was found: the ratio of days with adequate compliance, as assessed by electronic pill-boxes, was 90±13% in the GMP group and 76±19% in the GBC group ($p<0.0001$).
Conclusion: Apparent discrepancies in efficacy results appeared between 2 studies that aimed at comparing GMP and GBC. Our hypothesis is that, at the above-mentioned doses, the pharmacological activity of GBC is slightly superior to that of GMP (AGATE results). However, the better compliance observed with the once daily GMP in the clinical trial OBADE gave rise to a similar efficacy of both drugs. Then, in clinical daily practice where compliance is a major issue, it is likely that GMP offers a blood glucose control level at least equivalent to that of GBC.

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The European GUIDE-Study: head-to head comparison of efficacy and safety of two once daily sulfonylureas gliclazide MR and glimepiride in 845 Type 2 diabetic patients.

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Background and Aims: A progressive ß-cell failure is a characteristic feature of type 2 diabetes; consequently 5 years after diagnosis most patients need ß-cell secretagogues in order to achieve sufficient glycemic control. The European GUIDE-Study (**GI**ucose control in type 2 d**I**abetes : **D**iamicron MR versus glim**E**piride) is the first large scale head-to-head comparison of two sulfonylureas (SU) designed for once daily administration.
Materials and Methods: 845 type 2 diabetic patients from 12 European countries were randomised to either gliclazide modified release (MR) or to glimepiride in addition to their previous treatment (diet alone or diet in combination with metformin or α-glucosidase inhibitor) according to a double-blind, 6 months, parallel group design. Doses were increased stepwise from 30 to 120 mg gliclazide MR and from 1 to 6 mg glimepiride until metabolic control was achieved (fasting plasma glucose 5 - 7.8 mmol/l) or the dose maximum reached. Efficacy of both SU compounds was evaluated by HbA_{1c} values and safety by hypoglycemic episodes using the European Health Agency definition (EMEA guideline 2002). All patients were provided with a home blood glucose monitoring device and were instructed to measure blood glucose three times daily one day per week and at any time of symptoms suggestive of hypoglycemia. Blood glucose level (BGL) was obtained for 68% of all symptomatic events.
Results: The 2 treatment groups were comparable at baseline : age 61 years, diabetes duration 5.7 years, BMI 30.5 kg/m^2, HbA_{1c} 8.3%, 34% of patients on diet alone. HbA_{1c} decreased similarly in both groups from 8.39 to 7.24% (-1.15%) on gliclazide MR (n=388) and from 8.22 to 7.22% (-1.0%) on glimepiride (n=427). The mean difference between groups of the final HbA_{1c} adjusted on baseline and country was –0.06% CI 95% [–0.19 ; +0.08] (non inferiority threshold 0.5%; $p<10^{-15}$). No major hypoglycemic episodes (requiring external assistance with BGL<3mmol/l) occurred. Despite the fact that improvement of glycemic control was identical in both groups, minor hypoglycemic episodes (BGL<3mmol/l with or without symptoms) occurred significantly more frequently (Fisher's Exact test, p = 0.003) in patients treated with glimepiride (39 out of 439 patients (8.9%) with a total of 56 episodes) compared with those receiving gliclazide MR (15 out of 403 patients (3.7%) with a total of 22 episodes). Disposition of SU doses was similar in both groups.
Conclusion: Both once daily administered sulfonylureas showed almost identical efficacy in improving diabetes long-term control irrespective of their previous antidiabetic treatment. Remarkably, the safety of gliclazide MR was significantly better demonstrating about 50% less hypoglycemic episodes in comparison with glimepiride.

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Glimepiride and ischemic heart disease in diabetes mellitus Type 2: effects on the ischemic threshold.

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Background and Aims: The aim of this study was to compare the effect of glimepiride (Amaryl) and previous oral antidiabetic sulphonylurea drugs on the ischemic threshold during the exercise tests in patients with DM type 2 and ischemic heart disease.
Materials and Methods: An 8-week nonrandomized, crossover, controlled, open study investigating the effect of glimepiride on the ischemic threshold. 14 type 2 diabetics patients (8 males/6 females; aged 56.5+6.9 years) had reproducible positive Stress-Echo tests for myocardial ischemia. All patients underwent three computer-assisted treadmill Stress-Echo exercise tests, using Bruse protocol and direct measurement of oxygen consumption products. The first test (1st) was done with previous oral antidiabetic sulphonylurea drugs before starting glimepiride treatment, the second test (2nd) - four weeks after mono-therapy of glimepiride and before restarting initial oral antidiabetic sulphonylurea drugs, the third test (3rd) – four weeks after restarting initial oral antidiabetic sulphonylurea drugs. The ischemic threshold was assessed with: heart rate, blood pressure and oxygen consumption at the onset of 1.5 mm ST-segment depression and at peak exercise; at exercise duration and at the recovery of ST-segment depression and at the pain onset in seconds. Antianginal and antiischemic therapy was unchanged during the study.
Results: Changes in the kind of hypoglycemic therapy were not followed with significant changes of concentrations of HbA1c and glucose in blood. Exercise duration of the 2nd test after glimepiride treatment was greater than during the 1st and the 3rd tests at the initial oral antidiabetic sulphonylurea drugs (449.30±133.10 vs 415.40±162.50, *p<0.05*; 449.30±133.10 vs 400.70±152.60, *p<0.05*), as were peak rate pressure product (beats.min^{-1}.mmHg.10^2) (297.40±43.70 vs 285.90±51.50, *p<0.05* and 297.40±43.70 vs 263,30±54,70; *p<0.,05*) and peak oxygen consumption products (MET) (6.90±1.40 vs 6.30±1.20, *p<0.05*; and 6.90±1.40 vs 6.10±1.30, *p<0.05* respectively).
Conclusion: These findings suggest that the chronic treatment DM type 2 with high insulin-independent hypoglycemic activity sulfonylureas may lead to increase of ischemic threshold in diabetic coronary patients.

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Comparison of the effect of Repaglinide and Glimepiride on cardiovascular risk factors in patients with Type II diabetes.

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Background and Aims: Type 2 diabetes mellitus patients are exposed to an elevated number of cardiovascular risk factors which mainly depend on post prandial hyperglycemia. Our study aims at comparing the effects of two insulinotropic agents: Repaglinide (R) and glimepiride (G) on cardiovascular risk factors profile after a standard meal test.
Material and Methods: Our study was a 3-month randomised, cross-over parallel group trial of R (1 mg bid) vs G (2 mg qd) in patients with type 2 non insulin dependent diabetes mellitus (NIDDM) „naive" in diet treatment. R and G groups were matched for anthropometrics and metabolic characteristics. All patients had a mean age of 58 ± 6 years were slightly overweight (BMI=25.7 ± 0.8 Kg/m2) and a mean HbA1c of 6.7 ± 0.3 %. At the end of R and G treatment each patients underwent to a meal test.
Results: A significant difference in fasting of glucose (122±7.1 vs 131±6.6 mg/dl for R and 125±6.5 vs 131±6.6 mg/dl for G; p<0.05), total-cholesterol (181±7 vs 195±10 mg/dl for R and 187±9 vs 195±10 mg/dl for G; p<0.05), triglycerides (1.26±0.18 vs 1.49±0.14 mmo/L for R and 1.34±0.06 vs 1.49±0.14 mmo/L for G; p<0.05), PAI1(47.2±5.4 vs 55.2±6.4 ng/ml for R and 50.1±6.3 vs 55.2±6.4 ng/ml for G; p<0.05), PAP (409±51 vs 482±45 ng/ml for R and 436±49 vs 482±45 ng/ml for G; p<0.05) levels after both treatments was found. Indeed, a significant difference in fasting FFA (561±54 vs 618±60 µmol/L; p<0.05), plasma fibrinogenous (283±45 vs 331±37 mg/dl; p<0.05), TBARS (0.41±0.04 vs 0.46±0.04 nmolMDA/ml; p<0.05), TAT (3.23±0.51 vs 3.62±0.44 ng/ml; p<0.05), was found only after repaglinide treatment. At time 120'of meal test a significant difference in HDL cholesterol (1.49±0.05 vs 1.44±0.06 mmol/L; p<0.05), triglycerides (1.63±0.18 vs 1.77±0.15 mmol/L; p<0.05), FFA (610±69 vs 635±97 µmol/L; p<0.05), fibrinogen (299±41 vs 328±31 mg/dl; p<0.05), PAI-1 (49±4.9 vs 53.4±6 ng/ml; p<0.05), PAP (424±49 vs 463±46 ng/ml; p<0.05), TAT (3.25±0.25 vs 3.6±0.4ng/ml; p<0.05), TBARS (0.42±0.04 vs 0.45±0.04 nmolMDA/ml; p<0.05) plasma levels were found between R ad G group, respectively.
In R group a negative correlation between plasma TBARS levels at time 120 min and insulin secretion during 1st phase of meal test (r=-0.558, p=0.038) were found. Such correlation was lost after adjusted for changes in post-prandial (45 min) hyperglycaemia
Conclusion: Our results demonstrates that, in addition to its interesting effects, repaglinide may have beneficial effect reducing cardiovascular risk factors mainly through an antioxidant effect related to the antihyperglycaemic action.

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Repaglinide versus sulphonylurea in combination with bedtime NPH insulin in patients with Type 2 diabetes with secondary failure to oral treatment.

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Background and Aims: This multicentre, open label trial was designed to compare repaglinide vs. sulphonylurea in combination with bedtime NPH insulin in patients inadequately controlled on oral treatment.
Materials and Methods: After 3 weeks' run-in, 58 patients were randomised to two groups: group Rep+NPH (n=40; age 63.7 ± 8.87; diabetes duration 11.3±4.77; HbA_{1c} 8.9 ± 1.5%) was switched to repaglinide before meals and bedtime NPH insulin; group SU+NPH (n=18; age 61.7±8.57; duration 9.9±4.93; HbA_{1c} 8.9±1.5%) continued on same SU with addition of bedtime NPH. Treatment was 4 weeks' titration (NPH dose optimised to maintain FPG < 7.8 mmol/l) and 12-weeks' observation; 52 patients completed. Withdrawals were ineffective therapy (FPG > 7.8 mmol/l) in 5 cases (4 Rep+NPH; 1 SU+NPH) and ALT elevation in 1 case.
Results: Changes in HbA_{1c}, FPG and lipid levels from randomisation to end of study in completers, and incidence of hypoglycaemia, were analysed (ITT population). In Rep+NPH, HbA_{1c} decreased by 1.0%, from 8.9-7.9% ($p < 0.001$) while FPG decreased by 3.1 mmol/l, from 10.8-7.6 mmol/l ($p < 0.001$). Corresponding values in SU+NPH were 8.9-8.6% for HbA_{1c} (0.3%, non-significant decrease) and 10.5-7.6 mmol/l for FPG (2.8 mmol/l decrease, $p < 0.001$). HbA_{1c} and FPG were significantly lower in Rep+NPH compared to SU+NPH ($p < 0.05$ and $p < 0.001$, respectively). Insulin dose was similar in both groups. Parallel, significant decrease of triglycerides and significant increase of LDL cholesterol was found in both groups (no differences between groups). Body mass in Rep+NPH increased by 1.6 kg (80.9±14.0 to 82.5±14.1; $p < 0.05$) and in SU+NPH by 1.0 kg (78.9±8.6 to 79.9±14.2, non-significant). Incidence of hypoglycaemia was similar in both groups while number of night-time episodes was lower in Rep+NPH (0.3 vs. 0.61 episode/patient).
Conclusion: We conclude that repaglinide is more effective than sulphonylurea in combination with bedtime NPH insulin in treatment of patients with type 2 diabetes uncontrolled on oral hypoglycaemic therapy.

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Nateglinide versus glibenclamide in Maturity Onset Diabetes of the Young (MODY): lower post-prandial glucose but less hypoglycemia.

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Background and Aims: Pro291fsinsC mutation in the HNF1a gene is the most common cause of MODY3 diabetes. A defect in glucose-stimulated insulin secretion leads to postprandial hyperglycemia even with normal fasting glycemia. Hypoglycemia tendency due to high sensitivity to insulin and sulphonylurea preparations can hamper effective control of prandial glucose. In theory, a rapid-acting insulin secretagogue with short half-life could be the drug of choice. This pilot study was designed to evaluate the safety of a low dose of nateglinide in MODY3. We also compared its effectiveness in decreasing postprandial glucose and stimulating insulin secretion compared with a long-acting sulphonylurea glibenclamide and placebo.
Materials and Methods: We included 15 [5M/10F, median (interquartile range) age 41 (16) yrs, BMI 23.1 (2.4) kg/m2, HbA1c 6.7 (2.3) %, fasting plasma glucose (P-Gluc) range 4.3 - 16 mmol/l] subjects with MODY3 diabetes from the Botnia Study in a double-blinded study involving three visits for each patient. They received in random order test drug 1 [1.25 mg of glibenclamide (GB) or placebo (PL)] 30 min before and test drug 2 [30 mg nateglinide (NL) or placebo] 10 min before a standardised 450 kcal test meal including 70 g carbohydrates. 120 min after the 2nd drug, the subjects began light bicycle exercise (target pulse 80-110 /min with maximum mean pulse difference 5 /min between the visits)for 30 min. Samples were taken at 5-10 min intervals for measurement of P-Gluc, insulin, C-peptide and glucagon. Intra-individual differences between the visits were calculated (Matched signed rank test).
Results: P-Gluc at fasting was similar at the visits: NG 7.9 (5.6) vs. GB 7.8 (2.2) vs. PL 7.6 (2.7) mmol/l. Both the peak P-Gluc [NG 9.8 (8.0) vs. GB 11.5 (6.6) vs. PL 12.7 (7.2) mmol/l, P=0.030 NG vs. GB] and glucose area under curve at 140 min [NG 101 (294) vs. GB 210 (393) vs. PL 250 (348), P=0.036 NG vs. GB] were significantly lower at the nateglinide than at the glibenclamide and placebo visits. No hypoglycemia occurred at the nateglinide or placebo visits, while 6/15 patients had symptomatic hypoglycemia (P-Gluc <3.5 mmol/l) after glibenclamide (P=0.030, Fisher's 2-tailed test). In 4/6 cases with hypoglycemia, it occurred before exercise at 90-140 min after ingestion of the drug. The insulin, C-peptide and glucagon data are being analysed.
Conclusion: Acutely, nateglinide was more effective in controlling the prandial glucose than glibenclamide. No hypoglycemias occurred with nateglinide despite exercising after the test meal and inclusion of patients with mild diabetes. Long-term treatment study is needed to evaluate the usefulness of nateglinide in the treatment of MODY3.

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Comparison of effects of repaglinide and nateglinide on insulin secretion and postloading glucose excursions in patients with Type 2 diabetes.

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Background and Aims: To compare the effects of the rapid onset/short duration insulinotropic agents repaglinide (REP) and nateglinide (NAT) on postloading glucose excursions and insulin secretion in patients with type 2 diabetes mellitus.
Material and Methods: 29 type 2 diabetes patients were randomised to either REP (1 mg pre-meal 3x/day), NAT (60 mg pre-meal 3x/day) or placebo (PL) in combination with metformin (2000 mg/day) for 3 one-week crossover periods. On the final day of each period all subjects received intravenous glucose tolerance tests (IGTT) and liquid meal challenge tests (LMCT) with single preprandial doses of 120 mg NAT, or PL, or 2 mg REP. Average glucose, insulin and C-peptide responses were determined as the area under the curve (AUC_{xy}= integrated responce from hour x to y).
Results: In LMCT: Both REP and NAT reduced the maximal glucose peak in LMCT compared to PL (186.2±7.5, 185.3±9.1 and 207.8±8.3 mg/dl respectively, p<0.05). However, 3-4 hour post-dose mean glucose concentrations were lower with REP compared to NAT and PL (p<0.01). C-peptide response was greater with REP and NAT versus PL (p<0.05)(see Table of LMCT: α p<0.05 REP vs. PL, β p<0.05 NAT vs. PL in the paired two-sided Student's t test). In IVGTT: A significant reduction of blood glucose was achieved with REP at 10 min after i.v. glucose bolus (274.1±5.1 vs. 281.5±6.2 mg/dL in PL, p<0.05) and at 40 min in NAT (210.9±6.7 vs, 224.7±5.2 mg/dL in PL, p<0.05). The AUC_{gluc} of both drugs on glucose excursions over 175 min was similar (see Table of IGTT:α p<0.05 REP vs. PL, β p<0.05 NAT vs. PL in the paired two-sided Student's t test). Both drugs significantly enhanced early C-peptide release after the glucose bolus (see Table of IGTT).
Conclusions: The early insulinotropic effects of REP and NAT were similar in LMCT and similar in IGTT. The early glucose-lowering effects of REP in the IGTT were stronger than those of NAT. The effect of maximal glucose excursion was similar for both drugs in the LMCT.

LMCT

AUCgluc, mg/dl*h	0-1h	0-2h	0-3h	0- 4h
PL	38.13±2.85	98.38±6.56	109.23±10.20	90.44±12.75
REP	34.44±3.59	77.35±8.37α	63.08±12.74α	16.27±14.98α
NAT	32.72±2.98	65.13±7.37β	46.40±10.90β	1.58±12.65β
AUCc-peptide, ng/ml*h				
PL	1.17±0.13	3.82±0.37	5.78±0.50	6.65±0.55
REP	1.68±0.18α	6.26±0.60α	8.06±0.99α	9.69±1.25α
NAT	2.18±0.20β	5.54±0.47β	7.51±0.65β	8.17±0.79β

IGTT

AUCgluc, mg/dl*h	0-10 min	0-70 min	0-130 min	0-180 min
PL	23.82±0.76	110.55±2.94	129.71±5.98	120.03±8.01
REP	24.30±0.56	104.64±2.64α	103.39±5.40α	75.03±7.28α
NAT	24.24±0.66	99.00±4.15β	95.78±7.30β	67.70±8.78β
AUCc-peptide, ng/ml*h				
PL 0.03±0.01	1.10±0.15	2.18±0.32	2.59±0.44	
REP	0.17±0.04α	2.80±0.41α	5.52±0.82α	6.71±0.98α
NAT	0.16±0.03β	2.29±0.24β	4.43±0.33β	5.66±0.48β

PS 63
GLP-1 Analogues

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Cellular signaling in the GLP-1 and exendins action on lipid metabolism.

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Background and Aims: In rat adipocytes, GLP-1 is a dose-related lipogenic and/or lipolytic peptide, and stimulates glycosylphosphatidylinositol generation and/or cellular cAMP content; in both opposite actions of the peptide, activation of PI3K is required. In this work, we have studied, in normal rat adipocytes, whether other kinases – known to be involved in the signalling pathways of insulin action? participate in the effects of GLP-1 on lipid metabolism, and also the characteristics of the action of exendin-4 (Ex4) and its truncated form 9-39 (Ex9), both structurally related with GLP-1 and known to be lipogenic in rat adipocytes.
Materials and Methods: Cells were isolated by enzymatic digestion from the epididymal fat of normal Wistar rats. Lipogenesis – ^{14}C-Na acetate incorporation – and lipolysis – glycerol release – were determined in cells incubated during 1h, at 37ºC, in the absence (control) and presence of 10^{-9} M GLP-1, insulin, Ex4 or Ex9, and without and with $2.5x10^{-5}$ M PD98059 (PD) – MAP kinases inhibitor –, 10^{-7} M rapamycin (RAP) – p70s6k inhibitor – or 10^{-6} M wortmannin (W) – PI3K inhibitor –.
Results: In adipocytes from 4 rats, the lipogenic effect of either GLP-1 (123±5% of control, $p<0.02$), Ex4 (119±3%, $p<0.005$) or Ex9 (125±6% $p<0.001$) was blocked by the additional presence of PD (106±5%; 97±2% and 94±2%, respectively), while that of insulin (146±6%, $p<0.001$) was not modified (150±4%); RAP also abolished the increased acetate incorporation into lipids exerted by either exendin, but not that induced by GLP-1 or insulin. In cells from 4-8 rats, the stimulated lipolysis caused by either GLP-1 (180±7% of control, $p<0.001$) or Ex4 (178±6%, $p<0.001$) was completely prevented by the presence of PD ($p<0.001$ vs either peptide alone); RAP slightly reduced the lipolytic effect of GLP-1 (154±8%, $p<0.05$ vs GLP-1 alone) but failed to modify that of Ex4 (176±7%). As it is known to occur with GLP-1, inhibition of PI3K also blocked the lipolytic action of Ex4 (95±3%, $p<0.001$ vs Ex4 alone); Ex9 did not alter the lipolysis control value (95±5%), and completely antagonised the increment induced by either GLP-1 (99±6%, $p<0.001$ vs GLP-1 alone) or Ex4 (96±9%, $p<0.001$ vs Ex4 alone).
Conclusion: Ex4 and Ex9 share with GLP-1 the activation of the same initial kinases in their respective actions upon lipid metabolism in rat adipocytes, as besides that of PI3K, phosphorylation of MAPKs is required for the lipogenic action of GLP-1, Ex4 and Ex9 and for the lipolytic effect of GLP-1 and Ex4; also, activation of p70s6k mediates the lipolytic action of GLP-1 and the lipogenic effect of both exendins.

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Transplantation with an engineered cell line secreting GLP-1 and insulin restores euglycemia in STZ-rats.

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Background: Glucagon-like peptide-1 (GLP-1) is an incretin hormone derived from the proglucagon gene, capable of regulating the transcription of insulin, GLUT-2 and glucokinase. Aim: The aim of this study was to investigate the potential role of GLP-1 for the gene- and cell-therapy of diabetes.
Materials and Methods: We transfected mouse insulinoma (MIN-6) cells with a DNA fragment of the human proglucagon gene containing the nucleotide sequence encoding for human GLP-1, but lacking the coding region for glucagon. The GLP-1 sequence was under control of a glucose responsive promoter and linked to a secretory peptide. These cells were encapsulated and transplanted into the spleen of rats rendered diabetic by injection of streptozotocin (STZ). The cell line was characterized by northern blot, RIA, HPLC and immuno histochemistry analyses; and the rats studied for glucose tolerance for a forty-day post-transplant period.
Results: Northern blot, HPLC and RIA analyses confirmed that the minigene was transcribed and the protein appropriately translated, processed and secreted in the extracellular environment. Gene expression studies revealed that cells were capable of regulating the transcription of insulin and GLP-1 based on the concentration of glucose in the culture medium. GLP-1 action was mediated by an IDX-1-dependent

transactivation of the endogenous insulin promoter, as demonstrated by gel shift analysis. Transplant of these cells into the spleen of STZ-rats was able to normalize fasting blood glucose and was associated with normal glucose tolerance, as determined by an intra-peritoneal glucose tolerance test performed at four and eight weeks after transplant. No animal developed hypoglycemia for the entire duration of the study. Removal of the spleen, one day before the end of the study, led to the loss of glucose control suggesting that the transplanted cells were responsible for the metabolic improvement observed.
Conclusions: The present study lays the research foundation to investigate the potential use of GLP-1 for the gene or cell therapy of diabetes.

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Synergistic effects of a combination of DPPIV inhibitor with metformin on glycemic control, food intake and weight gain in Zucker *fa/fa* rats.
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Background and Aims: Our previous study showed that the combination of DPPIV (dipeptidyl peptidase IV) inhibitor (valine-pyrrolidide; val-pyr) with metformin synergistically increases plasma GLP-1 (glucagon like peptide-1) levels in normal rats, although neither metformin nor DPPIV inhibitor alone change basal GLP-1 levels, suggesting that this combination treatment could be expected to produce a GLP-1-induced favorable spectrum of antidiabetic actions(Biochem Biophys Res Commun 2002 Nov 15;298(5):779-84).The present study investigated the effect of a combination of a DPPIV inhibitor with metformin on glycemic control, food intake and weight gain in Zucker *fa/fa* rats (13-week- old).
Materials and Methods: d.H_2O (vehicle), metformin (300mg/kg), val-pyr (30mg/kg) or both compounds were administrated orally twice a day (at 10:00 and 16:00) for 14 days (n=10). An oral glucose tolerance test (OGTT) were performed on day 1 and 14. The compounds were administrated 30 min prior to glucose (2g/kg) load. Food intake and body weight were measured over the 14 days.
Results: Val-pyr and metformin improved the oral glucose tolerance in the OGTT on day 1. The combination treatment caused a synergistical improvement of oral glucose tolerance. It is likely that this synergistic effect results from the greater increase in GLP-1 levels caused by the combination.It is a notable that the combination treatment decreases food intake [cumulative food intake : 478.1±14.8 (vehicle), 491.8±11.1 (val-pyr), 484.4±13.3 (metformin) and 417.3±11.1* (g) (combination)] and body weight gain [66.0±4.2 (vehicle), 60.9±4.4 (val-pyr), 62.6±3.7 (metformin) and 39.5±4.7* (combination) (g)] over the 14 days, although neither metformin nor DPPIV inhibitor alone has any effect on either. After the 14-day treatment, the combination treatment showed an additive reduction of fasting blood glucose levels [116.9±4.3 (vehicle), 105.4±1.7 (val-pyr), 107.4±1.9 (metformin) and 97.3±3.8* (combination) mg/dl] and fasting plasma insulin levels [17.9±1.6 (vehicle), 16.0±1.6 (val-pyr), 10.7*#±1.1 (metformin) and 8.4*$±0.7 (combination) ng/ml], compared with that of metformin or DPPIV inhibitor alone group. (* vs vehicle, # vs val-pyr, $ vs metformin; $p < 0.05$)
Conclusion: These results suggest the possibility that the larger increase in plasma GLP-1 levels due to the combination therapy of DPPIV inhibitor and metformin causes the reduction in food intake and body weight gain and contributes to the greater improvement of glycemic control and insulin sensitivity than seen with monotherapy.

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Glucagon-like peptide-1 as a stimulator of pancreatic beta-cell insulin gene expression.
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Background and Aims: Glucagon-like peptide-1 (GLP-1) and its structurally-related analog Exendin-4 (Ex-4) are potent blood glucose-lowering agents when administered to type-2 diabetic subjects. Insulinotropic actions of GLP-1 at the pancreatic beta-cell include the glucose-dependent stimulation of insulin secretion as well as increased insulin biosynthesis. The aim of this study was to elucidate the signal transduction pathway by which GLP-1 stimulates insulin gene expression.
Materials and Methods: The activity of the rat insulin 1 gene promoter (RIP1) was studied in INS-1 cells transfected with a reporter incorporating –410 bp of RIP1 fused to the coding sequence of firefly luciferase (RIP1-Luc). 24 hr post-transfection the INS-1 cells were exposed to GLP-1 (1 nM) for 4 hr in the presence of culture medium containing 11.1 mM glucose. Luciferase activity was studied in lysates prepared from the transfected cells.
Results: The stimulation of RIP1-Luc by GLP-1 was blocked by the serine/threonine protein kinase inhibitor Ro 31-8220 (IC50 600 nM; $p < 0.001$) and resulted from interactions of the GLP-1-R with Gs-alpha GTP-binding proteins, as demonstrated by the failure of GLP-1 to act in cells treated with cholera toxin, but not pertussis toxin. Over expression of wild type Gs-alpha facilitated the action of GLP-1, whereas constitutively active Gs-alpha increased basal promoter activity in the absence of GLP-1. Introduction of inactivating mutations at the cAMP-response element (CRE) of RIP1 nearly abolished stimulatory actions of GLP-1, as did over expression of dominant negative A-CREB. The action of GLP-1 was not blocked by the cAMP antagonist 8-Br-Rp-cAMPS, and GLP-1 remained effective after treatment of cells with an inhibitor of adenylyl cyclase (MDL 12330A), or inhibitors of PKA (H-89, KT 5720, PKI). 8-CPT-2-O-Me-cAMP, a selective activator of cAMPGEFs (Epac1/2), failed to stimulate RIP1-Luc, and the action of GLP-1 was not affected by over expression of dominant negative Epac2. Importantly, calcium plays a significant role in determining the efficacy of GLP-1 in this assay. Depletion of intracellular calcium stores with thapsigargin (an inhibitor of the SERCA calcium ATPase) abrogated stimulatory actions of GLP-1 at RIP1-Luc.
Conclusions: These findings demonstrate that the stimulatory action of GLP-1 at RIP1 is mediated by interactions of the GLP-1 receptor with Gs-alpha. Surprisingly, the action of GLP-1 is shown to be cAMP-independent. A possible role for src kinase as a down stream effector of Gs-alpha is presently under investigation.

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Plasma protein binding of NN2211, a long-acting derivative of glucagon-like peptide-1, is important for its efficacy.
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Background and Aims: GLP-1 has a wide spectrum of biological effects and seems ideal for treatment of type 2 diabetes. The native hormone is unsuitable as a drug because it is broken down rapidly by DPP-IV and cleared by the kidneys. NN2211 is a stable derivative suitable for once daily administration. The mechanism of protraction is predicted to be binding to albumin, thereby escaping clearance in the kidneys, as well as stability towards DPP-IV and slow release from the injection site. This study shows the effect of NN2211 plasma binding on GLP-1 receptor activity.
Materials and Methods: In order to characterize the binding to plasma proteins, we have used in vitro assays with the human GLP-1 receptor.
Results: NN2211 and GLP-1 were equipotent (EC_{50} 61 ± 7 and 55 ± 19 pM) when analyzed in a buffer without the presence of plasma. The binding to plasma was measured as the difference between the EC_{50} of GLP-1 and NN2211 when analyzed in the presence of plasma. GLP-1 itself does not bind to albumin; however the assay itself changes upon addition of plasma. In the presence of 90% human plasma, the EC_{50} was 99 ± 8.5 nM and 1.7 ± 0.5 nM of NN2211 and GLP-1, respectively. This corresponds to a free fraction of 1.5% in 100% human plasma, assuming linearity. When porcine plasma was used, a free fraction of 1.0 % in 100% plasma was measured (EC_{50} was 127 ± 60 nM and 1.4 ± 0.5 nM). Pigs have been used to characterize the pharmacodynamics of NN2211. The half-life in pigs has been reported to be 14 hours, and total plasma levels of 6-8000 pM have been shown to be effective. The 1% free fraction corresponds to 84 pM free NN2211. Plasma concentrations of 50-100 pM have earlier been shown to be adequate for *in vivo* efficacy of natural GLP-1.
Conclusion: In conclusion, compared to natural GLP-1 relatively high total plasma concentrations of NN2211 are needed for full efficacy. However, the free concentration of NN2211 correlates to those obtained with GLP-1. Thus, the differences in efficacy between NN2211 and native GLP-1 can be explained by differences in free fractions.

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One week's treatment with NN2211, a long-acting GLP-1 derivative, significantly improves first phase insulin response and other markers of β-cell function, reduces endogenous glucose release, and ameliorates 24-h glycemia in Type 2 diabetic patients.

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Background and Aims: NN2211 is a long acting GLP-1 derivative designed for once daily administration in humans. The aim of this study was to explore the effect of one week's treatment with NN2211 on 24-hour glucose and hormone profiles, fasting endogenous glucose release (EGR) and β-cell function in type 2 diabetic patients.
Materials and Methods: Thirteen patients with type 2 diabetes ((means ± SD): age 56.4± 9.2 years, BMI 31.2± 3.6 kg/m^2, duration of diabetes 3.0± 2.6 years) were examined in a double-blind, placebo-controlled cross-over design. NN2211 6µg/kg was administered once daily for one week, whereafter 24-h profiles of glucose, insulin, glucagon and C-peptide were obtained. On the following day, fasting EGR was determined by tracer dilution technique. Fasting gluconeogenesis (GNG) was assessed using the ^2H_2O technique, and glycogenolysis (GLY) was calculated. First phase insulin response was evaluated by IVGTT (25g glucose), second phase insulin response by hyperglycemic clamp (16mM) and (near) maximal insulin secretory capacity by arginine stimulation test (5g). Insulin secretory rates (ISR) were estimated by C-peptide deconvolution analysis. Areas under curve (AUC) were calculated using the trapezoidal method. Statistical analyses were performed by ANOVA. Results are given as NN2211 versus placebo.
Results (mean±SEM)**:** *24-hour profiles:* NN2211 significantly reduced AUC for glucose (188±14 vs. 232±22 mmol/l*hr, p=0.014) and glucagon (2,179±118 vs. 2,371±135 pg/ml*hr, p=0.037), whereas AUC for insulin was unchanged. *Insulin secretion:* First phase insulin response was markedly increased after NN2211 administration (insulin AUC was 56±10 vs. 34±6 pmol/l*hr, p<0.01), and during hyperglycemic clamp mean insulin concentration and ISR were increased (930±263 vs. 272±53 pmol/l, p=0.015 and 15.0±2.7 vs. 6.9±1.1 pmol/kg/min*hr, p<0.001 respectively). During arginine stimulation test on top of hyperglycemia, AUC's for insulin and ISR were significantly improved (800±190 vs. 307±66 pmol/l*h, p<0.01, and 10.9±1.9 vs. 5.9±1.0 pmol/kg/min*hr, p<0.001). *Glucagon:* Both during hyperglycemia and after arginine exposure the glucagon responses were reduced by 17% (p<0.01 and p=0.012, respectively*). Fasting glucose production:* EGR was decreased (1.92±0.06 vs. 2.13±0.09 mg/kg/min, p=0.04), which was due to reduced GLY (0.83±0.04 vs. 1.02±0.07 mg/kg/min, p=0.01). GNG was unaltered (1.09±0.04 vs. 1.12±0.04 mg/kg/min, p=0.63).
Conclusion: One week's treatment with NN2211 significantly reduces 24-hour plasma glucose levels in type-2 diabetic patients. The β-cell function is substantially improved as demonstrated by first and second phase insulin responses as well as maximal insulin secretory capacity. Furthermore, glucagon secretion is suppressed. Fasting glycogenolysis is significantly reduced by NN2211, leading to suppressed fasting endogenous glucose release.

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The long-acting GLP-1 derivative, NN2211, restores beta cell sensitivity to glucose in subjects with Type 2 diabetes.

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Background and Aims: Glucagon-like peptide 1 (GLP-1) glucose-dependently stimulates insulin secretion, but its very short half-life limits its use as a therapeutic agent. NN2211 is a long-acting GLP-1 derivative suitable for once-daily administration.
Materials and Methods: We tested the effect of NN2211 on beta cell sensitivity in 10 subjects with type 2 diabetes, age 63 ± 8 years (mean ± SD), BMI 30.1 ± 4.2 kg/m^2, HbA_{1c} 6.5 ± 0.8%, in a randomized, double-blind, placebo-controlled, crossover study. A single subcutaneous injection of NN2211 (7.5 µg/kg) or placebo was administered in random order to subjects with type 2 diabetes 9 hours (equal to reported t-max) prior to testing. Beta cell sensitivity was assessed by a graded glucose infusion protocol with plasma glucose levels matched over the range of 5 to 12 mmol/L. Insulin secretion rates (ISR) were estimated by deconvolution of circulating C-peptide concentrations. Findings were also compared to responses of 10 healthy, nondiabetic volunteers to the same glucose infusion protocol.
Results: Compared to placebo, a single dose of NN2211 increased insulin and C-peptide levels, increased ISR area under the curve (AUC) (1130 ± 150 vs. 668 ± 106 pmol/min*kg; p < 0.001; mean ± SE), and increased slope of ISR vs. plasma glucose (1.26 ± 0.36 vs. 0.54 ± 0.18 pmol*L/(min*mmol*kg); p < 0.014), to values similar to nondiabetic controls who did not receive the drug (ISR AUC 1206 ± 99; slope of ISR vs. plasma glucose 1.44 ± 0.18). Insulin clearance and glucagon AUC were not significantly different between placebo treatment, NN2211 treatment, and healthy individuals. As expected, no hypoglycemic events occurred. Mild treatment adverse events (diarrhea and headache) were reported by 3/10 NN2211-treated and 2/10 placebo-treated diabetic subjects.
Conclusion: A single dose of the long-acting GLP-1 derivative, NN2211, restores beta cell sensitivity to physiological hyperglycemia in type 2 diabetes patients. These results substantiate the potential of NN2211 as a new therapeutic agent for the treatment of type 2 diabetes.

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No impairment of hypoglycemia counterregulation via glucagon with the long-acting GLP-1 derivative, NN2211, in subjects with Type 2-diabetes.

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Background and Aims: The GLP-1 derivative NN2211 is being evaluated as a new treatment for type 2 diabetes. Glucagon secretion is suppressed by GLP-1, therefore, NN2211 could disturb hypoglycemia counterregulation. This trial examined counterregulation during treatment with NN2211 vs. placebo.
Materials and Methods: Eleven subjects with type 2 diabetes (3F, age 56± 9 yrs, BMI 30.4±3.4 kg/m^2, diabetes duration 6±3 yrs, fasting plasma glucose (FG) 8.4±2.6 mM, HbA_{1c} 7.5±1.1 %) treated with diet (n=3) or with oral antidiabetic drugs (n=8) were studied in a placebo-controlled cross-over design. NN2211 (7.5µg/kg) was administered as a single s.c. dose at midnight. In the morning, regular insulin infusions (2 $mU \cdot kg^{-1} \cdot min^{-1}$) were given to achieve fasting euglycemia. Capillary glucose was consecutively clamped for 240 min at levels of 78, 66, 54, and 42 mg/dL for 60 min each. Glucose, insulin, C-peptide, glucagon, cortisol, growth hormone (GH) and catecholamines were determined. Insulin secretion rates (ISR) were derived by deconvolution of C-peptide profiles.
Results: NN2211 at mean concentrations of 7.9±1.8 nM reduced FG to 7.5±2.4 mM (placebo: 8.1±3.0 mM). At steady state insulin concentrations of ~1000 pmol/L, glucose infusion rates were similar with NN2211 vs. placebo (p=0.27). Exposure to hypoglycemia led to clear counterregulatory responses of glucagon (1.6 fold), cortisol (2.2 fold), GH (6.6 fold), adrenaline (14 fold) and noradrenaline (2.3 fold; all p<0.0001, ANOVA). Responses did not differ significantly for NN2211 vs. placebo (glucagon, p=0.76; cortisol, p=0.43; adrenaline, p=0.27, noradrenaline, p=0.57), except for GH (impaired response with NN2211, p=0.034). C-peptide concentrations were significantly higher with NN2211 at all clamp levels (p<0.0001). ISR was significantly different only at baseline and not at any hypoglycemic clamp level.
Conclusion: NN2211 reduces fasting glycemia but does not impair glucagon responses during hypoglycemia. NN2211, like GLP-1, does not impair hypoglycemia counterregulation, except for a reduction in GH response. The insulinotropic activity of NN2211 is glucose-dependent like that of GLP-1.

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Intravenous glucagon-like peptide 1 (GLP-1) is a feasible treatment after major surgery in patients with Type 2 diabetes.

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Background and Aims: The clinical course of patients with type 2 diabetes after major surgery is often complicated by the development of hyperglycemia. Recently, van den Berghe et al. demonstrated that intensive insulin with a continuous intravenous infusion reduces the mortality in critically ill patients on an intensive care unit. However, such an approach requires tight glucose control and poses the risk of hypoglycaemia. The insulinotropic gut hormone glucagon-like peptide 1 (GLP-1) has been shown to normalize blood glucose after intravenous infusion in patients with type 2 diabetes without causing hypoglycemia. Therefore, we studied the hypoglycaemic effect of intravenous GLP-1 in patients with type 2 diabetes after major surgical procedures.
Materials and Methods: 8 patients with type 2 diabetes (5 male, 3 female, age: 42 ± 12 yrs., BMI 28 ± 3 kg/m^2, HbA_{1c} 8.0 ± 1.9 %, fasting glucose: 180 ± 48 mg/dl, diabetes duration: 5 ± 4 yrs.), who had undergone major surgical procedures (abdominal surgery in 3, vascular surgery in 2, and bone surgery in 2 cases) were included between the second and the eighth post-operative day. The mean duration between the operation and the beginning of the experiments was 4 ± 2 days, the CRP levels at the beginning of the experiments were 49 ± 42 mg/dl (normal range < 5 mg/dl), indicating a systemic inflammation. On separate occasions, either GLP-1 (1.2 pmol · kg^{-1} · min^{-1}) or placebo were infused intravenously over 8 hours, in randomised order. Venous blood samples were drawn in the basal fasting state and in 30-min intervals during the experiments for the determination of glucose (glucose-oxidase) insulin, C-peptide, glucagon and GLP-1 (specific immunoassays).
Results: Following the intravenous infusion of GLP-1, GLP-1 rose to steady state plasma concentration of 140 ± 20 pmol/l, compared to 9 ± 0.4 pmol/l in the placebo group ($p < 0.001$). Blood glucose concentrations were significantly lowered during GLP-1 infusion and reached the normal fasting range (< 126 mg/dl) within two hours, whereas they remained in the hyperglycaemic range (> 140 mg/dl) over the whole study period with placebo ($p < 0.001$). No hypoglycaemic episode was recorded during the study period. Lowering of blood glucose was achieved by a significant rise of insulin secretion ($p < 0.001$ for insulin and C-peptide concentrations). Insulin secretion was stimulated by GLP-1 only in the presence of elevated glucose levels, but once glucose concentrations had reached the normal range, no further stimulation of insulin secretion was observed. Glucagon concentrations were significantly lowered by GLP-1 ($p = 0.042$). No patient reported adverse reactions during the infusion of GLP-1 or placebo.
Conclusion: These data for the first time provide evidence that intravenous GLP-1 has the potential to normalise glucose concentrations even in poorly controlled patients after major surgical procedures. Since intensive insulin therapy after major surgery is often complicated by the development of severe insulin resistance, GLP-1 therapy might offer a practical alternative.

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Patient reported outcomes (PROs) for the evaluation of diabetes drug therapy.

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Background and Aims: Patient-reported outcomes (PRO) data has become increasingly important in the evaluation of drug therapy. The objective of this study was to establish a set of standard patient-reported outcome measures for the evaluation of diabetes drug therapy for patients with type 2 diabetes
Materials and Methods: We administered the Short Form-36 Vitality Scale, Diabetes Treatment Satisfaction Questionnaire (DTSQ), Hypoglycemia Fear Survey Worry Subscale, and Diabetes Symptoms Checklist-Revised (DSCR) hyperglycemia, hypoglycemia, and fatigue subscales to 161 patients with type 2 diabetes participating in an international, randomized, dosing study of GLP-1 analog, an injectable diabetes treatment. We used baseline data to calculate descriptive statistics, reliability coefficients, and correlation coefficients. To explore whether scale items measured unique constructs, we performed an exploratory factor analysis with varimax rotation. We used data collected at baseline and end of study to explore the responsiveness of each PRO.
Results: Score distributions for the Worry Subscale were significantly skewed. Reliability coefficients for all PROs were acceptable (> 0.70). Correlations among PROs ranged from -0.12 to 0.61. An eight-factor solution determined 69% of the common variance. Using a factor loading criteria of > 0.40, all but 1 Worry Scale item loaded on Factor 1 or 3; Vitality Scale items loaded on Factor 2; DTSQ items loaded on Factors 5, 7, and 8; Hyperglycemia and Hypoglycemia items loaded on Factors 4 and 5, respectively. Fatigue items loaded on Factor 2 suggesting that fatigue is an aspect of vitality. At end of study, we observed significant improvements in vitality (p=.03) and hyperglycemic symptoms (p=.04) and a trend for improvement in hypoglycemic symptoms (p=.06). No significant deterioration in treatment satisfaction was observed, despite the introduction of injection therapy.
Conclusion: We provided evidence for the reliability and validity of 6 PROs for the evaluation of diabetes drug therapy. Factor analysis indicated that these scales measure one or more unique constructs. We also demonstrated the responsiveness of the Vitality Scale and DSCR subscales. The DTSQ did not demonstrate responsiveness in this trial, but has shown responsiveness in other clinical investigations. The significantly skewed distribution of the Worry Scale raises questions about its appropriateness for diabetes drug evaluation. Modifications, or use with selected populations (e.g., insulin users), may increase responsivity. The Vitality Scale, DTSQ, and the DSCR appear suitable as standard measures for the evaluation of diabetes drug therapy. Future research should explore the suitability of measures of other constructs (e.g., psychological well-being).

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Glitazones

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Thiazolidinedione-regulated polypeptides in the supernatant of human and rodent adipocytes.

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Background and Aims: Insulin-resistance is a hallmark in the pathophysiology of obesity and type 2 diabetes. It is commonly hypothesized that dysregulation of adipocyte derived polypeptides and hormones affects insulin-responsiveness of muscle, liver and adipose tissue. With the discovery of leptin and adiponectin as hormones regulating energy expenditure and insulin sensitivity the endocrine function of adipose tissue came into focus. Thiazolidionediones (TZD) exert an insulin-sensitizing effect on muscle and adipose tissue which is currently only incompletely understood. As transcriptional activators TZD regulate multiple genes involved in lipid and insulin-sensitizing pathways of which presumably only a limited number have been identified to date. Understanding disease-related changes in protein patterns holds the key to unique therapeutic and diagnostic approaches of the metabolic syndrome.
Materials and Methods: In order to discover novel drug targets from adipose tissue, human and rodent adipocytes were incubated with 0, 0.1, 1 and 10 µM troglitazone for 48 h. Peptides extracted from tissue homogenates and culture supernatant were separated by high performance liquid chromatography into 96 fractions. Each HPLC fraction was analyzed by matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry (MALDI-ToF-MS). Sequence information of peptides differentially regulated in reponse to troglitazone treatment was obtained by either nanoelectrospray or Edman sequencing .
Results: Peptide sequencing of rat adipose tissue identified protein precursors involved in lipid metabolism as well as secreted polypeptides and hormones. At least 15 signals in the supernatant of 3T3-L1 adipocytes were selectively and concentration dependently regulated by troglitazone. Increased troglitazone concentrations correlated with elevated adiponectin levels in the supernatant of 3T3-L1 cells and improved insulin-stimulated glucose utilization. A comparative study of the peptide pattern of human adipocytes derived from subcutaneous and omental fat depots revealed distinct alterations between the fat depots, which are subject to further analyses.
Conclusion: Our findings demonstrate the utility of our Peptidomics™ Technology to identify polypeptides implicated in the context of the pathophysiology of obesity. Profiling of the action of troglitazone on adipocytes revealed a distinct subset of peptides that were either up- or down-regulated. A comparative study of rodent and human adipose tissue is currently performed to confirm the clinical relevance of identified peptides. Sequence determination revealed novel peptides which are currently validated in the context of the metabolic syndrome. This may lead to peptides suitable as biomarker in profiling the action of novel compounds, or to novel biological peptides and drug targets for the treatment of obesity and diabetes.

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The efficacy of pioglitazone compared to metformin in drug naive patients with Type 2 diabetes.

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Background and Aims: Pioglitazone is an oral antidiabetic drug that increases the sensitivity of peripheral tissues to insulin and may provide an alternative first-line treatment in type 2 diabetes. This study compared the effects of pioglitazone and metformin on metabolic control in drug-naive patients with type 2 diabetes in several European countries.
Materials and Methods: We studied the effect of pioglitazone or metformin in 1199 patients with poorly controlled type 2 diabetes mellitus (glycosylated hemoglobin [HbA_{1c}]: 7.5-11%; normal, 4.3-6.1%) despite dietary advice. Patients were randomized to receive either pioglitazone up to 45 mg once daily or metformin 850 mg up to three times daily. HbA_{1c} and fasting plasma glucose (FPG) were measured.
Results: Eighty percent of patients completed the study. The adjusted mean HbA_{1c} values decreased similarly between the two treatment groups from baseline to Week 52. A greater mean reduction in FPG was observed in the pioglitazone group than in the metformin group. The overall frequency of adverse events was similar in the two treatment groups but pioglitazone patients reported more oedema and metformin patients more gastrointestinal adverse effects.
Conclusion: This study demonstrated that pioglitazone monotherapy is similar to metformin with respect to HbA_{1c} reduction but with a superior and durable effect on FPG over a 52-week period compared to metformin ($p<0.001$).

Time Course of Change from Baseline of HbA_{1c} and FPG over One Year for Pioglitazone and Metformin as Monotherapy

	HbA_{1c} (%)		FPG (mmol/L)	
	Pioglitazone	Metformin	Pioglitazone	Metformin
Mean at Baseline	8.69	8.68	11.8	12.0
Week 4	-0.26	-0.46	-1.3	-1.0
Week 8	-0.59	-0.85	-2.1	-1.9
Week 12	-0.94	-1.18	-2.4	-2.3
Week 16	-1.20	-1.41	-2.6	-2.5
Week 24	-1.43	-1.63	-2.8	-2.6
Week 32	-1.51	-1.66	-2.8	-2.5
Week 42	-1.49	1.61	-2.6	-2.4
Week 52	-1.42†	1.50	-2.5*	-2.1

Between group comparisons: †p = NS; *p = 0.016

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The long-term effects of pioglitazone (PIO) and glibenclamide (GLB) on glycemic control and Insulin Sensitivity (IS) in patients with Type 2 diabetes (T2D).

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Background and Aims: Previous studies with PIO have investigated its short-term (≤6 months) anti-hyperglycemic effect compared with placebo. Information on the long-term effects and degree of glycemic improvement by PIO vs other oral anti-hyperglycemic medications (OAM) is limited. We studied the long-term (1 year) effects of PIO and micronized GLB on glycemic control and IS in T2D patients.
Materials and Methods: In this double-blind, multicenter study in Scandinavian countries, patients with T2D (HbA1c >7.5% and ≤11% for those not on OAM, or HbA1c >7.5% and ≤ 9.5% for those on 1 OAM [sub-maximal dose]) were randomized to either PIO (initially 30 mg QD, n=91) or GLB (initially 1.75 mg QD, n=109) as monotherapy. Doses were titrated up to 10.5 mg (GLB) and 45 mg (PIO) to achieve glycemic targets during the next 12 weeks; it was recommended that this dose be maintained for the remainder of the study. Fasting plasma glucose (FPG), fasting serum insulin (FSI), and HbA1c were measured. HOMA-S was calculated to estimate IS. Data were analyzed by an analysis of covariance using (a) intention to treat (ITT) patients (last observation carried forward) (see Table) and (b) patients who completed the study.
Results: In each group, approximately 30% of the patients were OAM-naive at baseline. Body weight increased 1.1 ± 0.4 kg for GLB and 3.0 ± 0.5 kg for PIO (p=0.003). The incidence of hypoglycemic episodes was significantly less for the PIO group (4%) compared with the GLB group (29%) ($p<0.0001$).

Variable	Group (n)	Baseline mean±SD	LS Change mean±SEM	p-value vs baseline	p-value vs GLB
HOMA-S (%)	GLB (87)	99 ± 64	-13.0 ± 5.5	0 0.020	<0.001
	PIO (74)	84 ± 51	17.0 ± 6	0.006	
HbA1c (%)	GLB (96)	8.5 ± 0.8	-0.4 ± 0.14	0.002	0.787
	PIO (83)	8.4 ± 0.7	-0.5 ± 0.15	0.001	
FPG (mM)	GLB (94)	10.7 ± 2.0	0.3 ± 0.3	0.430	0.029
	PIO (82)	10.6 ± 2.4	-0.7 ± 0.4	0.036	
FSI (pM)	GLB (94)	76 ± 45	24 ± 6.4	< 0.001	0.007
	PIO (75)	87 ± 71	-1.3 ± 7.3	0.856	

For completers, PIO (n=55) significantly increased HOMA-S and decreased FPG and FSI compared with GLB (n=68). In addition, for completers, PIO significantly improved HbA1c (-1.2 ± 0.1% vs –0.7 ± 0.1%, p=0.002) compared with GLB.

Conclusion: For the ITT population, PIO and GLB had similar effects on HbA1c. For the Completer population, PIO produced a significantly greater reduction in HbA1c compared with GLB. Despite producing more weight gain than GLB, PIO increased HOMA-S (insulin sensitivity), whereas GLB decreased HOMA-S. In addition, PIO decreased FPG and FSI compared with GLB.

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The effects of pioglitazone, metformin and gliclazide as monotherapy or in combination on 3-hour OGTT investigations.

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Background and Aims: In patients with type 2 diabetes, there is an excessive rise in plasma glucose following ingestion of a meal compared with normal subjects. Postprandial glucose handling in diabetes has been implicated as a factor in excessive morbidity and mortality of this condition. We have examined the response of type II diabetic patients to several oral hypoglycaemic agents in four European randomised double-blind clinical trials.
Materials and Methods: In the the Quartet Studies, which consisted of two monotherapy studies (Pioglitazone [Pio] vs Metformin [Met] or Gliclazide [Glic]) and two combination therapy studies (sulphonylurea [SU] + Pio vs SU + Met and Met + Pio vs Met +Glic) postprandial glucose excursions were approximated using 75 g oral glucose tolerance testing.treatment. For plasma glucose and insulin measured during the OGTT, AUC (area under the concentration time curve) was calculated at baseline and Week 52 using the trapezoidal rule. The positive incremental AUC (i.e. the increase in glucose over the fasting value) was calculated for each patient.
Results: In total, 1056 patients from these trials were investigated. During a 3-hour glucose tolerance test performed without drug dosing in the morning, pioglitazone as monotherapy, as well as in combination therapy with sulphonylurea or metformin, significantly lowered post-load glucose with a decrease in the insulin level. In contrast, metformin and gliclazide showed no or much smaller reductions in glucose excursion accompanied by increases in insulin levels, which were particularly large in monotherapy.
Conclusion: The difference in the magnitude of insulin excursion between monotherapy and combination therapy may be due to patients on combination therapy having a greater degree of beta-cell deterioration and a hence less insulin-secretory reserve due to their longer duration of diabetes. The use of an insulin sensitizer either as monotherapy or combination therapy may have additional benefits on postprandial glucose disposal in type II diabetes.

Mean Change in incremental AUC of Glucose and Insulin in the Quartet Studies

Mean Change in incremental AUC	Pioglitazone	Metformin	Pioglitazone	Glicazide	Pioglitazone+ Sulphonylurea	Metformin + Sulphonylurea	Pioglitazone + Metformin	Glicazide + Metformin
Glucose mmol*h\L	-5.3	-2.0 p=0.001	-5.0	-0.4 p=0.001	-2.8	0.0 p=0.001	-3.7	-0.8 p=0.005
Mean Change in incremental AUC Insulin µIU*h\L	2.7	33.9 p=0.05	0.3	30.4 p=0.001	-2.7	3.1 p=NS	-6.2	14.8 p=0.05

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Long-term combination therapy with pioglitazone plus metformin for Type 2 diabetes: a randomised, comparative study with gliclazide plus metformin.

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Background and Aims: So far, a comparison of the combination of pioglitazone plus metformin with the established combination of a sulphonylurea plus metformin has not been carried out. The current study was therefore conducted to perform such a comparison over a period of 52 weeks to compare the efficacy and safety of the two combination therapies. As one of the most commonly prescribed sulphonylureas, gliclazide was chosen for use in the comparator arm.
Materials and Methods: Patients with type 2 diabetes that were inadequately controlled with metformin, used at a dose of at least 50% or greater than maximum dose, were recruited to the study. HbA_{1c}, FPG, fasting serum insulin, lipids and urinary albumin/creatinine ratio were measured at regular intervals throughout the study.
Results: A total of 630 patients received study treatment, 317 with pioglitazone plus metformin and 313 with gliclazide plus metformin. At Week 52, mean HbA_{1c} was reduced from baseline by 1.0% in both groups and mean FPG was reduced by 1.9 mmol/L (Pio+Met) and 1.7 mmol/L (Glic+Met). There were no significant differences between the groups, but there was some evidence of treatment deterioration with Glic+Met from Week 24 on. Changes in fasting insulin were -3.5 IU/mL with Pio+Met and -1.1 IU/mL with Glic+Met ($p<0.001$). The incidence of adverse events was similar in each group. There was more oedema with pioglitazone combination (6.3% vs 2.2%) and more hypoglycaemia with gliclazide combination (11.2% vs 1.3%).
Conclusion: Pioglitazone in combination with metformin provided an effective and well tolerated treatment for type 2 diabetes over a period of one year. Compared with the established combination of Glic+Met, there were benefits in terms of durability of glycaemic control. Pio+Met offers a valuable alternative to existing therapies due to the complementary effects on insulin resistance that is a core feature in Type 2 diabetes.

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The coefficient of failure for HbA_{1c} in drug naive patients treated with pioglitazone, metformin or gliclazide monotherapy.

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Background and Aims: The UKPDS and clinical experience have demonstrated that glycaemic control in type II diabetes declines overtime with most patients eventually needed combination therapy. The coefficient of failure for HbA_{1c} is a measure of disease progression and/or treatment failure. It is calculated as the increase in HbA_{1c} over time by performing a regression on the HbA_{1c} time course over one year for each patient.
Materials and Methods: Two one-year randomised double-blind trials (404 and 405) comparing the effects of pioglitazone vs metformin and pioglitazone vs gliclazide on metabolic control were conducted in Europe in drug-naive patients with type 2 diabetes uncontrolled by diet alone. In the first study, patients were randomized to receive either pioglitazone up to 45 mg once daily or metformin 850 mg up to three times daily. In the second study, patients were randomised to receive either pioglitazone up to 45 mg or gliclazide up to 320 mg daily. HbA_{1c} values were determined regularly throughout both studies. The last four time points during the studies, i.e. Weeks 24, 32, 42, and 52, were used to calculate the coefficient of failure.
Results: A total of 597 were randomised to metformin and 597 pioglitazone in the 404 study. 626 received glicazide and 624 aptients received pioglitazone in the 405 study. Calcuations of the coefficient of failure fomr the regressions lines were study 404:metformin 0.291; pioglitazone 0.057; and in study 405 glicazide 0.853: and pioglitazone 0.250
Conclusions: The coefficient of failure values obtained suggest that pioglitazone may have a more durable effect than either metformin or gliclazide in this group of patients. This apparent loss of effect of gliclazide over time may be due to the over stimulation and subsequent exhaustion of the beta-cell by sulphonylurea. After one-years therapy, the glycaemic control between the treatments was similar but this method may identify longer-term trends in control that may have implications for the management of type 2 diabetes and the potential value of an insulin sensitizer over conventional therapy.

Coefficient of Failure (% HbA1c/yr) for Pioglitazone, Metformin and Gliclazide as Monotherapy

Treatment	Pioglitazone	Metformin	Metformin	Glicazide
Coefficient of Failure (%HbA_{1c}/yr)	0.057	0.291	0.250	0.853

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Effects of pioglitazone versus fenofibrate treatment on glucose and fat metabolism in patients with Type 2 diabetes.

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Background and Aims: To compare the effect of pioglitazone (PIO) and fenofibrate (FENO) on glucose and fat metabolism, we studied 7 type 2 diabetic patients (age = 54±3 y, BMI = 30.0±0.9 kg/m^2, HbA_{1c} = 8.8±0.8%) before and after FENO treatment (200 mg/d) and 7 type 2 diabetic patients (age = 51±5 y, BMI = 31.9±2.1 kg/m^2, HbA_{1c} = 9.0±0.6%) before and after PIO treatment (45mg/d).
Materials and Methods: Subjects received a 75g OGTT and a 4h euglycemic insulin (80 mU/m^2 per min) clamp to determine insulin sensitivity (M value) before and after 3 months of PIO and FENO treatment.
Results: Following PIO, the fasting plasma glucose (207±23 to 137±9 mg/dl, p<0.05), mean plasma glucose during the OGTT (305±24 to 238±12 mg/dl, p<0.05) and HbA_{1c} (9.0±0.6 to 7.7±0.4%, p<0.01) decreased, while insulin sensitivity increased from 4.3±0.7 to 7.3±0.8 mg/kg-min (p<0.005) despite increased body weight (83.6±6.4 to 87.2±7.2 kg, p<0.01). Fasting plasma glucose (199±25 to 197±27 mg/dl), mean plasma glucose during the OGTT (273±22 to 287±18 mg/dl), HbA_{1c} (8.8±0.9 to 8.9±0.7%) and insulin sensitivity (4.7±1.0 to 5.2±1.0 mg/kg-min) did not change significantly after FENO treatment. PIO significantly reduced fasting plasma FFA (763±100 to 580±41 µM, p<0.05) and the mean plasma FFA concentration during the OGTT (586±59 to 375±21 µM, p<0.01); fasting plasma triglyceride concentration also decreased significantly (188±25 to 143±20 mg/dl, p<0.05). Following FENO treatment, fasting plasma FFA (718±51 to 812±109 µM) and the mean plasma FFA concentration during the OGTT (495±47 to 460±58 µM) did not change significantly; fasting plasma triglyceride concentration decreased (190±19 to 136±21 mg/dl, p<0.05) significantly.
Conclusion: Pioglitazone, a PPAR-gamma agonist, increases insulin sensitivity, reduces fasting plasma triglyceride concentration, improves glycemic control, and reverses the abnormalities in glucose and fat metabolism in patients with type 2 diabetes. In contrast, treatment with a PPAR-alpha agonist i.e. fenofibrate, does not significantly improve insulin sensitivity, glycemic control or reduce the elevated plasma FFA concentration in patients with type 2 diabetes despite similarly reducing plasma triglyceride concentration.

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Improved glycaemic control in individuals with Type 2 diabetes when treated with rosiglitazone plus 7.5 mg glibenclamide compared to increasing the glibenclamide dose to 15 mg.

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Background and Aims: This study evaluated the effect of adding rosiglitazone (RSG) to glibenclamide (GLB) on HbA_{1c} in individuals with type 2 diabetes mellitus (T2DM) when compared with uptitrating the GLB dose.
Materials and Methods: At baseline, all patients were receiving GLB 7.5 mg/day. Patients were randomised to receive RSG 4 mg bd plus GLB 7.5 mg/day, or had their GLB dose uptitrated to a maximum of 15 mg/day, for 26 weeks.
Results: At baseline, all patients had equivalent HbA_{1c} and fasting plasma glucose (FPG) values. The combination of RSG plus GLB produced significantly greater decreases in HbA_{1c} and FPG than uptitrating GLB (P < 0.0001 in both cases). The number of patients with hypoglycaemia and oedema was 18.5% and 9.5%, respectively, in the RSG + GLB group (n = 168) and 4.1% and 2.9%, respectively, in the GLB group (n = 172). Six patients (3.6%) in the RSG + GLB group were withdrawn due to hypoglycaemia, and three patients (1.8%) were withdrawn due to oedema. There was one withdrawal due to a serious adverse event in the RSG + GLB group, and none in the uptitrated GLB group.

	Treatment group RSG + GLB	Uptitrated GLB
HbA_{1c} (%)	n = 160	n = 154
Mean baseline	7.9	8.0
Mean Δ from baseline ± SD	-0.91* ± 0.99	-0.14** ± 0.92
Difference from uptitrated GLB	-0.81*	NA
% with reduction ≥ 0.7%	61.3%	24.0%
FPG (mmol/l)	n = 165	n = 170
Mean baseline	9.38	9.61
Mean Δ from baseline ± SD	-2.15* ± 2.20	+0.18 ± 2.32
Difference from uptitrated GLB	-2.43*	NA
% with reduction ≥ 1.67 mmol/l	55.2%	14.1%

*P < 0.0001, **P = 0.0532, P = 0.3023

Conclusion: The addition of rosiglitazone (4 mg twice daily) to glibenclamide (7.5 mg/day) is safe and well-tolerated and is significantly more effective at improving glycaemia than increasing the dose of GLB monotherapy to 15 mg/day.

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Benefits beyond glycaemia of adding rosiglitazone rather than glibenclamide to metformin monotherapy in Type 2 diabetes mellitus.

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Background and Aims: The UKPDS has demonstrated that there is progressive loss of glucose control on monotherapy within 3 years of diagnosis of type 2 diabetes mellitus (T2DM). Subsequently, combination therapy is often employed to maintain therapeutic effect and reduce side effects. Rosiglitazone (RSG) and metformin (MET) lower plasma glucose concentrations by different mechanisms of action, and when used in combination may offer an advantage in control of glycaemia over other agents.
Materials and Methods: In this multicentre, randomised, double-blind study, the effects of two different glucose-lowering agents on cognitive function, efficacy and safety in T2DM were evaluated. As cognitive function was the primary objective of this study (data not presented), equivalence of glycaemia was required. Patients on MET combination therapy were kept on the same dose of MET, while the second oral anti-diabetic medication was washed-out for 8 weeks. Patients remained on background MET and were randomly assigned to receive either RSG (n = 69) or glibenclamide (GLB) (n = 72) for 24 weeks. If fasting plasma glucose (FPG) ≥ 7.77 mmol/l (140 mg/dl), the dose of blinded medication was uptitrated. RSG was initiated at 4 mg od and could be titrated up to 4 mg bd. GLB was initiated at 2.5 mg od and could be titrated up to 7.5 mg bd.
Results: Baseline FPG in both treatment groups was similar, 9.88 ± 0.37 mmol/l (178 ± 6.6 mg/dl) (MET + RSG) and 9.66 ± 0.31 mmol/l (174 ± 5.5 mg/dl) (MET + GLB). At the end of treatment, MET + RSG and MET + GLB showed similar reductions in FPG of 2.12 ± 0.30 mmol/l (38.2 ± 5.4 mg/dl) and 2.31 ± 0.29 mmol/l (41.6 ± 5.2 mg/dl), respectively. As insulin resistance is considered an important risk factor for cardiovascular disease, improving insulin sensitivity is thought to provide cardiovascular benefit. Insulin sensitivity as measured by HOMA-S increased by a median of 10.4%S in the MET + RSG group compared to a median decline of 2.6%S in the MET + GLB group. Episodes of hypoglycaemia were ~4.5 fold more common in the MET + GLB group (13.3%), compared to the MET + RSG group (2.9%). Gastrointestinal adverse experiences were more frequently reported in the MET + GLB group compared to the MET + RSG group, 25.3% vs. 14.3%. Diarrhoea was reported in 8.0% of patients in the MET + GLB group compared to 1.4% in the MET + RSG group. No significant difference in weight gain after 24 weeks of treatment was observed between MET + GLB (1.5 ± 0.4 kg) and MET + RSG (1.0 ± 0.4 kg).
Conclusions: The addition of RSG to MET effectively lowered FPG, improved insulin sensitivity, and was associated with fewer episodes of hypoglycaemia and gastrointestinal related adverse experiences when compared to MET + GLB in patients with T2DM who were previously on other metformin combination therapies.

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Antiatherogenic effect of thiazolidinediones in Type 2 diabetic patients, irrespective of the responsiveness to its antidiabetic effect.
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Background and Aims: Thiazolidinediones (TZDs), a class of insulin-sensitizing agents used clinically to treat type 2 diabetes, are also antiatherogenic. Studies with cultured cells in vitro and those with animal models in vivo showed that the antiatherogenic effect of TZDs is mediated through their direct action on the vasculature. This study was designed to elucidate the relationship between the antiatherogenic and antidiabetic effects of pioglitazone, a TZD, in type 2 diabetic patients.
Materials and Methods: A total of 106 Japanese type 2 diabetic patients (49 men and 57 women, mean age 59.9 years) were included and divided into two groups; the pioglitazone-treated group (30 mg daily for 3 months) (n = 70) and untreated control group (n = 36). Prior to this study, the 42 patients in the treatment groups and 16 in the control group had been treated with sulfonylureas, which were continued at fixed dosages throughout this study.
Results: A total of 106 Japanese type 2 diabetic patients (49 men and 57 women, mean age 59.9 years) were included and divided into two groups; the pioglitazone-treated group (30 mg daily for 3 months) (n = 70) and untreated control group (n = 36). Prior to this study, the 42 patients in the treatment groups and 16 in the control group had been treated with sulfonylureas, which were continued at fixed dosages throughout this study. Overall, the pioglitazone treatment significantly reduced hyperglycemia, hyperinsulinemia, glycosylated hemoglobin (HbA1c) levels, and increased plasma adiponectin concentrations relative to the control group ($P < 0.01$). It also significantly decreased plasma high-sensitivity C-reactive protein (CRP) levels, a marker of inflammation, and pulse-wave velocity (PWV), a direct measure of arterial distensibility ($P < 0.01$). The antiatherogenic effect was observed in both the nonresponders showing less than 1% of reduction in HbA1c (n = 30) and responders showing more than 1% of reduction (n = 40). Analysis of covariance revealed that treatment with pioglitazone was associated with a low CRP and PWV, independent of the changes in parameters related to glucose metabolism, *i.e.* FPG, IRI, HbA1c, and HOMA-IR.
Conclusion: This study represents the first demonstration of the antiatherogenic effect of pioglitazone in both nonresponders and responders with respect to its antidiabetic effect, and suggests that pioglitazone can exert its antiatherogenic effect independently of its antidiabetic effect.

PS 65
PPAR Agonists

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Short term treatment with pioglitazone improves glucose tolerance in Type 2 diabetes by improving suppression of endogenous glucose production.
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Background and Aims: The mechanisms by which pioglitazone (PIO) improves oral glucose tolerance were investigated in 12 diet/sulfonylurea treated T2DM (age=54±8 y, BMI=30.6±2.8 kg/m^2; HbA1c=9±2.6%).
Materials and Methods: Tissue glucose disposal (Rd) and endogenous glucose production (EGP) were quantitated before and after 12 wk of PIO (45 mg/day) using the double tracer (oral 1-14C-glu/IV 3-3H-glu) OGTT (75 g) technique.
Results: PIO decreased fasting plasma glucose (9.4 to 7.4 mM, p<0.01) and HbA1c (9.0 to 7.3%, p<0.01) despite increased body weight (82 to 86 kg, p<0.01). Following PIO, fasting plasma insulin (10±1 to 8 ±1 uU/ml) and mean plasma insulin during OGTT (19±4 to 14±4 uU/ml, p<0.05) decreased, while the insulinogenic index (DI/DG) during the OGTT increased slightly (16±4 to 22±3, p=NS). Basal EGP decreased (2.6±0.2 to 2.2±0.3 mg/kg*min), while the basal hepatic insulin resistance index (EGPxFPI) declined (16±3 to 12±2) after PIO (P<0.10). Suppression of EGP during OGTT decreased from 0.75±0.14 to 0.37±0.10 mg/kg*min (P<0.05); Rd rose slightly (29 to 32 mg/kg*min), while the glucose clearance (1.5 to 1.6 ml/kg*min) and tissue insulin sensitivity index (Rd glu/mean PI) rose (70 to 81, p<0.01) during the OGTT. No difference was found in exogenous glucose appearance rate after PIO (688 vs. 652 mg/kg*min). This study represents the first evaluation of the mechanisms via which PIO improves oral glucose tolerance.
Conclusions: PIO improves OGTT by augmenting tissue glucose uptake and enhancing suppression of endogenous (hepatic) glucose production.

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Effects of pioglitazone versus diet and exercise on free fatty acid clearance.
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Background: Suppression of lipolysis, and thereby free fatty acid (FFA) concentrations by insulin plays an important role in modulating insulin's effects on glucose metabolism. With insulin resistance increased FFA are thought to play a central role in the metabolic defects. Thiazolidinediones (TZD) improve insulin sensitivity, but their effect on FFA is unknown. We compared the effects of pioglitazone (PIO) with classical insulin sensitization (diet and exercise; Diet/ex) on FFA kinetics in insulin resistant upper body obese (UBOb) adults.
Methods: 39 UBOb volunteers (28-36 kg/m^2) underwent: an intravenous glucose tolerance test to measure insulin sensitivity (Si) using Bergman's minimal model; an FFA turnover study ([9,10-^{3}H]oleate) during either a saline infusion (n=20) or during a hyperinsulinemic (1.0 mU/kg FFM/min), euglycemic clamp (n=19). Subjects were randomized to receive PIO 30 mg daily (18 ± 0.4 weeks) or Diet/ex (20 ± 0.6 weeks), after which all studies were repeated.
Results: The Diet/ex group lost 11.7 ± 3.2 kg and the PIO group gained 2.2 ± 1.1 kg. Si improved (P< 0.05) with both Diet/ex (5.3 ± 1.0 to 10.3 ± 1.9) and PIO (4.2 ± 0.6 to 6.6 ± 1.1). FFA (oleate) kinetic data are provided in the table. Oleate kinetics were unchanged in saline studies, but during the insulin clamp concentrations were lower after treatment. In the Diet/ex group greater suppression of oleate release by insulin was seen after treatment. In the PIO group oleate clearance increased during the insulin clamp to account for the lower concentrations.
Conclusion: Diet/ex enhances insulin sensitization of lipolysis resulting in lower FFA concentrations during hyperinsulinemia. In contrast, PIO does not enhance suppression of lipolysis but increases FFA clearance, thereby lowering FFA concentrations during hyperinsulinemia. This is a potentially novel mechanism that may contribute to the improved Si seen with TZD's.
*p<0.05 cf pre-treatment values

	CLAMP Pre	Post	SALINE Pre	Post
Oleate (umol/l)				
Diet/ex	24 ± 5	11 ± 2*	238 ± 10	239 ± 12
PIO	35 ± 15	20 ± 4	230 ± 18	230 ± 14
Oleate flux(umol/min)				
Diet/ex	39 ± 6	21 ± 5*	209 ± 14	188 ± 9
PIO	45 ± 11	43 ± 7	189 ± 19	210 ± 21
Oleate clearance(l/min)				
Diet/ex	1.8 ± 0.2	1.9 ± 0.2	0.9 ± 0.1	0.8 ± 0.05
PIO	**1.7 ± 0.2**	**2.4 ± 0.2***	0.8 ± 0.1	0.9 ± 0.11

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A comparison of the effect of pioglitazone and gliclazide monotherapy onlLipid profiles in drug-naïve patients with Type II diabetes.

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Background: Lipid abnormalities especially high triglycerides, low HDL and moderately elevated LDL, and cardiovascular comorbidity and mortality are more prevalent in type 2 diabetes. Improving lipid levels in order to achieve target values is important in the overall management of these patients

Materials and Methods: A total of 1270 patients with type 2 diabetes were randomized in a European parallel-group, double-dummy, double-blind study. Patients with poorly controlled type 2 diabetes mellitus (HbA_{1c} 7.5–11%) received either pioglitazone up to 45 mg once daily or gliclazide 160 mg up to two times daily.

Results: Lipid parameters were measured at intervals during the study. Mean triglycerides were 17% lower than baseline in the pioglitazone group compared with 14% in the gliclazide group; HDL-cholesterol increased by 20% in the pioglitazone group compared with 6% in the gliclazide group; LDL-cholesterol increased by 3% in the pioglitazone group compared with a 5% decrease in the gliclazide group; and total cholesterol increased by 4% in the pioglitazone group compared with a 5% decrease in the gliclazide group. At Week 52, the mean total cholesterol/HDL-cholesterol ratio was higher in both treatment groups (pioglitazone: 14%; gliclazide: 10%), and the improvement observed with pioglitazone was significantly greater on the key lipid disturbances in diabetic patients than that with gliclazide. Decreases from baseline levels of free fatty acids were also more pronounced in the pioglitazone group (0.13 mmol/L) compared with the gliclazide group (0.03 mmol/L) (mean treatment difference –0.09 [95% CI: -0.12, -0.06 mmol/L]; P<0.001).

Conclusions: Pioglitazone reduced triglyceride, which may indicate a potent anti-atherogenic effect, and HDL-C increases were three times greater than with gliclazide. LDL-cholesterol was slightly increased with pioglitazone treatment. Although data suggest that increases in LDL-C correlate with cardiovascular risk, the exact nature of the relationship is uncertain in patients with diabetes where compositional differences in LDL-cholesterol may be more important. Pioglitazone treatment produced greater reductions in total cholesterol/HDL-cholesterol ratio than gliclazide. Both pioglitazone and gliclazide reduced free fatty acid levels, decreases were three times greater with pioglitazone. Reduction of free fatty acid levels is associated with decreases in insulin resistance, a recognized cardiovascular risk marker. The overall change in lipid profile support the concept that pioglitazone improves diabetic dyslipidemia and may reduce the risk of cardiovascular disease

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Results of liver safety testing in 3713 Type 2 diabetic patients treated for one year in double blind controlled trials with pioglitazone, metformin or gliclazide.

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Background: Type 2 diabetic patients are more likely than non-diabetic patients to show abnormalities in liver tests. They can also suffer from more severe liver pathology, including non-alcoholic steatohepatitis (NASH). The aetiology of NASH has been suggested to reside in the increase in hepatic insulin resistance seen in type 2 diabetics and drugs such as thiazolidinediones, which reduce insulin resistance, have been reported to be decrease liver fat.

Material and Methods: Two monthly liver testing was performed as part of four European randomised double-blind trials comparing treatment of type 2 diabetes, with a pioglitazone (pio), metformin (met) and gliclazide (glic) over one year. Two trials used monotherapy treatments (pio vs met and pio vs glic) and two combination therapies (pio + met vs glic + met and pio + sulphonylurea vs met + sulphonylurea). Patients with known liver disease or with ALT levels 3 ULN were excluded from participation.

Results: Mean HbA_{1c} was 8.7% at baseline and was reduced similarly with pio and non-pio treatments. HOMA-S analyses showed insulin sensitivity increased 9-15% with pio, about 5% with met and decreased by about 7% with glic. Pio resulted in reduction of liver enzymes compared to non-pio treatments. These results are also reflected in the number of patients showing large increases in any liver test at any time during treatment. In comparator groups met treatment resulted in some small reductions of mean levels of liver enzymes, whereas with glic treatment no changes or small increases were seen.

Conclusions: The results suggest that reduction of insulin resistance with pioglitazone does indeed lead to improvement of liver function.

Change in Liver Enzymes

		AST	ALT	GGT	Alkaline Phos.
Pioglitazone	Mean Change	-3%	-17%	-18%	-12%
	% patients >3ULN	0.6%	0.9%	3.5%	0%
Non-Pioglitazone	Mean Change	2%	4%	-1%	-4%
	% patients >3ULN	0.5%	1.5%	4.4%	0%

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Cardiac safety of pioglitazone in comparison with metformin and gliclazide.

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Background: Patients with type 2 diabetes are at risk of increased cardiovascular morbidity compared to non-diabetic patients. It is therefore of vital importance to assess and compare effects of different drugs to treat type-2 diabetes, not only on metabolic control but also on cardiovascular outcomes. This is particularly the case for new classes of agents such as the thiazolidinediones.

Aims and Methods: Fatalities and hospitalisations for cardiovascular events reported from four large double-blind, randomised, active comparator controlled European trials in over 3700 patients have been combined to compare cardiovascular profiles of 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. Although patients with recent MI or stroke were excluded, about 15% of patients had known cardiac disease and about 50% were reported to be hypertensive. Pioglitazone treatment was not associated with any excess of cardiovascular morbidity compared to non-pioglitazone treatment and cardiovascular mortality rates were higher in non-pioglitazone treated patients. Deaths from all causes occurred in 7 pioglitazone patients and 10 non-pioglitazone patients. There was no evidence of heart failure associated with pioglitazone treatment. Reports of all heart failures (hospitalised and non-hospitalised) occurred in 12/1857 pioglitazone patients and 10/1856 patients randomised to metformin or gliclazide. Studies of longer duration in at risk patients such as the ProActive trial- a cardiovascular outcome study in type II diabetes- will determine whether over longer periods treatment with pioglitazone confers further protection against cardiovascular complications of type 2 diabetes.

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Rosiglitazone is effective and safe in daily practice.

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Background and Aims: Evaluation of efficacy and safety of rosiglitazone (RSG) when used in daily practice in Germany.

Materials and Methods: 22,808 patients newly initiated on RSG were enrolled from July 2000 until November 2001 in a non-interventional, quality controlled observational study (AOCS = Audited Observational Cohort Study). The mean observational period per patient was 6 months, giving a total of 11,131 patient-years for the whole study.
Results: The patients represented a typical, insufficiently controlled type 2 diabetic population: age 62 years, diabetes duration 5 years, male:female 1:1, BMI 29 kg/m^2, HbA_{1c} 8.2%, fasting blood glucose (FBG) 9.9 mmol/l (179 mg/dl) (all data are medians). During the observational period, the median HbA_{1c} was significantly reduced by 1.3% ($P = 0.0001$) and FBG by 2.8 mmol/l (50 mg/dl) ($P = 0.0001$), there were no relevant differences in glycaemic efficacy between patients treated with RSG + metformin (MET) and RSG 4 mg + sulphonylurea (SU). Overall, at the end of the observational period an HbA_{1c} responder rate (defined as a reduction in HbA_{1c} of > 0.7%) of 72.0% and a FBG responder rate (defined as a reduction in FBG of > 1.67 mmol/l [30 mg/dl]) of 67.6% was reached. The percentage of patients with an $HbA_{1c} \leq 6.5\%$, $\leq 7.0\%$ or $\leq 7.5\%$ increased from 6%, 14% and 27% at the beginning of the observational period to 34%, 56% and 72% at the end, respectively. Mean blood pressure was reduced from 144/84 to 138/82 mmHg ($P = 0.0001$). Mean body weight was reduced by 1 kg ($P = 0.0001$) from 84.9 kg to 83.9 kg, (RSG + MET: 87.2 to 85.8 kg; RSG 4 mg + SU: 80.7 to 80.0 kg). Overall, RSG was safe and well tolerated. Adverse experiences (AEs) were reported in 3.1% of the patients, serious AEs in 1.1% of the patients.
Conclusions: The results of this observational study show that the therapy with rosiglitazone in daily practice is effective, safe and well tolerated.

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The PPARα/γ agonist tesaglitazar inhibits fatty acid-induced changes of smooth muscle cells proteoglycans: a potential anti-atherogenic effect.

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Background and Aims: The dyslipidaemia of insulin resistance and type 2 diabetes exposes arterial cells to high levels of VLDL, small LDL and albumin-bound fatty acids (NEFA). This dyslipidaemia is a strong risk factor for cardiovascular disease. PPAR agonists show anti-atherosclerotic effects in mouse models by mechanisms that remain to be established. Entrapment of LDL by proteoglycans (PGs) of the arterial intima that are secreted by smooth muscle cells (SMC) appears to be a critical initial step for their accumulation at lesion-prone sites. Versican, a PG rich in glycosaminoglycans (GAGs) containing chondroitin sulphate (CS), is the most prominent of those binding LDL. It has been proposed that, in diabetes, changes in GAG composition of major arteries may contribute to atherogenesis. Furthermore, we showed previously that NEFA can modulate matrix PGs in SMC and speculated that constant exposure to NEFA could be one of the causes contributing to alteration of arterial cells in individuals with insulin resistance and/or diabetes. Here we explored whether tesaglitazar, a dual PPARα/γ agonist in clinical development, could block alterations induced by linoleate in the GAGs of the matrix of human arterial SMC. We also evaluated whether tesaglitazar might alter the affinity of LDL for the secreted GAGs.
Materials and Methods: ^{35}S-GAGs were isolated from human arterial SMC cultured with 10 nM insulin that were exposed for 48 hours to 100 and 300 mM of albumin-bound linoleate (alb-LO). Expression of genes was measured by quantitative PCR and binding of human LDL was measured by electrophoretic band shift.
Results: Alb-LO treatment increased the CS/heparan sulphate (HS) ratio of secreted PGs by 30-40% ($p<0.02$). These changes were reflected in the expression levels of the genes for the core proteins of CS and heparan PGs. The changes in the CS/HS ratio caused by alb-LO were abolished by 5 and 10 mM tesaglitazar. In addition, tesaglitazar decreased 3-4 fold ($p<0.05$) the affinity (Kd) of human LDL for the isolated GAGs when compared to GAGs synthesized in the presence of alb-LO. BIM I, a protein kinase C (PKC) inhibitor, at 10 mM also reverted the alb-LO provoked GAGs alteration.
Conclusion: NEFA increase secreted CS-PGs, for which LDL has higher affinity. NEFA probably do this by activation of PKC, which in turn opposes insulin action. Tesaglitazar has an inhibitory effect on the changes induced by overexposure of arterial smooth muscle to NEFA. If present in vivo, this inhibition may form part of the potential anti-atherogenic effect of tesaglitazar and other PPAR agonists.

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The pharmacokinetics of tesaglitazar (GALIDA™) in healthy male subjects.

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Background and Aims: Tesaglitazar (GALIDA™) is an enantiomer-pure novel PPARα/γ agonist that is being developed to target insulin resistance-related glucose and lipid abnormalities associated with type 2 diabetes and metabolic syndrome. This two-period, open-label crossover study (SH-SBC-0002) evaluated the pharmacokinetics of tesaglitazar in healthy males.
Materials and Methods: Eight subjects received a single oral (1 mg) or iv (1 mg) dose of ^{14}C-tesaglitazar (1st dose) and tesaglitazar (2nd dose) in randomised order. Serial blood samples and complete urine and faeces collections were taken up to 336 h post-dose. Radioactivity in plasma, urine and faeces was measured by liquid scintillation counting. Tesaglitazar in the plasma and urine was determined using reverse-phase liquid chromatography and mass spectrometry. The enantiomer of tesaglitazar was analysed by normal-phase liquid chromatography on a chiral column and mass spectrometry.
Results: Absorption of tesaglitazar was rapid, Cmax at ~1 h post-dose, and the absolute bioavailability was ~100%. Mean plasma clearance was 0.16 L/h and the volume of distribution at steady state was 9.1 L. After either dose the plasma concentration-time profiles of radioactivity and tesaglitazar were virtually identical, indicating low systemic metabolite concentrations and that the elimination of the metabolite(s) was formation rate limited. The half-lives of both radioactivity and tesaglitazar were ~45 hours. The recovery of radioactivity was quantitative in all subjects: 99.9% (i.v.) and 99.6% (oral). With both regimens, 20% of the dose was recovered unchanged in urine, resulting in a renal clearance of 0.030 L/h. The remainder of the radioactivity recovered in the urine, ~70% of the dose, was identified as the glucuronic conjugate of tesaglitazar. Both i.v. and oral doses were well tolerated. There was no indication of partial inversion or metabolism of the S-enantiomer to the R-form. Phase I metabolism was not detected, which suggests that tesaglitazar has a low potential for clinically significant drug-drug interaction by inhibition of CYP450.
Conclusion: Tesaglitazar has a favourable pharmacokinetic profile, including complete bioavailability, low plasma clearance and metabolism by glucuronidation. This favourable PK profile will allow once-daily administration of tesaglitazar, thereby potentially aiding patient compliance.

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A PPAR-alpha activator, K-111, improves skeletal muscle insulin sensitivity in obese rhesus monkeys.

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Background and Aims: A selective activator of peroxisome proliferator activated receptor alpha (agent K-111, Kowa Company, Ltd. Tokyo, Japan), improves whole-body insulin sensitivity as determined by the euglycemic hyperinsulinemic clamp method in obese, insulin-resistant rhesus monkeys. Our purpose was to determine whether improvement in whole-body insulin sensitivity was related to changes in glycogen and triglyceride storage in skeletal muscle.
Materials and Methods: We obtained ex-situ freeze-clamped vastus lateralis muscle samples before (basal) and during (insulin-stimulated) a euglycemic hyperinsulincemic clamp after vehicle (baseline) and again after 6-8 weeks of dosing (3 mg/kg body weight/day) in 6 obese insulin-resistant rhesus monkeys.
Results: K-111 treatment resulted in enhanced action of insulin during a euglycemic hyperinsulinemic clamp to increase glycogen synthase total activity (GST) (basal vs. insulin-stimulated GST: 33.3 ± 6.4 vs. 50.1 ± 10.5 nmol/min/mg protein, $p<0.05$). An increase in GST in response to insulin during vehicle was not observed (basal vs. insulin-stimulated GST: 37.1 ± 7.0 vs. 33.0 ± 4.8 nmol/min/mg protein). Glycogen content increased during K-111 treatment clamp (basal vs. insulin-stimulated glycogen: 0.52 ± 0.14 vs. 0.81 ± 0.21 µmol/mg protein, $p=0.10$, p=ns) but not during vehicle clamp (basal vs. insulin-stimulated glycogen: 0.89 ± 0.27 vs. 0.65 ± 0.19 µmol/mg protein). With K-111 treatment, the effect of insulin (corrected for basal) on GST activity was positively correlated to the effect of insulin on glycogen content ($r=0.95$, $p<0.005$). In addition, during K-111 treatment, triglyceride content decreased significantly during the clamp (basal vs. insulin-stimulated: 35.0 ± 9.1 vs. 16.6 ± 3.2 nmol/mg dry weight, $p<0.05$) and the effect of insulin to lower triglyceride content during the K-

111 treatment clamp was significantly greater than during vehicle clamp (vehicle vs. K111 treatment: -0.08 ± 0.17 vs. –0.44 ± 0.09 nmol/mg dry weight, p<0.05).
Conclusions: We conclude that the PPAR-alpha activator, K-111, which improves whole-body insulin sensitivity, also improves skeletal muscle insulin sensitivity as determined by an increase in glycogen synthase activity and by triglyceride content lowering during a euglycemic hyperinsulinemic clamp in rhesus monkeys.

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Improvement of the metabolic syndrome by a PPAR-alpha activator, K-111, through mitigation of insulin resistance and dyslipidemia rather than improved glucose tolerance and ß-cell hyperresponsiveness.
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Background and Aims: The peroxisome proliferator activated receptors (PPARs) belong to the family of ligand-activated nuclear receptors involved in regulation of lipid and carbohydrate metabolism with direct relevance for metabolic diseases including obesity, the metabolic syndrome, and Type 2 diabetes mellitus.
Materials and Methods: Obese, insulin-resistant rhesus monkeys (n=6) with the metabolic syndrome were administered orally vehicle and then K-111 (Kowa Co. Ltd., Tokyo, Japan), a PPAR-alpha specific activator (3 mg/kg/day p.o. for 7 weeks). Fasting glucose and insulin concentrations, lipid fractions, intravenous glucose tolerance tests, and euglycemic hyperinsulinemic clamps were carried out at vehicle and again at the end of 7 weeks of dosing.
Results: Results showed significantly decreased hyperinsulinemia (vehicle vs. K-111 mean±SE: 249±75 vs. 163±33 µU/min; p=0.02) and concurrent increased peripheral glucose uptake rate (6.4±0.9 vs. 7.8±1.6 mg/kgFFM/min; p=0.07) consistent with improved insulin sensitivity. Plasma triglycerides were significantly decreased (287±92 vs. 159±43 mg/dl; p=0.03) with significantly reduced large VLDL fractions (V5+V6; p=0.06) as determined by NMR; additionally, HDL cholesterol subclasses (H3+H4+H5) were significantly increased (52±9 vs. 71±8 mg/dl); p=0.02). Glucose tolerance and ß-cell acute insulin response to glucose as measured by intravenous glucose tolerance testing were not significantly improved during K-111 administration (p's=0.2).
Conclusions: We suggest that ß-cell failure and impaired glucose tolerance are significantly less responsive to the actions of a PPAR-alpha activator. The major therapeutic effects of K-111 include its antidiabetic and lipid-modulating effects, primarily evidenced by mitigation of hyperinsulinemia, improvement in insulin sensitivity and substantial improvement in dyslipidemia.

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Stevioside in combination with soy-based dietary supplement exerts a beneficial effect on Type 2 diabetic GK-rats – multifactorial treatment of Type 2 diabetes and the metabolic syndrome.
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Background and Aims: The Stevia rebaudiana Bertoni (SrB) plant has been used in traditional medicine by the Guarani Indians in Paraguay and Brazil in the treatment of diabetes. We have recently demonstrated that stevioside, a diterpene glycoside isolated from the plant SrB, possesses insulinotropic, glucagonostatic, anti-hyperglycaemic and anti-hypertensive effects in animal studies. We have also found that a dietary supplement of soy protein, isoflavones, and cotyledon fibre (Abalon®) has beneficial effects on cardiovascular risk markers in type 2 diabetes.To investigate if the combination of stevioside and a dietary supplement of soy protein possesses beneficial qualities in the treatment of type 2 diabetes and the metabolic syndrome.
Materials and Methods: Diabetic GK rats were fed for 4 weeks with 4 different test diets. A) Standard carbohydrate rich lab diet (Chow), B) Chow + stevioside (0.03 g/kg BW/day), C) 80 % soy (Abalon®) + 20 % Chow (adjusted for vitamins and minerals) and D) 80 % soy (Abalon®) + 20 % Chow + stevioside 0.03 g/kg BW/day. An intra-arterial catheter was inserted in the carotic artery of rats after 3 weeks and at week 4 the conscious rats underwent an intra-arterial glucose tolerance test (GTT) (2.0 g/kg BW).
Results: **Stevioside** exerts beneficial effects in the mild type 2 diabetic GK rat i.e.: **1)** lowers blood glucose (incremental area under the glucose curve (IAUGC)) (group A vs. B a 31 % reduction and group C vs. D a 86 % reduction, p<0.00005), respectively; **2)** Increase of the first phase insulin secretion (IAUIC) (0-30 min): (group A vs. B a 80 % increase and C vs. D a 163 % increase; p<0.003), respectively; **3)** Suppresses glucagon (IAUGC): (group A vs. B by 28% and group C vs. D by 49 %, p< 0.0004), respectively; **4)** After 2 weeks of treatment with stevioside a 10 % suppression of the systolic blood pressure was observed (p<0.0002). **Abalon®** had a beneficial effects on CV risk markers i.e : **1)** Lowers total-cholesterol (group A vs. C by 15%, p<0.0043); **2)** Reduces Triglycerides (group A vs. C by 47%, p< 0.0028); **3)** Reduces FFA : (group A vs. C by 13 %, p<0.02) .
Conclusion: The combination of stevioside and Abalon® appears to possess the potential as effective treatment of a number of the characteristic features of the metabolic syndrome i.e. hyperglycaemia, hypertension and dyslipidaemia. However a long-term proof of concept study in type 2 diabetic subjects is needed to verify these promising results.

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A new herbal combination (Hyponidd) in the management of Type 2 diabetes.
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Background and Aims: There is a growing interest in the use of traditional medications in the therapy of type 2 diabetes. This study was carried out to ascertain the benefits of combining several herbal ingredients (which are individually used for diabetes and dyslipidemia) on glycemic and lipid profiles of type 2 diabetic subjects uncontrolled on oral sulfonylurea with or without metformin.
Materials and Methods: In a double blind placebo controlled study, one hundred type 2 diabetic subjects unresponsive to maximal doses of sulfonylurea with or without metformin were randominzed to receive either drug (n=50) or placebo (n=50). Subjects on insulin therapy were excluded . The patients of both groups contunued their oral drugs throughout the study. HbA1c was measured at baseline, 90 days and 180 days. Plasma glucose and serum lipid profiles were assesed on a monthly basis.
Results: Over a 6 month follow up period, drug therapy resulted in reduction in the fasting and postprandial plasma glucose levels as well as HbA1c as compared to placebo at each visit. There was also a significant (p<0.05) improvement in the lipid paramenters (decreased LDL cholesterol and triglyceride and a rise in HDL cholesterol; see table) when compared to placebo at each visit. The drug was well tolerated, and no significant

change in weight occured in both groups. At the end of 6 months, the plasma glucose (fasting and postprandial) and HbA1c in the treatment group decreased from 202.1± 47 to 101.0±16 mg/dL, 305.2±54 to 170.8±24 mg/dL and 8.25 to 6.88% respectively as compared to no significant changes in the placebo group. There was a significant ($p<0.05$) fall in the serum fasting and postprandial insulin levels in the treatment group, while values in the placebo group did not change significantly.
Conclusion: This study demonstrates the benefits of a herbal combination "Hyponidd" as an adjunctive to oral sulfonylurea and/or metformin therapy in type 2 diabetes. The combination of several drugs was found to be safe and effective in correcting hyperglycemia and dyslipidemia, Therapy did not result in weight gain, and the insulin level too was favourably reduced. Further studies are needed to establish it's use as a first line agents, as well as in identifying the active principle in the combination, as well as it's mechanism of action.

Table.Effects of the drug on lipid parameters (* p< 0.05 vs Baseline)

mg/dlmean (sd)	Drug Group Baseline	Drug Group 6 months	Placebo group Baseline	Placebo group 6 months
Triglycerides	219.9 (67)	162.9 (31)*	199.5(79)	202.1(74)
LDL Cholesterol	152.8(43)	137.1(37)*	124.9(41)	118.6(38)
HDL Cholesterol	45.3(20)	55.0(26)*	50.6(25)	53.7(35)

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The effect of sibutramine on insulin secretion and insulin resistance in obese Type 2 diabetic patients.
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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, glycaemic control, insulin secretion and insulin resistance in obese type 2 diabetic patients.
Materials and Methods: 53 diabetic patients, of mean age 46.2±7.4 years and mean BMI 33.9± 2.5kg/m^2 were treated with sibutramine at a mean daily dose of 12.7± 2.8mg for three months. 42 age- and BMI-matched type 2 diabetic patients only on hypocaloric diet were also followed-up for three months and served as a control group. 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. Insulin resistance was assessed by the HOMA-IR index.
Results: We have found a significant reduction in body weight in the sibutramine-treated group - 6.8%, while in the control group it was 2.9% ($p<0.001$ between the groups). This was accompanied by a significant reduction in body fat mass in the treated group ($p<0.0001$). 73.6% of sibutramine-treated patients achieved weight loss > 5%, compared to 13.1% in the control group. We have established a significant decrease in waist circumference in the treated group ($p<0.01$). HbA_{1c} decreased significantly in the sibutramine-treated patients ($p<0.05$ as compared to the control group). Insulin resistance (HOMA-IR) decreased by 21.9% in the sibutramine-treated patients (from 9.35±2.9 to 7.3± 2.4mU/L.mmol/l), which was significantly different as compared to the control group (from 8.93±3.1 to 8.31±2.9mU/L.mmol/l) ($p<0.001$). There was a significant correlation between weight loss and decrease in insulin resistance ($r=0.58$, $p<0.01$). 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 was no significant change in the SPIS and AUC for total insulin secretion during IVGTT following sibutramine therapy.
Conclusions: The results of this controlled, randomized, open-label study demonstrate that sibutramine contributes to a significant reduction in body weight, body fat mass and waist circumference and improves glycaemic control and insulin sensitivity in obese diabetic patients. There is an increase in FPIS in type 2 diabetic patients following sibutramine therapy with no change in the overall insulin secretion. Thus sibutramine appears to be an effective and well-tolerated drug in the treatment of obesity in type 2 diabetic patients.

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Orlistat increases serum adiponectin levels and decreases body weight in Type 2 obese diabetic subjects.
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Background and Aims: Lower circulation levels of adiponectin are observed in subjects with obesity and diabetes and positively associated with whole-body insulin sensitivity. The goal of this study was to examine the effects of orlistat on adiponectin levels in type 2 obese diabetes.
Materials and Methods: Thirty-three newly diagnosed type 2 obese diabetic subjects who had never accepted antihyperglycemic medications were recruited for a randomized double-blind placebo-controlled trial for 24 weeks with orlistat or placebo(360 mg/day). Sixteen nonobese control subjects participated in the study. Informed written consent was obtained from each subject. The investigations had been carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. The serum adiponectin, fasting serum insulin, biochemical measurements, oral glucose tolerance tests(OGTT), and weight and waist circumference measurements were performed before and after 12 and 24 weeks' treatment based on the caloric restriction.
Results: Fasting and postprandial serum glucose significantly decreased in the orlistat group after treatment compared with baseline. Compared with control subjects, baseline fasting insulin and insulin sensitivity (HOMA-IR) were higher (all $P=0.0001$) and serum adiponectin levels were significantly lower($P<0.001$) in the diabetic subjects. Weight loss was observed within four weeks of initiation of treatment with orlistat, and continued to reduced during 24 weeks($P<0.001$). After 24 weeks of orlistat treatment, mean body fatty mass, waist and hip circumference, and W/H were significantly reduced. Orlistat treatment caused a greater improvement in body fatty mass and waist circumference reduction than placebo($P=0.002$ and $P=0.006$, respectively). Fasting insulin and HOMA-IR were significantly improved than baseline after orlistat treatment, but remained unchanged in the placebo group. Adiponectin levels were rose uniformly in all subjects after 24 weeks with orlistat treatment ($P=0.0003$), but no significant difference detected among the placebo group. The changes of adiponectin levels after orlistat treatment were positively correlated with body weight($r=0.57$, $P=0.018$), BMI($r=0.60$, $P=0.010$), waist($r=0.55$, $P=0.023$), W/H($r=0.71$, $P=0.001$) and body fatty mass($r=-0.54$, $P=0.024$).
Conclusion: Our findings show that orlistat treatment could increase adiponectin levels and reduce body weight and fatty mass, improve insulin sensitivity in type 2 obese diabetic subjects. Measurement of adiponectin levels might serve as a convenient biomarker for drug administration and orlistat could become a new therapeutic tool in management or prevention diabetes.

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Effects of lipase inhibition on gastric emptying of, and the glycaemic and incretin responses to, an oil/aqueous drink in Type 2 diabetes.
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Background and Aims: It is now recognised that the rate of gastric emptying is a major determinant of postprandial glycaemia; there is also increasing evidence that the latter is an independent risk factor for the macrovascular complications of diabetes mellitus. Postprandial glucose and insulin levels are also influenced by the incretin (glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)) responses to a meal. The use of the lipase inhibitor, orlistat, in obese patients with type 2 diabetes results in a modest improvement in overall glycaemic control, as assessed by glycated haemoglobin, which may be attributable to weight loss. Information relating to the effects of orlistat on postprandial glycaemia is limited. The aims of this study were to determine the acute effects of orlistat on gastric emptying of a drink containing oil and glucose components, postprandial glycaemia and incretin levels in type 2 diabetes.
Materials and Methods: Seven type 2 subjects (aged 58 ± 5 years) managed by diet alone consumed 60ml olive oil (labelled with 20 MBq ^{99m}Tc-V-thiocyanate) and 300ml water containing 75g glucose (labelled with 6 MBq ^{67}Ga-EDTA), on two separate days, with and without 120mg orlistat (Xenical, Roche Products Pty Ltd), while lying in the left lateral decubitus position against a gamma camera. Venous blood samples, for measurement of blood glucose and plasma insuin, GLP-1 and GIP were obtained immediately before, and after, the drink at regular intervals.
Results: Results are shown in the table as mean ± SEM. Gastric emptying of both oil ($P<0.0005$) and glucose ($P<0.0005$) was faster and postprandial

glucose (P<0.005) and insulin (P<0.005) concentrations substantially greater, after orlistat when compared to control. In contrast, plasma GLP-1 (P<0.001) and GIP (P<0.05) concentrations were less after orlistat.
Conclusion: We conclude that inhibition of fat digestion by orlistat may exacerbate postprandial glycaemia as a result of more rapid gastric emptying and a diminished incretin response. These observations have implications for the use of lipase inhibition to promote weight loss in type 2 diabetes.

Table

T = 120 min	Control	Orlistat	P value
Intragastric retention of oil (%)	59.8 ± 7.1	19.0 ± 3.9	< 0.0005
Intragastric retention of glucose (%)	80.8 ± 4.5	33.7 ± 6.0	< 0.0005
Blood glucose (mmol/L)	8.6 ± 1.2	14.8 ± 1.5	< 0.005
Plasma insulin (mU/L)	17.2 ± 4.8	33.4 ± 7.9	< 0.005
Plasma GLP-1 (pmol/L)	51.4 ± 6.7	14.2 ± 2.7	< 0.001
Plasma GIP (pmol/L)	92.1 ± 12.6	37.6 ± 8.0	< 0.05

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The human amylin analog, pramlintide, reduces body weight in insulin-treated patients with Type 2 diabetes.
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Background and Aims: There is increasing evidence that the beta cell hormone, amylin, which is co-secreted with insulin (ins) in response to meals, may be involved in the central regulation of feeding behavior and body weight. For instance, in some recent animal studies, administration of amylin reduced food intake and body weight, whereas administration of an amylin antagonist increased food intake and body fat stores. Insulin-requiring patients with type 2 diabetes are deficient in amylin. Two long-term, randomized, placebo-controlled trials with no dietary interventions have shown that mealtime amylin replacement with pramlintide (pram) lowers HbA_{1c} in conjunction with weight loss in insulin-treated patients with type 2 diabetes.
Materials and Methods: To further examine this weight effect we conducted a pooled analysis of the two trials. For 26 weeks, 284 patients [baseline HbA_{1c} 9.3%, weight 91.3kg] were treated with ins+placebo (pbo), and 292 (HbA_{1c} 9.1%, weight 92.4kg) with ins+pram (120µg BID).
Results: Despite a greater reduction in HbA_{1c} (-0.4% vs. pbo, p=0.0001), pram+ins patients had significant weight loss compared to the pbo+ins patients (-1.8kg (2%), p=0.0001). The proportion of patients losing ≥5% of body weight was 3 to 4 times greater with pram+ins than with pbo+ins (11% vs. 3%, p=0.0005). The change in weight with pram was unrelated to the change in HbA_{1c} (r=0.05, n.s.), but positively related to the change in subjects total daily insulin use (r=0.45, p<0.0001). Stratification by baseline BMI revealed that the greatest weight loss occurred in patients with a BMI >40kg/m^2, who achieved a mean weight reduction of 3.2kg (3%, p=0.0009) that was associated with a 10% reduction in the total daily insulin dose.
Conclusion: These findings support further evaluation of the weight-lowering potential of pramlintide in obese patients with type 2 diabetes, such as when used in conjunction with behaviour modification.

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AZD7545, a novel PDHkinase inhibitor which activates PDH in vivo, improves glucose tolerance in obese (fa/fa) Zucker rats.
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Background and Aims: Pyruvate dehydrogenase (PDH) is a key enzyme in controlling the rate of glucose oxidation, and the availability of gluconeogenic precursors. The proportion of PDH in the active (dephosphorylated) form is controlled by the balance of specific kinase and phosphatase activites. AZD7545 is a PDHkinase (PDHK) inhibitor which is of potential benefit for the treatment of Type 2 diabetes.
Materials and Methods: The functional effect of inhibition of phosphorylation was evaluated using native porcine PDH in the presence of recombinant human PDHK2. PDH activity was measured in a primary culture of rat hepatocytes by determining the rate of release of $^{14}CO_2$ from 1-^{14}C-pyruvate. For in vivo assessment of PDH activation, samples of liver and gastrocnemius muscle were taken 2 hours following a single, oral dose of compound to male Wistar rats. PDH activity was determined spectrophotometrically in a coupled assay linked to arylamine aminotransferase. % PDH activation was assessed by determining the activity before and after full activation using porcine PDH phosphatase. For evaluation of the effect on insulin resistance, male obese (fa/fa) Zucker rats were dosed for 7 days. An oral glucose tolerance test (OGTT) (2g/kg glucose) was carried out in 7-hour fasted animals, 2 hours following the final dose of AZD7545.
Results: In the presence of PDHK2, AZD7545 increased PDH activity with EC_{50} 5.2nM (95% confidence limits 3.2-8.5nM). In rat hepatocytes, the rate of pyruvate oxidation was stimulated 2-fold; EC_{50} 105nM (69-160nM). In vivo, 30mg/kg AZD7545 increased the proportion of liver PDH in its active, dephosphorylated form from 24.7±6.2% to a maximum of 70.3±2.6% (p<0.001) and skeletal muscle from 21.1±1.9% to 53.3±4.0% (p<0.001). After 7 days' administration to obese (fa/fa) Zucker rats, a dose-related decrease was observed in the glucose AUC following an oral glucose challenge, from 15.22±0.38mM.h in vehicle-treated animals to 12.30±0.11mM.h (p<0.001) in rats given 20mg/kg AZD7545. Peak blood glucose concentration measured after 20 minutes was significantly (p<0.001) reduced from 12.2±0.5mM to 8.1±0.3mM.
Conclusion: AZD7545 increased PDH activity in both liver and muscle in Wistar rats and improved glucose tolerance in obese (fa/fa) Zucker rats. These results suggest that PDHkinase inhibitors may be of benefit in the treatment of Type 2 diabetes.

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The novel pyruvate dehydrogenase activator AZD7545 is a selective inhibitor of pyruvate dehydrogenase kinase 2.
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Background and Aims: The pyruvate dehydrogenase multienzyme complex (PDH) plays a key role in the control of glucose metabolism. Phosphorylation of the PDH E1 subunit by PDH-kinase (PDHK) inactivates the enzyme. Thus the balance between phosphatase and kinase activities in a tissue determines enzyme activity. AZD7545 elevates PDH activity *in vivo* in liver, skeletal muscle and heart and has been developed as a potential treatment for Type 2 diabetes. In the present study we have investigated the effects of AZD7545 on the activity of the PDHK isoforms PDHK1 (predominantly expressed in heart), PDHK2 (ubiquitously expressed) and PDHK4 (expressed in heart and skeletal muscle).
Methods: Human PDHK1, PDHK2, PDHK4, E1 and E2 were expressed in E. Coli. Kinase activity was determined by following ^{33}P incorporation from [^{33}P]-ATP into the E1 subunit of PDH in the presence of the E2 subunit. PDH activity in tissue extracts was determined spectrophotometrically and % PDH activation was determined by measuring the activity before and after full activation with PDH phosphatase.
Results: Incubation of PDHK2 with AZD7545 led to complete inhibition of kinase activity (IC50 6.1 ± 1.2 nM). AZD7545 also inhibited PDHK1 activity (IC50 36.8 ± 18 nM) but in this case complete inhibition could not be achieved. In contrast AZD7545 failed to inhibit PDHK4 activity. No difference was observed in the IC50 for inhibition of PDHK2 measured at 10 µM ATP (Km concentration; IC50 6.1 ± 1.2 nM) and that measured at 100 µM ATP (IC50 6.4 ± 2.2 nM). Thus AZD7545 is a non-ATP competitive inhibitor of PDHK2. This was confirmed by molecular modelling studies which reveal that AZD7545 binds at a site distinct from the ATP- binding site. A series of PDH activators, structurally related to AZD7545, showed similar properties with respect to inhibition of PDHK isoforms. The selectivity displayed for PDHK2 over PDHK4 correlates with the observation that following administration of a maximally effective dose of compound to rats a near complete activation of PDH activity in liver (low levels of PDHK4 expression) is consistently observed (from 35.3 ± 3.9 % active in the absence of compound to 90.2 ± 2.2 % active following treatment, p<0.0001), while only partial activation of PDH activity is achieved in both skeletal muscle (from 23.3 ± 1.4 % to 64.3 ± 2.3 % active, p<0.0001) and in heart (from 35.7 ± 2.3 % to 61.7 ± 4.3 % active, p<0.0004), tissues which express relatively high levels of PDHK4. Furthermore in 24 h starved rats, when PDHK4 expression is upregulated in skeletal muscle but not liver, the ability of AZD7545 to activate PDH activity in skeletal muscle is severely blunted while activation of liver PDH is retained.
Conclusions:AZD7545 and related compounds are inhibitors of PDHK2 and PDHK1 but do not inhibit PDHK4 activity. This selectivity profile correlates with the ability of these compounds to activate PDH to a greater extent in liver than in both skeletal muscle and heart and with the observation that starvation blunts their ability to elevate PDH activity in skeletal muscle .

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Chronic administration of the novel PDH kinase inhibitor AZD7545 to Zucker (fa/fa) rats gives glucose-lowering activity without adverse effects on body weight.

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Background and Aims: Some recently developed anti-diabetic drugs have the disadvantage of causing weight gain. Pyruvate dehydrogenase (PDH) is a key enzyme involved in the regulation of glucose oxidation. Activation of PDH by inhibition of PDHkinase is a potential approach to the treatment of Type 2 diabetes without the complication of associated weight gain. AZD7545 is a PDH activator which significantly increases liver and muscle PDH activity *in vivo*. In the obese Zucker rat, an insulin resistant model of the early stages of Type 2 diabetes, we have compared the chronic effects of AZD7545 on body weight and anti-diabetic activity to those of rosiglitazone, a PPARγ agonist known to cause weight gain.
Materials and Methods: Male Zucker (fa/fa) rats, received vehicle, AZD7545 10mg/kg or rosiglitazone (RSG) 3mg/kg p.o. once daily for 28 days. Body weight and food intake were measured daily. Blood glucose measurements were taken post-prandially on day 15, and also during an oral glucose tolerance test (OGTT) (2g/kg glucose) on day 25. Terminal 5hr fasted plasma glucose and insulin concentrations were measured on day 28. Results were compared to vehicle controls and significance of data was ascertained using a 2-tailed unpaired t-test.
Results: After 15 days' dosing, both AZD7545 and rosiglitazone significantly ($p<0.01$) reduced fasting (2hr) plasma glucose concentrations (vehicle: 8.9±0.7 cf. 6.7±0.2 (AZD7545) and 5.9±0.3mM (RSG)). A reduction in peak post-prandial glucose concentrations was also observed (15.0 ±0.8 cf. 9.6±0.5 and 7.1±0.1mM respectively). Furthermore, after 28 days, rosiglitazone significantly ($p<0.01$) lowered 5-hour fasted plasma glucose concentrations (6.7±0.2 cf. 5.8±0.1mM) and plasma insulin concentrations were significantly reduced by both AZD7545 ($p<0.01$) and rosiglitazone ($p<0.001$) (43.4±4.6 cf. 21.3±3.6 and 5.2±0.6 ng/ml respectively). Furthermore, both AZD7545 and rosiglitazone significantly reduced ($p<0.05$) peak glucose concentrations during an OGTT (10.1±0.5 cf. 8.6±0.4 and 8.0±0.6mM respectively). Rosiglitazone administration caused a highly significant ($p<0.001$) increase in average food intake (39.4±1.9 cf. 27.1±1.2g/day), which was maintained for the duration of the study. Body weight gain over the 28 days was also significantly ($p<0.001$) increased (31.2±2.4 cf. 11.5±1.5%). In contrast, AZD7545 had no significant effect on food intake (26.9±1.0g/day) or body weight gain (13.1±1.2%).
Conclusions: Consistent with published data, rosiglitazone reduced plasma glucose and improved glucose tolerance but also caused a significant increase in food intake and body weight of Zucker rats. In contrast, AZD7545 improves glucose disposal without adversely affecting food intake or body weight.

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AJ-9677, a specific human β_3-adrenoceptor agonist, improves obesity and insulin sensitivity in dietary-obese mice.

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Background and Aims: Obesity is a disorder in energy balance caused by excess food intake, lack of exercise and hereditary abnormalities. In this study, we investigated whether AJ-9677, a specific human β_3-adrenoceptor agonist under clinical development, improves obesity and insulin sensitivity in dietary-obese mice.
Materials and Methods: Male CD-1 (ICR) mice were made obese by a 30% high-fat diet given over a period of 7 weeks. They were then treated with AJ-9677 (0.01~ 0.1 mg/kg/day p.o.) for 21 days. Obese mice body weight and food consumption were monitored daily, and their body fat mass was estimated from homogenized carcasses. AJ-9677 effects on uncoupling proteins (UCPs) and adipocytokines mRNA levels in the epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were evaluated using RT-competitive PCR method, and its acute and chronic effects on oxygen consumption and respiratory quotient (RQ) were measured using a calorimetric apparatus. Plasma samples for determination of AJ-9677 effects were examined using ELISA (adiponectin, insulin and TNF-α), affinity HPLC (HbA1c) and an enzymatic method (NEFA).
Results: AJ-9677 (0.01~ 0.1 mg/kg/day p.o.) dose-dependently decreased body weight by up to 9% ($p<0.001$) and specifically reduced body fat mass in obese mice (46%, $p=0.002$) without affecting food consumption. In addition, AJ-9677 markedly increased UCP-1 mRNA levels (170-fold, $p=0.008$) in WAT, but unexpectedly not in BAT of obese mice, and up-regulated UCP-2 mRNA levels in BAT (1.6-fold, $p=0.006$). Moreover, AJ-9677 restored the impaired expression of β3-adrenoceptor to normal levels in WAT (3-fold, $p=0.01$). AJ-9677 decreased TNF-α mRNA levels (32%, $p=0.02$) in WAT and normalized the lowered adiponectin (1.9-fold, $p=0.04$) and GLUT4 mRNA levels (4.5-fold, $p=0.003$) in WAT of obese mice. Acute administration of AJ-9677 increased oxygen consumption (3 hour peak, 1.9-fold, $p<0.001$) and decreased RQ indicating an enhancement in energy expenditure and fat metabolism. Chronic treatment with AJ-9677 enhanced the increase in oxygen consumption induced by its acute treatment (3 hour peak, 1.5-fold, $p=0.001$). Finally, AJ-9677 increased plasma adiponectin level (1.4-fold, $p=0.007$) and decreased plasma HbA1c (from 3.7% to 3.5%, $p=0.046$), insulin (69%, $p=0.046$), NEFA (33%, $p<0.001$) and TNF-α levels in obese mice.
Conclusion: Our results indicate that AJ-9677, a specific human β_3-adrenoceptor agonist, improves obesity by promoting energy expenditure through stimulation of UCP-1 in BAT as well as WAT, and ameliorates insulin sensitivity by controlling the expression of adipocytokines in WAT. It is therefore suggested that AJ-9677 has great potential in the treatment of obesity and diabetes.

PS 67
Metformin

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Acute effect of metformin on gene expression of glucose 6 phosphatase in rat hepatoma cells.

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Background and Aims: Metformin is a widely used drug for treatment of type 2 diabetes and it has been suggested that its primary action on glucose metabolism is an inhibition of glucose production of the liver. Hepatic glucose production is mainly regulated by glycogenolysis and glyconeogenesis. The hepatic mechanism of metformin is not yet fully elucidated because some studies showed that metformin had no effect on glyconeogenesis and another showed the effect of metformin on insulin signaling. In this study, to clarify the effect of metformin on glyconeogenesis in liver, we examined the gene expression of a key enzyme of glyconeogenesis, glucose 6 phosphatase (G6P) using quantitative real-time PCR.
Materials and Methods: We used insulin sensitive rat hepatoma cell line, H4IIE in this study. mRNA levels of G6P were quantitated using real-time PCR. Because G6P mRNA transcription level is low in the absence of any stimulus, we studied the ability of metformin to repress the induction of G6P mRNA stimulated by a combination of a nonhydrolyzable cAMP analog (8-Br-cAMP) and dexamethasone (Dex). The cells were treated with the various concentrations of metformin in the presence of 500 nM dexamethasone and 500 μM 8-Br-cAMP. Furthermore, to evaluate the role of MAP kinase and protein kinase C, the effect of pretreatment with MEK-1 inhibitor PD98059 or PKC inhibitor GF109203X (GFX) for 1 h was tested. To elucidate the role of Akt, phosphorylation of Akt were examined using anti-phosphoAkt antibody in Western blot.
Results: The expression of G6P was induced by dexamethasone/cAMP and these induction were significantly suppressed by 100 nM insulin (88% inhibition, $p<0.001$). Metformin also suppressed dexamethasone/cAMP-induced expression of G6P in a dose-dependent manner. Metformin significantly inhibited at 250 μM (40%, $p<0.05$), and reached a maximun effect at 2 mM (79%, $p<0.005$). Pretreatment with PD98059 or GFX showed no effect on both insulin- and metformin-induced suppression. In Western blot analysis, metformin did not induce the phosphorylation of Akt. Taken together, metfomin suppressed gene expression of G6P through the insulin-independent pathway.
Conclusion: Metformin can reduce hepatic glucose production through suppression of G6P gene expression without modification of MAP kinase, protein kinase C and Akt.

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Obligatory role of membrane events in the modulatory effect of metformin on the mitochondrial function.

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Background and Aims: From recent findings on hepatocytes about the indirect effect of metformin (MET) targeted on the respiratory chain complex I, we revisited this issue and tried to determine the causality of any alteration at this enzymatic level using the *Xenopus laevis* oocyte model.
Materials and Methods: Complex I activity from isolated mitochondria (differential centrifugation) or mitochondria in living cells was followed according to the mode of drug application (MET injected and incubated, either as a free form or encapsulated into liposomes, at both ambient and cold temperature), including competition studies with a structural analog, asymmetrical dimethylarginine (ADMA). The uptake of MET and its subcellular distribution was parallely examined with the help of the radioactive drug.
Results: Addition of 50 μM or 10 mM MET reduced by 40-50% the rotenone-sensitive activity of complex I only in incubating intact oocytes but not in isolated mitochondria. The drug prior injected inside these cells had also no measurable effect. In spite of this and the negligible binding of MET to the mitochondrial fraction, there was a fairly good correlation between the pronounced inhibitory action of MET on complex I and its progressive appearence within the oocyte cytosol, which took longer to be observed with 50 μM than with 10 mM. More interestingly, MET as a liposomal form was again able to alter specifically this enzymatic site when added directly to organelles (-48%, $P < 0.005$). Furthermore, a temperature-dependent effect was clearly shown. At 4°C, oocytes failed to take up efficiently the free drug (50 μM) and accordingly its subsequent action on respiration was therefore lost. Likewise, MET transport was significantly hindered ($P < 0.01$) and inhibition of complex I totally disappeared when ADMA was placed together with MET either at the same concentration (50 μM) or in excess (2.5 mM). On the other hand, both the uptake of MET at the supra-therapeutical dose (10 mM) and its corresponding effect on cell respiration were neither attenuated in cold conditions nor counteracted by the presence of 2.5 mM ADMA.
Conclusion: These intriguing data are strongly in accordance with a membrane-mediated uptake and vesicular routing for MET. They also support the view that MET may recognise some specific membranous sites, likely belonging to effector systems, before penetrating the cell in a bound state through a yet obscure endocytosis mechanism which still has to be identified.

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Direct effect of metformin on vascular contractility.

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Background and Aims: The biguanide metformin reduced macrovascular complications when used as an initial antidiabetic therapy in overweight type 2 diabetic patients during the United Kingdom Prospective Diabetes Study (UKPDS). This action has been shown to be independent of its effects on blood glucose, lipid and insulin concentrations. This study examines the direct effects of metformin on vascular contractility and passive tension.
Materials and Methods: Using isolated segments of thoracic mouse aorta, responses to noradrenaline (NA) and acetylcholine (Ach) were investigated with a Mulvany Halpern Myograph. The aortic section was tensioned at 1g. An initial adaptation period of ~ 45 minutes was given to equilibrate the tissue. This simulates the tension exerted by circulating blood. The passive tension was also investigated using a Small Vessel Myograph. The tissue was exposed to the presence and absence of metformin 10^{-5}M for 1 and 4 hours at 37ºC in oxygenated physiological buffer.
Results: At 1 hour metformin 10^{-5}M caused a 49% increase in the maximum contractile response at NA 10^{-6}M ($P < 0.001$) compared to control. At 1 hour the effect of Ach was not significantly different in the presence of metformin, although there was an additional 8% relaxation compared to control.
At 4 hours metformin 10^{-5}M again shifted the concentration response curve with NA to the left. At this time there was a 221% increase in the contractile response to NA 10^{-6}M ($P < 0.001$) compared to control. Also at 4 hours maximum NA contracted aorta showed an increased relaxation in response to Ach, increasing the relaxation response by 36% to Ach 10^{-6}M ($P < 0.01$) compared to control.
The passive tension generated by transverse stretching of the aorta showed that after 1 hour there was ~2-3 % increase in the tension in the tissue treated with metformin, but this was not statistically significant. At 4 hours, a larger pretension was seen in the metformin treated tissue, being ~6-10 % greater. ($P < 0.03$ v control at 3000μm).
Conclusion: These studies show that metformin can act directly on the aortic wall to increase NA-stimulated contraction and Ach-stimulated relaxation. These effects indicate a direct influence on the interaction of NA with vascular smooth muscle and Ach with endothelium. Metformin also directly increased passive tension of the vessel wall.

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Acute lipid-induced insulin resistance in the liver is ameliorated by metformin and rosiglitazone via distinct mechanisms.

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Background and Aims: We have recently shown that thiazolidinedione pre-treatment protects normal rats from insulin resistance induced by an acute intravenous lipid load. In this study, we investigated whether

metformin (Met) can also prevent the development of insulin resistance in the same model, and compared its mechanism of action with that of rosiglitazone (RSG).
Materials and Methods: Male Wistar rats (~360g) were given RSG (4mg/kg.day), Met (120mg/kg.day) or vehicle (Con-) by gavage for 7d. Subsequently, either Intralipid (0.5ml/h, -Lip) or glycerol (-Gly), as control, were infused for 5h in some animals (n=13-15 per group). Of these, n=7-9 per group underwent euglycemic-hyperinsulinemic clamping (70pmol/l insulin) between 3-5 h, and [^{14}C]-2DG and [^{3}H]-R-bromopalmitate tracers were administered 45 min prior to euthanasia.
Results: Clamp glucose infusion rates were maintained at levels similar to Con-Gly in both RSG-Lip and Met-Lip groups, and were 58% and 67% greater than that of Con-Lip respectively (both p<0.01 vs Con-Lip). The insulin-sensitising effects of RSG were relatively greater in peripheral tissues than those of Met, accompanied by enhanced systemic fatty acid clearance (by 62%, p<0.01 vs Con-Lip) and increased adipose tissue fatty acid uptake (2-fold, p< 0.01 vs Con-Lip). In contrast, systemic fatty acid clearance was not affected by Met. Although both drugs substantially prevented impaired insulin suppressibility of hepatic glucose output (Clamp HGO Con-Lip 7.7±0.8, RSG-Lip 2.2±2.3, Met-Lip -2.1±1.0 mg/kg/min), only RSG significantly reduced liver fatty acid uptake (by 40%, p<0.05 vs Con-Lip), and liver triglyceride storage after clamp (Con-Lip 33.3±3.31, Met 34.3±2.91, RSG 22.9±2.83µmol/g; p=0.02 vs control). In addition only RSG elevated plasma adiponectin (RSG 10.6+1.7 vs Con-Lip 5.2±0.7µg/ml; p<0.01). Both drugs increased basal liver AMP-activated protein kinase (AMPK) activity but the effect was much greater for Met than for RSG (Met, 3.8 fold, p=0.002 vs RSG 2.3 fold vs control, p=0.08).
Conclusion: RSG and Met clearly both oppose insulin resistance induced by an acute lipid load, but this effect is achieved through different mechanisms. The insulin-sensitising effects of RSG are accompanied by a partitioning of lipids into adipose tissue rather than the liver (Lipid steal hypothesis), and elevation of adiponectin. In contrast, the effects of Met are not mediated by altered fatty acid turnover or increased adiponectin, but instead may be related to greater phosphorylation of target molecules by AMPK. Thus, Met and RSG demonstrate different but complementary mechanisms of action, supporting their use in combination for therapy of lipid-induced insulin resistance.

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Metformin does not increase homocystein in pregnant PCOS women.
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Background and Aims: Many women with polycystic ovary syndrome (PCOS) have difficulty to achieve pregnancy. To facilitate menses, ovulation and pregnancy, metformin has gained increasing popularity. However, metformin might increase plasma homocysteine by reducing vitamin B_{12} absorption and possibly also folate absorption. Elevated maternal homocysteine levels is a risk factor for pregnancy complications and poor pregnancy outcome. Recently the use of metformin in pregnant women with PCOS has been reported. These studies have been retrospective or non-randomised and the effect on maternal homocysteine levels has not been reported. In a pilot study we have investigated the effect of metformin on maternal plasma homocysteine levels in pregnant PCOS women.
Materials and Methods: 23 pregnant women with PCOS were included in a randomised double blind placebo controlled pilot study before gestational week 9. All partisipants had received the diagnosis of PCOS before the present pregnancy. They were treated with metformin 850 mg bid or identical placebo capsules. In addition they were all given one tablet a day of folate 1mg and one multivitamin tablet (vit A 800 µg, vit B_1 1.4 mg, vit B_2 1.6 mg, vit B_6 2 mg, vit B_{12} 1 microg, folic acid 200 µg, niacin 18 mg, pantotenic acid 6 mg, vit C 60 mg, vit D 5 µg, vit E 10 mg, Fe^{++} 14 mg, Zn^{+} 15 mg, Cu^{++} 2 mg, iodine 150 µg, Mn^{++} 2.5 mg, Cr^{+} 50 µg, and Se^{+} 50 µg) a day. Mann-Whitney statistics were used to compare groups.

Results:
Plasma homocysteine values according to study group

		Week 8		Change to week19		Change to week 32		Change to week 36
		n Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Homocysteine (µmol/L)	P	10 5.0 (1.6)	10	-1.5 (1.1)	8	-1.4 (0.9)	8	-1.3 (0.8)
	M	10 5.6 (1.6)	10	-2.1 (0.9)	10	-2.1 (1.3)	10	-2.0 (1.2)
Folate (nmol/L)	P	10 22 (9)	10	7 (9)	8	4 (15)	8	7 (16)
	M	9 18 (13)	9	10 (12)	9	10 (12)	9	16 (15)
Vitamin B_{12} (pmol/L)	P	10 250 (97)	10	-45 (51)	8	-82 (94)	8	-80 (78)
	M	11 276 (110)	11	-77 (66)	11	-119 (76)	11	-111 (70)

There were no significant differences between the placebo (P) and metformin (M) group at any time during the study.
Conclusion: To our knowledge this is the first prospective randomised double blind study on the effect of metformin in pregnant women with PCOS. Contrary to our hypothesis we were unable to demonstrate a homocysteine increasing effect of metformin in pregnant women with PCOS. As shown in previous studies maternal homocysteine levels fell during the first trimester. However, there was no tendency towards increasing homocysteine levels in the third trimester. This might be due to the substitution with B-vitamins throughout pregnancy. Our finding is encouraging as there seems to be a trend towards using metformin in pregnant PCOS women.

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Effects of metformin in nondiabetic male subjects with nonalcoholic steatohepatitis (NASH).
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Background and Aims: Insulin resistance has been implicated in the pathophysiology of NASH, a common disorder often associated with obesity and diabetes. Since metformin can improve insulin resistance, it has been tested in NASH, with some evidence for improved liver function. We wished to test its efficacy, with and without prescribed diet change, another intervention that has been suggested to be useful.
Materials and Methods: We studied 5 nondiabetic male subjects (BMI 33.1 ± 2.5 kg/m^2), before and during 12 mo metformin, with weight reduction during the last 6 mo using a behavioral program. Dose was increased stepwise to 2 g/d based on GI tolerance. Adherence was excellent based on monthly visit attendance and pill counts.
Results: With metformin, weight decreased 1.56 ± 0.57 kg and FFM 0.44 ± 0.13 kg ($P \leq 0.05$) at 6 mo, with no change in fasting plasma glucose, insulin, HbA_{1c} or lipids. A further 3.8 ± 1.5 kg loss occurred at 12 mo. Hyperinsulinemic (40 mU /m^2·min), euglycemic clamps showed marked baseline peripheral insulin resistance of glucose metabolism: glucose Rd at 825 ± 62 pM insulin was 3.5 ± 0.5 mg/kg/min, Ra was suppressed 88% and FFA decreased 76% (632 to 152 µM, P<0.01). None of these clamp responses were different from those of a comparable group of obese male subjects without NASH [n=7, BMI 33.2 ± 1.9], although fasting insulin levels were higher in the NASH patients: 182 ± 28 pM vs 96 ± 18 pM, P=0.02. None of these clamp responses changed post 6 mo metformin, though postabsorptive Ra increased from 1.55 to 1.76 mg/kg.min (P=0.03). Serum ALT declined 10 U/L at 6 mo and 36 U/L at 12 mo (P = 0.03 by ANOVA). Mean serum AST, ALP and GGT did not change at 6 or 12 mo. Neither liver biopsy morphology (n=4, 6 mo) nor estimated liver fat by ultrasound (12 mo) showed changes. An unexpected finding was a small decline in ALP and GGT during the clamps ± metformin (P=0.047) and in ALT during the clamp before metformin (P=0.047).
Conclusion: 1. The 6 and 12 mo treatment with maximum metformin doses (with 1.6% and 5.7% weight loss, respectively) in this small, male NASH sample showed even less than expected improvement of insulin resistance and minimal evidence for *improvement* in liver dysfunction. However in *no* case was there evidence of *progression*. The variable response to metformin in available data suggests that a multicentre, randomized trial is required. (The largest response in liver tests and glucose metabolism we have observed was in a woman with previously untreated DM2, implicating a possible additional effect of diabetes and its treatment, in NASH patients.) 2. Our NASH patients did not appear to have more insulin resistance during the clamp than that due to obesity.

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Effects of a single-tablet metformin-glibenclamide[a] combination treatment (Glucovance®) on fasting and postprandial plasma insulin in diet-failed Type 2 diabetic patients: data from two randomised, double-blind trials.

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Background and Aims: Pharmacokinetic studies suggest earlier absorption of glibenclamide from a single-tablet metformin-glibenclamide combination treatment vs. metformin and glibenclamide co-prescribed. The insulin responses to these treatments thus require evaluation. We analysed data on fasting and postprandial insulin in patients receiving metformin-glibenclamide combination tablets or standard glibenclamide from 2 randomised, double-blind trials in a total of 1292 type 2 diabetic patients. Published studies show that the combination tablets controlled blood glucose more effectively than glibenclamide alone, despite lower mean glibenclamide dosages, and that the incidence of hypoglycaemic symptoms was lower or comparable with the combination tablets vs. glibenclamide.
Materials and Methods: Patients had hyperglycaemia despite previous diet and exercise therapy. In Study 1, metformin-glibenclamide 250 mg/1.25 mg combination tablets were evaluated in comparison with treatment based on glibenclamide 2.5 mg or metformin 500 mg. In study 2, patients were randomised to metformin-glibenclamide 250 mg/1.25 mg or 500 mg/2.5 mg, glibenclamide 2.5 mg, metformin 500 mg or placebo, titrated to up to 4 tablets/day. Data on metformin are omitted for clarity.
Results: Increases in fasting insulin were smaller with metformin-glibenclamide 250 mg/1.25 mg tablets, vs. glibenclamide. In study 2, glibenclamide, but neither single-tablet combination, increased fasting insulin vs. placebo. Insulin concentrations 2 h after a test meal were significantly larger for the 250 mg/1.25 mg combination tablet than for glibenclamide alone in Study 2; 2 h postprandial insulin excursions were larger in the combination groups vs. glibenclamide alone, though this was not statistically significant.
Conclusions: Single-tablet metformin-glibenclamide combinations enhanced postprandial insulin release, without affecting fasting insulin significantly. Glibenclamide increased both. The differences in insulin secretion may result from earlier absorption of glibenclamide, or enhanced beta-cell function following greater improvements in glycaemia with the single-tablet combination.

Mean changes from baseline in plasma insulin

Study		Mean daily glibenclamide dose (mg)	ΔFI (ΔIU/mL)	Δ2 h PPI (µIU/mL)	Δ2 h PPIE excursions (µIU/mL)
1	M-G 250 mg/1.25 mg	3.7	1.3*	20.3	19.2
	Glibenclamide 2.5 mg	7.6	4.5	16.3	12.0
2	Placebo	–	1.4	0.9	-0.2
	M-G 250 mg/1.25 mg	2.8	3.8*	29.7*	25.3
	M-G 500 mg/2.5 mg	4.1	4.9	25.0	18.9
	Glibenclamide 2.5 mg	5.3	7.2†	15.1	7.4

*Significant difference vs. glibenclamide alone; †significant difference vs. placebo;
FI/PPI: fasting/postprandial insulin; PPIE: postprandial insulin excursions; M-G: metformin-glibenclamide.
[a]Glyburide in the US

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Improved blood glucose control in patients switched from metformin and glibenclamide* co-administered to a single-tablet metformin-glibenclamide combination treatment (Glucovance®): a retrospective chart review from four clinics.

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Background and Aims: Metformin combined with a sulphonylurea is an effective and widely used therapeutic strategy for patients with hyperglycaemia on 1st- or 2nd-line pharmacologic therapy. We have evaluated the implications for efficacy and tolerability of switching type 2 diabetic patients from metformin plus a sulphonylurea co-administered to a single-tablet metformin-glibenclamide combination.
Materials and Methods: This was a retrospective chart review of data from 4 US hospitals. Patients had received metformin plus a sulphonylurea for ≥6 months prior to the period of analysis, and then received the single-tablet metformin-glibenclamide combination for a mean of 283 days. Baseline HbA_{1C} measurements were made between 35 days before and 3 days after the beginning of follow-up. Final measurements were made after a mean of 195 days of combination treatment.
Results: Records from 72 patients (97% male) were evaluated. Demographic details at baseline were (means): age 62 years, HbA_{1C} 8.3%, duration of diabetes 7.6 years, body mass index 32.9 kg/m^2. Most patients (72%) were Caucasian, 11% were African-American, 4% were Latino/Hispanic, and 13% were of other/unknown ethnicity. Average daily dosages of metformin increased significantly after the switch (1607 mg to 1750 mg, $p<0.05$), while the mean glibenclamide dosage decreased (17 mg to 15 mg, $p<0.01$). The switch to single-tablet combination therapy was associated with a mean reduction in HbA_{1C} of -0.6% ($p<0.01$). Larger mean changes in HbA_{1C} were observed in 37 patients with HbA_{1C} ≥8% at baseline (-1.3%, $p<0.001$) vs. 35 patients with HbA_{1C} <8% (+0.1%, p=NS). Plasma lipid profiles (total-, LDL-, or HDL-cholesterol and triglycerides) were unchanged, as was mean body weight (104 kg before and after the switch). Subgroup analyses suggest that changes in the dosages of drugs, additional medications, or adherence to regimens could not explain the magnitude of the decrease in HbA_{1C} following the switch. A slight increase in reports of hypoglycaemia was observed after the switch, compared with before (11% vs. 4%).
Conclusions: In this retrospective chart review, switching patients from metformin and glibenclamide co-administered to a single-tablet metformin-glibenclamide combination was associated with a statistically and clinically significant improvement in HbA_{1C}. An earlier absorption of glibenclamide with the single-tablet combination, compared with metformin and glibenclamide co-administered, may have contributed to the observed differences in efficacy.
*Glibenclamide is known as glyburide in the US.

PS 68
New Compounds

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Effects of a novel thiazolidinedione (TZD) analogue, MCC-555, and metformin on gene expression of phosphoenolpyruvate carboxykinase (PEPCK) and phosphorylation of AMP-activated protein kinase (AMPK) in cultured rat hepatocytes.

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Background and Aims: In diabetic patients, increased expression of a hepatic gluconeogenic enzyme, PEPCK, contributes to an acceleration of hepatic glucose output resulting in fasting hyperglycemia. The expression of PEPCK is inhibited by insulin and is regulated at the level of gene transcription. Previous studies have shown that TZDs inhibit gene expression of PEPCK by potentiating the action of insulin or by direct insulin-like actions through a cascade from insulin receptor to Akt/PKB. However, it has been recently reported that TZDs and metformin as an insulin-sensitising agent exert their antidiabetic actions by stimulating AMP-activated protein kinase (AMPK) that would not be involved in the insulin-signaling cascade. Although we previously reported that MCC-555 , a novel TZD analogue accelerates phosphorylation of Akt/PKB in cultured hepatocytes, its precise action mechanisms are not well investigated. In this study, effects of MCC-555 on hepatic PEPCK gene expression and phosphorylation of AMPK were examined using primarily cultured rat hepatocytes to evaluate the molecular mechanisms of its antidibetic actions, and were compared with those of metformin and insulin.
Materials and Methods: Hepatocytes from male Wistar rats were isolated by the collagenase perfusion method. Primarily cultured hepatocytes were treated for 8 hrs with 500 μM dibutyryl cyclic-AMP (cAMP), 100 nM insulin, 30 μM MCC-555, 500 μM metformin, or various combinations thereof. Cells were harvested and total cellular RNA and protein were isolated. Northern analyses of PEPCK and western blot analyses of phosphorylated AMPK were performed.
Results: MCC-555 and metformin decreased the PEPCK mRNA expression in cultured hepatocytes as insulin did. cAMP-induced increase in PEPCK mRNA expression of hepatocytes was also decreased by MCC-555, metformin or insulin. The expression of phosphorylated AMPK was increased by treatment with MCC-555 or metformin, which was not prevented by an inhibitor of PI3-kinase-Akt cascade, wortmannin.
Conclusion: These observations suggest that MCC-555 and metformin may exert their antidiabetic actions by decreasing PEPCK gene expression not through the PI3-kinase-Akt cascade but through phosphorylation of AMPK.

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A nonadipogenic compound BLX-1002 induces glucose uptake in 3T3-L1 cells and has strong anti-hyperglycemic effect in *db/db* and *ob/ob* mice.

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Background and Aims: BLX-1002 is an amino acid conjugated, novel small molecule (MW<500) synthesized by using a Rational Drug Design Approach. It is being developed for the treatment of type-II diabetes and associated complications.
Materials and Methods: In vitro ^{14}C Deoxyglucose uptake, adipogenesis, aP2 (fatty acid binding protein) expression assays were carried out to characterize BLX-1002 in 3T3-L1 adipocytes and in human preadipocytes. BLX-1002 was compared side by side with rosiglitazone, a known marketed drug for type-II diabetes. In vivo efficacy of BLX-1002 was determined in *db/db* and *ob/ob* mice. Several acute and sub-acute toxicological studies were conducted to evaluate its toxicological reactions.
Results: After 72 hours of incubation, BLX-1002 dose dependently increased basal glucose uptake (1.4 and 2.3 fold of basal at 0.1 and 1.0 μM respectively) in 3T3-L1 adipocytes. In the same experiment 0.1 μM of rosiglitazone induced 2.8 fold of basal uptake. These uptakes are glucose transporter mediated as addition of Cytochalasin B (25 μM) completely abrogated the drug effect. In fatty acid binding protein (aP2) expression assay, in human subcutaneous pre-adipocytes, it was observed that incubation of cells with 10 μM BLX-1002 for 72 hours did not change the expression level of aP2 protein (3.1 ± 0.3 RLU) compared to untreated cells (2.98 ± 0 RLU). In contrast, more than 25-fold increase in aP2 expression (80.3 ± 4 RLU) was observed when cells were exposed to equimolar concentrations of rosiglitazone, a strong agonist of PPARγ receptor. In adipogenesis experiments, total cellular lipids accumulated after BLX-1002 or rosiglitazone treatments were measured after Oil red O treatment. Rosiglitazone induced a 4.5 fold induction of cellular lipid accumulation during adipogenesis process, whereas BLX-1002 treatment remained unaltered (0.6 fold of basal at 10 μM). Oral treatment of diabetic *db/db* and *ob/ob* mice at a dose range between 10-50 mg/kg body weight, resulted in a 40-50% decrease in blood glucose concentrations. In *db/db* mice within 13 days of treatment it significantly reduced fasting blood glucose (39%), Glycosylated hemoglobin (1 %) and increased the Acrp30/Adiponectin levels in these animals compared to the vehicle treated group. Treatment with BLX-1002 resulted in significant improvement in serum insulin (30-70%), triglycerides (30-60%), free fatty acids (20-30%). Unlike other drugs, BLX-1002 did not increase the body weight gain compared to vehicle group in *db/db* and *ob/ob* mice. Several toxicological studies revealed that there is no adverse reaction /or increase in serum transaminases, BUN or creatinine levels after treatment of the BLX-1002 with its highest tested dose of 200mg/kg body weight for 14 days.
Conclusion: These results suggest that BLX-1002 is a novel, non-adipogenic, orally active anti-diabetic compound which may act through a non PPARγ mediated pathway. BLX-1002 is currently being evaluated for clinical development.

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EML 257 restores a glucose-dependent effect on pancreatic hormonal secretion in rat models of Type 2-diabetes.

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Background and Aims: A major defect in type 2- diabetes is the markedly impaired insulin release in response to glucose (GSIS). Similar beta-cell dysfunction is present in two models of type 2 diabetes: the neonatal streptozotocin-treated (N0STZ) rat and the Goto Kakisaki (GK) rat. EML 257 (4-fluoro-γ-oxo-α-phenylmethyl benzenebutanoic acid ([+])) is a new oral antidiabetic compound chemically distinct from the glinides, sulfonylureas, biguanides and thiazolidinediones. EML 257 exerts its antihyperglycemic activity through both increase in insulin secretion and inhibition of hepatic glucose production. The aim of this study was to further characterize the pancreatic impact of EML 257 especially in diabetic situations.
Materials and Methods: The direct effect of EML 257 (10 μM) on hormonal pancreatic secretion was assessed in vitro using isolated islets and perfused pancreases from non diabetic Wistar (W), N0STZ and GK rats. EML 257 effects were compared to those of repaglinide (10 μM).
Results: EML 257 did not induce insulin secretion in the absence or at low (2.8 mM) glucose concentration (G) while it potentiated GSIS at G ≥ 5.5 mM (171% at G 8 mM vs controls, $p<0.001$) in W isolated islets. A similar potentiation of GSIS with no effect in the absence of glucose was observed in perfused pancreas studies. In N0STZ rats as well as in GK rats, EML 257 improved GSIS from isolated islets. In perifused GK islets, EML 257 induced a first phase of insulin release similar to that obtained in the W group while it elicited only a modest insulin release under basal conditions (G 5.5 mM). In diabetic perfused pancreases, percent increase in GSIS (16.5 mM) was 396% for N0STZ (1428 ± 315 vs 362 ± 52 μU/min in controls, $p<0.02$) and 307% for GK rats (927 ± 251 vs 302 ± 107 μU/min in controls, $p<0.05$). In both diabetic rat models, repaglinide induced insulin secretion in the absence of glucose but was unable to improve GSIS. Glucagon secretion from W and N0STZ perfused pancreases was potentiated by EML 257 in the absence of glucose while the physiological inhibitory effect of glucose was not blunted by EML 257. By contrast, repaglinide blocked glucagon secretion independent of the presence of glucose in the perfusion medium.
Conclusion: These observations in type 2 diabetic animal models suggest that EML 257 activity differs from marketed insulin secretagogues since it is able to restore physiological glucose control of insulin and glucagon secretion. The strict glucose dependence of EML 257 activity may optimize daylong glycemic control and protect the diabetic patient from hypoglycemic risk.

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Safety and dose proportionality of EML 257, a new dual-acting oral antidiabetic compound, in healthy adult males.

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Background and Aims: EML 257 [4-fluoro γ-oxo-α-phenylmethyl benzenebutanoic acid (+)], a new dual-acting oral antidiabetic compound, combines a glucose-dependent increase in insulin secretion with an inhibitory effect on hepatic glucose production in animal models of diabetes. Dose-ranging studies were conducted in healthy male subjects to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of ascending single and multiple doses of EML 257.
Materials and Methods: Healthy adult males (18–40 years; n=64) were randomised to receive a single oral dose of EML 257 ranging from 25 to 1600 mg. In a second study, 42 healthy male subjects (18–40 years) received a single oral dose (Day 1) of treatment (EML 257 [50, 100, 200, 400 or 600 mg] or placebo) following an ascending-dose design. After a 3-day washout, the same dose was administered twice daily for 6 days (Days 4–9), with a final single dose given on Day 10. All adverse events (AEs) were recorded throughout the study period. Pharmacokinetic parameters were calculated and effects on plasma glucose and insulin levels were monitored pre- and post-dosing.
Results: EML 257 was generally well tolerated at single doses up to 1600 mg/day, and following twice-daily oral administration over 7 days (up to 1200 mg/day). No serious AEs were recorded following repeated administration, and all treatment-emergent AEs were of mild to moderate intensity and assessed as unlikely to be or not related to study treatment. Following the twice-daily administration of high doses of EML 257 to healthy subjects fasted from12 hours pre-dose to 4 hours post-dose, there were no episodes of severe hypoglycaemia, and dose-dependent blood glucose decreases were mild, of short duration and resolved spontaneously. Absorption of EML 257 was rapid, reaching peak plasma levels within 0.5–4 hours of dosing, with both C_{max} and AUC increasing in a dose-proportional manner after single or repeated administration. Bi-exponential elimination of drug from plasma occurred with a mean $t_{1/2}$ of 5.54–7.03 hours after a single dose and 6.88–8.45 hours after repeated dosing. Less than 2% of the dose was excreted unchanged in the urine. Steady state was achieved within 4 days of repeated twice-daily dosing. Mean accumulation ratios (AUC Day 10 vs Day 1) increased in a dose-dependent manner, ranging between 1.20 and 1.30 for the 50–400 mg dose groups, increasing to 1.45 for the 600 mg dose group. Single and multiple administrations of the 400 and 600 mg doses of EML 257 resulted in a rapid increase in plasma insulin levels concomitant with a mild short-lived decrease in blood glucose from baseline levels. Despite the glucose-dependent mechanism of action of EML 257, the pharmacodynamic evidence of efficacy that was observed in these healthy, normoglycaemic, fasted subjects, implies the potential for clinical efficacy at doses less than 400 mg twice daily in diabetic patients.
Conclusion: EML 257 was well tolerated at all dose levels. Exposure was dose proportional resulting in a dose-dependent increase in insulin levels with a small decrease in plasma glucose concentration.

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LM 4156, a novel, balanced PPAR α and γ activator improves glucose tolerance and triglyceride clearance.

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Background and Aims: LM 4156 is a novel, balanced peroxisome proliferator-activated receptor (PPAR) α and γ activator that is being developed to target insulin resistance-related glucose and lipid abnormalities associated with type 2 diabetes and metabolic syndrome. The study examined the effects of LM 4156 on glucose tolerance and HbA1C in db/db mice and its activity on triglyceride (TG) clearance in fa/fa Zucker rats.
Materials and Methods: Ten-week-old male db/db mice were dosed orally with LM 4156 (100 mg/kg/d) for 7 days. On Day 7, an oral glucose tolerance test was performed after an overnight fast. Blood samples were collected from the tail before (basal value) and 30, 60 and 120 minutes after the glucose load (80 mg/ mouse) for determination of glycaemia and insulinaemia. In a separate study, nine-week-old male db/db mice were orally dosed orally with LM 4156 (100 mg/kg/d) for 5-week to determine chronic effects on hyperglycaemia and HbA1C. TG clearance was assessed by an intravenous fat tolerance test in 12-week male fa/fa Zucker rats after 7 days of treatment with LM 4156 (100 mg/kg/d). On Day 7, Intralipid® (100 mg TG/rat) was injected 2 hours after food withdrawal and 1 hour after treatment. Blood samples were collected under anaesthesia before and 2, 5,10, 20, 30 and 60 minutes after the fat load for determination of TG.
Results: LM 4156 significantly decreased both basal hyperglycaemia (-35%, p<0.01 vs placebo) and hyperinsulinaemia (-51%, p<0.01 vs placebo) and reduced the postprandial response assessed by area under incremental curve (AUIC) for glucose ($AUIC_{0\text{-}120min}$: -37%, p<0.01 vs placebo) and insulinaemia ($AUIC_{0\text{-}60min}$: -81%, p<0.05 vs placebo) in db/db mice. This improvement of glucose tolerance was confirmed, in the chronic (5-week) study, by a significant reduction of hyperglycaemia (-66%, p<0.01 vs placebo) and of HbA1C (-2.8% in LM 4156 group vs +2.1% in placebo group, p<0.01). In fa/fa Zucker rats, LM 4156 decreased basal TG (-60%, p<0.01 vs placebo) and restored TG clearance (5.42 µmol TG/min vs 4.17 µmol TG/min in placebo, p<0.01) to the level of lean rats (5.09 µmolTG/min).
Conclusion: The dual PPAR α and γ activator LM 4156 induces a potent decrease of hyperglycaemia, HbA1C and plasma hypertriglyceridaemia in animal models of diabetes and insulin resistance. These properties are associated with an improvement in insulin sensitivity and TG clearance and offer a potential monotherapy in the management of both hyperglycaemia and dyslipidaemia in type 2 diabetic patients.

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LM 4156, a novel, balanced PPAR α and γ activator decreases hypertriglyceridaemia without effect on body weight and adipose tissue in fa/fa Zucker rats.

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Background and Aims: LM 4156 is a novel, balanced peroxisome proliferator-activated receptor (PPAR) α and γ activator that is being developed to target insulin resistance-related glucose and lipid abnormalities associated with type 2 diabetes and metabolic syndrome. As overweight is now considered as a major feature associated with this syndrome, therefore we studied the effect of LM 4156 on body weight and adipose tissue in insulin-resistant obese Zucker rats.
Materials and Methods: Obese male Zucker rats (fa/fa) were treated orally for 15 days with LM 4156 (100 mg/kg/d) or rosiglitazone (10 mg/kg/d). Body weight and food consumption were measured during the study. At Day 15, serum lipids were assessed enzymatically, and epididymal and retroperitoneal white adipose tissues (WATs) were removed to measure adipocyte size by morphometry using an image analysis system.
Results: There was no modification of food consumption during the study in placebo- (-2g/d) and LM 4156- (–1g/d) treated rats, but rosiglitazone induced a marked increase: +10g/d over the 15 days of treatment. Body weight gain and epididymal fat weight were similar in the placebo- (+65g and 6.3g respectively) and in the LM 4156-treated groups (+63g and 5.9g respectively, NS vs placebo) but they were significantly increased with rosiglitazone (+113g and 8.5g respectively, both p<0.01 vs placebo). The increase in body weight was directly related to adipose tissue increase as supported by the positive correlation between body weight gain and epididymal fat weight (r^2=0.77). Both compounds induced a potent decrease in triglycerides levels: -52% and -65% vs placebo (both p<0.01 vs placebo), for LM 4156 and rosiglitazone respectively. Morphometric analysis of adipocyte distribution along with their size (sectional area) demonstrated that LM 4156 did not exhibit the profile of a specific PPAR γ activator, which is characterized by a marked increase in the population of smaller-sized adipocytes in both epididymal and retroperitoneal WATs (area range: 2000-4000 μm^2) as observed with rosiglitazone.
Conclusion: These data demonstrate that LM 4156, which is a balanced PPAR α and γ activator, decreases plasma triglycerides without the major effects shown by a specific PPAR γ activator on adipocyte size distribution, body weight gain and food consumption, in fa/fa Zucker rats. This lack of a detrimental effect on body weight and adipose tissue would be of added value in overweight diabetic patients.

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In vitro and in vivo metabolism of LM 4156 in rat, dog, monkey and human: potential clinical consequences.

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Background and Aims: LM 4156 is a novel balanced peroxisome proliferator-activated receptor (PPAR) α and γ activator that is being developed to target insulin resistance-related glucose and lipid abnormalities associated with type 2 diabetes and metabolic syndrome. This study was conducted to determine the *in vitro* and *in vivo* metabolic profiles

of LM 4156 in different species and to predict the risk of *in vivo* drug-drug interactions in humans.
Materials and Methods: LM 4156 *in vitro* metabolism was assessed in seeded cultured hepatocytes from Sprague-Dawley rats, beagle dogs, cynomolgus monkeys and humans. The *in vivo* metabolism studies were conducted in Wistar rats, beagle dogs, cynomolgus monkeys and humans after administration of [^{14}C]LM 4156. A liquid chromatography/tandem mass spectrometric method was used to identify the metabolites. The enzymes responsible for the metabolism of LM 4156 in humans were identified using supersomes expressing individual cytochromes P450 (CYPs) and UPD-glucuronyl transferases (UGTs). The inhibitory and induction potential of LM 4156 towards several CYPs were investigated using human hepatic microsomes and hepatocytes.
Results: After a 24-hour incubation with hepatocytes, LM 4156 was extensively metabolised, with phase II metabolism accounting for 62%, 100%, 93% and 82% of total metabolism in rat, dog, monkey and human hepatocytes, respectively. Seven metabolites were identified: two oxidative and five conjugated derivatives. The two conjugated metabolites plus the oxidative derivative observed in human hepatocytes were also found after rat and monkey hepatocyte incubation. These *in vitro* results were confirmed *in vivo*. Excretion balance was similar in the three animal species, faecal excretion being predominant and ranging from 66% in monkeys to 70% in rats. In humans, 57% of the radioactivity was recovered in faeces and 40% in urine. Metabolic profiles indicated that all the metabolites identified *in vitro* were found *in vivo* in dogs, monkeys and humans; in rats, some differences were observed.
Identification of the enzymes involved in LM 4156 biotransformation showed that five CYPs and four UGTs contribute to LM 4156 metabolism, CYP2C8 and 2D6 being the main oxidative enzymes and UGT1A1 and 1A3 being predominant in phase II metabolism.
Inhibitory and induction potential studies showed that LM 4156 was a weak inhibitor of CYP1A2 and 2C9 (IC_{50} values >100 μM) but not of CYP3A4. LM 4156 did not induce CYPs, including CYP3A4.
Conclusion: These data indicate that LM 4156 metabolism was well characterised in the different species used during its development. The results demonstrated that many enzymes are involved in LM 4156 metabolism but not CYP3A4, and that LM 4156 has neither inhibitory nor induction potential toward P450 enzymes including CYP3A4. Therefore, it can be concluded that there is little risk of *in vivo* drug-drug interaction involving LM 4156 and other co-administrated compounds, particularly CYP3A4 substrates like statins.

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PPARpan improves glycemic control without weight gain or hemodilution: nonclinical data in diabetic rodents and non-human primates.

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Background and Aims: PPARpan agonists bind and activate the three peroxisome proliferator-activated receptor (PPAR) subtypes a , g, and d modulating the metabolism, storage, and transport of fatty acids and their metabolites. The three subtypes are co-expressed in nearly all metabolically active tissues, suggestive of an interactive relationship for normal energy homeostasis. The objective of these studies was to profile a potent PPARpan agonist in both diabetic rodents and insulin resistant non-human primates.
Materials and Methods: Compound 1, a PPARpan agonist was evaluated as an oral solution in Zucker Diabetic Fatty fa/fa rats (0.3-3 mg/kg) and in 6 middle-aged obese, hyperinsulinemic rhesus monkeys (dose-escalation, 0.03-0.3 mg/kg) for 4-weeks per dose. A PPARg agonist was included in the ZDF rat study as a reference agent with post-prandial measurements taken at 1-week intervals. The colony of spontaneously obese monkeys exhibit symptoms resembling Metabolic Syndrome. They present with obesity (18.8 ± 1.9 kg, normal lean (nl) 9 kg), elevated fasting glucose (95 ± 6 mg/dL, nl 60), elevated insulin (253 ± 87 μU/mL, nl 45) and TGs (267 ± 59 mg/dL, nl 48), with low HDLc (64 ± 7 mg/dL, nl 48). Euglycemic clamps were performed during vehicle and again after treatment with the PPARpan agonist. Serum clinical chemistries were obtained at 2-week intervals following a 16-hour fast.
Results: Compound 1 decreased serum glucose (-68%), insulin (-84%), TGs (-32%) and NEFAs (-72%) in diabetic ZDF fa/fa rats to a similar degree as the reference PPARg agonist. There was no change in food consumption, and no increase in bodyweight or decrease in hematocrit associated with efficacy. In obese hyperinsulinemic rhesus monkeys, dose-dependent reductions relative to vehicle were observed. At the 0.3 mg/kg dose: glucose -27%, insulin -80%, and TG -82%, HDLc increased by 91% and was associated with significant increases in Apo-AI and -AII. Non-HDLc decreased by 32%. LDLc decreased 27% by NMR analysis, with an associated increase in LDL particle size. Insulin sensitivity improved as measured by euglycemic clamps from 6.4 ± 3.0 to 9.8 ± 2.9 mg/kg FFM/min. (p=0.04) following treatment with compound 1 .
Conclusion: Insulin sensitivity improved in both diabetic rodents and non-human primates with normalization of glucose and insulin, combined with improvements in lipid profiles as assessed by increased HDLc and lowering of TGs and LDLc. Notably, hemodilution and weight gain were not observed. These findings suggest that a molecule possessing PPARpan agonist activity may be effective therapy for treating the insulin resistance, hyperglycemia, and dyslipidemia associated with type 2 DMand Metabolic Syndrome.

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Antidiabetic properties of a new potent glycogen synthase kinase 3 inhibitor.

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Background and Aims: We have previously reported that potent and selective pyrimidine based glycogen synthase kinase 3 (GSK3) inhibitors stimulate glycogen synthase activities, promote insulin-mediated glucose uptake and increase glucose disposal in rodent models of diabetes. These are the only selective GSK3 inhibitors to have reported in vivo antidiabetic activities thus far. We now report the in vivo efficacy of a potent GSK3 inhibitor from a distinct chemical class.
Materials and Methods: Compound CT21022 was tested for its ability to inhibit a diverse panel of kinases (including GSK3, P70S6 kinase, PDK1, ERK2, PI3 kinase, Akt1 and PKC zeta). Glycogen synthase (GS) activity in isolated liver and muscle tissues from ZDF rats was also monitored following a single oral dose of CT21022, and its abilities to reduce blood glucose and improve oral glucose tolerance (OGT) were tested in ob/ob mice and ZDF rats.
Results: CT21022 potently inhibited GSK3 with half maximum inhibitory concentrations (IC50) of 28 nM. The compound showed selectivity for GSK3 with IC50 for P70S6 kinase: 3.8 μM, PDK1: 5.8 μM, ERK2: >10 μM, PI3 kinase: >10 μM, Akt1: >10 μM, and PKC zeta: >10 μM. CT21022 also activates GS activity ex vivo in isolated muscle tissue from ZDF rats and in liver and muscle from animals treated orally with this compound. Upon a single oral dose of CT21022 to ZDF rats, a 172% and 55% increase of GS activity in liver and muscle tissues, respectively, was observed. In ob/ob mice, single oral dose of CT21022 elicited a rapid reduction of non-fasted blood glucose. The reduction achieved 44% by 7 hours and was sustained at 24 hours post dose (30%). Following 4 days of once a day oral treatment with CT21022, overnight fasting blood glucose was reduced by 47% compared with the vehicle group and plasma insulin was also significantly reduced (70% reduction). When a single oral dose was administered to ZDF rats, CT21022 also elicited a rapid reduction of non-fasted blood glucose. The reduction reached 20% by 7 hours. After continued dosing once a day for 3 days, CT21021 significantly improved OGT.
Conclusion: These studies demonstrate that a GSK3 inhibitor from a new chemical series possesses a favorable pharmacological profile, with certain advantages over the previous pyrimidine series. These findings support the notion that GSK3 plays a significant role in the pathophysiology of type 2 diabetes and suggests that GSK3 inhibitors in general may represent a novel approach to treat the disease.

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The insulin sentiziser S15511-1 operates in a muscle fibre type specific manner.

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Background and Aims: S15511-1 is an original compound with demonstrated effects on insulin sensitivity in animal models of insulin resistance. However, its molecular mechanism of action remains to be established in more detail. The aim of our study was therefore to investigate the effect of long-term administration S15511-1 on the insulin stimulated glucose uptake and on the total GLUT4 content in rat skeletal muscles with different fibre type compositions.
Materials and Methods: Male Wistar rats were allocated to two groups. The treatment group received injections twice a day for 2 weeks, with 2 × 1 ml/kg of S15511-1 (5 mg/ml). Sedentary rats injected with corresponding volumes of saline served as control animals. The two groups were pair fed. In vitro insulin stimulated glucose uptake and total GLUT4 content in muscles displaying different fibre type composition were measured.
Results: Long-term S15511-1 administration resulted in a fibre type specific increase in glucose transport with the most pronounced effect on epitrochlearis muscles expressing predominantly type IIb fibres (5.50 ± 0.39 vs 3.80 ± 0.22 µmol/ml/hour, $p < 0.01$) while soleus expressing primarily type I fibres showed no significant increase in glucose transport (6.09 ± 0.18 vs 5.90 ± 0.21 µmol/ml/hour). S15511-1 administration concurrently resulted in an increased amount of total GLUT4 in the white parts of the gastrocnemius muscles (113.4 ± 9.5 vs 100.0 ± 2.8 (arb. units), $p < 0.05$) but no change was observed in the red parts of gastrocnemius.
Conclusion: S15511-1 is a potential promising novel insulin sensitizer that is capable of enhancing glucose transport in the relative less insulin sensitive type 2 muscle fibres. This enhanced glucose transport is mirrored by a fibre type specific increase total GLUT4 amount and thereby resembles the improved glucose homeostasis observed after long-term exercise. The role of AMP-activated protein kinase behind this effect remains to be determined.

PS 69
Hypoglycaemia (I)

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Recall of severe hypoglycaemia and consistency of self-estimated state of awareness in Type 1 diabetes.

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Background and Aims: Ability of patients with insulin-treated diabetes to remember severe hypoglycaemia and consistency of their self-estimated awareness of hypoglycaemia are not well-documented but important in clinical practice. The aim of this study was to assess recall of severe hypoglycaemia in patients with type 1 diabetes and to evaluate the feasibility of a simple method for clinical classification of awareness of hypoglycaemia.
Materials and Methods: One-year prospective study of a cohort of patients with type 1 diabetes (n=230). Rate of severe hypoglycaemia reported retrospectively at the end of the study was compared to prospectively recorded rate during the study period. Self-estimated awareness was explored in questionnaires at baseline and at end and assessments were evaluated by occurrence of severe hypoglycaemic episodes.
Results: Almost 90% of the participants correctly recalled whether or not they have had severe hypoglycaemia. However, those with high prospectively recorded numbers had incomplete recall, resulting in 15% underestimation of the overall rate. Based on the answer to the question "Do you recognise symptoms, when you have a hypo?" the cohort was classified in three groups: 40% with normal awareness, 47% with impaired awareness and 13% with unawareness. The groups with impaired awareness and unawareness had 5.1 and 9.6 times higher rates of severe hypoglycaemia, respectively, compared to the group with normal awareness ($p<0.001$).
Conclusion: Patients with type 1 diabetes generally remember severe hypoglycaemic episodes during a one year period. The usefulness of a simple method for classification of state of awareness in clinical practice is suggested.

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Modelling hypoglycaemic count data with extra observed zeros.

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Background and Aims: Severe hypoglycaemia is a major life-threatening complication of type I diabetes in children. All children with type 1 diabetes are at risk of experiencing an episode of severe hypoglycaemia due mainly to intensive insulin therapy. Hypoglycaemic count variables indicate how many times a severe hypoglycaemic episode has occurred within a defined observation period. These counts take the form of non-negative integers (0,1,2,3,4 etc). In order to examine risk factors associated with a severe hypoglycaemic episode, it is crucial to use models specifically designed for count outcomes that fit the overall structure of the data set. We determine if the commonly used Poisson regression model fits severe hypoglycaemic count data when there are excess zero counts present and we investigate the use of alternative models.
Materials and Methods: A total of 1229 children with type 1 diabetes (mean age 11.7 years and sd 4.1) of which 605 (49.2%) were males were studied. Prospective assessment of severe hypoglycaemia (an event leading to loss of consciousness or seizure or resulting in a hospital admission) was made over the nine-year period, 1992-2001. Patients were seen with their parents every three months. Data were analysed using the Poisson regression, zero-inflated Poisson (ZIP) and zero-inflated negative binomial (ZINB) regression models. The over dispersion and likelihood ratio statistics were calculated to assess and compare the model fits.
Results: Our cohort of 1229 diabetic children had an average number of clinic visits to be 18.9 (sd=11.9), with more than 70% of them having ten or more visits. Less than 30% (171 males and 159 females) experienced at least one episode of severe hypoglycaemia over the whole follow-up period. Amongst those who had experienced a SH event, the average number was 2.5 events (ranging from 1 up to 15 events per child). The average age of a child who never had a SH event was 11.9 years compared with 11.4 years for children who had one or more SH event. In total there were 834 SH events. A comparison of all three models was made. The Poisson regression

model did not fit the data well. The ZIP model fitted the data much better than Poisson, however ZINB fitted significantly better than ZIP or Poisson.
Conclusion: The use of Poisson regression models may lead to biased parameter estimates as it does not adequately account for the large number of zero counts . We recommend the use of the ZINB model to examine any risk factors associated with severe hypoglycaemia. These models have not been used previously in diabetes epidemiology. Statistical software is available to fit these models.

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Recall of severe hypoglycaemic episodes and course of hypoglycaemia awareness in insulin-treated Type 2 diabetes in one year follow-up.
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Background and Aims: Most studies reporting severe hypoglycaemia (SH) are based upon retrospective data. Ability of patients with type 2 diabetes to recall SH is not known. The aim of this study was to assess recall of SH by insulin-treated patients with type 2 diabetes over a period of one year.
Materials and Methods: 129 consecutive out-patients with insulin-treated type 2 diabetes (mean age 62 ±11 years, diabetes duration 15 ±7 years, duration of insulin use 9 ±5 years, HbA1C 8.4 ±1.2 %,) were included in a one-year prospective observational study. An identical questionnaire was completed at the base line and at the end of study, on occurrence of SH in the preceding year. SH was defined by assistance from other persons and rates of SH reported retrospectively at the end of study were compared to prospectively recorded rates during the same period. Self-estimated hypoglycaemia awareness was scored on a 4 point scale.
Results: The overall rates of prospectively and retrospectively recorded SH were similar (0.41 and 0.47 episodes per patient-year, respectively, p=0.60). There was a high degree of agreement between prospective and retrospective data (R^2=0.62, p<0.001). At baseline 69% had normal awareness, 25% reported impaired awareness and 6% had hypoglycaemia unawareness. At the end of the study, awareness was scored similar to baseline by 74% of the participants (p=<0.001). The rate of SH was 0.20, 0.81 and 1.2 episodes per patient-year in groups with normal awareness, impaired awareness and unawareness of hypoglycaemia, respectively (p<0.005).
Conclusion: Recall of severe hypoglycaemia is well-preserved during a one-year period in patients with insulin-treated type 2 diabetes. State of hypoglycaemia awareness estimated by a simple question is reproducible and has significant influence on prospectively recorded rate of SH.

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Predicting occurrence of severe hypoglycemia from routine self-monitoring blood glucose data.
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Background and Aims: Severe hypoglycemia (SH) is a dangerous potential consequence of intensive insulin therapy. However, accurate prediction of SH has been unsuccessful. The DCCT was able to account for 8% of its long-term occurrence based on history of SH and HbA_{1c} and this was increased to 18% by a newer structural-equation model. We have reported that the Low Blood Glucose Index (LBGI), a risk measure of how frequently and how low BG goes, in combination with history of SH accounted for 46% of the variance of SH events over the next six months. We have also observed and reported the following patterns occurring during the 48-24 hours prior to SH: 1) average BG decreased due to recurrent mild hypoglycemic episodes; 2) BG swings increased over the whole BG range, and 3) LBGI increased, most notably in the 24 hours preceding an SH episode. Based on these previous observations we now report mathematical algorithms predicting both long-term risk of SH and imminent (next 24 hours) SH episodes.
Materials and Methods: Data from two groups of subjects with Type 1 diabetes (T1DM) were used for prediction of long-term risk and imminent SH. Group 1 consisted of 96 subjects (58 females) with average age = 35 (SD=8) years, duration of T1DM = 16 (SD=10) years and HbA_{1c} = 8.6% (SD=1.8), who performed SMBG for a month (3-4 readings/day) and then completed 6 months of SH diaries. These data were used to design Algorithm 1 using the LBGI and history of SH to predict long-term risk of SH. Group 2 included 85 subjects (41 females) with average age = 44 (SD=11) years, duration of T1DM = 26 (SD=11) years and HbA_{1c} = 7.7% (SD=1.1), who completed 6 months of SMBG (4 readings/day) and parallel diaries of SH. These data were used to design Algorithm 2 predicting imminent (within 24 hours) SH events. Algorithm 2 utilizes 30-day moving values of the LBGI and its variance, and low BG risk values over the last 24 hours. When these risk values exceed certain combination of thresholds, Algorithm 2 signals increased risk of SH in the next 24 hours.
Results: Subjects in Group 1 reported on average 2.2 SH episodes per person over 6 months, with more than half of all subjects reporting no SH. Algorithm 1 accounted for 49.5% of the total variance of SH and computed the theoretical probability of each subject experiencing at least one SH in the next 6 months. The agreement between this probability and the prospectively observed frequency of SH was 95%. Subjects in Group 2 reported 4.7 SH episodes per person for 6 months. Algorithm 2 predicted (within 24 hours) 50% of these episodes regardless of subjects' frequency of SMBG. For subjects who had at least 4 readings during the day preceding an SH episode, this prediction increased to 57%.
Conclusion: Computational algorithms using routine SMBG data have the potential to accurately evaluate long-term risk of SH and predict at least half of imminent SH episodes, thus helping reduce the occurrence of such episodes in T1DM.

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Confirmed lower risk of hypoglycaemia with insulin glargine versus NPH insulin: a meta-analysis of 2304 patients with Type 2 diabetes.
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Background and Aims: Insulin glargine (LANTUS®) is a once-daily basal insulin analogue that provides glycaemic reductions equivalent to NPH insulin, with reduced hypoglycaemia risk (particularly nocturnal), in patients with Type 2 diabetes. The 'Treat-to-Target' study showed that more patients on insulin glargine reached HbA_{1c} ≤7.0% without confirmed or severe nocturnal hypoglycaemia versus NPH insulin. The purpose of this analysis was to substantiate these findings in a larger patient population.
Materials and Methods: A meta-analysis of hypoglycaemia profiles from all Phase III/IIIb controlled trials for insulin glargine versus once- or twice-daily NPH insulin in adults with Type 2 diabetes, including the 'Treat-to-Target' study, was performed. All studies were 16–28 weeks long except one 52-week study, for which interim 20-week data were used.
Results: Patient demographics were similar between the insulin glargine (n=1142) and NPH insulin (n=1162) groups; mean baseline HbA_{1c} was 8.8 ± 1.1% versus 8.7 ± 1.1%, respectively. Insulin glargine and NPH insulin treated patients reached similar endpoint HbA_{1c} (7.8 ± 1.3% vs 7.7 ± 1.2%). For the incidence of all symptomatic and nocturnal hypoglycaemia, and for the incidence of episodes confirmed by plasma glucose (PG) levels, there was a consistent, significant risk reduction of hypoglycaemia associated with insulin glargine versus NPH insulin (Table), with a most notable 29% risk reduction of confirmed nocturnal hypoglycaemia.
Conclusion: These results are consistent with those of the 'Treat-to-Target' study. The lower hypoglycaemia risk with insulin glargine may facilitate treatment to lower blood glucose targets, allowing more patients with Type 2 diabetes to reach HbA_{1c} ≤7.0%.

Hypoglycaemia type	Insulin Glargine	NPH insulin	p value	Insulin Glargine % risk reduction
All symptomatic	54.2%	61.2%	0.0006	11
Confirmed symptomatic (PG ≤4 mmol/L [≤72 mg/dL])	46.0%	53.3%	0.0004	14
Confirmed symptomatic (PG ≤3.1 mmol/L [≤56 mg/dL])	29.9%	37.0%	0.0002	19
All symptomatic nocturnal	28.4%	38.2%	<0.0001	26
Confirmed symptomatic nocturnal (PG ≤4 mmol/L [≤72 mg/dL])	23.9%	33.9%	<0.0001	29
Confirmed symptomatic nocturnal (PG ≤3.1 mmol/L [≤56 mg/dL])	16.3%	23.1%	<0.0001	29

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A meta-analysis of phase III/IIIb studies comparing insulin glargine with human NPH insulin in Type 2 diabetes: severe hypoglycaemia is less common with insulin glargine.

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Background and Aims: Randomized, controlled studies comparing insulin glargine (LANTUS®) with NPH insulin in patients with Type 2 diabetes have consistently shown at least equivalent glycaemic control, with a trend towards less severe hypoglycaemia (a symptomatic event requiring assistance from another person, and plasma glucose <56 mg/dL [3.1 mmol/L] or prompt recovery after oral carbohydrate, intravenous glucose or glucagon administration) for insulin glargine. The aim of this analysis was to investigate the occurrence of severe hypoglycaemia in a large population of patients with Type 2 diabetes.
Materials and Methods: A meta-analysis was carried out on all Phase III/IIIb studies conducted to date with insulin glargine versus NPH insulin (four studies). These studies were of 16–28 weeks' duration, except for one study of 52 weeks' duration, where interim 20-week data were used. Insulin glargine (n=1142) was administered once daily, and NPH insulin (n=1162) once- or twice-daily.
Results: At baseline, there were no significant differences between the insulin glargine and NPH insulin groups in mean (± standard deviation) age (58.0 ± 9.8 vs 58.4 ± 9.3 years, respectively), body mass index (30.5 ± 4.9 vs 30.5 ± 6.4 kg/m^2, respectively), diabetes duration or HbA_{1c} (8.8 ± 1.1 vs 8.7 ± 1.1%, respectively). Mean endpoint HbA_{1c} was similar with insulin glargine (7.8 ± 1.3%) and NPH insulin (7.7 ± 1.2%). Compared with NPH insulin, insulin glargine was associated with a significantly lower incidence of severe hypoglycaemia (1.4% of patients experiencing 24 episodes versus 2.8% experiencing 42 episodes; p=0.0237). This equates to a 50% risk reduction in the incidence of severe hypoglycaemia with insulin glargine versus NPH insulin use. There was also a significantly lower incidence of severe nocturnal episodes with insulin glargine versus NPH insulin (0.7% of patients experiencing 14 episodes vs 1.8% experiencing 23 episodes; p=0.0159). This equates to a 61% risk reduction in the incidence of severe nocturnal hypoglycaemia with insulin glargine versus NPH insulin.
Conclusion: These findings indicate that the incidence of severe symptomatic hypoglycaemia is lower with insulin glargine versus NPH insulin in patients with Type 2 diabetes; this is important for the safe management of these patients. This analysis also suggests that insulin glargine is especially suited to treat-to-target algorithms with stringent blood glucose targets.

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Driving performance of patients with Type 2 diabetes mellitus during euglycaemia and moderate, symptomatic hypoglycaemia.

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Background and Aims: The incidence of type 2 diabetes mellitus is mounting throughout the world. Although driving privileges are progressively restricted, no clear data are available on driving performance or the occurrence of road traffic incidents.
Materials and Methods: We studied 20 type 2 diabetic subjects with normal awareness of hypoglycaemia, and 24 healthy controls, in a state-of-the-art driving simulator. Driving was studied in different environments during clamped euglycaemia (5.0 mmol/L) and moderate hypoglycaemia (2.7 mmol/L). In addition to uneventful driving, some unexpected events were programmed to occur. Also, a continuous peripheral detection task was applied to monitor driver's workload. Various driving parameters were measured, including reaction time, standard deviation of lateral position (swerving), standard deviation of steering angle (quality of steering), lane exceedance, time to line crossing (lane keeping) and speed. For several subgroups post-hoc analyses were performed.
Results: Driving performance during euglycaemia was slightly poorer in the study group in a small number of conditions. Under all other euglycaemic conditions driving performance was better nor worse than performance in the control group. During hypoglycaemia driving performance was not further affected. When comparing to known standards of good driving, driving performance remained within limits of safe driving in all conditions, both euglycaemic and hypoglycaemic. No subgroup with poorer driving performance could be identified. However, the effort needed to drive was higher in a number of circumstances.
Conclusion: Patients with type 2 diabetes and normal awareness of hypoglycaemia can drive safely during euglycaemia. For reasons unclear, more effort is required to drive safely as compared to non-diabetic drivers. During moderate, symptomatic hypoglycaemia safe driving is maintained. Therefore, when hypoglycaemia occurs during driving, patients have the opportunity to take proper action before driving performance will inevitably decrease at lower blood glucose levels.

PS 70
Hypoglycaemia (II)

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Type 1 diabetic patients with hypoglycemia unawareness have decreased plasma metanephrine levels, reflecting reduced adrenomedullary capacity.

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Background and Aims: Counterregulatory failure in diabetic patients with hypoglycemia unawareness is thought to be caused by resetting of the glycemic threshold for hormonal activation to lower levels rather than reduced secretory capacity. However, observations of reduced adrenaline responses to other stimuli (e.g. exercise) suggest a contribution of attenuated adrenomedullary capacity. Since >90% of circulating metanephrine is derived from adrenomedullary conversion of adrenaline, plasma metanephrine may be used as a measure of adrenomedullary capacity. We measured plasma metanephrine levels in type 1 diabetic patients with hypoglycemia unawareness to assess their adrenomedullary capacity.
Materials and Methods: Ten type 1 diabetic patients (5 men, 5 women) and 10 age-, sex- and BMI-matched nondiabetic controls were enrolled. All patients had clinical hypoglycemia unawareness and were specifically selected for adrenomedullary failure during experimental hypoglycemia (adrenaline responses, 2.5±0.3 versus 7.6±0.9 nmol/L), but had no signs of autonomic neuropathy. Plasma metanephrines and catecholamines were measured at baseline and after obtaining euglycemia by a hyperinsulinemic (360 pmol·m^{-2}·min^{-1}) glucose clamp.
Results: At baseline, diabetic patients had higher fasting plasma glucose (12.2±1.0 versus 5.2±0.1 mmol/L, P<.001) and plasma insulin levels (337±76 versus 65±6 pmol/L, P=.003) than nondiabetic controls. After insulin infusion, glucose levels were similar (5.0±0.0 versus 4.9±0.1 mmol/L, P=NS), yet plasma insulin levels were still somewhat higher in patients (836±92 versus 559±24 pmol/L, P=.01). There were no differences between patients and controls in plasma adrenaline or noradrenaline levels at baseline or at clamped euglycemia. However, plasma metanephrine levels were significantly lower in diabetic patients both at baseline (178±9 versus 243±17 nmol/L, P=.01) and at clamped euglycemia (157±13 versus 204±14 nmol/L, P=.02). Consequently, the metanephrine/adrenaline ratio was lower in diabetic patients than in controls throughout the study (P=.03).
Conclusion: Type 1 diabetic patients with hypoglycemia unawareness have lower plasma metanephrine levels than matched controls, indicating reduced adrenomedullary capacity. Further research is required to elucidate to what extent this reduced adrenomedullary capacity contributes to hypoglycemia unawareness.

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Intake of CNS-active drugs and substances by patients with diabetes prior to severe hypoglycaemia.

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Background and Aims: Ingestion of excessive amounts of alcohol increases the risk of severe hypoglycaemia in diabetes by compromising hepatic glucose mobilisation and by interfering with hypoglycaemic awareness. The latter effect may be shared by other agents with effect on the central nervous system (CNS). The aim of this study was to explore exposure to CNS-active substances prior to episodes of severe hypoglycaemia in patients with insulin treated diabetes.
Materials and Methods: One-year prospective study including events of severe hypoglycaemia in patients with insulin treated diabetes requiring prehospital or emergency room treatment. Participants were interviewed concerning circumstances of the event including exposure to alcohol, pharmaceutical and other drugs with CNS effects. Blood tests were screened for alcohol, CNS-active pharmaceutical agents and substances of abuse by HPLC combined with mass spectroscopy.
Results: A total of 148 cases in 115 patients (91 type 1 diabetes, 19 type 2 diabetes, 5 diabetes due to chronic pancreatitis) were included. Alcohol intake was reported in 57 (39%) and positive serum-ethanol was present in 24 (17%) cases. In 23 (16%) both alcohol history and blood test was positive. Intake of CNS active drugs was reported in 12 (8%) of cases and 10 (6%) reported exposure to cannabis. Screening of blood samples was positive in 16 cases (11%) with antidepressants, benzodiazepines, opioids and amphetamine (6, 4, 2 and 2 cases, respectively) as the most frequently occurring drugs. Only 2 blood samples were positive for both alcohol and drugs. Overall, some kind of CNS active substance was detected in 38 (26%) of the samples.
Conclusion: History of and biochemical verification of ingestion of CNS active agents prior to severe hypoglycaemia is present in a large proportion of episodes requiring medical emergency treatment. This suggests that ingestion of such substances may play a role for the most severe episodes of hypoglycaemia.

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Angiotensin II receptor gene polymorphisms and occurrence of severe hypoglycaemia in Type 1 diabetes.

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Background and Aims: We have previously shown a strong relationship between high plasma angiotensinogen concentration, high serum ACE activity and occurrence of severe hypoglycaemia (SH) in type 1 diabetes. This study was undertaken to test the hypothesis that genotypes for angiotensin II receptor (ATIIR) subtypes 1 and 2 are also related to rate of SH.
Materials and Methods: A cohort of 171 unselected patients with type 1 diabetes, untreated with ACE inhibitors or angiotensin II receptor antagonists, were followed for one year by monthly questionnaires and immediate reporting of episodes of SH (defined as episodes needing assistance from others). ATIIR subtype 1 (A1166C) and 2 (G1675A) genotypes were determined by PCR. Subjects were characterised by C-peptide status, hypoglycaemic awareness, HbA1c, and clinical data.
Results: Subjects homozygous for the A-allele of the ATIIR subtype 2 reported a 2.6 (95% CL: 1.3-6.3) times higher rate of SH compared to those not carrying the allele (p=0.015). There was no significant relationship between ATIIR subtype 2 genotype and rate of mild (p=0.79) or biochemical hypoglycaemia (p=0.68) or other risk factors as self-reported state of awareness (p=0.15), C-peptide status (p=0.17) or HbA1c (p=0.11). There was no influence of ATIIR subtype 1 on occurrence of SH, awareness, mild or biochemical hypoglycaemia.
Conclusion: The A-allele of ATIIR subtype 2 at codon 1675 that confers low expression of the receptor is associated with occurrence of severe hypoglycaemia compared to the G-allele. This provides further evidence for a critical role of the renin-angiotensin system in predisposition of patients with type 1 diabetes to severe hypoglycaemia.

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Effects of insulin-induced hypoglycaemia on energy intake and food choice at a subsequent test meal.

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Background and Aims: Hypoglycaemia is a common complication of diabetes treatment. It is widely believed to cause an increase in food intake, but surprisingly there is little data on the magnitude of this effect, or whether subjects make appropriate food choices to correct hypoglycaemia as rapidly as possible. We have therefore undertaken a preliminary study to investigate the effects of insulin-induced hypoglycaemia on food intake at a test meal.
Materials and Methods: Sixteen healthy volunteers (mean age 29.8years) enrolled for a double-blind cross over study: either insulin (0.05units/kg) or saline was given as a bolus intravenously. Blood glucose was measured

every 5 minutes for 20 minutes following the injection and thereafter every 20 minutes till 120 minutes. Subjects were given a tray of foods at 20 minutes after insulin or saline, presented in an ad-libitum style and were asked to eat as much or as little food as they wished. Food was weighed before and after consumption.
Results: Blood glucose was unchanged following saline (4.3 ± 0.4 to 4.4 ± 0.3 mmol/l) (mean ± SD). There was a transient decline in blood glucose after insulin with a nadir at 20 minutes (4.31± 0.34 to 2.41 ± 0.45 mmol/l), which returned to baseline at 40 minutes. Energy intake during the meal was increased by 17% (7117.8 ± 3746 kJ vs 5973.6 ± 3410 kJ, p=0.026) following insulin dose compared to saline. Subjects consumed all the high fat foods (muffins) (69.2 ± 54.1 vs 29 ± 42.3 gm, p=0.009) after the insulin dose. A non-significant increase in high carbohydrate food consumption was also observed.
Conclusions: Transient insulin-induced hypoglycaemia increases energy intake. Subjects consumed more high fat foods after insulin compared to saline. High fat foods have the potential to induce passive overconsumption and also have a low glycaemic index, which might therefore prolong hypoglycaemia, both of which might lead to weight gain in individuals with recurrent hypoglycaemia.

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Hypoglycemia warning: a simulation based on blood glucose data.
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Background and Aims: The availability of continuous glucose monitoring (CGM) systems in principle allows to detect hypoglycemic episodes in due time. A recent publication by Choleau et al. (Diabetes 2002 Nov;51(11):3263-73) using a glucose electrode for CGM in rats showed, that activating a hypoglycaemic warning signal, when a low glucose level is reached is obviously too late. It is necessary to introduce a trend analysis of blood glucose (BG) changes to prevent the occurrence of hypoglycemic events.
Materials and Methods: BG profiles of five patients with diabetes were evaluated for at least 24 hours, by means of regular BG measurements with a laboratory method and in parallel with a non-invasive, continuous glucose sensor (Pendragon NI-CGMD). The patients were instructed to follow their normal daily activities, injecting insulin according to their requirements during this period. A numerical analyses of the BG profiles, namely of slopes, second differentials thereof and curvatures of glucose changes, was performed. This analysis allowed developing a model that predicts BG values 20 minutes in the future. By employing this model on the data recorded from the five patients, we evaluated the applicability of the model for hypoglycemic warnings in time. Recorded BG profiles showed that in total 22 hypoglycemic events took place with BG values lower than 60 mg/dl.
Results: The model identified all hypoglycemic events. Additionally, in 5 cases the warning signal was also triggered when BG decreased rapidly to values below 80 mg/dl but BG then started to increase again before reaching the hypoglycemic level. The occurrences of hypoglycemic events were detected by the model 20 min before they took place.
Conclusion: Our simulation suggests that a continuous glucose monitoring system can be a reliable warning system for hypoglycemic events in patients with diabetes.

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The long term impact of severe hypoglycaemia on cognitive function in early onset Type 1 diabetes mellitus.
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Background and Aims: It has been suggested that the developing brain is susceptible to hypoglycaemic insult. However the question as to whether hypoglycaemia is responsible for the cognitive impairment documented in children with early onset Type 1 Diabetes Mellitus remains unproven. The aim of this study was to examine this question by studying a large population based sample of children in whom hypoglycaemia frequency had been closely monitored and recorded from diagnosis.
Materials and Methods: Children who had been diagnosed with Type 1 Diabetes Mellitus prior to the age of 6 yrs (3.2±1.5 yr) were included in the study. When studied, all subjects were older than 6 yrs (6-15yr, mean 10.1±2.4 yr). In this sample, hypoglycaemia frequency (episodes of coma or convulsions) had been monitored prospectively in all subjects from diagnosis and recorded, along with HbA_{1c} and other clinical parameters at 3 monthly intervals. Children with a history of coma or convulsion (n=42) were compared with a sample of children with no history of severe hypoglycaemia (n=43) matched for age of diabetes onset, diabetes duration and age. As part of an assessment that also included cerebral magnetic resonance imaging and quantitative electroencephalogram measures, a single investigator blind to subject group administered a comprehensive test battery of learning and memory (Children's Memory Scale: Cohen 1997) and a general intellectual assessment (Weschler Intelligence Scale for Children: Third Edition). No significant cognitive differences were found between hypoglycaemia and non-hypoglycaemia groups in any of the assessed domains of memory or intellectual function and no evidence of cognitive impairment was found in diabetic subjects compared to nondiabetic population controls. Subgroup analysis of those experiencing severe hypoglycaemia prior to age 6 yrs revealed similar findings. Furthermore, examination of those memory sub-tests considered to be most sensitive to hippocampal compromise did not show any significant hypoglycaemia related affects. Mean HbA_{1c} from diagnosis was not associated with any differences in the assessed intellectual functions.
Conclusion: In this sample, severe hypoglycaemia in children with early onset diabetes was not associated with impaired intellectual and memory function. Compromised cognitive function in children with early onset diabetes may be due to other diabetes related effects, rather than hypoglycaemia.

PS 71
Devices

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Glucose monitoring self-testing:evaluation of five last generation different devices performance.
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Background and Aims: Considering that blood glucose self-monitoring is a source of clinical data that are increasingly important in therapeutic decisions, aim of the present study was to assess the accuracy and reproducibility of different glucose meters.
Materials and Methods: The following glucose meters were evaluated: Optium (Medisense - USA), Induo (Lifescan - USA), Accu-chek Comfort (Roche - CH), Accu-chek Active (Roche - CH), Accu-chek Comfort (Bayer- USA), Ascensia Elite (Bayer - USA). Blood glucose was determined twice with each instrument, and with glucose oxydase as reference method (CX3 Beckman - USA), on a venous blood sample drawn in the morning, after overnight fast, in a consecutive series of 66 diabetic outpatients. All determinations were performed by trained laboratory technicians. HbA1c was determined using an HPLC method (Menarini Diagnostic Italy, upper value of the reference range=6.2%) on the same blood samples, in our certified laboratory.
Results: Mean glycaemia value (glucose-oxydase) was 176.7+ ± 51.2 mg/dl (range 92-372), and mean HbA1c value was 7.1 ± 1.4 % (range 3.3 - 12.7). Correlation coefficient between the first and the second determination of blood glucose with each meter was: 0.98 for Optium, 0.99 for Induo, 0.98, for Accu-chek Comfort, 0.99 for Accu-chek Active and 0.99 for Ascensia Elite, while correlation of the mean of the two measurements with laboratory determinations was: 0.94, 0.97, 0.96, 0.96 and 0.96 respectively. A best fitting analysis was performed, showing that linear regression was the best fitter for correlation of each meter values and laboratory determination. When applying a linear regression model (Y=Ax) for conversion of meter values (x) into laboratory values (y), A was 0.92 for Optium, 0.90 for Induo, 0.75 for Accu-chek Comfort, 0.97 for Accu-chek Active, 0.92 for Ascensia Elite (Bayer - USA). Correlation (r) with HbA1c was 0.77 for laboratory-determined glycaemia, 0.68 for Optium, 0.90 for Induo, 0.75 for Accu-chek Comfort, 0.99 for Accu-chek Active, 0.92 for Ascensia Elite.
Conclusions: All the meters tested showed a satisfactory reproducibility, and measured values were closely correlated with laboratory findings. However meters systematically underestimated in different measure blood glucose levels with respect to reference method, suggesting the need for some form of correction. Present results could be used as a basis for the definition of such correction equations, facilitating the comparison of measures obtained using different meters.

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Self-monitoring of blood glucose (SBGM) using a glucometer with alternative site glucose testing: are all recomended testing site reliable?
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Background and Aims: SBGM using a glucometer, allowing alternative site glucose testing, has recently been a widely discussed. The recommended alternative testing site is the forearm, whilst some devices make it possible to obtain blood from other sites. The aim of the study was to compare results of measurements of all recommended blood glucose testing sites made at glucometer FreeStyle with fingertip values combined with laboratory tests.
Materials and Methods: A total of 640 blood glucose measurements were performed in patients hospitalized at our Diabetes Center. As a rule, 2 measurements were made at a time: sampling from an alternative site (thenar, hypothenar, forearm, arm, thigh, calf; n=320) and follow-up fingertip blood sampling (n=320). The blood sample obtained from an alternative site was examined using the glucometer; whereas the fingertip blood sample was analyzed simultaneously using the glucometer and the laboratory (glucometer Free Style, TheraSense and Beckman Analyser, Beckman Instruments). Statistical analysis was undertaken using regression analysis. Further, we determined the number of qualifications exceeding the admissible tolerance of error of measurements (Bland - Altman method, 1986). The result is expressed as the number of determinations in excess of the mean ± 2SD (SD = standard deviation) of the values measured.
Results: Of the 320 blood samplings from an alternative site (thenar in10%, hypothenar in 9,7%, forearm in 42.5%, arm in 20.6%, thigh in 9.4%, and thigh in 7.8%). The method of linear regression revealed the following correlations (alternative site vs fingertip; alternative site vs laboratory; fingertip vs laboratory): thenar (n=32) r=0.989 ;0.988; 0.988, hypothenar (n=31) r =0.984; 0.988; 0.983, forearm (n=136) r=0.981; 0.970; 0.987, arm (n=66) r=0.972; 0.912; 0.988, thigh (n=30) r=0.941; 0.919; 0.976, calf (n=25) r=0.989; 0.965; 0.993. The differences between the study sites were not statistically significant. Likewise, none of the study sites exceeded significantly the allowed deviation (the mean ± 2 SD of the measured values).
Conclusion: Comparative measurements revealed a good correlation between blood glucose values from the blood sample obtained from an alternative sampling site compared with values obtained in fingertip sampling. Also, a good correlation was seen in results obtained during fingertip blood sampling when compared with blood glucose values using the glucometer versus laboratory tests.
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The effects of skin temperature and testing site on blood glucose measurements taken by the InDuo™ integrated system.
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Background and Aims: Modern blood glucose (BG) monitoring devices (e.g. InDuo™) require very low blood volumes, allowing for testing at sites other than the traditional fingertip, but the reliability of such testing has not been fully elucidated. This randomised study aimed to compare the effects of cold/warm skin temperature combined with alternate site (forearm) testing versus conventional finger tip measurements.
Materials and Methods: Nineteen patients who had previously used InDuo™ for 6 weeks participated. Four simultaneous (within 1 minute) BG readings (left and right forearms and fingertips) were obtained from each patient 15, 10 and 5 minutes before eating. Ten minutes before eating, the patient immersed one arm in cold water (T: 15.5°C) and the other in warm water (T: 35.0°C). At time = 0 minutes arms were removed from water baths and the patient was offered a standard meal (of ≤15 minutes duration). Arms were again immersed in water baths and BG measured from the same locations 20 minutes after eating and at subsequent 15-minute intervals for 185 minutes. The effects of site testing and temperature were assessed in this period by identifying maximum BG concentration (C_{max}) and time to C_{max} (T_{max}).
Results: Significantly lower C_{max} values were observed for: cold forearm versus cold fingertip (-28.58 mg/dL; $p < 0.001$), warm forearm versus warm fingertip (-11.95 mg/dL; $p = 0.028$), cold fingertip versus warm fingertip (-17.16 mg/dL; $p = 0.002$), and cold forearm versus warm forearm (-33.74 mg/dL; $p < 0.001$). Significantly longer T_{max} were reported for cold forearm versus warm forearm (-22.37 minutes; $p < 0.001$) and cold forearm versus cold fingertip (-20.00 minutes; $p < 0.001$).
Conclusion: These results demonstrate that cold skin and forearm conditions significantly underestimate BG and delay time to maximal value compared with warm skin and fingertip readings.

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Alternative-site glucose measurement in clinical random samples: effect of skin temperature and time from antecedent meal.
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Background and Aims: Alternative-site glucose testing has earlier been evaluated mainly in special experimental conditions. We studied its reliability in random clinical samples with reference to skin temperature and time from antecedent meal.
Materials and Methods: We measured plasma glucose in samples from fingertip, palm and forearm in 50 insulin-treated diabetic subjects at the end of a routine outpatient visit. A FreeStyle meter (TheraSense, USA) was used and the stick was made with the device provided by the manufacturer. Forearm glucose was tested and skin temperature measured before and after 15 sec rubbing. Time from previous meal was registered. To obtain an

adequate blood sample a second attempt was needed in 4%, 22%, 34% and 26% of the patients in sampling from fingertip, palm and forearm without and with rubbing, respectively. In seven patients even the second attempt failed in at least one area and a third attempt was not undertaken. Thus the final study population consisted of 43 patients (mean age 35 yrs, BMI 23.3 kg/m2, HbA1c 8.7%, duration of diabetes 15 yrs).
Results: The mean (SD) glucose value from finger, palm, unrubbed forearm and rubbed forearm were 10.4 (4.5), 10.5 (4.4), 10.4 (4.2) and 10.2 (4.2) mmol/L (NS). In samples taken <2 hrs from previous meal (mean finger glucose 11.5 mmol/L, n=20) the finger-arm difference was +0.28 (NS) before and +0.60 mmol/L (p=0.06) after rubbing. In samples taken >2 hrs after meal the finger-arm difference was -0.18 (NS) before and -0.15 (NS) mmol/L after rubbing. In patients with low initial forearm temperature (<33.0 degrees C, n =20) rubbing increased it by 0.73 (p<0.001) and the finger-arm difference was -0.20 mmol/L (NS) before and +0.02 mmol/L (NS) after rubbing. In patients with high initial forearm temperature (>33.0 degrees C, n=23) rubbing increased it by 0.25 and the finger-arm difference was +0.23 (NS) before and +0.33 (p=0.12) after rubbing.
Conclusion: The overall reliability of forearm measurements with fingertip values as reference was satisfactory. Shortly after meal forearm values are somewhat lower than fingertip values. Forearm skin temperature slightly modifies the results: compared with fingertip glucose low arm temperature tended to associate with higher or similar forearm glucose whereas higher arm temperature tended to associate with lower arm glucose values.

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Evaluation of a new photoacoustic non-invasive continuous glucose monitor.
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Technology: Photoacoustics involves ultrasonic waves created by the absorption of light. These ultrasound waves are generated by illuminating the tissue with laser pulses at several selected wavelengths. Optical wavelengths are selected to provide specificity to glucose and remove the influence of other substances present in the blood. Analysis of the acoustic signals can map the depth profile of the absorbance of light in the tissue. The Photoacoustic effect is used to measure the influence of the glucose on the optical properties of the blood inside the vein and remove the influence of the surrounding tissue. An advanced prototype was evaluated in both in-vitro and on diabetic patients. The unique ability to non-invasively measure the optical properties directly from inside the blood vessel, enables accurate simulation of the In-Vivo effects of the glucose in a phantom model.
Materials and Methods: *Ex-Vivo*: A specially designed vein simulating phantom, which consisted of a tubing perfused with human whole blood (simulating vein) and surrounded by a light-scattering gel simulating the tissue, was developed to test the sensitivity of the non-invasive glucose monitor to blood glucose levels and to assess the interfering effect of different blood constituents and environmental parameters on the system calibration. Glucose was present in the tested blood samples at 60 to 600 mg/dl. The potential interferents were added at 3 to 5 times of the upper normal levels. *In-Vivo*: Seven, type I, diabetic individuals were tested using a prototype device attached to the wrist's vein. The blood glucose level was varied using glucose load and insulin treatment intermittently with 2 to 3 maxima and minima peaks during 4-6 hours of observation.
Results: *Ex-Vivo*: The PA measurements demonstrated linear correlation with blood glucose: r = 0.99 and mean absolute deviation (MAD) of 17 mg/dl. When one of the potential interferents (urea, bilirubin, the triglyceride mimic Triacetin or the hypoglycemic drug Metformin) were present, the MAD varied from 23 to 30 mg/dl. *In-Vivo*: 103 data points were collected from 7 subjects using finger sticks. The range of glucose level variations for the tested individuals was 100 to 450 mg/dl. The Clark error grid analysis showed that 100% of the measurements fell within zones A and B (88.3% in zone A and 11.7% in zone B). MAD was 25.0 mg/dl, and the correlation coefficient r was 0.93.
The proposed technology signifies an important step towards the implementation of real-time non-invasive continuous blood glucose monitoring.

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Postprandial glucose monitoring in Type 1 diabetes mellitus.
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Background and Aims: Assesment of blood glucose regulation in Type 1 DM currently relies on capillary glucose measured just before meals. These measurements give little information on patterns of glycemic changes such as postprandial hyperglycemic peaks and fluctuations. We aimed therefore to test a continuous subcutaneous glucose monitoring device (GlucoDay ®, A. Menarini Diagnostics) to describe postprandial glucose changes in T1DM.
Materials and Methods: Twenty-three T1DM patients on intensive insulin treatment (12 male/11 female; mean ± SD age 42 ± 9 y; duration DM 17 ± 8 y; HbA_{1c} 7.7 ± 0.8 %; daily insulin 50 ± 13U; BMI 23.8 ± 2.2 kg/m^2) were hospitalised for 1 day in the metabolic ward. After an overnight registration and about 14 hours after insertion of the subcutaneous microfibre of GDay, patients received a standard breakfast B (870 kcal, 61 energy% as fat, 28% as carbohydrate) and 3 hours later lunch L (670 kcal, 46 energy% as fat, 28% carbohydrate). Habitual self-glucose monitoring and insulin administration were not modified. GDay was monitored continuously online via an infrared connection. Intravenous blood samples were taken fasting and every hour for a total of 8 hours after breakfast.
Results: When calibrated by linear regression, GDay levels correlated well with intravenous plasma glucose (r = 0.96), with 92.6 % of the data falling within the A region of the error grid analysis and none in the C, D and E regions. The data thus recorded every 3 minutes was used to calculate parameters which describe the postprandial glucose changes in each patient. The total (8 hour) area under the curve AUC was related to duration of diabetes (r = 0.51, p = 0.016). This relationship was stronger in the postbreakfast period (30 min- and 3h-AUC, r = 0.62, p = 0.002). Similarly, maximum peak and 2h- glucose were related to diabetes duration only in the postB period (r = 0.57, p = 0.005). There was no significant relationship with age, BMI, insulin dose or HbA1c even when AUC was corrected for insulin dose. Although total insulin dose at B (14 ± 8 U) was higher than at L (10 ± 5 U, p = 0.030), 2h-glucose was higher after B (243 ± 69 vs 180 ± 79 mg/dL after L, p<0.0001) but both were lower than the respective maximum postprandial peaks (313 ± 105 mg/dL after B and 304 ± 119 after L, p < 0.0001) which were reached 1h25min after B and 4h8min after L. 3h-AUC was lower but 30-min AUC was higher after L (7,488 ± 2,208 vs 5,725 ± 2,414 min.mg/dL after B, p = 0.004). Glucose spikes (maximum peak minus fasting plasma glucose) were similar after B and L but the difference between maximum and minimum values was bigger after L (219 ± 115 vs 165 ± 110 mg/dL after B, p = 0.020). Duration of hyperglycemic periods >200, 140 or 126 mg/dL were not different after B or L, but time spent at glucose <100 mg/dL was longer after L (p < 0.0001).
Conclusion: These results illustrate the quality and possible uses of subcutaneous glucose registration to monitor and characterise postprandial glucose patterns in T1 DM. Application of these methods to evaluate glucose patterns in this and other clinical situations in DM can lead to therapeutic and dietary adjustments and ultimately improve glycemic control.

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Stability, response time, and inter-sensor variability of the Medtronic Minimed subcutaneous glucose sensor.
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Background and Aims: Subcutaneous glucose sensors require more frequent calibration if the signal is not stable, the response may be slowed due to delays in glucose transport to the interstitial fluid space, and signals may differ between sensors inserted in the same subject. The present study was designed to assess the stability, response time, and inter site variability of the Medtronic MiniMed SC glucose sensor.
Materials and Methods: Sensitivity (S), response time (τ), and variability were evaluated in 14 non-diabetic subjects (13 M 1 F, aged 52.7± 1.8 years, BMI=26.8± 0.9) who were admitted to the UCLA general clinical research center for 3 days. Two sensors were inserted in the abdominal area at ~6 am on day 1, and intravenous lines were started. At ~9:00 am on each day 5 mg/kg/min of 20% glucose was infused for 30 min and samples taken every

10 min for 30 min prior to and every 5 min during and for 30 min following the infusion. Plasma glucose (PG) was determined with a Beckman glucose analyzer. S and τ were estimated by fitting sensor current (I) during the test to a differential equation model ($dI_m/dt = -p_1I_m + p_2PG + OS$; $=1/p_1$, $S=p_2/p_1$, OS=offset current). Variability was assessed by comparing the 2 sensor signals (correlation, mean absolute difference).
Results: Of the 84 profiles (14 subjects x 2 sensors x3 tests per subject) 12 could not be identified due to an inadequate sensor response ($r^2<0.36$) or technical failure. Sensitivity was not different between days (0.105±0.01, 0.1130± .011 and 0.109± 0.009 nA per mg/dl; p=NS, repeated-measures-ANOVA) but the response time was longer on day 1 (10.5± 1.2 min) than on days 2 or 3 (6.9± 0.84 and 6.7± 0.87 min; p<0.05). The correlation (r^2) between the sensors averaged 0.9 (range 0.6 to 1.0) and the mean-absolute-difference between sensors was 4.9± 0.2%.
Conclusions: Sensor sensitivity was stable over the 3-day period, the sensor response time was 6 to 10 minutes and intersite variability between sensors in the abdominal area was less than 5%.

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Safety of continuous subcutaneous insulin infusion and multiple doses insulin therapy assessed with the use of continuous glucose monitoring system (CGMS).
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Background and Aims: Short-term intensive insulin therapy might help achieve improvement of metabolic control in poorly controlled type 2 diabetes patients. Multiple doses insulin therapy (MDI) and continuous subcutaneous insulin infusion (CSII) are two recommended methods for this purpose. Safety of these treatment modalities has been extensively studied, however the clinical experience is confined only to clinically overt hypoglycemic periods. With the arrival of new technologies like Continuous Glucose Monitoring System (CGMS) it has become possible to fully assess the safety features of given insulin therapies. The aim of the study was to determine the safety of MDI and CSII with the 24-hour glucose monitoring enabling to detect both symptomatic and symptom-free periods of hypoglycemia.
Materials and Methods: The study group was 40 poorly controlled insulin-treated type 2 patients (mean age 58.5±3.4 years, body mass index 27.4±2.8 kg/m^2, duration of diabetes 5.8±1.0 years, glycated hemoglobin A_{1c} 10.8±1.7%) who were randomized to one of the treatment modalities: MDI or CSII, both with the use of insulin analogue lispro. 48-hour CGMS measurement was started on the second day of the therapy and. Hypoglycemic events (defined as blood glucose <3.5 mmol/l) and clinical symptoms or lack of thereof were noted.
Results: Mean duration of a hypoglycemic event was 50 min. Number of the events per day in MDI patients was almost twice as high as in CSII patients: 1 versus 0.57 event per person, respectively (p<0.001). Mean duration of the episodes of hypoglycemia unawareness was 32 min in MDI, and 18 min in CSII patients (p<0.05). The incidence of these events was similar in MDI and CSII group (28 vs 23% of all events, p>0.05).
Conclusion: We conclude that MDI may be associated with longer duration of the episodes of hypoglycemia unawareness than CSII in type 2 diabetes patients.

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Clinical evaluation of an hand-held meter for self monitoring of blood 3betahydroxybutirate (3BOH) during interruption of continuous subcutaneous insulin infusion (CSII) in Type 1 diabetics.
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Background and Aims: Diabetic ketoacidosis (DK) is a relatively frequent and insidious complication of (CSII). In CSII treated patients DK can lead to important metabolic disturbances without being accompanied by a concomitant marked increase of blood glucose(BG). The evaluation of urinary ketones do not allow for a prompt detection of insulin deficiency during CSII due to the time delay for their clinically significant appearance. The availability of an hand-held meter for self monitoring of blood 3betahydroxybutirate (3BOH) could improve metabolic control during CSII and minimise the related risks.The aim of the study was to define the clinical relevance of capillary 3BOH measurement (Electrochemical sensor Optium, MediSense/Abbott Laboratories) for the early detection of metabolic deterioration and for the managemnt of the recovery phase during CSII.
Materials and Methods: Open clinical trial conducted on 8 type 1 diabetic patients already on CSII therapy. After an overnight fast; at 8 am (T0) the insulin infusion was interrupted for a period of four hours. At noon (T240) the CSII was re-established with a bolus followed by basal infusion according to the level of metabolic deterioration. At 4 pm (T480) the study ended. The following parameters were measured: a) plasma glucose, capillary and plasma 3BOH at 30 minutes interval, b) plasma insulin at 1 hour interval, c) urinary ketones at 2 hours interval and d) blood pH at T0, T240 and T480.
Results: CSII interruption caused a rapid and significant decrease in mean plasma insulin levels (15 ± 13 at T0 vs 3 ± 3 microU/ml at T240, p =0.02). A concomitant deterioration of metabolic control was indicated by the increase of mean blood glucose from 149 ± 54 at T0 to 224 ± 56 mg/dl at T240. (p<0.001) and of mean capillary 3BOH from 0.1 ±0.1 at T0 to 0.9 ± 0.6 mmol/L at T240 (p<0.001). Both BG and capillary 3BOH showed a linear correlation with the degree of insulin deficiency (r = -0.82 and r = -0.83 respectively) but capillary 3BOH increased earlier and more markedly than BG (clinical significant change respect to basal at 141 ± 25 minutes after T0 for 3BOH and at 197 ± 21 minutes after T0 for BG; p=0.05).The restoration of CSII produced a rapid and significant increase of insulinaemia in respect to T240 (plasma insulin at T480 11 ± 5 microU/ml; P< 0.05 vs T240). Mean BG and mean capillary 3BOH at T480 were 119 ± 24 mg/dl and 0,2 ± 0,2 mmol/L respectively (p <0.05 for both parameters vs T240). The time to significant changes for BG and capillary 3BOH was 167 ± 29 and 86 ± 21 minutes respectively (p=0.0006). The analysis of plasma ketones showed a good correlation with the capillary values, confirming data already available in literature. The comparison with the urinary ketones showed a more rapid increase of capillary ketones and a faster recovery.
Conclusion: Capillary 3BOH represents a more rapid indicator than BG and urine ketons for insulin deprivation . In the recovery phase capillary 3BOH reacts more rapidly than glucose and can be used as a useful parameter for targeting insulin therapy avoiding hyperinsulinisation and risk of later hypoglycaemia.

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A classical control systems model of β–cell insulin secretion for use in a closed-loop insulin delivery algorithm.
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Background and Aims: Models that describe insulin secretion as a function of plasma glucose (PG) can potentially be used for closed-loop insulin delivery. We evaluate 3 models presently being considered for a closed-loop insulin delivery system linking Medtronic MiniMed glucose sensors and insulin pumps.
Materials and Methods: Models were evaluated for the ability to decribe insulin secretion during rapidly rising and falling glucose dynamics (hyperglycemic clamps) and by closed-loop simulation. Clamps were performed in 14 non-diabetic subjects (11 M 3 F, aged 482 years; BMI 25.70.8 kg/m2). Simulations were based on the minimal model of glucose kinetics. Model 1 (M1) separated insulin secretion into static (delayed response to plasma glucose (PG)) and dynamic (reacts to rate of increase in PG) components; model 2 (M2) described the response in terms of a classical proportional, integral, derivative controller; and model 3 (M3) separated the response into proportional and derivative components, with the proportional component enhanced by prior PG exposure (glucose potentiation).
Results: M1 fit the response during the rise but failed to fit during the fall (delay time from the rise-and-fall longer than when estimated from the rise alone, 45±11vs. 25±3 min; p<0.05). M2 under- then over-estimated the response during the rise (residual run), predicted the initial suppression during the fall, but failed to remain suppressed once PG had stabilized (integrel windup). M3 fit the insulin response during rise and fall but remained in a potentiated state after glucose had normalized. Closed-loop simulation studies indicated M2 and M3 could compensate for 50% decreases in insulin sensitivity and/or a 50% increases in endogenous glucose appearance without error - M1 resulted in fasting hyperglycemia. M3 could be made to go unstable with repeated short term glucose exposure. M2 was stable across a wide range of gains and meals but required integrel reset after prolonged exposure to hyperglycemia.
Conclusions: M2 is most suited for a closed-loop insulin delivery algorithm. The model is stable across a wide set of meal and minimal model paraemeters and emulates the β-cell under all but the most extreme conditions. Variations of M3 may still be adapted into the closed-loop design.

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Correlation between some cardiovascular risk factors and cardiac autonomic neuropathy in Type 2 diabetes.

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Background and Aims: It has been demonstrated extensively that the cardiac autonomic neuropathy (CAN) is an independent predictor of increased cardiovascular (CV) morbidity and mortality in diabetic population. It seems that CAN is connected with other CV risk factors. Various studies suggest that cardiac autonomic mechanisms are involved in the etiology of hypertension and hypertension, itself, may contribute to more severe forms of CAN. Correlation between increased blood pressure (BP) and microalbuminuria (MA) is well established. The aim of the present study was to evaluate whether BP and MA values are connected with severity of CAN.
Materials and Methods: A five standard CV reflex test battery as proposed by Ewing, was performed totally in 143 type 2 diabetic patients 54 of them then were selected for the present study (age 40-65, without known ischemic heart disease, mean HbA_1c - 7.7 % \6.8-9.4 %\). According to severity of CAN (Jermendy et al., 1995) patients were divided into 3 groups: Gr. 1- with mild (n=20), Gr.2 - moderate (n=18) and Gr.3 - severe (n=16) CAN. In each group resting BP, pulse pressure (PP), MA, QTc interval (on surface ECG, using Bazzete`s formula) and QTc dispersion ($QTcd=QTc_{max}-QTc_{min}$) were assessed.
Results:

Parameter	Gr. 1	Gr. 2	Gr. 3
systolic BP (mm Hg)	133 ±14.1	153.4 ±18.2	159.6 ±22
diastolic BP (mm Hg)	86.2 ±11.3	86.7 ±9.8	87.4 ±15.9
PP (mm Hg)	46.2 ±9.0	69.5 ±13 .5	72.2 ±11.6
MA (mg/l)	32.4 ±1.1	54.5 ±2.1	79.2 ±5.1
QTc (sec)	0.391 ±0.14	0.402 ±0.13	0.411 ±0.12
QTcd (msec)	36.8 ±10.87	39.3 ±10.22	48.7 ±16.81
diabetes duration (years)	6.35 ±2.6	10.27 ±1.8	26.6 ±1.9

Conclusion: These data provide evidence of a relationship between the severity of CAN and PP, MA, QTcd and diabetes duration. Correlation does not exist between CAN and systolic, diastolic blood pressure or QTc. It is concluded that CAN is associated with some of CV risk factors and may carry an increased risk of mortality among diabetic patients.

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Type 1 diabetic patients age and the results of spectral heart rate variability.

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Background and Aim: Autonomic dysfunction of cardiovascular system is an important sudden death risk factor in diabetic patients. Heart rate variability (HRV) has been shown to be a reliable non-invasive technique for the analysis of two components of the autonomic nervous system. The aim was
a) to assess the autonomic balance in type 1 diabetic patients without (N0; 34) and with diabetic late complications (N1; 55) (retinopathy, peripheral neuropathy, nephropathy)
b) to assess the influence of age on the parameters of spectral and frequency analysis.
To evaluate the pattern of cardiovascular autonomic balance standard cardiovascular reflex tests (Ewing battery tests) and short heart rate variability were performed using computer assisted method (ProSciCard).
The following parameters were assessed:
1. HRV in 5 min period at rest, during standing (S), after cold pressure test (CPT), after deep breathing test (standard deviation of RR interval, coefficient of variation, root mean square of successive differences - RMSSD).
2. HRV during deep breathing (standard deviation of RR interval, CV, RMSSD, E/I ratio, MCR),
3. the Valsalva index and modified 30:15 ratio.
Absolute and relative (%) spectral HRV power were assessed in 3 standard frequency bands: very low frequency (VLF) 0.01-0.05 Hz, low frequency (LF) 0.05-0.15 Hz, high frequency (HF) 0.15-0.5 Hz a) at rest (R), b) after deep breathing test (DBT), c) during standing 0-5 min (S) and d) after cold pressure test (CPT). The autonomic balance was estimated by ratios: VLF/LF and LF/HF.
Results: The following parameters had the highest strength of discrimination between the group N0 and N1:
LnVLF-R/LF-R (0.05 vs 0.55; p<0.001); ln VLF-S/LF-S (-0.08 vs 0.50;p<0.001);
LnVLF-DBT/LF-DBT (-0.003 vs 0.69; p<0.001)

• In group N0 patients age significantly influenced the following parameters:

	VLF (beta; p)	LF (beta; p)	HF (beta, p)	LF/HF (beta, p)
Rest	0.06; 0.02	-0.09; 0.03	-0.11; 0.0004	0.05; 0.009
DBP	-0.05; 0.049	-0.08; 0.003	-0.09; p=0.00006	0.09; 0.02
S	-0.12; 0.02		-0.04; 0.03	
CPT		-0.07; 0.03	-0.09; 0.00006	0.05; 0.05

• In group N1 patients age significantly influenced the following parameters:

	VLF (beta; p)	LF (beta; p)	HF (beta; p)	LF/HF (beta; p)
Rest		-0.03, 0.009	-0.07; 0.002	
DBP		-0.03; 0.003		
S	-0.04; 0.03	-0.06, 0.007		
CPT			-0.03; 0.03	

Conclusions: Spectral heart rate variability results should be assessed in regarding the patients age.

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Differential role of Type 2 diabetes and cardiovascular autonomic neuropathy in impaired control of skin microcirculation.

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Background and Aims: The purpose of our study was to determine whether microvascular dysfunction is related to Type 2 diabetes and in particular to diabetic cardiovascular autonomic neuropathy (CAN).
Materials and Methods: Microvascular function was compared between a control group (C) consisting of 23 healthy adults and a group of 20 patients with Type 2 diabetes (DM2) matched for age and between two age-matched groups of 10 patients with Type 2 diabetes and CAN (CAN+) and 24 Type 2 diabetic patients without CAN (CAN-). (mean age, range: C: 47.70, 40-68 y; DM2: 51.7, 40-59y ; CAN-: 58.38, 49-66y; CAN+: 59.90, 54-66y , HbA1c: DM2:7.66±1.75%; CAN-:8.09±1.60%; CAN+: 10.41±1.88%).Cutaneous microvascular function was assessed at the forearm level using laser Doppler flowmetry. Superficial blood flow (BF) reactivity was measured in 2 and 6 mm depth after iontophoresis of acetylcholine (ACH) and sodium nitroprusside (SNP) as well as after a deep breath and ice-cold water immersion of the contralateral hand for 30 sec. Results are expressed as the ratio between stimulated and basal BF.
Results: The ACH-induced increase in BF was lower in DM2 than in C in both 2 mm (DM2: 3.01±2.08 (mean±SD), C: 4.48±1.86; p<0.05) and 6 mm depth (DM2:1.72±0.45, C:2.27±0.67; p<0.01). Contralateral cold-water-immersion induced BF reduction was exacerbated in DM2 as compared to C in 6 mm depth (DM2: 0.54±0.20, C: 0.69±0.18; p<0.05). In both groups, no difference in BF reactivity was noted to SNP or deep breath. Blood flow reduction following a deep breath was impaired in CAN+ in both 2mm (CAN+: 0.70±0.31, CAN-: 0.47±0.21 (p<0.05)) and 6 mm depth (CAN+: 0.77±0.27, CAN-: 0.60±0.20 (p<0.05)). No further differences were noted between the two groups.
Conclusion: Type 2 diabetic patients not only show evidence of endothelium-dependent cutaneous microvascular dysfunction, but also enhanced vasconstriction to cold pressor testing, suggesting increased sympathetic activity. By contrast, in patients with cardiovascular autonomic neuropathy, microcirculation reactivity is impaired due to diminished sympathetic tone.

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Unilateral numbness in diabetic patients: electrophysiological study.

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Background and Aims: Diabetic polyneuropathy is a symmetrical disorder. However, some diabetic patients complain of asymmetric foot numbness. It is not known if unilateral sensory change in the foot is a part of diabetic polyneuropathy, or not. We, therefore, evaluated the nerve conduction changes in the legs of diabetic patients by means of F-wave analysis in addition to conventional nerve conduction technique, since minimal F-wave latency is the most reproducible and reliable measure of the nerve conduction.
Materials and Methods: Forty-five diabetic patients (Male 26, Female 19), mean age 60years, were included in the study. Twenty-five patients had bilateral numbness of the feet, 14 showed no sensory changes, and 6 had unilateral foot numbness. Motor nerve conduction study was performed by using standard surface electrodes, and 16 to 20 F-waves were recorded successively from the bilateral tibial nerves to compare difference between the both sides. Similar studies were carried out in 50 young healthy volunteers aged 19-35years associated with neither low back pain nor neurological abnormalities. Older subjects were not included to the control group, since they might have asymptomatic lumber problems. All studies were carried out in an air-conditioned EMG laboratory to maintain the skin temperature of the leg more than 32°C.
Results: In normal subjects, upper limits of difference (mean+3sd) in conduction parameter between the bilateral tibial nerves were defined as 1.2ms for distal motor latency, 6.2m/s in MCV, and 1.7ms in minimal F-wave latency. CMAP amplitudes were never less than a half of the other side. In diabetics, we found 12 patients showing unilateral F-wave conduction block or F-latency difference more than 2ms, up to 7ms, one of which had a prolonged distal latency and a fall in CMAP amplitude in the side of prolonged F-latency. Eleven patients had no abnormal difference in MCV at all. All of 6 patients with unilateral numbness showed abnormal difference in F-latency, prolonged on the symptomatic side. In patients with bilateral and no sensory changes, 85% showed no F-latency difference, and 15% showed mild asymmetry of F-wave conduction. Except for one case with possible traumatic nerve damage in the foot, all of the patients showed symmetric MCV values distal to the knee bilaterally.
Conclusion: Asymmetric prolongation of F-wave latency with symmetric peripheral MCV value strongly suggests that changes in and around the nerve roots are responsible for unilateral sensory changes of the foot in diabetics.

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Early spinal cord involvement in diabetic peripheral neuropathy.

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Background and Aims: Distal symmetrical polyneuropathy (DSP) is a common complication of diabetes, the pathogenesis of which is poorly understood and there are no effective treatments. Hitherto considered a disease of the peripheral nerve, involvement of the spinal cord has been largely overlooked. We have recently reported extensive involvement of the spinal cord with significant reduction in cord-cross sectional area in eastablished DSP. However, the relevance to the pathogenesis of DSP depends on whether these changes occur early in the course of the disease.
Materials and Methods: A total of fifty seven male, type 1 diabetic patients were randomly selected, and underwent detailed neurological evaluation to stage the severity of DSP (NIS(LL)+7). They were then divided into 3 groups: 17 with diabetes but no DSP (Non-DSP; Dyck's Stage N0), 15 with Sub-clinical DSP (Stage N1a) and 25 with Established DSP (Stage N1b/N2), and underwent T2 weighted MRI of their cervical spine. The images were post processed using a semi-automated computerised technique to measure the cross-sectional area of the cervical spine at disk space C2/C3, by a blinded assessor.
Results: Spinal cord area was significantly lower in both the subjects with Sub-clinical and Established DSP compared to Non-DSP controls (ANOVA, p<0.001). However, there was no significant difference in cord area between the two DSP groups (Sub-clinical DSP Vs Established DSP; p=0.16). Spinal cord volume measurements also showed significant volume reduction in both the Sub-clinical and Established DSP groups compared to the Non-DSP group (ANOVA, p<0.001). There was no significant difference in cord volume between the two DSP groups. Further analysis showed an early reduction in the medio-lateral (width) diameter of the cord compared to the antero-posterior diameter (p=0.037), suggesting an earlier involvement of the spinothalamic (pain and temperature) tracts.
Conclusion: In conclusion, spinal cord involvement occurs early and provides further evidence of concomitant central nervous system involvement in DSP. Furthermore, this non-invasive, rapid test may serve as an early marker for the identification of subjects that are more likely to respond to therapeutic intervention.

DSP Groups	Mean Area (SD)	Mean Vol (SD)
Non DSP	65.93 (5.6)	490.85 (42.4)
Subclinical DSP	57.29 (8.1)	429.57 (59.5)
Established DSP	54.05 (3.8)	410.43 (26.6)

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SET – a new device for the measurement of pain perception threshold for detection of peripheral diabetic neuropathy in diabetic patients.

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Background and Aims: Impairment of pain perception threshold is believed to be a major predictor in the development of neuropathic foot ulceration in patients with diabetes mellitus. The aim of our study was to evaluate a new handheld device (SET) for quantitative measurement of pain perception threshold, in comparison to established methods for detection of diabetic neuropathy.
Materials and Methods: Sixty patients with diabetes mellitus (age 61.7±11.7 years; BMI 29.3±4.8) received measurement of pain perception threshold using SET at 4 defined areas of each foot. In addition, warm-, cold-, heat pain- and vibration perception thresholds were determined by the use of a computer based peltier thermode and a vibration stimulator (medoc, TSA 2001, Ramat Yishai, Israel).
Results: Using the new SET device, patients with established small nerve fibre dysfunction (heat- and pain perception) showed significantly elevated pain perception thresholds at the different skin areas in both feet (table 1). A significant correlation could be observed between plantar and dorsal measuring sites (r=0.78; p<0.0001) and between both extremities (r=0.85, P<0.0001). No significant association could be found with the vibration perception threshold as a parameter of large nerve fibre function.
Conclusion: Measurement of pain perception threshold using the SET device is a reliable method for identifying patients with deterioration of small nerve fibre function. Further studies have to clarify the prognostic value and the cut off limits for patients with special risk for developing diabetic foot ulceration.

Comparison of SET Measurements

	Control	Neuropathy	Significance
Area 1 (N. saphenus)	8.0±6.2	11.6±6.3	p<0.01
Area 2 (N. saphenus)	7.5±5.8	10.4±6.2	p<0.01
Area 3 (N. tibialis post.)	8.0±6.1	10.0±6.5	p=0.08
Area 4 (N. plantaris med.)	6.0±5.6	8.0±6.2	p=0.08

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A semi-quantitative model of normal and neuropathic diabetic human sural nerve and its sorbitol content.

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Background and Aims: Metabolism of excess glucose to sorbitol is implicated in the pathogenesis of diabetic neuropathy, but quantitative aspects of neural sorbitol metabolism *in vivo* are poorly defined. We describe here the first semi-quantitative model of the structure and composition of idealized human sural nerve segments, both normal (N) and

with moderate diabetic neuropathy (DN). In addition, the structural models are combined with nerve biopsy metabolite data and experimentally measured enzyme kinetic constants to create a steady state metabolic model of sural nerve sorbitol (nS) as a function of nerve glucose (nG).
Materials and Methods: In Zopolrestat Protocol 078-107, a clinical diabetic neuropathy study, nS and nG were assayed in placebo-treated baseline sural nerve biopsies from 54 fasted type 1 and 2 diabetic patients with moderate neuropathy. Plasma glucose (pG), nS, nG and kinetic constants for human aldose reductase (AR) and sorbitol dehydrogenase (SDH) were determined by standard methods. All µm data are diameters. Water density was taken as 1.0 g/cc. Literature analysis guided construction of a 1 mm^3 cylindrical nerve segment with 0.5 mm^3 epineurial (EP) and fascicular (F) compartments, and estimation of EP and F compositions (% v/v) in N and DN nerves. Structural and compositional data were linked and optimized for consistency with Microsoft® Excel spreadsheets. Structural and compositional data were merged with biochemical and literature histochemical data to model nS in a 3-compartment steady state metabolic model.
Results: Model parameters that best fit available data were: whole nerve (WN) H_2O (75%, N, DN), cells (N, 18%; DN, 8%); Schwann cells (N, 11%; DN, 5%); axons (N, 5%; DN, 2%), collagen (N, 17%; DN, 26%), myelin (N, 6%; DN, 3%), interstitial fluid (N, 58%; DN, 62%); in F: myelinated fibers (MF) (N, 8500/mm^2; DN, 4000/mm^2; frequencies, 63% 4-µm, 37% 10-µm, N, DN), unmyelinated fibers (UF)(N, 34,000/mm^2, 1.1-µm; DN, 16,000/mm^2, 0.6-µm). Regenerating clusters (RC) in DN were modeled as 7-µm with 2.5 2.4-µm MF, 3 0.6-µm UF/cluster, 166 RC/mm^2 (0.3% WN) and intravascular space as < 1% WN (N, DN). In DN, average nG (7.7±3.0 (53) mM), when corrected to glucose-accessible space (10.5±4.0 (53) mM)), was not different from pG (9.8±3.8 (50) mM) ($p>0.3$), and the two correlated ($r=0.57$, $p<0.0001$), as did nG with nS ($r^2=0.18$, $p<0.002$). Steady state assumptions combined with measured enzymatic kinetic constants (AR, K_{mG}=80 mM, k_{cat}=49 min^{-1}; SDH, K_{mS}=1.5 mM, k_{cat}=193 min^{-1}) and a computer-selected [AR]/[SDH] ratio = 25.6 resulted in a comparable fit ($r^2=0.17$) of nS from the nG data.
Conclusion: These results define for the first time in a quantitative way the idealized average structure and composition of a human sural nerve segment in N and DN states. In conjunction with metabolite data from nerve biopsies of fasted diabetic patients, the model is consistent with a steady state relationship, on average, between glucose and sorbitol in normal and diabetic neuropathic sural nerves. These results lay groundwork that will help advance our understanding of the possible role of excess polyol pathway metabolism in the pathogenesis of human diabetic neuropathy.

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The tissue IGF-1 gene expression abnormalites of auto/paracrine origin and diabetic peripheral neuropathy in diabetic rats.
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Background and Aims: The pathogensis of peripheral neuropathy ,one of most common chronic complications of diabetes mellitus, remains to be investigated .Insulin like growth factor-1 (IGF-1) is one of important neurotrophic factors essential for nerve growth and maintenance.We explore IGF-1 gene expression in sciatic nerve tissues with longer duration of diabetic rats and its relation to peripheral neuropathy.
Materials and Methods: Diabetic was induced in Sprague Dawley rats. The parameters were measured as follows: IGF-1 mRNA by reverse transcriptase- polymerase chain reaction (RT-PCR). IGF-1 peptide by enzyme-linked immunosorbent assay (ELISA); electrophysiological parameters of nerves by evoked electromyogram ; morphometric evaluation of sciatic nerves under light microscope .
Results: During early diabetic stage (week 2)before occurence of electrophysiological abnormality,IGF-1 mRNA (0.430 ± 0.031 vs. 0.370 ± 0.016 P<0.01, vs. 0.280 ± 0.010 P<0.001, respectively),IGF-1 peptide contents [(38.44 ± 3.60)ng/mg vs. (30.06 ± 2.41)ng/mg P<0.01, (38.44 ± 3.6)ng/mg vs. (3.71 ± 2.70)ng/mg P<0.001), respectively]in sciatic nerve tissue reduced in diabetic rats with hyperglycemia ,varing with severity of diabetic state compared with non-diabetic control rats,and further gradually down-regulated in the diabetic rats with duration of diabetes(month 3).Furthermore, they correlated with nerve functional(sensory nerve conduction velocity:r=0.74, P<0.001) and structural abnormality(axonal areas r=0.81,P< 0.001) of sciatic nerve. No difference was found in the above parameters between diabetic rats with euglycemia and non-diabetic control group.
Conclusion: It suggested that IGF-1 gene expression in nerve tissues was down-regulated from early diabetic stage, and varied with the severity and duration of diabetic state. The decrement in IGF-1 level might contribute to the initiation and development of diabetic neuropathy at least via autocrine or paracrine pathway.

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Cardiac autonomic neuropathy predicts overall mortality in Type 1 diabetic patients with and without diabetic nephropathy.
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Background and Aims: Cardiac autonomic neuropathy has been associated with poor prognosis, however the mechanisms remain a matter of debate. Since abnormal cardiovascular reflexes is far more prevalent in patients with another devastating complication: diabetic nephropathy, the aim of the present study was to evaluate the predictive value of cardiac autonomic neuropathy in type 1 diabetic patients with and without diabetic nephropathy.
Materials and Methods: In a prospective observational follow-up study 197 type 1 diabetic patients with overt diabetic nephropathy (120 men, age (mean(SD)) 41 ± 9 years, duration of diabetes 28 ± 8 years) and a matched control group of 191 patients with longstanding type 1 diabetes and persistent normoalbuminuria (117 men, age 43 ± 10 years, duration of diabetes 27 ± 8 years) were followed for 9.2 (0.0-9.5) years (median(range)). At baseline, mean RR interval during deep breathing consisting of six deep breaths for one minute in a lying position were determined.
Results: At baseline, severe (<5 beats/min) and mild (5-10 beats/min) impairment of heart rate control was seen in 39% (95%CI: 32-46) and 38% (31-45) of patients with nephropathy and in 10% (6-14) and 24% (18-30) of normoalbuminuric patients respectively. During 9.2 years of follow-up 51 patients with and 15 patients without nephropathy died, p<0.001. Among patients with diabetic nephropathy Kaplan-Meier survival curves revealed that presence of severe/mild cardiac autonomic neuropathy was associated with a poorer prognosis, log rank test p=0.01, with a relative risk of dying of 3.5 (95%CI 1.2-10.3). Similarly, in patients with normoalbuminuria, severe impairment of heart rate control predicted a higher rate of overall mortality (log rank p<0.001), relative risk: 6.1 (1.9-18.9).
Conclusion: Cardiac autonomic neuropathy, as assessed by a simple bedside test, is an important risk factor for early mortality in type 1 diabetic patients with and without diabetic nephropathy.

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Aldose reductase gene polymorphisms and peripheral nerve function in Type 2 diabetic patients.
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Background and Aims: The aim of the study was to screen the promoter region and the 10 exons of the human aldose reductase gene, a candidate gene for diabetic microvascular complications, for DNA sequence variants in type 2 diabetic patients and non-diabetic control subjects. We also investigated two previously reported variants, the C(-106)T polymorphism and the -2.1 kb (CA)n dinucleotide repeat marker, in association with neurophysiologic deterioration in diabetic subjects. For that, data from a 10-year follow-up study were used.
Materials and Methods: The study population included 85 newly diagnosed, middle-aged type 2 diabetic patients and 126 population-based non-diabetic control subjects from eastern Finland. The genetic analyses were performed using the PCR, SSCP, RFLP, and automated laser fluorescence scanning analyses. The neurophysiologic analyses included measurements of conduction velocities and amplitudes of the deep peroneal motor and superficial peroneal, radial, and sural sensory nerves at the time of diagnosis and at the 10-year examination in patients with type 2 diabetes
Results: The genetic screening identified two novel polymorphisms (C(-11)G and A11370G), and two previously reported ones (C(-106)T and C19739A), none of which occurred in the coding region of the gene. The C and Z-2 alleles of the C(-106)T polymorphism and the (CA)n repeat marker, respectively, were found to be more frequent in type 2 diabetic subjects than in the non-diabetic subjects. The Z-2 and C alleles were also

found to be in 60% linkage disequilibrium in diabetic subjects. At baseline, the diabetic subjects with the T allele of the C(-106)T polymorphism had lower sensory response amplitude values in the peroneal (p=0.025), sural (p=0.007) and radial (p=0.057) nerves than the subjects with the CC genotype. During the 10-year follow-up, the diabetic subjects with the T allele had a greater decrease in the conduction velocity of the motor peroneal nerve than those with the CC genotype (p=0.016), but smaller decrease in the response amplitudes in the sensory peroneal (p=0.021), sensory sural (p=0.076) and motor peroneal (p=0.088) nerves.
Conclusion: The present study suggests that the C(-106)T polymorphism of the aldose reductase gene could contribute to the development of neurophysiologic deterioration in type 2 diabetic patients.

PS 73
Neuropathy - Treatment

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Suppression of oxidative stress with proanthocyanidine ameliorates peripheral neuropathy in spontaneously diabetic GK rat.
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Background and Aims: Oxidative stress (OS) is implicated in the pathogenesis of diabetic complications. However, it is still unclear how the OS is involved in the development of diabetic neuropathy. Proanthocyanidines (PA), a polyphenol derived from grape seed, has been shown to have a strong anti-oxidative effect. In this study, we therefore examined if PA can prevent the development of neuropathy in the GK rat, a model of spontaneous non-obese type 2 diabetes.
Materials and Methods: GK rats were given a diet containing 0.04% PA or phloridzin (PH, 100mg/kg, s.c.) twice daily for 15 wks, beginning at 8wks of age. Normal Wistar rats fed a standard diet served as normal control. During the experiment, glycated hemoglobin (HbA1C) and motor nerve conduction velocity (MNCV) were regularly monitored. For the marker of OS and endothelial injury, plasma TBARS and von Willbrand factor (vWF) were measured at the end of the experiment. Biochemical analysis on Po mRNA expression by quantitative RT-PCR was also conducted on the excised sciatic nerves at wk 0, 6, 15, respectively.
Results: Elevated HbA1c levels in GK rats were not affected by PA throughout the experimental period, whereas PH normalized the levels (4.9±1.2% for PA, 4.6±0.5% for untreated GK vs 3.4±0.1% for PH at wk 15, $p<0.01$). Plasma TBARS values were increased up to 2.4 fold in GK rats at wk 15 compared to normal control ($p<0.01$) and both PA- and PH-treatment suppressed the levels by 40 % and 30 %, respectively (both $p<0.01$ vs untreated GK rats). vWF levels were augmented 2 fold in GK rats at wk 15 compared to baseline levels ($p<0.01$) and both PA and PH inhibited this elevation about 50 to 60 %. Age-dependent increase in MNCV was depressed in GK rats and the average values were significantly smaller than those in normal controls at wk 10 and 15 ($p<0.01$). These delays were near normalized in both PA- and PH-treated groups ($p<0.01$). P0 mRNA expression in sciatic nerve progressively declined to 70 % at wk 6 and 45 % of baseline levels by wk 15 (both $p<0.01$). But in PA-treated group, such decline was not observed at wk 6, and its level at wk 15 was still significantly higher than untreated GK rats ($p<0.05$).
Conclusion: These results indicate the inhibition of excessive OS by PA is beneficial for the prevention of the development of peripheral neuropathy in type 2 diabetes and the effects were associated with inhibition of endothelial injury and improvement of myelin synthetic process. Alternatively, impaired myelin metabolism concurrent with vascular injury may account for the delay of MNCV and contribute to the development of neuropathy in type 2 diabetes.

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C-peptide prevents the molecular abnormalities of the paranode in Type 1 diabetic polyneuropathy (DPN).
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Background and Aims: Axoglial dysjunction is a characteristic structural change in type 1 human and experimental DPN and accounts for the more severe functional deficits in type 1 DPN. The disruption of the paranodal barrier allows for lateralization of nodal Na-channels resulting in conduction block. The high affinity insulin receptor colocalizes with paranodal axoglial junctions which consist of caspr and cytoskeletal proteins like actin and contactin and are joined by cell adhesive β1 Na-channel subunit which in turn interacts with RTPTβ. RTPTβ is dependent on insulin and NGF signaling. The SH3 domains of caspr mediate protein-protein interaction by binding to p85 of PI-3 kinase a key intermediary of these signaling pathways.
Materials and Methods: To examine the possible molecular abnormalities underlying axoglial dysjunction in type 1 DPN, we examined 8 mo type 1 BB/Wor- and type 2 BB/Z-rats and type 1 rats replenished with proinsulin C-peptide.
Results: In type 1 rats the expression of caspr , contactin and β1 Na -channel were significantly ($p<0.02$ or less) downregulated, whereas actin and the β2 Na-channel were not. These abnormalities did not occur in

hyperinsulinemic and isohyperglycemic type 2 BB/Z-rats and were prevented by insulinomimetic C-peptide in type 1 rats, in whom the paranodal barrier remain intact.
Conclusion: From these data we conclude that impaired insulin action in type 1 DPN not only downregulates the expression of several key paranodal molecules , but also interferes with their assembly and that these abnormalities are preventable by replenishing C-peptide levels in type 1 diabetes.

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Effect of bFGF on diabetic neuropathy.
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Background and Aims: Demyelination and decreased nerve blood flow (NBF) have been recognized as pathopysiologially characteristic features of diabetic neuropathy. Basic fibroblast growth factor (bFGF) is a multifunctional protein and stimulates angiogenesis and proliferation of various cells, including neural cells. Although the beneficial effects of treatment with bFGF have been reported in an ischemic model and degenerative peripheral neuropathy, effects of bFGF on diabetic neuropathy have not been precisely discussed. Therefore, this study was conducted to investigate effects of bFGF on neural cell growth and nerve functions under diabetic condition, using immortalized mouse Schwann cells (IMS32 cells) and STZ-diabetic rats, respectively.
Materials and Methods: 1) IMS32 cells were cultured in 5.5 (NG) or 40 mM glucose (HG) for 3 days, and were treated with various concentrations of human recombinant bFGF under serum free condition for 24 hours. The proliferation activities by assay of [^{3}H]-thymidine uptake and MAPK (p42/44 and p38) activities by the ratio of phosphorylated to total MAPK protein expression were measured. 2) Diabetes was induced by intraperitoneal injection of STZ (60mg/kg BW) to 8-week-old male Wistar rats. After 3 weeks, local treatment with bFGF (50μg) in fibrin gel was performed on the right sciatic nerves and the left sciatic nerves were treated with fibrin gel (F) alone. Five days later, motor nerve conduction velocity (MNCV) and NBF were measured.
Results:1) Decreased proliferation activities of IMS32 cells under the HG condition were ameliorated by bFGF in a dose dependent fashion (NG: 100%, NG + 0.05 ng/ml bFGF: 125.0±12.5, NG + 0.5 ng/ml bFGF: 163.1±7.3, NG + 5 ng/ml bFGF, 273.2±12.4, HG: 50.6±3.3, HG + 0.05 ng/ml bFGF: 75.2±5.0, HG + 0.5 ng/ml bFGF: 104.5±9.5, HG + 5 ng/ml bFGF: 194.9±14.9). 2) MAPK activities of IMS32 cells were increased by bFGF. 3) Decreased MNCV in diabetic rats was ameliorated by bFGF treatment (control rats (C) + F: 48.5±0.7 m/s, C + bFGF: 50.1±2.2, diabetic rats (D) + F: 34.4±1.3, D + bFGF: 43.0±1.4). 4) A reduction in NBF of diabetic rats was improved by bFGF treatment (C + F: 14.3±0.4 ml/min/100g, C + bFGF: 15.0±0.5, D + F: 7.8±0.3, D + bFGF: 12.2±0.6).
Conclusion: These results suggest that bFGF would have therapeutic effects on diabetic neuropathy through enhancing not only angiogenesis but also regeneration of Schwann cells.

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Effects of poly(ADP-ribose)polymerase inhibition on large and small nerve fibre function in experimental diabetes.
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Background and Aims: Poly(ADP-ribose)polymerase (PARP) is a nuclear enzyme activated by DNA damage, which initiates an energy-consuming process depleting cellular NAD and ATP. PARP is stimulated as a result of oxidative stress effects in diabetes and may, therefore, constitute a downstream mechanism pertinent to diabetic complications. The aim was to assess whether PARP inhibitor treatment could correct nerve dysfunction in experimental diabetes.
Materials and Methods: After 6 weeks of untreated streptozotocin-diabetes, rats were treated with the PARP inhibitors, GPI6150 (Guilford Pharmaceuticals; 30 mg/kg) or 3-aminobenzamide (3-AB; 30 mg/kg) for 2 weeks. For the GPI6150 study, measurements were made on nerve conduction velocity, nerve perfusion, and nociceptive thresholds, in vivo. The 3-AB study focussed on the relaxant responses of the nitrergic autonomic innervation of gastric fundus, in vitro.
Results: Sciatic motor and saphenous nerve sensory conduction velocities were reduced by 21.1±1.0% (mean±SEM) and 15.4±1.2%, respectively with diabetes ($p<0.001$); GPI6150 treatment completely corrected these deficits ($p<0.001$). Sciatic nerve endoneurial blood flow was 49.1±6.0% reduced by diabetes ($p<0.001$) whereas with GPI6150 treatment, perfusion was in the upper half of the nondiabetic range ($p<0.001$). Touch allodynia was 44.6±6.8% elevated by diabetes ($p<0.001$) and this was completely corrected by GPI6150 ($p<0.001$). Mechanical hyperalgesia to a pressure stimulus on the surface of the foot was 46.9±2.3% increased by diabetes ($p<0.001$), GPI6150 treatment attenuated this defect by 59.7±16.1% ($p<0.01$). Foot withdrawal latency to a noxious thermal stimulus was 33.2±2.9% reduced by diabetes ($p<0.001$), indicating hyperalgesia, and this was completely corrected by GPI6150 ($p<0.001$). Non-adrenergic non-cholinergic relaxation responses to electrical stimulation of gastic fundus strips precontracted with 5-hydroxytryptamine were examined. Relaxation was depressed by diabetes ($p<0.01$) in the frequency range 1-16Hz, maximum relaxation being attenuated by 42.5±9.0% ($p<0.001$). Treatment with 3-AB completely corrected ($p<0.001$) this diabetic deficit such that relaxation was in the upper half of the nondiabetic range. With prolonged nerve stimulation, relaxation of control fundus was well maintained (91.9±5.1%) whereas in diabetic rats relaxation markedly declined after 30s (to 1.8±14.3%; $p<0.001$); 3-AB gave partial correction such that relaxation only fell to 51.6±13.5% ($p<0.01$).
Conclusion: PARP makes a wide-ranging contribution to somatic sensory and motor and autonomic nerve dysfunction in early experimental diabetes and the use of PARP inhibitors could represent a novel therapeutic approach to diabetic neuropathy, which requires further investigation.

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Efficacy of duloxetine in the treatment of the pain associated with diabetic neuropathy.
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Background and Aims: Serotonin (5-HT) and norepinephrine (NE) are implicated in mediating endogenous analgesic mechanisms via descending inhibitory pain pathways in the brain and spinal cord. Duloxetine is a potent, selective and balanced dual reuptake inhibitor of serotonin (5-HT) and norepinephrine (NE). The aim of these studies was (1) to evaluate potential pain relieving properties of duloxetine in pre-clinical models of pain in rats and (2) to extend these observations further to examine the efficacy, safety and tolerability of duloxetine in the treatment of pain associated with diabetic neuropathy in man.
Materials and Methods: *Preclinical* - Male SD rats (200-250g,Harlan Labs, IN) were evaluated for effects of duloxetine (10, 20 and 30 mg/kg, oral) in (a) persistent pain behavior in the formalin model (b) mechanical allodynia behavior in two models of neuropathic pain, L5/L6 spinal nerve ligation and partial sciatic nerve ligation models. *Clinical* - In a 12-week multicenter double-blind study, 457 patients with diabetic neuropathy were randomly assigned to treatment with duloxetine 60 mg BID, 60 mg QD, 20 mg QD, or placebo. The average age was 60 and duration of diabetes and diabetic neuropathy was 11.3 and 3.7 years respectively.
Results: *Preclinical* - Duloxetine reversed persistent pain behavior in the formalin model and significantly improved withdrawal thresholds in the L5/L6 spinal nerve and partial sciatic nerve ligation models. The effects were dose-dependent and occurred at doses that showed no neurological side effects. *Clinical* - Duloxetine 60mg QD and BID demonstrated significant improvement compared to placebo on the average pain severity score, beginning 1 week after randomization and continuing through the study period. Duloxetine also separated from placebo on nearly all of the secondary efficacy measures. The most commonly reported treatment-emergent adverse events were nausea, somnolence, dizziness, constipation, and dry mouth. Duloxetine did not differ from placebo on HgbA1c and lipid profile changes from baseline to endpoint and in the weekly average number of significant hypoglycemic episodes.
Conclusion: These pre-clinical and clinical data are consistent with the proposed role of 5-HT and NE as being key mediators of descending pain pathways. Further, these results suggest that 5-HT and NE reuptake inhibition by duloxetine may offer an effective and safe alternative for treatment of persistent pain states in man. Importantly, the clinical results provide definitive evidence that duloxetine at 60 mg QD and 60 mg BID was safe and effective in the treatment of pain associated with diabetic neuropathy.

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Ruboxistaurin (RBX) mesylate improves Diabetic Peripheral Neuropathy (DPN) symptoms and signs.

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Background and Aims: Hyperactivation of protein kinase C β (PKC β) is believed to play a pivotal role in development of diabetic microvascular complications (DMC) including DPN.
Materials and Methods: To assess the impact of RBX, a specific inhibitor of PKC β, on human DPN, 205 patients with type 1 or 2 diabetes mellitus (DM) and DPN were randomized in a phase 2, multicenter, clinical trial evaluating placebo, 32 mg RBX or 64 mg RBX administered once daily for 58 weeks. DPN was identified by a vibration detection threshold (VDT) ≥ 97th percentile for age and anthropometric measures, and established using the criteria of Dyck. Symptoms were evaluated using the Neuropathy Total Symptom Score-6 (NTSS-6), which assess the intensity and frequency of positive sensory neuropathy symptoms (numbness, prickling sensation, aching pain, burning sensation, lancinating pain, and allodynia) at baseline, 1, 3, 6 and 12 months after randomization. Signs were quantitated by the Neuropathy Impairment Score (NIS[LL]), an assessment of the neurological examination of the lower limb and composite scores (CS) of nerve function (NIS[LL] + 4 attributes of peroneal and tibial nerve electrophysiology) at baseline and after one year.
Results: Change in NTSS-6 for patients with clinically significant positive sensory symptoms symptoms (NTSS–6 >6; max score=22; N=83) is shown. In all patients, there was a statistically significant improvement for the change from baseline when placebo was compared both the NIS and NIS(LL) + 4 for the 32 mg RBX group ($p< 0.05$) but not the 64 mg RBX group (p = N.S.). The change in the NTSS-6 score correlated with the NIS(LL) ($p =0.007$) and the NIS(LL) + 4 ($p = 0.012$).
Conclusion: In conclusion, RBX relieves the positive neuropathic sensory symptoms and improves the underlying pathophysiology of diabetic peripheral neuropathy.

Change in NTSS-6

Treatment Group (n)	Base-line	Δ 1 month	Δ 3 months	Δ 6 months	Δ 12 months
Placebo (35)	9.99	-0.97	-1.57	-1.23	-1.69 32
mg RBX (22)	11.33	-2.34	-1.70	-2.31	-3.32 (p=.064)
64 mg RBX (26)	10.35	-2.15	-2.60	-3.59 (p=.017)	-3.55 (p=.014)

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Effects of α–lipoic acid on heart rate variability, QTc interval parameters and antioxidant status in Type 2 diabetic patients with cardiovascular autonomic neuropathy.

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Backgrounds and Aims: The present study had examined the effect of α-lipoic acid (ALA) on heart rate variability (HRV) and QTc interval parameters, on superoxide dismutase (SOD), glutathione peroxidase (GPO) activities and reduced glutathione (GSH), malonaldegide (MDA) contents in the RBCs' of Type 2 diabetic patients with CAN assessed by reduced HRV.
Methods: 31 patient with Type 2 diabetes mellitus (DM) and CAN (57.0±6.8 years, 17m/14f) were allocated in two treatment groups. All patients were randomized to receive either daily 600 mg ALA i/venously for 2 weeks and then orally 600 mg ALA (n=22) or placebo (n=9) during 2 months. The following short-term HRV measures were assessed: coefficient of variation (CV), root mean square successive difference (RMSSD), spectral power in the low-frequency (LF) and high-frequency (HF) bands; standard 5 tests. Statistics: one way analysis of variance (ANOVA).
Results: The assigning of ALA by the Type 2 DM with subclinical stage of CAN positively influences parameters of HRV. In particular, it increases RMSSD (7.75±0.53 ms was observed before the treatment; 10.32±0.38–after treatment, t-criterion=63.27, $p<0.001$), LF (964.5 ±9.33 mc^2 and 1082.0±33.08 mc^2, t-criterion=46.7, $p<0.001$), HF (859.7±12.63 mc^2 and 977.5± 26.96 mc^2, $p<0.001$), CV (7.7±0.35% and 7.12±0.17%, $p<0.05$) parameters. Intravenous infusions of ALA with its subsequent oral assigning by the Type 2 DM patients with clinical stages of CAN were accompanied by legibly expressed positive dynamic changes of SOD activity (7.38±0.19→8.85±0.38 (after completion of intravenous infusions),$p<0.05$; 10.13±0.84 (on completion of a treatment course), $p<0.001$), authentic ascending of GPO activity on completion of a treatment course (267.76±19.21 mcmol GSH/min Hb, $p<0.001$). The authentic increasing of the GSH concentration and decreasing of a kept in MDA repairing were observed. The increasing of RMSSD parameters (4.72±0.53 mc, 7.4±0.75 and 9.02±0.27 mc, $p<0.01$ and $p<0.001$), LF and HF, LF/HF ratio and CV decreasing are simultaneously established. The obtained data can testify the increase of nervous impulses realization rate in visceral parasympatic and sympatic fibers. The authentic decreasing of an QTc interval parameters (0.57±0.069→0.52±0.057→0.47±0.044, $p<0.05$ and $p<0.01$, respectively) were observed. In diabetic patients of monitoring group, positive changes of investigated parameters after completion of a conventional therapy course were not detected ($p<0.05$).
Conclusions: Usage of ALA is accompanied by decreasing of CAN clinical symptoms, improvement of HRV, QTc interval, antioxidant defense parameters. ALA may be used for the prevention and treatment of cardiovascular autonomic neuropathy.

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Effect of antioxidant treatment with α-lipoic acid on symptomatic diabetic polyneuropathy: a meta-analysis of four randomized placebo-controlled trials.

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Background and Aims: To obtain a precise estimate on the efficacy and safety of 600 mg of α-lipoic acid given i.v. over 3 weeks in diabetic patients with symptomatic polyneuropathy.
Materials and Methods: We searched the database of VIATRIS GmbH, Frankfurt, Germany, for clinical trials of α-lipoic acid according to the following prerequisites: randomized, double-masked, placebo-controlled, parallel-group trial using α-lipoic acid infusions of 600 mg i.v. per day for 3 Weeks, except for weekends, in diabetic patients with positive sensory symptoms of polyneuropathy which were scored by the Total Symptom Score (TSS) in the feet on a daily basis. Four trials (ALADIN I, ALADIN III, SYDNEY, NATHAN II) comprising n=1258 patients (α-lipoic acid: n=716; placebo: n=542) met these eligibility criteria and were included in a meta-analysis based on the intention-to-treat principle. Primary analysis involved a comparison of the differences in TSS from baseline to the end of i.v. treatment between the groups treated with α-lipoic acid or placebo. Secondary analyses included daily changes in TSS, responder rates (≥50% improvement in TSS), individual TSS components, Neuropathy Impairment Score (NIS), NIS of the lower limbs (NIS-LL), individual NIS-LL components, and the rates of adverse events.
Results: After 3 weeks the relative difference in favor of α-lipoic acid vs placebo was 24.1 (13.5-33.4)% (geometric mean with 95% confidence interval) for TSS and 16.0 (5.7-25.2)% for NIS-LL (both $p<0.05$). The responder rates were 52.7% in patients treated with α-lipoic acid and 36.9% in those on placebo ($p<0.05$). On a daily basis there was a continuous increase in the magnitude of TSS improvement in favor of α-lipoic acid vs placebo which was noted first after 8 days of treatment. Among the individual components of the TSS, pain, burning, and numbness decreased in favor of α-lipoic acid as compared with placebo (all $p<0.05$). Among the NIS-LL components the relative differences in favor of α-lipoic acid after 3 weeks were 57 (21-105)% for pin-prick sensation on the great toe, 35 (5-75)% for touch-pressure sensation on the great toe, and 69 (22-135)% for the ankle reflexes (all $p<0.05$). The rates of adverse events did not differ between the groups.
Conclusion: These data provide evidence that treatment with α-lipoic acid (600 mg/day i.v.) over 3 weeks is safe and significantly improves both neuropathic symptoms and deficits to a clinically meaningful degree in diabetic patients with symptomatic polyneuropathy.

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Insulin therapy improves cardiac autonomic function in Type 2 diabetic patients.

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Background and Aims: There is scintigraphic evidence that insulin improves cardiac autonomic innervation in diabetic patients. The effects of insulin on ECG-based cardiac autonomic parameters, however, have not been studied. The aim of the study was to assess the effects of insulin therapy on parameters of cardiac reflex tests in diabetic patients, who had been treated previously with oral antidiabetic agents.
Materials and Methods: 30 type 2 diabetic patients with a HbA1c of > 7.5% under oral antidiabetic agents were included into the study. Clinical

characteristics: HbA1c 8.9±0.6 % (X±SD), 59.4±5.0 yrs, BMI 26.8±1.8 kg/m^2, duration of diabetes 5.8±2.3 yrs. Insulin therapy was applied according to international guidelines. Parameters of five cardiac reflex tests were assessed to study cardiac autonomic function: Coefficient of variation (CV) of heart rate variation (HRV) at rest and during deep breathing, HRV in response to standing (max/min 30:15-ratio), Valsalva ratio, systolic blood pressure response. Cardiac autonomic neuropathy (CAN) was defined as the presence of more than one abnormal test. Rate-corrected QT(QTc)-interval was also assessed in the patients.
Results: At 4-months follow-up, mean insulin dosage in the patients was 25±8 IU/day. HbA1c was 7.2±0.6 % (p <0.0001 vs. mo 0) and BMI 27.2±1.8 kg/m^2 (p <0,05, vs. mo 0). Three parameters of cardiac autonomic function significantly improved from month 0 to month 4: CV of HRV at rest 2.79±0.84 vs. 2.96±0.85 (p <0,001), CV of HRV during deep breathing 4.15±1.46 vs. 4.38±1.68 (p=0.002), 30:15 ratio 1.06±0.05 vs. 1.09±0.09 (p=0.02). Valsalva ratio and systolic blood pressure response did not change significantly (1.16±0.04 vs. 1.17±0.04; 14.1±4.0 vs. 14.6±3.9 mmHg). CAN was present in 7 patients at month 0 and in 6 patients at month 4 (n.s.). Length of QTc-interval changed significantly from 417±12 ms at month 0 to 398±11 ms at month 4 (p<0.05).
Conclusion: The study demonstrates that insulin therapy improves cardiac autonomic function in type 2 diabetes mellitus. Whether these mechanisms, i.e. contribute to improvement of prognosis of diabetic patients with acute myocardial infarction has to demonstrated in future studies.

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Vardenafil (Levitra®) improved patient satisfaction with erectile hardness, orgasmic function, and sexual experience in men with erectile dysfunction and diabetes irrespective of level of glycemic control.

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Background and Aims: Erectile dysfunction (ED) occurs with higher frequency and may be more severe and is more difficult to treat in men with diabetes. ED severity has been reported to correlate with HbA1c. Vardenafil (Levitra®), a highly selective and potent phosphodiesterase type 5 inhibitor, was previously shown to improve erectile function (EF) [International Index of Erectile Function-Erectile Function [IIEF]-EF domain, erection penetration, maintenance, and quality in men with diabetes irrespective of HbA1c. Here we report the influence of HbA1c on additional parameters relative to erection quality and sexual experience satisfaction in men with ED and diabetes.
Materials and Methods: In a multicenter, double-blind, fixed dose, phase III trial, 452 men with type 1 or 2 diabetes and ED >6 months were randomized to placebo, vardenafil 10 mg, or 20 mg for 12 weeks. Efficacy variables included diary questions regarding per-patient success rates of satisfaction of erectile hardness and overall sexual experience, and IIEF-intercourse satisfaction/orgasmic/overall function domains (analyzed by ANCOVA).
Results: At baseline, mean IIEF-EF domain scores (ITT population) ranged from 11.0-12.4 (moderate ED). Over 12 weeks, vardenafil significantly improved success rates in satisfaction with erectile hardness, sexual experience, and IIEF-intercourse satisfaction/orgasmic/overall function domains irrespective of HbA1c levels. The most commonly reported treatment emergent adverse events (AEs) ≥5% were headache (11-13%) flushing (9-10%) and rhinitis (5-10%) and were reported to be mild to moderate in intensity. Serious AEs were reported in 3%, 2%, and 3% for placebo, vardenafil 10, and 20mg, respectively.
Conclusion: In this study, vardenafil significantly improved patient satisfaction with erectile hardness and overall sexual experience relative to placebo over 12 week treatments in patients with diabetes irrrespective of HbA1c. Thus, vardenafil improves satisfaction with erection hardness and sexual experience in patients with diabetes independently of glycemic control.

Efficacy Variables **HbA1c**	Placebo ≤6	>6-≤8	>8	Vardenafil 10 mg ≤6	>6-≤8	>8	Vardenafil 20 mg ≤6	>6-≤8	>8
Satisfaction with erection hardness# (mean per patient success rate, %[n])	6.7 [3]	12.0 [56]	9.5 [77]	45.7 [5]	35.1** [65]	30.8** [76]	39.4 [4]	44.3** [64]	42.6** [71]
Satisfaction with sexual experience# (mean per patient success rate, %,[n])	5.8 [3]	17.8 [56]	16.8 [77]	53.9 [5]	41.5** [65]	37.2** [76]	64.1 [4]	52.8** [64]	51.5** [71]
IIEF Domain: Intercourse satisfaction *** (score, [n])	6.4 [3]	6.1 [58]	6.4 [76]	11.2 [4]	7.8* [64]	8.1† [76]	10.6 [4]	8.7** [65]	9.1** [69]
IIEF domain: Orgasmic function*** (score,[n])	2.3 [3]	4.9 [58]	5.3 [76]	5.7 [4]	6.4* [64]	6.3 [76]	8.2 [4]	6.7† [65]	7.0† [69]
IIEF domain: Overall function*** (score, [n])	3.6 [3]	4.2 [58]	4.6 [75]	8.4 [4]	6.2† [64]	5.9† [76]	7.9 [4]	6.6** [64]	6.8** [69]

Intent-to-treat population; # - Overall population; *** - last observation carried forward, *p<0.005 vs PLA, †-p<0.002 vs PLA, **p<0.0001 vs PLA, treatment*HbA1C for all parameters = NS

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Neuroprotective effects of novel neurotherapeutic topiramate in diabetic patients.

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Background and Aims: The degree of in vitro neuronal cell death in response to pro-apoptotic factors in sera from patients with diabetic neuropathy correlates with specific fiber losses in vivo. In addition to pain and impaired pain/thermal perception, C-fiber damage adversely affects skin blood flow (SKBF). Severely impaired SKBF precedes hyperglycemia in patients with type 2 diabetes (T2DM) and co-segregates with features of the dysmetabolic syndrome. Topiramate (TPM) is a neurotherapeutic that appears to prevent neuronal apoptosis and stimulate neuronal growth. In in vitro studies with N1E–115 murine adrenergic neuroblastoma cells, we found that 100 ng/mL TPM reduced diabetic sera-induced apoptosis (from 16.5% to 4.1%); apoptosis was significantly reduced in 8 of 8 patient sera-treated cultures (14.2 ± 4.7% reduction). 100 ng/mL TPM also significantly increased cell growth: Day 1, 176 ± 19% without TPM vs. 217 ± 23% with TPM (p<0.01); Day 2, 226 ± 24% vs. 275 ± 31% (p<0.03). We subsequently evaluated in vivo effects of TPM on nerve growth and function in patients with diabetic neuropathy.
Materials and Methods: In an 8-week open-label trial in 11 patients with T2DM (60 ± 2 yrs; BMI 32 ± 2; C-peptide 2.35 ± 0.5 mg/mL), C-fiber neuropathy was diagnosed by total neuropathy scores, nerve symptom scores, and quantitative sensory tests (QST). Intra-epidermal nerve fiber density (IENF) was determined by immunohistochemical staining. TPM was titrated to 400 mg/day or maximum tolerated dose. Baseline and 8-wk evaluations: metabolic parameters, QST, SKBF (laser Doppler), skin biopsies. Blinded observer performed histological evaluations. Significance was assessed using within subjects ANOVA.
Results: After 8 weeks' treatment, TPM significantly increased IENF (P<0.04), dendrite length (P<0.04), and conduction amplitude (P<0.04), and improved symptoms of C-fiber dysfunction (P=0.04). Metabolic parameters (HbA1c, P<0.04; total cholesterol, P=0.002) and diastolic blood pressure (P=0.006) were also significantly improved. Side effects were consistent with clinical experience, with no serious side effects reported.
Conclusion: These findings suggest that TPM may reverse cytotoxic effects of sera and improve/restore neuronal function in patients with diabetic neuropathy. TPM may also positively effect components of the dysmetabolic syndrome.

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Diabetic Foot - Measurement and Clinical Intervention

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The role of metabolism L-arginine and transforming growth factor beta 1 in the pathogenesis of diabetic foot ulcers.

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Background and Aims: The aim of this study was to test the hypothesis that abnormalities of L-arginine metabolism may be involved in the pathogenesis of diabetic foot ulcers.
Materials and Methods: There were 20 controls (C), 25 diabetic patients with neuropathy (DN) and 25 with neuropathic diabetic foot ulcers (DNU). Patients with severe ischaemia (ancle brachial pressure index 6/10 and vibration perception threshold (VPT) >25 volts using a biothesiometer (Biomedical Instrument Co., Newbury, OH). Blood (10ml) was taken from the antecubital vein. Plasma was prepared by centrifuging samples and frozen in liquid nitrogen till use. Plasma nitrate (stable product of nitric oxide) concentration was measured using HPLC. Skin biopsies were taken from the dorsum of the foot of non-diabetic subjects and diabetic patients and from the ulcer edge of DNU. The tissue was bisected and one half placed immediately in liquid nitrogen for biochemical analysis. Activity of nitric oxide synthase (NOS) was measured as the ability of tissue homogenates to convert [3H] L-arginine to [3H] L-citrulline. Activity of arginase was determined as its ability to convert L-arginine to urea. Concentration of transforming growth factor beta 1 (TGF-beta1) was measured too.
Results: Patients with recurrent foot ulcers (n = 13) had higher VPT values compared to subjects with non-recurrent foot ulcers (n = 12): (47,4 ± 6,5 volts versus 32,6 ±7,3 volts respectively, $p < 0,05$). DNU had significantly higher plasma nitrate values compared to DN and C groups: 79,68 (55,41; 95,08) mM versus 20,98 (13,46; 29,79) mM and 39,03 (28,94; 41,32) mM respectively, $p < 0,05$. Patients with recurrent foot ulcers had significantly higher plasma nitrate than subjects with non-recurrent ulcers: 95,08 (86,16; 98,75) mM and 54,83 (47,9; 59,87) mM, $p < 0,001$. The results showed increased inducible NOS activity in DNU (5,92 B/T/mg protein) compared with DN (1,59 B/T/mg protein) and C (3,36 B/T/mg protein), $p < 0,01$. Arginase activity was increased in DNU (1,96 mg urea/mg protein) compared with DN (0,52 mg urea/mg protein) and C (0,28 mg urea/mg protein), $p < 0,01$. Concentrations of TGF-beta1 was reduced in DNU (0,88 ng/mg protein) compared with C (2,48 ng/mg protein), $p < 0,01$.
Conclusion: Severity of neuropathy and higher plasma nitrate in diabetic patients with recurrent neuropathic foot ulcers may be important factors associated with the impaired healing and recurrent neuropathic foot ulcers. The reduced concentrations of TGF-beta1 in diabetic foot ulcer patients may account for the raised and sustained activity of NOS as the normal homeostatic cytokine control mechanism is impaired. The increased activity of arginase could account for the characteristic callus formation around these ulcers.

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Local treatment with protease-inhibitors promotes wound healing in diabetic patients, but does not affect the expression of matrix-metalloproteases.

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Background and Aims: Wound healing in diabetes is impaired and non-healing ulcerations are relevant complications. 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. Therefore the topical use of protease-inhibitors should influence the wound healing and promote the transition from a chronic to an acute wound.
Materials and Methods: We included 33 patients with chronic diabetic foot lesions (stage Wagner 2) in this study. 15 received standard "good wound care". 18 patients were additionally treated with a protease-inhibitor (Promogran®, Ethicon) and the dressings were changed daily. At the first visit and after four and eight days two 3mm punch biopsies were taken from the center of the wound. One sample was immediately frozen at -20°C, the second biopsy was stored in RNAlater RNA stabilization reagent (Quiagen) and also at -20°C. Biopsies were analysed by ELISA for MMP-1, -2, -8, -9, Tissue Inhibitor of MMP 2 (TIMP-2) and Interleukin 1-β (IL1-β) levels. We also analysed the mRNA levels of MMP-1, -9,-13,-14 as well as IL1-β and TNFα by RT-PCR (TaqMan).
Results: We observed an significant reduction of the wound area in the treatment group (16%) when compared with the placebo group (1,6%) during the short treatment period of eight days ($p=0.045$). Both groups were not different for age (treatment group 64±11 years vs. placebo group 62±12 years), duration of diabetes (treatment group 15±11 years vs. placebo group 16±11 years) HbA1c (treatment group 7,4±1,1 % vs. placebo group 7,7±1,9 %) and initial size of the lesion (treatment group 1237 mm^2 [range: 25-7200] vs. placebo group 1132 mm^2 [range360-3600]). All these factors had no significant influence of the MMP expression or wound healing dynamic. Levels of MMP mRNA as well as IL1-β and TNFα were not different between both groups and at the three different time-points. The levels of MMP's in wound tissue (analysed by ELISA) were also not significant different. IL1-β was at day 8 increased in the treatment group ($p=0.01$) only.
Interestingly, we found a significant reduction of the MMP-9/TIMP-2 ratio in the group exhibiting a more rapid healing course ($p<0.03$).
Conclusion: The local treatment with a protease-inhibitor beneficially affects wound healing. In contrast to data of wound fluid, our study demonstrated for the first time unchanged mRNA levels of MMP's during treatment with a protease-inhibitor. At the level of cell-tissue, MMP's were also not statistically different. The more relevant ratio of MMP-9/TIMP-2 was decreased in the treatment group.
Our data did not show an effect on the absolute expression of MMP's and growth factors by local treatment with protease-inhibitors, while the MMP-9/TIMP-2 ratio in wound tissue was decreased. Equally important, we did not find a compensatory increase in the MMP-RNA expression since wound size was clearly reduced. Protease-inhibitor might thus be useful tools for treating chronic diabetic wounds.

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Oxidative injury and antioxidant status in diabetic foot.

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Background and Aims: Oxidative stress plays an important role in the etiology and progression of diabetic complications including diabetic foot. This study was carried out to assess the oxidant stress, antioxidant status and the tissue nitric oxide synthase activity in patients with diabetic foot
Materials and Methods: Thirty two diabetic patients with foot ulcer formed the study group (Group A), fifteen diabetic patients without evidence of diabetic neuropathy or peripheral vascular disease formed Group B, and 15 healthy age and sex matched non-diabetic individuals formed the control group (Group C). All the subjects had given written informed consent for inclusion into the study. A detailed history regarding duration of diabetes, type of therapy and triggering factor for foot ulcer was noted. Clinical examination was carried out with careful assessment of peripheral and autonomic neuropathy (monofilament and tuning fork test) and pedal pulses (ankle brachial pressure index). Glycemic control was achieved by multiple subcutaneous insulin injections. Laboratory Investigations. Apart from the routine hematological and biochemical ivestigations, wound swab was sent for bacteriogram and sensitivity test. Blood samples were collected for estimation of serum malondialdelyde (MDA) serum tocopherol (vitamin E) and superoxide dismutase (SOD) levels after achieving good glycemic control. A punch biopsy was taken from the edge of foot ulcer after informed consent of 10 patients from Group A and from two non-diabetic subjects with surgical wounds The tissue sample was collected in normal saline and immediately frozen at -70° C for subsequent processing and histochemical staining for NADPH diaphorase activity.
Results: All the three groups were matched for age and sex. In the study, 16 (50%) of the 32 patients had neuropathic ulcers 3 patient (9.3%) had an ischaemic ulcer and 13 patients (40.7%) had neuroischemic ulcers. Serum malondialdehyde levels were significantly higher in the Group A patients as compared with the diabetic controls (1.164 ± 0.371 vs. 0.710 ± 0.277 nmol/ml; $p<0.001$) and non diabetic controls (1.164 ± 0.371 vs 0.357 ± 0.134 nmol/ml; $p<0.001$).
Superoxide dismutase levels in patients of group A was significantly lower than that of group B (16.42 ± 2.38 vs 20.82 ± 3.02; $p<0.001$). However, this difference was not significant between group B and C (20.82 ± 3.02 vs 21.44 ± 3.37; ns)

Tocopherol (vitamin E) levels were significantly lower in patients of group A in comparison with patients of group B and C. (Group A 5.04 ± 1.76 vs Group B 9.10 ± 2.83 vs Group C 10.68 ± 2.58). The levels of malonaldehyde or the antioxidants among the various types of ulcers within Group A were comparable. The tissue section obtained from 10 diabetic wound stained for NADPH diaphorase activity show intense staining while the control section did not show staining of the tissue.
Conclusion: Patients with diabetic foot ulcers have higher oxidative stress and have lower antioxidant defenses. The NO synthase activity is increased, favouring NO production, which is however rendered ineffective, possibly by conversion to peroxynitrile due to the high pro-oxidant activity and lowered antioxidants.

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Reduction of plantar pressure using a prototype pressure relieving dressing.
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Background and Aims: The purpose of the study was to investigate the effectiveness of a prototype pressure relieving dressing (Coloplast A/S Denmark) to reduce plantar pressures in neuropathic diabetic patients.
Materials and Methods: Eighteen diabetic patients with neuropathy (without foot ulceration) and peak plantar pressures > 500 kPa were studied. Subjects wore the prototype dressing on the metatarsal head (MTH) with the highest pressure. Peak plantar pressure was measured (using the optical pedobarograph) on 3 consecutive days, with the same MTH on the contralateral foot used as the control site. Peak pressure was also analysed on the MTH's lateral and medial to the dressing.
Results: A mean pressure (SD) reduction of 30% was observed at the dressing site [817.2 (136.8) v 573.2 (165.7) kPa, p<0.0001]. A pressure reduction of 26% was maintained over the next two days. Mean pressure returned to baseline levels once the dressing was removed [764.3 (177.3) kPa]. The lateral MTH showed a mean pressure reduction of 16% [439.3 (154.5) v 370.0 (91.7) kPa, p<0.05] whilst the medial MTH showed a mean pressure reduction of 20% [491.5 (192.3) v 393.9 (155.4) kPa, p<0.05]. There was no difference in peak pressure at the control MTH [601 (184) v 599 (169) kPa].
Conclusion: A mean pressure reduction of 30 % compares favourably with other ulcer treatment methods (off-loading devices). In addition to foot ulcer treatment, this type of dressing could also have a role in preventative treatment where individual high-risk MTH sites can be identified and targeted with minimal change to the patients' lifestyle. The results of this pilot study indicate that further studies on active ulcer patients are required.

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Toe systolic pressure in diabetic patients during hemodialysis.
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Bakground and Aims: The incidence of amputations is increased 15-fold among diabetic patients. In patients receiving hemodialysis, HD, due to diabetic nephropathy this increase is elevated even further.It is therefore of utmost importance to improve diabetic foot care in this high risk population. The aim of the study was to measure toe systolic pressure before and during HD and to evaluate a new light plethysmographic screening method.
Material and Methods: 19 diabetic patients receiving HD at Sahlgrenska University Hospital were studied during a standard HD treatment (Age63±15 years, male 74%, type 1 58%, foot ulcer history 52%). Brachial blood pressure (BBP), ankle systolic pressure (ASP) and toe systolic pressure(TSP) were measured before HD. BBP and TSP were measured at thirty minutes before the end of the dialysis treatment and fifteen minutes after the HD. Ankle pressure was measured with a hand held Doppler (Vasculoscope™, 820) and an ordinary blood pressure cuff. Toe systolic pressure was determined with a combined blood pressure cuff and pulsoximeter probe (ArterioTest™).A pulsoximeter was used for the pletysmographic readings (Biox-Ohmeda™ 3800). Neuropathy was evaluated with a monofilament (Semmes-Weinstein 5.07) at six plantar sites. All measurements were done on both sides and in duplicate.
Results: Before dialysis, brachial systolic pressure (BSP), ASP and TSP were 161±24, 192±75, 108±50 mmHg. ASP was significantly higher than TSP, p<0.001. Ankle brachial index (ABI) was significantly higher than toe brachial index (TBI), 1.22±0.5 versus 0.67±0.28,p<0.001. Eight patients (43%) had ABI< 1.3 indicating media sclerosis in one or both legs. Ten patients (52%) had neuropathy according to the monofilament test. There were no significant differences between the two legs. During the dialysis BSP was significantly reduced, 137±19 mmHg, p<0.001. After the dialysis it returned towards the predialytic values, 149±23 mmHg. TSP was reduced as well during dialysis, 79±49 mmHg, p<0.001, but did not return to the predialytic values after the dialysis 87±48 mmHg, p<0.001. The reduktion in TSP (26%) induced by dialysis was more pronounced than that of BSP (12%), p<0.05. In four legs, the systolic pressure reduction during dialysis resulted in toe systolic pressures below 20 mmHg (The detection limit for the pulsoximeter method).
Conclusion: Diabetic patients on HD have a high incidence of media sclerosis making ankel pressure measurements less suitable for screening. Toe systolic pressure is reduced during HD. As a consequence, critical limb ischemia can be induced or worsened. Moreover, the systolic pressure reduction during HD was more pronounced in the peripheral compared to the central circulation, reflecting a paradoxical vasoconstrictor response. The high incidence of critical limb ischemia in this population suggests the use of TSP screening in all diabetic patients on dialysis. Intensive foot care may then be initiated to prevent ulcerations and amputations.

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Bone mineral density in patients with diabetic osteoarthropathy.
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Background and Aims: The consequence of pathologic bone changes in diabetic foot is diabetic osteoarthropathy (DOAP). It is unclear, is DOAP a late outcome of systemic osteoporosis or a result of influence of local bone destruction to other skeleton. The aim of the study was to assess bone mineral density (BMD) in lumbar spine (L1-L4) and femoral neck in patients with acute and chronic DOAP. Find the possible correlation between BMD in femoral neck and bone remodeling markers levels.
Materials and Methods: 50 patients with diabetes mellitus were studied. 25 patients had chronic DOAP (group 1), 10 patients had acute DOAP (group2) and 15 patients had diabetic neuropathy (group 3). All patients were comparable by age, sex, duration of diabetes mellitus and body mass index (BMI). Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DEXA) in lumbar spine (L1-L4) and femoral neck. Diabetic neuropathy was measured by graduated tuning folk. Diabetes control was assessed by glycosylated haemoglobin A1c level. As a markers of bone formation we measured the levels of bone specific alkaline phosphatase (BSAP), and osteocalcin (OC). The levels of type 1 collagen carboxy-terminal telopeptide (1CTP), and tartrate-resistent acid phosphatase (TRAP) in blood serum were measured as markers of bone resorption.
Results: All patients with DOAP had osteopenia in the femoral neck more frequently then in lumbar spine (p<0,05). Acute DOAP followed low BMD in the hip (p<0,001). Patients with chronic DOAP had significant differences in BMD between affected and nonaffected limb. BMD in affected limb was lower (p<0,05). We found positive correlation between BMD and vibration sensivity (r=0,67, p<0,001). No relationships were found between BMD and HbA1c levels. Positive correlation between OK and BSAP levels and BMD in the femoral neck were found in patients with chronic DOAP (group 1) (r=0,5, p<0,01), and negative correlation between TRAP levels and BMD (r= -0,6, p<0,002). At the same time we found negative correlation between BSAP and BMD in the femoral neck in patients with acute DOAP (group 2) (r= -0,4, p<0,02).
Conclusion: That results demonstrate the influence of local bone destruction in the foot (DOAP) to the BMD in the hip. Low limb immobilization of affected foot decrease BMD in the femoral neck. We conclude that cortical bone mass in the feet is reduced in severe diabetic neuropathy. Patients with DOAP are group of risk of femoral neck fractures.

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The importance of minimal inhibition concentration and pharmacokinetic parameters of antibiotics used in the treatment of diabetes foot infections.

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Background and Aims: Evaluation of minimal inhibition concentration (MIC_{90}) and individual pharmacokinetic parameters may help to optimize antiinfective therapy in type II diabetic patients suffering from neuropathic foot ulcers (Wagner II-IV) infection.
Materials and Methods: Prior to antibiotic (ATB) administration, wound swab for microbiological examination including MIC_{90} (dilution method) was performed. Standard dosing regimen of amoxicillin/clavulanate (AMO/CLA) was used in 12 patients, whereas standard dosing regimen of clindamycin (CLI) and ciprofloxacin (CIP) combination was used in other 12 patients. Firstly, the antibiotics were administered as i.v. infusion (AMO/CLA 1 g /200 mg over 30 min every 8 hrs, or CLI 300 mg/30 min /8 hrs plus CIP 200 mg/30 min /12 hrs, resp.). Then, in convalescence, the therapy was switched to oral (AMO/CLA 875 mg/125 mg every 12 hrs, and CLI 300 mg /8 hrs plus CIP 500 mg /12 hrs, resp.). For the pharmacokinetic analysis at steady state, five blood specimens were taken on the 3rd day of the infusion phase (before the next i.v. infusion and after 30 min, 1 hr, 2 hrs, and 4 hrs) and, similarly, six specimens on the 3rd day of the oral phase (prior to dose and at 15 min, 30 min, 2 hrs, 4 hrs, and 6 hrs after the dose), resp. Determination of plasma concentration of ATB was performed using validated HPLC method. Concentration/time data were assessed using MW\Pharm 3.30 and EDSIM 2.03 by the 2-compartmental model with 1st-order absorption. Pharmacodynamic parameters t(C>MIC) and Cmax/MIC, resp., were evaluated. Paired Wilcoxon test was used for the statistical evaluation of infusion vs oral differences.
Results: (in x±SEM) are presented in table.
Conclusions: Our preliminary results proved the MIC-value as important parameter in the optimization of the antiinfective dose regimen . By the assessed kinetic and dynamic parameters (in correlation with wound healing), both infusion and oral administration of CLI and CIP combination in the standard dose regimen could secure the sufficient therapy. On the contrary, oral administration of amoxicillin-clavulanate could not guarantee the therapy of diabetes food infection.

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	F(oral)	C(av-ss) i.v. inf.	[mg/l] oral	t(C>MIC) i.v. inf.	[%] oral	Cmax/MIC i.v. inf.	oral
AMO	0,79±0,05	9,92±1,28	4,39±0,54*	61±10	45±8 *	-	-
CLI	0,69±0,07	3,56±0,68	2,59±0,31	67±21	64±20	-	-
CIP	0,73±0,10	0,84±0,07	1,35±0,21	-	-	18,5±4,7	17,4±5,5

F = bioavailability; C(av-ss) = average plasma concentration in the steady state; t(C>MIC) = time of the drug plasma concentration over the MIC, in percent of the dosing interval; * = p<0,05

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Lower extremity amputation in diabetic patients.

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Background and Aims: Lower extremity amputation (LEA) is a costly and disabling complication of diabetes mellitus (DM) related mainly with peripheral vascular disease. Although significant improvement in the treatment of these disorders has taken place in the last years, this hasn´t always been translated in a reduction of the amputation rates.The purpose of this study was to evaluate the amputation rate of diabetic patients (D) versus non diabetics (ND) in a large universitary hospital in north of Portugal between 1989 and 2002. In the diabetic group we also evaluated the presence of ischemia and the amputation types, major, minor (any transverse loss of limb through or below tarso-metartasal joint), above and below knee.
Materials and Methods: We retrospectively analyzed data from all patients with and without DM, with the diagnosis of non traumatic LEA, according to the criteria of ICD9. Statistical analysis was done with Student's t test, Chi-Square or Fisher exact test. A two tailed p value <0.05 was considered significant.
Results: During this period there were a total of 615209 admissions and of these 50016 were diabetics. A total of 1727 non traumatic LEAs were done, 759 (44%) in D (Males=420; Females=339) and 968 (56%) in ND (M=688; F=339). The risk of amputation was significantly higher in D than in ND (odds ratio=8.98; 8.15<OR<9.89; p<0.0001). There was a female predominance of amputation in diabetics (45% vs 29%;p<0.0001). D were older than ND (67.6±1.13 vs 65.5±1.31 years; p<0.001) and had longer hospitalizations (median: 28 vs 23 days; p=0,02). In both groups F are significantly older than M (D:70.3 vs 65.5 years; ND:72.7 vs 62.9 years; p<0.001) but M have longer hospitalization than F (medians: D:31 vs 24 days, p<0.01; ND:24 vs 18, p=0.01). In hospital mortality was similar in D and ND (14% vs 13%; p=0.57). In diabetics the presence of ischemia was significantly associated with an increased risk of amputations (64% vs 36%; odds ratio=3.17; 2.56<OR<3.93; p<0.0001) and with a longer hospitalization (median: 30 vs 23 days;p=0.004). There were no differences in age (67.4±1.13 vs 65.9±2.52 years; p=0.057) sex distribution (F:46% M:54% vs F:42% M:58%; p=0.40) and in hospital mortality (13.99% vs 13.91%) between D with and without ischemia. Amputations types in D were: minor 322, below knee 118, above knee 348, not specified 10. From 1989 to 2002, the absolute number of LEAs in D increased from 34 to 81. One possible explanation for this huge difference may be probable undercoding during the first years.
Conclusion: There are significant variations in the characteristics of diabetics amputated during this period: women are older and suffered more amputations than men and ischemia is associated with a 3 fold increased risk of amputation. The rates of LEAs in diabetics have increased between 1989 and 2002. Thus in order to reduce them more specific interventions are needed.

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Transcutaneous oxygen tension as an index of successful revascularization in diabetic patients with ischaemic foot ulcers.

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Background and Aims: The aim of the present study was to assess the efficacy of transcutaneous oxygen tension (TcPO2) for the assessment of successful percutaneous transluminal angioplasty (TPA) in diabetic patients with ischaemic foot ulcers.
Materials and Methods: Six diabetic patients with ischaemic foot ulcers (stage IV Fontaine) underwent peripheral revascularization by TPA in our unit. In all cases, angiography was previously performed. TcPO2 was recorded before (T0) and 1, 2, 3 and 4 weeks after TPA.
Results: As shown in the table, a progressive improvement of cutaneous oxygen tension (pO2) was observed after TPA, which reached its peak 3 weeks after surgery. At the same time, a decrease of cutaneous carbon dioxide tension (pCO2) was observed 1 week after PTA which reached a plateau during the following weeks. In agreement with TcPO2 findings, none of our patients had complications after PTA (100% limb salvage rate) or needed to undergo a second revascularization procedure.
Conclusion: Measurement of transcutaneous oxygen tension is a valid tool to assess successful revascularization after percutaneous transluminal angioplasty. Our results also suggest that the best timing to perform a more aggressive surgical debridment in diabetic patients with ischaemic foot ulcers who underwent PTA is about three weeks after the surgical procedure, as indicated by the pO2 curve.

	T0	1-week	2-week	3-week	4-week
pO2 (mmHg)	8.97 ± 9	26.83 ± 17	34.12 ± 22	41.6 ± 22	40.93 ± 27
pCO2 (mmHg)	71.2 ± 27	43.75 ± 10	39.7 ± 14 34	18 ± 8	33.28 ± 7

mean ± SD

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Can we reliably assess the neurological status of the foot in children using currently available methods?

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Background and Aims: There is substantial evidence that early neuropathic changes in patients with Type 1 diabetes begin in childhood.However, there is a huge gap in our understanding regarding the natural progression of neuropathy.Our current understanding of diabetes demonstrates that good glycaemic control can ameliorate or reverse early neuropathic changes.Behaviour modification (involving patient empowerment) is more effective in younger than in older people.Indeed irrespective of age we are obligated to empower or patients with the information regarding their risk status.The value of neuropathy assessment underpinned with education from an early age is currently understated in diabetes management.However, before we can apply existing neuropathy testing to children we need to explore the sensitivity and reliability of such methods.
Materials and Methods: We investigated three methods of neurological assessment (1g and 10g monofilaments, Neurothesiometer and CASE IV thermosensor). Methodologies were qualitatively explored in two focus groups of children aged 8 – 12y. Normative data were collected in 100 normal children aged 5 – 14y (mean age 8.0y).
Results: There was 100% sensitivity and differentiation for 8 sites using 1g and 10g monofilaments. VPT (mean 2.5V, range 1-4V) produced highly reproducible responses (r >, 0.8) consistent with previous data. Children of all ages could maintain attention for reliable use of these tests.The CASE IV produced variable warm / cool thresholds irrespective of the childs age.Several factors may be responsible for this : 1. The CASE IV demands significant prolonged concentration and has not previously been validated for use in children. 2. During the tests children became easily distracted and bored. 3. The existing probe was designed for use in adults and difficulties occurred maintaining contact between the probe surface and the dorsum of the foot. 4.The baseline temperature of the probe increased during use.
Conclusion: Existing methods of sensory testing can be reliably applied to children as young as 5y.The 1g monofilament may provide a better discriminater of early sensory changes than the 10g.However, a reliable, portable and children friendly approach to thermal sensory assessment needs to be developed if we are to detect neuropathic changes early on in childhood.
Our further work involves devising such a method and assessing it in children with diabetes

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Diabetic Foot - Outcome and Clinical Aspects

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Outcome of in-patients with diabetic foot lesions seen at Muhimbili National Hospital, Dar es Salaam, Tanzania, 1997-2002.

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Background: Foot complications cause significant morbidity and mortality in diabetes patients, and are the leading cause of non-traumatic lower limb amputation (LLA). However, there are limited published data regarding this problem from developing countries.
Aim: To determine the prevalence rate, presenting features, associated risk factors, and clinical outcome of foot ulcers in diabetes patients admitted to Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania, during January 1997-December 2002 (study period).
Materials and Methods: During the study period, non-selective, consecutive, adult diabetes patients hospitalised with foot ulcers were enrolled after informed consent. Detailed clinical and epidemiologic data were recorded for each patient followed by a comprehensive physical examination. Clinical outcome was documented.
Results: Of 2134 diabetes patients admitted to MNH during the study period, 350 (16%) had foot ulcers. Outcome data were available for 312 patients (89%). 147 (47%) of the 312 patients were managed without amputation, while 141 (45%) underwent amputation (70 minor, 71 major, 2 bilateral). Compared to non-amputees, amputees were older (56 ±12 vs 53±13 years, p=0.05), more likely to present with angiopathy (P <0.001) and gangrene (P <0.001). Time lost until initial presentation to the hospital was significantly longer in patients finally suffering amputation than in non-amputees (4.2±3.9 vs 3.4±4.0 weeks, P=0.003). Mean duration of hospital stay was 59 days for amputees compared to 31 days for non-amputees (p<0.001). Death as an endpoint during the follow-up period was significantly more prevalent in patients with ischemic lesions compared with pure neuropathic lesions (44% vs 25%, p=0.004) and in amputees compared with patients treated conservatively (33% vs 25%, p=0.03). Furthermore twenty-four patients selected for amputation died from sepsis before surgery was initiated.
Conclusion: Diabetic foot ulcers are a common cause of hospital admission in Tanzania and result in long periods of hospitalisation and high mortality. Education of patients should underscore the importance of foot care and consulting a doctor during the early stages of the foot ulcer disease.

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Duration of ulcer-free time after healing of foot ulcers in diabetes.

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Background and Aims: Those who suffer foot ulcers are often elderly, have other complications of diabetes and vascular disease, and have poor overall prognosis. Healing of ulcers is delayed and uncertain; and is frequently followed by recurrence. The overall unadjusted rates of repeat ulceration in our own practice is 46.5%. Since early death was the reason why ulcers did not recur in 42.7% of the remainder (22.8% of the total), it follows that duration of ulcer-free days is a more meaningful measure of the effectiveness of patient care than crude ulcer healing time or healing rate.
Materials and Methods: We have therefore examined our comprehensive ulcer-management database in order to determine how many patients ever become ulcer-free, and for how long they remain so.
Results: Of 370 patients (with 1031 ulcers) referred to our specialist out-patient service between 1/1/2000 and 31/07/2002, 131 (35.4 %) have never become ulcer-free during the period of follow-up: 56 (15.1% of the total) remain unhealed, 9 (2.4%) had amputations and 49 (13.2%) have died. Outcome in 17 (4.6%) was not known. In only 239 (64.6%) did all their ulcers heal at some stage. The overall prognosis was better in this group: only 3 died in the period of follow-up. However 91 of those who healed (38.1% of 239) had a recurrent or new ulcer, with a median interval to onset of 126 (14-903) days. Of those 148 patients who healed but have not had a recurrence, 12 had been managed by major amputation and were excluded

from analysis, while 136 (56.9% of those who healed; 36.8% of the total) have remained ulcer free for a median time of 456 (7-1043) days.
Conclusion: 64.6% of patients became ulcer-free at some stage but only 56.9% of these survived free of recurrence. Kaplan-Meier analysis suggests that recurrence is relatively unlikely if a patient has remained ulcer free for more than one year after healing.

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Mortality in diabetic foot ulcer patients cannot be predicted with conventional models and is currently undertreated by medical teams.
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Background and Aims: The increasing realisation that cardiovascular disease is the main cause of death in diabetic patients has prompted changes in management, particularly of Type 2 diabetes. Foot ulcer patients have long been recognised to carry a poor prognosis. This study examined the survival of a cohort of patients from 1995 to 1999, and then a more recent cohort of patients, to determine the true mortality rate, the predicted mortality rate and extent of prophylactic intervention.
Materials and Methods: 348 consecutive diabetic foot ulcer patients, comprising 155 neuropathic patients, (102 males, 55% type 2, age first visit 56.9 (SD 15.4) years, duration, median 13.8 years); and 193 neuroischaemic patients, (119 males, 80.8% type 2 ($p<0.02$ vs N), age 68.2 (SD 12.7) years ($p<0.001$ vs N), duration 13.0 years, were seen between 1995-1999, and followed-up until 2002, median five years. Multiple sources were examined for date of death, cause, and cardiovascular risk modifying drugs. To assess the impact of recent guidelines on cardiovascular risk reduction 100 patients from 2002, matching the original group, had primary cardiovascular risk determined using the highest value of Framingham, UKPDS and New Zealand tables. Secondary prevention mortality risk was determined from a meta-analysis of the control groups of intervention trials.
Results: 30 patients underwent amputation (8%). Over 50% of neuroischaemic and 25% of neuropathic foot ulcer patients had died by 3.5 years after first visit at a multidisciplinary clinic. Amputation did not increase mortality. Cardiovascular risk calculations only predicted a quarter of the observed mortality. For neuropathic patients the calculated 3.5 year risk was 6.8%, and for neuroischaemic patients 12.9%. In 1995-1999 under 20% were on aspirin, 10% on other therapies. In 2002 this improved, 80% of „secondary prevention" patients on aspirin vs. 39% „primary", but under half on statins, 38% ACE-inhibitors, 20% Beta-blockers. However prescribing for „secondary" prevention equalled „primary".
Conclusion: Mortality in foot ulcer patients is not identified by conventional means and exceeds deaths post myocardial infarction. Therefore, „Secondary prevention" measures should be advocated for all foot ulcer patients, especially those with neuroischaemic ulcers.

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Comparison of outcome in different types of foot ulcer using a detailed classification system at first referral.
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Background and Aims: There are few data available on the outcome anticipated for all ulcers encountered in clinical practice, and on the case-mix of those referred to specialist units. Such data are essential for analysis of comparative performance. The main barrier to obtaining such information has been the lack of a precise, but reasonably simple classification system
Materials and Methods: We have reviewed the spectrum of ulcers referred to a specialist unit serving a population of 300,000 over 12 months. Ulcers were classified by area, depth, infection, and degree of ischaemia and neuropathy. We compared outcomes at 3 months (healed, unhealed, amputation, death) in different ulcer types.
Results: 389 ulcers were classified at the time of referral. AREA: 33% of those with cross-sectional area $<1cm^2$ (N=208) and 41% of those 1-$3cm^2$ (N=100) healed by 3 months, respectively. 2% and 5% were amputated, compared with 16% in ulcers $>3cm^2$. DEPTH: 305 (78.4% of 389) ulcers involved skin and subcutaneous tissue only; 42 (10.8%) extended to tendon and periosteum and 28 (7.2%) involved bone. Rates of healing in these three groups were 37%, 19% and 14%; rates of amputation were 2%, 10% and 29%. INFECTION: 255 (65.6% of 389) ulcers were clean, while 69 (17.7%) were superficially infected, 45 (11.6%) had cellulitis and 20 (5.1%) osteomyelitis. Healing occurred in 35%, 28%, 27% and 45%, respectively. ISCHAEMIA: There was no ischaemia in 141 (36.2%), but it was moderate in 75 (19.3%) and severe in 157 (40.4%); 16 (4.1%) had gangrene. Healing rates in these groups were 50% (no ischaemia), 37% (moderate), 19% (severe) and 6% (gangrene). Rates of amputation were 1%, 5%, 6% and 25%, respectively, while 0%, 1%, 4% and 13% died within 3 months. NEUROPATHY: Protective sensation was intact, moderately and severely impaired in 68 (17.5%), 112 (28.8%) and 197 (50.6%), respectively. 12 (3.1%) had Charcot osteoarthropathy. Healing was observed in 33%, 36%, 33% and 8% respectively. OVERALL: 129 of 389 ulcers (33%) healed by 91 days, 19 (4.9%) had been resolved by amputation (nine major; 10 minor) and 10 (2.6%) by death, while 231 (59.4%) persisted unhealed. There were strong correlations between outcome and baseline classification for area, depth and arteriopathy ($p<0.001$, Spearman), but none with infection or neuropathy.
Conclusion: Use of a detailed classification enables audit of clinical practice and could facilitate comparison between centres. Such an approach could be used to provide benchmarks of acceptable care.

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Relationship between lesion duration at referral and outcome in diabetic foot ulcers.
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Background and Aims: In order to investigate whether delayed referral contributes to adverse outcome in diabetic foot ulcers, we have examined the relationship between outcome and ulcer duration at time of first expert assessment, as well as a number of other potentially predictive variables.
Materials and Methods: A retrospective review of comprehensive clinical data base.
Results: 772 ulcers were referred to our specialist multidisciplinary out-patient clinic between 1st January 2000 and 1st July 2002, and followed for a minimum of six months or until death. Median ulcer duration between estimated onset and first specialist assessment was 35 days (range 1-516). 513 (66.5%) healed without surgery; 99 (12.5%) were unhealed; 81 (10.5%) were resolved by amputation, and 79 (10.2%) by death. The population was divided into four groups on the basis of lesion duration at referral: 1 – same month (N=340, 44.0% of 772); 2 – preceding month (N=249, 32.3%); 3 – two months earlier (N=110, 14.2%); 4 – longer (N=73, 9.5%). There was a significant difference between these four lesion duration groups in terms of outcome (healed, unhealed, amputation, death), $p<0.001$ (Chi square). However, there were also significant differences between the lesion duration groups in ischaemia ($p<0.001$, Chi Square), neuropathy ($p<0.001$), depth ($p=0.001$), infection ($p=0.029$), but not area ($p=0.062$). Significant correlations ($p<0.001$, Spearman rho) were observed between outcome type and area ($r=0.226$), depth ($r=0.180$) and ischaemia ($r=0.355$). Logistic regression analysis revealed that the model best able to predict outcome type consisted of ischaemia and area (Chi Square = 124.98, df 2, $p<0.001$).
Conclusion: We have found no evidence of an independent effect of delayed referral on outcome, although median ulcer duration at referral was 35 days in this population. It is possible that if increased speed of referral is beneficial, it may need to be very much quicker. The benefit of an urgent assessment service for all ulcers needs to be formally assessed.

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A model based calculation of treatment outcomes and economic effects of intensified foot care in an outpatient clinic setting.
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Background and Aims: There is sufficient evidence, that intensified foot ulcer treatment is capable to reduce amputation and mortality rates among diabetics. We wanted to varify if higher short term costs for intensified care can be compensated by reduction of amputations in an Austrian cost setting.
Materials and Methods: Data of 119 ulceration histories of patients in a specialized Austrian outpatient clinic have been collected retrospectively, whereas standard treatment data were derived from internationally published papers. We assessed the outcome as rates for healing, ulceration according to the San Antonio Wound Classification, amputation and mortality. Data for intensified care and standard treatment were extrapolated using a Markov model. The average treatment costs for intensified care have been calculated with respect to outcome, focused on

data about medications, dressings, diagnostic methods, hospitalization and visits. Costs for standard treatment were estimated in cooperation with physicians and health insurances.
Results: Baseline mean age was 66 years, the mean diabetes duration was 16 years. 22.7 % of the patients of the outpatient clinic had a superficial ulceration, 38.7% had an ulceration with infection, 10.9 % had an ulceration with contributing ischaemia and 27.7% with both infection and ischaemia. The table shows the rates for healing, amputation and mortality for intensified care as well as the average costs (discount rate: 5%) per patient and corresponding data for standard care as results of the Markov model simulation.

		1 year (extrapol.)	2 years (extrapol.)	3 years (extrapol.)	5 years (extrapol.)	10 years (extrapol.)
Intensified care	Healed without amputation (%)	83,2 %	80,2 %	75,5 %	67,6 %	51 %
	Rate of amputations (%)	12,6 %	14,8 %	16,4 %	20,9 %	30,9 %
	Mortality (%)	4,2 %	8,6 %	13,4 %	33 %	40,3 %
	Overall costs (Euro, per patient)	*4971*	*6142*	*7340*	*9585*	*13860*
Standard care	Healed without amputation (%)	45 %	42,8 %	35 %	24,4 %	9,9 %
	Rate of amputations (%)	33 %	35,8%	42,8%	53,7%	71,4%
	Mortality (%)	22 %	28,8 %	36,8%	50,2 %	73,4 %
	Overall costs (Euro, per patient)	*6522*	*8100*	*10060*	*13200*	*18009*

Conclusion: Intensified care is able to reduce amputations by more than 60%, which has also been shown by other studies. The results indicate that treatment costs for intensified care are lower due to less amputation. Markov models are useful tools to virtually compare different treatments within patients and represent a practicable alternative, if real life studies are impossible to be performed. The robustness of the results depends on the quality of the estimated parameters. To reduce variance of results and possible wrong estimations sensitivity analysis are to be performed.

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Descriptive study of the Diabetic Foot Clinic in Costa Rica: 5 years of follow-up.
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Background and Aims: To describe the management and characteristics of the diabetic patients evaluated during 5 years at the Diabetic Foot Clinic in Hospital México, San José, Costa Rica.
Research Design and Methods: Clinical files of all the patients attended in the diabetic foot clinic during the years 1997-2002 were reviewed and clinical data were collected. Age, gender, type of diabetes,duration, treatment, characteristics and location of lesions, HbA1c levels , pulses, treatment and management of evaluated cases were all included in the analysis. Means and standard deviations were calculated for continuous variables and percentages for categorical variables. SPSS software for windows version 8.0 was used for the data management. The local committee for bioethics approved the study.
Results: A total of 377 patients were evaluated in the clinic during five years. Mean age was 60±4 years. 56% of patients were women. Mean time of diabetic evolution was 16,6 ± 3,2 years. Mean time of foot lesion presentation was 33 ± 5 days. 92% of patients were Type 2 diabetics. 63% of the patients were using insulin for treatment . 20% of the patients had prior history of foot or lower limb amputation. Foot ulcer was the predominant lesion in 23% of the patients, followed by foot abscess in 21% of the cases, and 10% of cases presenting Neuroarthropathy of Charcot. The first toe and the sole region were the most common location of lesions (25% and 29% of cases respectively). There were absence of pedial and/or posterior tibial pulses in 32% of patients. Angiography was conducted in 20% of the patients, while major and minor amputations were performed in 27% and 18% of patients respectively. 45% of lesions were considered to be neuropathic and 37 % were described as ischemic lesions. Of the 20% of patients that went to angiography, 40% of those patients had artery bypass graft surgery for lower limb rescue. Mean HbA1c levels of these patients at the moment of presentation at the clinic was 9,89± 0,60 %.
Conclusions: Foot ulcers and amputations are the main problems in the Diabetic Foot Clinic in Costa Rica. Metabolic control of the Diabetic Foot Clinic patients is alarming. Only a few percentage of patients have the option of an artery bypass graft surgery for limb salvage.

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Evaluation of the impact of chiropodist care in the secondary prevention of foot ulcerations in diabetic subjects.
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Background and Aims: To evaluate the influence of regular chiropodist care on the recurrence rate of diabetic foot ulcers within one year.
Materials and Methods: Ninety-one diabetic outpatients with healed foot ulcers (age 65±11 years, 40 women and 51 men, diabetes type [1/2] 6/85, BMI 28.5±4.4, diabetes duration 16±11 years, HbA1c 8.4±1.6 %) were randomized to a group that received monthly remunerated routine chiropodist care (n=47) or a control group.
Results: Within a median follow up of 386 days, ulceration recurred in 18 patients in the chiropodist group and 25 patients in the control group, Hazard Ratio (HR) 0.60 95% CI [0.32, 1.08], p=0.09. Analysis of ulceration per foot demonstrated a significant reduction (20 vs. 32 ulcerations, Cox Relative Risk [Cox RR] 0.52, 95% CI [0.30, 0.93], p=0.03) in favour of chiropodist care. Per protocol analysis of patients who actually underwent chiropodist foot care on a regular basis also indicates the beneficial influence of chiropodist care, with ulceration in 13 vs. 30 patients (HR 0.53 95% CI [0.30–1.01], p=0.05) and in 15 vs. 37 feet (Cox RR 0.46, 95% CI [0.24–0.90], p=0.02) for the intervention and control groups, respectively. Minor amputation was required in 2 patients in the intervention group and 1 patient in the control group. Four patients in the control group and 2 patients in the intervention group died during the trial.
Conclusion: These data suggest that secondary preventive measures by a chiropodist may reduce recurrence of foot ulcers in diabetic patients.

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Teleconsultation (TC) of patients with diabetic foot syndrome (DFS).
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Backround and Aim: The aim of the study is to analyse the practicability and feasibility of TC in the diagnosis and treatment of patients with diabetic foot syndrome. Two diabetic centres with long experience were involved.
Patients and Methods: Group A: synchronous TC of patients with diabetic foot syndrome were performed using Vtel Smart Station version 4.0 and an ISDN line for data transfer. Video consultations were carried out using a mobile camera and a document camera and were repeated 14 days later. Group B: asynchronous TC were performed by sending 1-2 digital photos of the diabetic foot together with demographic and clinical data as well as x-ray pictures by e-mail. Patients were treated by one centre and consulted by the other. The consultant and the treating doctor independently worked out the strategy for each patient. A structured questionnaire was used to quantify practicability and agreement with the advice for diagnosis and treatment.
Results: Synchronous TC lasted longer than asynchronous TC. The synchronous TC however offers advantages like receiving further informations (more pictures, immediate answer of question while the patient is still present) and an immediate beginning of therapeutic intervention. Asynchronous TC needs less time and organisation on both sites but important data may be missing for planning the strategy and the intervention is always delayed.
Conclusion: TC is practicable and feasible in the treatment of patients with diabetic foot syndrome and can be implemented in routine care. TC opens new possibilities of communication in the highly specialized interdisciplinary care of DFS. In less experienced physicions but even among specialists a rapid second opinion can be obtained and standardisation of care can be developed. Although the initial investment for the equipment is rather high the telemedicine approach is much cheaper and highly efficient.

Patients

	n	Age (y)	Diabetes	Duration of diabetes (y)	Wagner classification grade: 1	2	3	4
Group A	10	median: 71 range: 46-84	type 1: 0 type 2: 10	median:13 range: 2-25	0	5	4	1
Group B	15	median: 66 range:47-80	type 1: 2 type 2: 13	median:15 range: 1-31	2	7	5	1

Results

	n	Duration of consultation	Agreement in diagnisis strategy		Feasibility	Efficiency
Group A	10	median: 20 min. range: 5-60 min.	10/10	8/10	Good:16 Moderate:3 Poor:1	Good:19 Moderate:1 Poor:0
Group B		15 median: 7 min. range: 4-10 min.	12/15	10/15	Good:12 Moderate:3 Poor:0	Good:12 Moderate:3 Poor:0

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The diabetic foot inclusion as an academic activity for physiotherapy graduating students.

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Background and Aims: It has been well recognized that an interdisciplinar approach is fundamental to reduce the devastating outcomes of foot problems. Thus, basic information should be available during the graduation period of academic activities of those professionals who shall be involved with diabetic patients. Physiotherapists have been playing an important role on how to diagnose and rehabilitate patients with limited joint mobility (LJM) and diabetic neuropathy (DN). The aims of this pilot study are: 1) to train physiotherapy students on the diagnosis of DN and LJM, 2) to use techniques to improve joint amplitude, and 3) to set up an integrated physiotherapy clinical activity for immediate referral of diabetic patients.
Materials and Methods: Assessment of DN was performed by using a 128 Hz tuning fork, 10 g monofilament, hammer, pin, cotton wool, goniometer. A visual pain scale(VPS)was applied to evaluate neuropathic pain score. DN evaluation followed the Practical Guidelines of the International Consensus on the Diabetic Foot(PGIC), LJM goniometry was performed in accordance to the American Medical Association protocol, and neuropathic pain was evaluated by means of a 10 cm lenght VPS. 41 consecutive diabetic patients were enrolled in the study during 3 months after being referred by the doctor to a 20 physiotherapy graduating student group, who made the clinical evaluation under qualified supervision of a university professor.
Results: 82.92% had type 2 diabetes, mean age was 54.50 yr, 68.29% had hypertension, 14.63% had active ulcer and 21.95% reported previous ulcer history (66% on the forefoot) and 24.39% had amputation (70% minor), which allocated them as Risk 3 of the PGIC risk classification system. Neuropathic symptoms were present among 56.09% (52.17% VPS score ranged 1-5 cm). As for the goniometry, mean dorsiflexion amplitude were 11.46° and 12.19° for the right and left limbs, amplitude loss found to be 8.8° and 7.8°, respectively, while mean plantar flexion amplitude were 33.20° and 35.12°, amplitude loss found were 6.70° and 4.87° for both right and left limbs.
Conclusion: There has been a significant amplitude reduction among the diabetic patients referred for goniometric evaluation to detect LJM and that finding placed them in a twice-a-week physiotherapy clinical session. The diabetic foot centre has been considered a very positive means of integrating the academic graduating activity to clinical grounds and to the community. This pioneer approach has now been included in the curriculum programme of the Catholic University of Brasilia physiotherapy graduation course, therefore, representing a great achievement for both students and the diabetic community.

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Low ankle-brachial index is associated with high mortality among Type 2 diabetic subjects in Taiwan.

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Background and Aims: Low ankle-brachial index (ABI) was associated with high mortality for old age population in previous studies. But it has not been reported specifically for those with type 2 diabetes.
Materials and Methods: There were a total of 2023 type 2 diabetic patients, 1006 males and 1017 females with 60.98 ± 9.47 years old (mean ± SD), recruited for peripheral vascular disease screening by the measurement of ankle-brachial index from Jul 1996 to Dec 1999. Demographic characteristics (age, sex, duration of diabetes, smoking, body mass index) and metabolic parameters (systolic blood pressure, hemoglobin A1c, total cholesterol, triglycerides) were also obtained. Alive status was checked till Dec 31, 2000. Kaplan-Meier and Cox regression model were chosen for outcome evaluation.
Results: The follow-up period was 2.52 ± 1.02 years. There were 1905 patients with ABI >= 0.9 (94.1 %), 78 patients (3.9 %) between 0.9 and 0.7 and 40 patients (2.0 %) with ABI<0.7, respectively. On Dec 31, 2000, the mortality number for three groups was 62, 9, 8, respectively. The odds ratios [95% Confidence Interval] were 3.88 [1.85, 8.12] (p< .0001) for those ABI between 0.7 and 0.9 and 6.68 [2.97, 15.0] (p< .0001) for ABI below 0.7. In Cox regression model, older in age and low ABI were significant risk factors for all-cause mortality, where total cholesterol (p=0.062) and smoking (p=0.096) were of borderline significance. Life expectancy was also significantly shorter as ABI decreased.
Conclusion: Low ankle-brachial index is a risk factor of all-cause mortality among type 2 diabetic patients. Early screening and proper intervention may be beneficial.

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Nephropathy - Epidemiology

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The prevalence of microalbuminuria in a nationally representative sample of primary care attendees: focus on patients with diabetes.

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Background and Aims: The HYDRA study is a large-scale epidemiological research program in primary care, that allows identifying the size and impact of recognized and unrecognized high risk patients especially with regard to the prevalence of microalbuminuria. We aimed to determine the prevalence of a positive dipstick test for microalbuminuria in patients in primary care depending on age, gender and severity of the disease and to correlate these positive tests with comorbid conditions.
Materials and Methods: On the study day, 45,125 patients attending their primary care physician completed a study questionnaire. Doctors completed for each patient a standardized clinical appraisal, supplemented by lab tests. Microalbuminuria sticks were used to determine the rate of albumin excretion in the microalbuminuric range among attendees. Data presented were adjusted for cluster and response bias effects as well as for age and gender.
Results: (1) 9.1% of all patients (point prevalence) were diagnosed by the doctor to have diabetes plus hypertension; (2) 12.5 % of these patients with diabetes and hypertension were diagnosed to have (diabetic) nephropathy (vs pateints without hypertension or diabetes: odds ratio 4.6); (3) roughly three times as many (37.8%), were tested positive for albuminuria on the day of examination; (4) compared with patients having neither diabetes nor hypertension, comorbid patients with both conditions and a positive dipstick test in the microalbuminuric range had markedly increased rates of comorbid illnesses, such as peripheral arterial disease (21.3%), heart failure (27.0%), coronary artery disease (12.3%) and retinopathy (20.2%).
Conclusion: This study provides not only further insight into the high prevalence of patients with both diabetes and hypertension, but also into the high burden of comorbidities associated with these patients. It also emphasizes the clinical utility of dipstick tests for (micro-) albuminuria as an important marker for identifying patients at high risk for further comorbid diseases.

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Renal disease in patients with Type 2 diabetes with and without diabetic retinopathy.

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Background and Aims: Several recent studies had suggested that non-diabetic renal disease (NDRD) is common among patients with DM type 2 with renal involvement, particulary in the absence of diabetic retinopathy (DR). The aim of our study was to evaluate the cause of renal disease in a cohort of patients with DM type 2, with clinical suspicion of a NDRD, with or without DR.
Materials and Methods: Sixty-six patients were included in the study. All these patients had the signs of renal impairment and underwent renal biopsy. Renal tissue specimens were processed according to the standard procedures for light, immunofluorescence and electron microscopy techniques. DR was determined on fundoscopy by ophthalmologist.
Results: There were 51 males and 15 females, mean age 58.6 ± 10.7 years, mean DM duration 7.5 ± 7.9 years, in 8 pts DM was diagnosed at the time of renal biopsy, mean proteinuria 5.4 ± 4.2gr/L, mean s-creatinine 262 ± 177.8 umol/L. The patients were treated with insulin -11 patients, oral hypoglycemic agents -33 pts and with diet alone -14 pts, in 8 pts DM was not treated. Twenty-five included pts had DR (37.9 %) and 41 pts had no DR (62.1 %). DR-positive group (n=25) included 22 pts with DN (DN alone-9 pts, DN in combination with NDRD -13 pts) and 16 pts with NDRD (NDRD alone-3 pts, NDRD in combination with DN -13 pts). DR-negative group (n=41) included 26 pts with DN (DN alone-9 pts, DN in combination with NDRD -17 pts) and 32 pts with NDRD (NDRD alone -15 pts, NDRD in combination with DN -17 pts). The most common form of NDRD was glomerulonephritis (85 % of patients with NDRD). Patients with DR had DN in 88 % and NDRD in 64 %, alone or in combination. Patients without DR had DN in 63.4 % and NDRD in 78 %, alone or in combination.
Conclusion: Our results suggest that in our group of patients with DM type 2 and clinical suspected nondiabetic renal disease, the possibility of diabetic nephropathy or nondiabetic renal disease can not be predicted by the presence or absence of diabetic retinopathy. Glomerulonephritis was the most common form of NDRD. Therefore, for identifying those patients with a treatable renal disease and for better determining of renal prognosis, we recommend renal biopsy in type 2 diabetes patients with renal impairment.

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Prevalence of microvascular complications in a cohort of young persons with Type 1 diabetes mellitus.

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Background and Aims: Type 1 diabetic patients have a pronounced risk of nephropathy and retinopathy. Is the risk declining? We are investigating the prevalence of nephropathy, retinopathy, hypertension and risk factors for microvascular complications in a national cohort.
Materials and Methods: In 1973-1982, 1914 children (age <15 years) were diagnosed with Type 1 Diabetes Mellitus in Norway. In 1989-1990, a representative subset of these (n=371) were examined for nephropathy, retinopathy and hypertension. We are re-examing this cohort. Eight persons are deceased, 5 are emigrated, leaving us with 358 persons. So far 255 (71%) are examined. The mean age of the subjects is 31,8 years (range 20-42,3) and the mean duration of diabetes 22,5 years (range 18,2-29,3). Arterial blood pressure is measured twice in sitting position after 15 min. rest with a standard mercury sphygmomanometer. Fundus photography is performed in mydriasis by a nonmydriatic 45 Canon camera (45NM-CR), using a 35 mm film. Two photographs are taken of each fundus. Overnight timed urine samples are collected at home. Persistent microalbuminuria was defined as urinary albumin excreation rate (AER) > 15 ug/min in at least two out of three consecutive urine samples and overt nephropathy as AER > 200 ug/min in at least two out of three consecutive urine samples. Smoking habits, antihypertensive treatment and lipid-lowering medication were registered.
Results: 255 persons are examined, 141 men and 114 women.
Nephropathy: Complete urine data (n=245). 48 (19,6%) subjects have nephropathy, either microalbuminuria (n=31), proteinuria (n=16) or are on dialysis (n=1). 23 (48%) of these 48 are on hypertensive treatment.
Hypertensive treatment: 38 (15%) are on hypertensive treatment. 14 of them have normoalbuminuria.
Retinopathy: 44 (17,2%) have had laser treatment. The fundus photographs are yet not read.
Smoking: 106 (41,5%) are current smokers.
Lipids: 14 (5,5%) are on lipid-lowering medication.
Conclusion: Compared to other studies it is noteworthy that only 7% of the subjects with a mean duration of diabetes more than 20 years, have overt nephropathy.

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Incidence and management of end stage renal disease (ESRD) in diabetic patients in Franche-Comté.

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Background and Aims: A prospective epidemiological study was conducted from January 1 to December 31 , 2002 in the region of Franche-Comté (FC) in the east of France, to determine the incidence, the characteristics and to analyse the management of diabetic patients (DP).
Materials and Methods: All patients newly accepted for renal replacement therapy (RRT) in 2002 were included. We collected demographical, clinical and biological data every month. Statistical analysis was conducted with using the epi info software and the chi square and the Mann Whitney's tests to compare DP and non diabetic patients (NDP).
Results: 142 patients started a RRT of whom 45 (31.6%) were diabetic including 42 type 2 and 3 type 1. The mean diabetes duration was 16.4 ±11.3 years. Diabetes (27.5%) and hypertension (23.9%) were the main causes of ESRD and 33% of patients were referred to nephrologists less than 6 months before starting RRT in the 2 groups. 53.3% of DP were treated by haemodialysis, 44.4% by peritoneal dialysis and 2.2% by pre-empted renal transplantation. 24.5% of DP were dialysed in emergency conditions mainly because of cardiac failure. The DP and NDP were not different for age, sex ratio, smoking, haemoglobin (Hb) level, proportion of patients treated by erythropoietin or reached Hb level more than 11 g/dl, lipids levels, nutritional parameters, CRP, 24h-proteinuria, hepatitis B serologic and social status. Proportion of patients with controlled blood pressure (<130/85 for NDP and < 130/80 for DP) were not different, but DP used more antihypertensive drugs (2.9 ±1.5 vs 2.3 ±1.5. p=0.02). Diuretics and calcium channel blockers were the most used drugs in DP and NDP. ACE inhibitors and β-blockers were used only in 50% and 38% of DP respectively. The use of anti-platelet agents (54%) was insufficient too. DP were more often treated by lowering-cholesterol drugs (35.6% vs 18.6%. p=0.03) than NDP, and only 55% of lipid levels have been fully measured. The mean glomerular filtration was significantly less deteriorate in DP, meaning that DP probably began RRT earlier than NDP. Mean HbA1c of DP was 7.2 ±1.5 %, 60% had a follow-up by a diabetologist and 66.7% an eye examination within the year preceding RRT, 26.3% had a previous screening for coronary artery disease (CAD), 68% a retinopathy, 16% a CAD, 33% foot complications and 11% a previous stroke.
Conclusion: Our study shows that diabetes is the leading cause of ESRD in FC and this burden is still rising. Management of DP and NDP is not different and not optimal, especially cardiovascular management. Extensive cooperation between multidisciplinary teams working on a network (including general practitioners, cardiologists, diabetologists and nephrologists) should improve this situation.

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Effects of chronic kidney disease and end stage renal failure on mortality in Pima Indians with Type 2 diabetes.

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Background and Aims: We examined the effects of chronic kidney disease (CKD) and end stage renal failure (ESRF) treated by renal replacement therapy on overall and cause-specific mortality in diabetic Pima Indians between 1965 and 1998.
Materials and Methods: Diabetic subjects (726 men, 1034 women) ≥ 35 years old were participants in an epidemiologic study of diabetes and its complications. Underlying causes of death were determined by reviewing all available clinical records, autopsy reports, medical examiners' findings and death certificates. Causes were classified by ICD-9. Deaths and person-years (pyrs) of follow-up were stratified in a time-dependent fashion into categories of normal serum creatinine (SCr), CKD (SCr ≥ 133 µmol/L (1.5 mg/dl) in men, ≥ 124 µmol/L (1.4 mg/dl) in women) or ESRF. Subjects with CKD for whom renal replacement therapy was unavailable or refused were classified as having CKD, and all died of diabetic nephropathy.
Results: During a median follow-up of 10 years (range 0.04-34 years) 756 subjects died; 71 of the deaths occurred in persons with CKD and 171 in those with ESRF. The age-sex-adjusted death rate for all natural causes (n = 679) was 20/1000 pyrs (95% confidence interval = 18-22) in subjects with normal SCr, 102/1000 pyrs (62-142) in those with CKD and 197/1000 pyrs (146-249) in those with ESRF. As shown in the table, death rates from all natural causes, diabetic nephropathy, ischemic heart disease (IHD), stroke and infectious diseases, adjusted for age, sex and diabetes duration in a proportional-hazards model, were significantly higher in subjects with kidney disease than in those with normal SCr.
Conclusion: In addition to the expected excess due to diabetic nephropathy, CKD without progression to ESRF was associated with increased mortality from IHD and infectious diseases. With progression to ESRF mortality from stroke also increased.

Death rate ratios (95% CI) in CKD and ESRF relative to normal SCr for various causes of death

Underlying cause	CKD	ESRF
All natural causes	4.0 (3.1 - 5.1)	7.6 (5.9 - 9.6)
Diabetic nephropathy	25 (16 - 40)	27 (16 - 45)
IHD	3.1 (1.7 - 5.6)	8.2 (4.9 - 14)
Stroke	1.2 (0.3 - 5.3)	6.3 (2.2 - 18)

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Prevalence of microalbuminuria in recently diagnosed Type 2 diabetes mellitus and its relationship to non-traditional risk factors: observations from the ADOPT study.

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Background and Aims: This study assessed the prevalence of microalbuminuria (MA), a risk factor for cardiovascular (CV) disease and early mortality in type 2 diabetes mellitus (T2DM).
Materials and Methods: The prevalence and associations of MA, defined as albumin:creatinine ratio (ACR) > 30 mg/g, were studied in 4,134 drug-naive T2DM patients (FPG ≤ 9.99 mmol/l) diagnosed within 3 years upon entering a randomised double-blind comparative drug intervention trial (ADOPT).
Results: The overall prevalence of MA was 15.2% and was independent of disease duration or age. Patients diagnosed with MA (MA+) were more frequently male, significantly more obese ($P < 0.0001$), and had a significantly higher white blood cell count (WBC) ($P < 0.001$). Additionally, MA+ patients had higher blood pressure (BP) and prevalence of hypertension (HTN), as well as worse metabolic control than patients with normoalbuminuria (MA-).

Risk Factor	**MA+**	**MA-**	***P*-value**
ACR, mg/g	87.2, 43–138	4.0, 3.5–10.0	
Male, %	62.5	57.3	0.0148
Age, yr	56.5 ± 10.6	56.6 ± 9.9	NS
Waist circ, cm	108.6 ± 14.8	104.8 ± 14.5	< 0.0001
HbA_{1c}, %	7.5 ± 0.99	7.3 ± 0.92	< 0.0001
FPG, mmol/l	8.64 ± 1.59	8.39 ± 1.45	< 0.0001
Systolic BP, mmHg	137.0 ± 16.4	132.1 ± 15.2	< 0.0001
Diastolic BP, mmHg	81.1 ± 9.3	79.4 ± 8.7	< 0.0001
Dx HTN+*, %	83.3	76.3	< 0.0001
WBC x 10^9/l	7.1	6.5	< 0.001

Mean ± SD, or Geometric Mean, IQR for ACR, *prior diagnosis of HTN or BP ≥ 130/85

Treatment with ACE inhibitors and/or AII receptor blockers was also more frequent in MA+ (21.5%) vs MA- (17.7%) patients ($P < 0.024$). LogACR significantly correlated with HbA_{1c} (r = 0.056, $P = 0.0004$), FPG (r = 0.054, $P = 0.0006$), SBP (r = 0.110, $P < 0.0001$), DBP (r = 0.085, $P < 0.0001$) and WBC (r = 0.086, $P < 0.0001$).
Conclusion: This study shows that MA was significantly related to traditional and non-traditional CV risk factors. In this cohort, the prevalence of MA was high, and similar to the 12.3% reported by the UKPDS. This emphasises the need for more aggressive, comprehensive treatment of MA, hyperglycaemia, hypertension and other associated CV risks in T2DM.

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Prevalence and degree of anemia in diabetic patients with declining renal function.

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Background and Aims: Patients with diabetic nephropathy have an excessive risk for cardiovascular morbidity and mortality which is not explained by classic risk factors such as hypertension, dyslipoproteinemia , smoking etc..In patients with nondiabetic kidney disease it has been shown, that anemia which usually develops in preterminal renal insufficiency may be a further reason for cardiovascular complications. So far there are only few reports about prevalence and degree of anemia in diabetic nephropathy. Therefore we investigated these questions in a large sample of diabetic patients with different degrees of impaired kidney function.
Material and Methods: Diabetic patients (n= 119.730) were screened in 125 offices of GPs and diabetologists with respect to prevalence of renal insufficiency, i.e. serum creatinine > 1.3 mg/dl. The following parameters were measured/stated in these patients: age, gender, hemoglobin level (g/dl; HB), serum creatinine concentration (mg/dl), creatinine – clearance (ml/min; CCL) calculated by the Cockgroft formula.
Results: A decreased kidney function defined as creatinine – clearance < 90ml/min was found in 3507 patients (= 2.93%). Most of the patients (58.9%) showed a creatinin-clearance between 30 – 60 ml/min. The prevalences of anemia in male and female patients defined by different cut-off of HB - levels were: HB <13 g/dl: 39.6% / 65.7%; HB <12 g/dl: 25.3 % / 43,9%; HB <11 g/dl: 17.2% / 25.8%; HB < 10g/dl: 11,6% / 14.5%. There was a close correlation between creatinine - clearance and hemoglobin concentration in both, male and female patients: CCL 60 – 89 ml/min HB 13.8/ 12.2 g/dl; CCL 30 – 59 ml/min HB 13.3/12.3 g/dl ; CCL 15 – 29 hb 11.3 /11.6g/dl; CCL < 15 ml/min HB 10.3/9.8 g/dl. The mean hemoglobin concentration in female patients was lower than in male patients at any given creatinine – clearance. However the decrease of hemoglobin level from CCL 60 – 89 ml/ min to < 15 ml/ min was substantially higher in males (- 3.5 g/dl) than in females (-2.4 g/dl).
Conclusions: Diabetic patients show a high prevalence of anemia with declining kidney function. In contrast to findings in patients with nondiabetic kidney disease, anemia develops at an earlier stage of renal insufficiency. Prevalence of anemia is higher in female patients, the decrease of hemoglobin concentration is faster in male patients with declining renal function. Anemia represents a frequent complication in patients with diabetic nephropathy and may be an additional cause of the high rate of cardiovascular complications.

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Association of dyslipidemic phenotypes and nephropathy in Type 1 diabetes: the Italian cohort of the EURODIAB IDDM complication study.

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Background and Aims: Elevated serum cholesterol acts as an independent promoter of progression of diabetic nephropathy in type 1 diabetes. Furthermore, a close relationship has been described between triglycerides and microalbuminuria. The aim of this study was to assess the prevalence of raised AER in type 1 diabetic patients stratified by dyslipidemic phenotypes in the Italian Cohort of the EURODIAB IDDM Complications Study.
Materials and Methods: A total of 978 type 1 diabetics (52% M, 48% F) were studied. Mean age was 32±10 yrs, diabetes duration (DD) 14±9 yrs; BMI 23.2±2.7 kg/m2, HbA1c 8.1±1.8%. Cutoff points for dyslipidemia were: total cholesterol >160, triglyceridemia >200 mg/dl, HDL-C <35 mg/dl for men and <45 mg/dl for women. Dyslipidemic phenotypes (Ph) were IIa, IIb, IV, and isolated low HDL-C. Triglycerides and total cholesterol were centrally measured by fully enzymatic methods on overnight fasting samples. HDL-C was determined after PEG precipitation. LDL-C was estimated by the Friedewald formula. HbA1c was measured with an enzyme immunoassay, urinary albumin by an immunoturbidimetric method.
Results: Total cholesterol (205±39 vs 194±41 mg/dl) but also HDL-C (63±16 vs 52±14 mg/dl) were higher in females (p<0.0001). Triglyceride were higher in males (94±56 vs 85±45 mg/dl, p<0.005); no difference were in LDL-C (123±36 vs 125±35 mg/dl, M vs F). A normal lipid profile occurred in 763 subjects (78.3% M, 78.0% F). Ph IIa was in 118 patients (10.9% M, 13.3% F), Ph IIb in 21 (2.4% M, 1.9% F), Ph IV in 17 (2.6% M, 0.8% F). Isolated low HDL-C emerged in 57 subjects (5.8% M, 5.9% F). A dyslipidemia was present in 16.5% of subjects with HbA1c7.9%(p<0.0001). By median HbA1c, Ph IIa was in 8.2% and 16.0%, respectively (p=0.0002); Ph IIb in 0.4% and 3.9% (p=0.0002); Ph IV in 1.2% and 2.3% (p=0.22); isolated low HDL-C in 6.6% and 4.9% (p=0.27). Prevalences of Ph IIa, IIb, and IV dyslipidemias, but not that of low HDL-C phenotype increase with HbA1c (test for linear association, p=0.0013). Ph IIa and IIb were older (p<0.0001), had longer DD (p=0.0007); Ph IIa had higher BMI (p<0.0001). Ph IIa, IIb and IV had higher waist circumference (WC), HbA1c, sBP and dBP (all p<0.001). AER (median value) was higher in Ph IIa (12.8), IIb (44.6) and IV (14.6) than in normolipidemics (8.9) and in low HDL-C subjects (10.2) (p<0.0001). Differences among phenotypes persist covariated by sBP and dBP, age and DD, WC and HbA1c. Prevalence of raised AER and that of overt nephropathy were higher (p<0.0001) in Ph IIa (36.3 and 15.9%), IIb (65 and 35%) and IV (47.1 and 11.8%) than in normolipidemics (22.8 and 4.9%) and in low-HDL-C (30.9 and 3.6%). Stepwise regression analysis includes as independent correlates of raised AER: sBP, HbA1c, dBP, DD and dyslipidemic phenotype.
Conclusion: Glycemic control is an important determinant of lipid phenotypes in type 1 diabetes. Several dyslipidemic phenotypes (IIa, IIb and type IV) emerge as independent correlates of nephropathy in type 1 diabetes.

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Cystatin C is a sensitive marker of glomerular filtration rate in Type 1 and Type 2 diabetes.

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Background and Aims: Sensitive methods to detect early changes in renal function are urgently needed in diabetes. Cystatin C is suggested as a reliable candidate marker of glomerular filtration rate (GFR) and might more readily detect subtle decrements of GFR than does serum creatinine. The aim of our study was to investigate whether cystatin C can serve as a more sensitive indicator of early impairment of renal function in both DM1 and DM2. Serum cystatin C (Cys-C) was compared with serum creatinine (sCr) and the Cockcroft-Gault formula (C-G) in estimating GFR. GFR has been measured, as reference method, by the iohexol plasma clearance (I-GFR).
Materials and Methods: Ninty diabetics (54 DM1, 36 DM2; 49 M, 41 F, age 20-72 yrs; DD 1-39 yrs; BMI of 19-36 kg/m2) were recruited. Among DM1 (age 36±9 yrs, HbA1c 8.8±1.6%), 23% had normal AER, 26% microalbuminuria, and 51% overt nephropathy. The respective features for DM2 (age 60±9 yrs, HbA1c 8.6±1.6%) were 22%, 23% and 55%. Hypertension was in 49% and 69%, respectively. All subjects with raised AER were on ACE-inhibitors or AT1 antagonists. Iohexol (Omnipaque 300 - Nycomed, Oslo) plasma concentrations were measured by HPLC, sCr was measured enzymatically and Cys-C by the Dade/Behring PENIA test on a BN II analyzer.
Results: In DM1 sCr was 1.37±0.65 mg/dl (range 0.67-3.40), Cys-C 1.14±0.61 mg/l (0.47-3.18), C-G 74±26 (27-132) ml/min, and I-GFR 77±37 ml/min/1.73 m2 (16-167). In DM2, the respective values were 1.56±0.75 (0.65-3.69) mg/dl, 1.26±0.66 (0.58-3.32) mg/l, 57±25 (15-115) ml/min and 67±34 (19-154) ml/min/1.73m2. In both groups the relation between I-GFR and the reciprocal of Cys-C was stronger than those with the reciprocal of sCr and C-G (DM1: r=0.89, r=0.82, r=0.71; DM2 r=0.85, r=0.76, r=0.70 respectively, all p<0.001). Diabetics were then divided as “high” or “low” renal function (above/below median I-GFR). In “low” function all parameters were related to I-GFR. On the contrary, in “high” function patients, correlation between I-GFR and sCr and C-G were lost, while the correlation with cys-C remained significant (see table). By ROC analysis, Cys-C showed better accuracy than sCr or C-G both in DM1 (0.90 vs 0.88 and 0.78) and in DM2 subjects (AUC: 0.98 vs 0.94 and 0.90). Comparisons remained significant when ROC (I-GFR cut-off 100 ml/min for DM1 and 80 ml/min for DM2) was limited to subjects with “high” GFRs: AUC for Cys-C was greater than those for sCr and C-G both in DM1 (AUC: 0.72 vs 0.59 and 0.58) and DM2 (0.59 vs 0.49 and 0.45).
Conclusions: In subjects with both DM1 and DM2, cystatin C may be a more accurate serum marker than serum creatinine and Cockcroft and Gault formula for an early identification of subjects with slight reduction of renal function.

	DM1 GFR<median	DM1 GFR>median	DM2 GFR<median	DM2 GFR>median
sCr	r=0.83, p<0.0001	r=0.08, p=0.70	r=0.84, p<0.0001	r=0.20, p=0.44
C&G;	r=0.76, p<0.0001	r=0.30, p=0.02	r=0.56, p=0.01	r=0.24, p=0.34
Cys-C	r=0.85, p<0.0001	**r=0.49, p<0.01**	r=0.69, p<0.001	**r=0.45, p<0.05**

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Relationship between serum cystatin C as an early marker of renal dysfunction and urinary albumin excretion, transferrin and Type IV collagen in Type 2 diabetic patients.

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Background: In type 2 diabetes, not all patients with microalbuminuria progress to overt nephropathy, and some patients progress without antecedent microalbuminuria. Although measurements of microalbuminuria are the established predictor of risk for progressive disease, glomerular filtration rate (GFR) is widely believed to be the best overall index of renal function. Little information, however, is available concerning the relation of serum cystatin C (Cys-C) as a better GFR marker in the initial renal function impairment with indicators for glomerular permeability/permselectivity.
Aim: To investigate the relationship between serum Cys-C and urinary albumin excretion rate (AER), transferring (TF) and type IV collagen (IV-C) excretion.
Methods: The study consisted of 115 inpatients with type 2 diabetes (aged 28 - 80 years). All blood samples were measured under standard condition in the overnight fast: plasma glucose, HbA1c, serum creatinine(s-Cr), Cys-C and serum lipids. Serum Cys-C levels were determined by latex immunoagglutination method. 24-h urine samples for measurement of 24-h creatinine clearance (CCr) indicative of GFR, AER, TF and IV-C were collected.
Results: A significant positive correlation was found between serum Cys-C and s-Cr, CCr, AER, TF and IV-C in all patients (n=115; p<0.01, respectively). Patients were divided into 3 groups according to the grade of CCr as follows: Group A (n=66); 70 ml/min <CCr, Group B(n=26); 50<CCr < or = 70ml/min and Group C(n=23); CCr< or = 50 ml/min. Serum Cys-C concentrations in the Group A, B, and C increased in that order(mean±SD; 0.71±0.14 , 0.96±0.44 , and 1.50±0.77, respectively), with significant differences between the groups (p<0.05, respectively), while no differences were observed for s-Cr, AER, TF and IV-C between Group A and B. When data from Group A and B were pooled, a significant correlation was found between serum Cys-C and AER or IV-C (r=0.211, p<0.05; r=0.435, p<0.01, respectively), although no significant correlations were observed between serum Cys-C and other variables.
Conclusion: These results suggest that serum Cys-C is a more sensitive marker than s-Cr, especially for type 2 diabetic patients with small to moderate reduction in GFR, and simultaneously that a combination of serum Cys-C with AER or IV-C has advantages over early clinical measures in different theoretical conditions of glomerular damage.

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Increased pasma trombin-activatable fibrinolysis inhibitor levels in normotensive Type 2 diabetic patients with microalbuminuria.

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Background: Hypofibrinolysis is a common finding in patients with diabetes mellitus and a risk factor for diabetic nephropathy. Recently, a new potent inhibitor of fibrinolysis, the thrombin-activatable fibrinolysis inhibitor (TAFI) has been isolated from human plasma. The possibility that TAFI also participates in the mechanism of hypofibrinolysis has not been appraised in diabetic patients with microalbuminuria.
Aims: In the present study, we investigated the plasma levels of TAFI and its relation to urinary albumin excretion in normotensive diabetic patients with normo- and microalbuminuria.
Subjects and Methods: Thirty-nine normotensive non-obese type 2 diabetic patients (27 with normoalbuminuria, 12 with microalbuminuria, age 54.1±1.8 years, BMI 22.5±0.4 kg/m ^{2}, duration 8.8±1.2 years, systolic blood pressure 129.6±2.3 mmHg, diastolic blood pressure 76.7±1.6 mmHg, HbA1c 9.3±0.3 %, Mean± SEM) and 20 age-matched normal subjects were enrolled in this study. The plasma levels of thrombin-antithrombin complex (TAT) were measured by an enzyme immunoassay (EIA). The plasma levels of D-dimer were measured using commercial EIA kit . The plasma levels of soluble thrombmodulin were measured by EIA kit. The plasma levels of TAFI were also measured using a commercially available EIA kit . The interassay and intraassay coefficients of variability were less than 10%.
Results: The plasma levels of TAT were significantly increased (22.1± 2.6 vs. 8.3 ±1.0 nmol/l, p<0.05) whereas, the D-dimer /TAT ratio was significantly decreased (15.7 ± 1.4 vs. 26.5 ± 2.2, p<0.05), showing the occurrence of hypercoagulability and hypofibrinolysis in diabetic patients. The plasma levels of TAFI in diabetic patients with microalbuminuria were significantly higher than those in diabetic patients with normoalbuminuria (194.1 ± 24.5 vs. 128.8 ± 12.3 %, p<0.02) or normal subjects (194.1 ± 24.5 vs. 99.5 ± 4.9 %, p<0.005). Univariate analysis showed that the plasma TAFI levels are significantly and proportionally correlated with urinary albumin excretion rate (r=0.58, p<0.005) and with plasma soluble thrombomodulin levels, a marker of endothelial cell damage, in all diabetic patients (r=0.42, p<0.01) and inversly correlated with glomerular filtration rate (r= -0.38, p< 0.05). Multivariate analysis also showed that the plasma

levels of TAFI are signifycantly correlated with urinary albumin excretion rate (p< 0.05) and weakly correlated with plasma levels of soluble thrombomodulin (p = 0.06).
Conclusion: These data suggest that increased plasma level of TAFI may be involved in the mechanism of vascular endothelial damage in patients with type 2 diabetes mellitus.

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Elevated plasma asymmetric dimethylarginine as a marker of cardiovascular morbidity in early diabetic nephropathy in Type 1 diabetes.
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Background and Aims: An increased plasma concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, has been associated with endothelial dysfunction and atherosclerosis in non-diabetic populations. In patients with end stage renal failure circulating ADMA is raised and a strong and independent predictor of cardiovascular outcome. We aimed to investigate the relation between ADMA and diabetic micro- and macrovascular complications in a large cohort of type 1 diabetic patients with or without early diabetic nephropathy.
Materials and Methods: ADMA concentrations in plasma were determined by a HPLC method in 408 type 1 diabetic patients with overt diabetic nephropathy (252 men, age (mean(SD)) 42.7 (11.0) years, duration of diabetes 28 (9) years, serum creatinine (median(range)) 102 (52-684) µmol/l). A comparable group of 192 patients with longstanding type 1 diabetes and persistent normoalbuminuria served as controls (118 men, age 42.6 (10.2) years, duration of diabetes 27 (9) years).
Results: In patients with overt diabetic nephropathy plasma ADMA concentration was elevated 0.46 (0.08) µmol/l (mean(SD)) versus 0.40 (0.08) µmol/l in normoalbuminuric patients, p<0.001. Circulating ADMA increased in nephropathic patients with early declining kidney function, and thus in patients with serum creatinine in the upper quartile (>136 µmol/l) the ADMA level was 0.50 (0.08) µmol/l as compared with 0.46 (0.07), 0.45 (0.07), and 0.44 (0.07) µmol/l in patients with serum creatinine levels between 103 and 136 µmol/l, between 83 and 103 µmol/l, and below 83 µmol/l, respectively (p<0.001 ANOVA). Mean ADMA levels were similar in patients presenting with or without diabetic retinopathy, NS. However, in 44 patients with nephropathy and a history of non-fatal myocardial infarction or stroke ADMA was significantly elevated, 0.48 (0.08) µmol/l as compared with 0.46 (0.08) µmol/l in patients without major cardiovascular events, p=0.05.
Conclusions: Elevated circulating ADMA may contribute to the excess cardiovascular morbidity and mortality, even early in the course of diabetic nephropathy.

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Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetic patients in the FinnDiane Study.
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Background and Aims: Increased levels of CRP and IL-6, a finding suggestive of the presence of inflammation and insulin resistance, have been observed in type 2 diabetic patients. In such patients, CRP was also predictive of diabetic nephropathy. Two cross-sectional studies assessing the association between low-grade inflammatory markers and diabetic nephropathy in type 1 diabetic patients have shown conflicting results. Therefore, the aim of the study was to assess whether low-grade inflammation is associated with diabetic nephropathy in type diabetic patients.
Materials and Methods: We studied 194 type 1 diabetic patients from the ongoing nationwide, multi-centre Finnish Diabetic Nephropathy Study (FinnDiane), divided into three groups (67 patients with normal albumin excretion rate (AER), 64 with microalbuminuria, and 63 with macroalbuminuria) based on their AER in two out of three consecutive overnight or 24 hour urine collections. Patients with normal AER were required to have neither antihypertensive medication nor signs of cardiovascular disease. Patients with microalbuminuria or macroalbuminuria were all treated with ACE-inhibitors, drugs that may attenuate low-grade inflammation. The three groups had similar gender and disease duration. Thirty-one healthy blood donors served as controls. In addition to standard clinical and laboratory measurements, we calculated as a measure of insulin sensitivity the glucose disposal rate (eGDR) with an equation developed by Williams et al. CRP was measured by radioimmunoassay with a detection limit of 0.05 mg/l and IL-6 by high sensitivity enzyme immunoassay with a detection limit of 0.1 ng/l.
Results: CRP and IL-6 levels were higher in diabetic patients than in healthy controls. In the diabetic patients CRP increased in parallel with the severity of the disease (NORMO 2.0±1.7, MICRO 2.6±1.7, MACRO 2.9±2.5 mg/l; p=0.016) as was also true for IL-6 (1.9±1.5, 2.9±3.3, 3.6±3.1 ng/l; p<0.001). The difference in IL-6 remained significant even after adjustment for waist-hip ratio. In univariate analyses there were significant associations between CRP and diastolic blood pressure, eGDR, AER, and triglycerides. IL-6 correlated positively with duration of diabetes, waist-hip ratio, eGDR, AER, creatinine, HbA_{1c}, triglycerides, and negatively with HDL-cholesterol. In multiple regression analyses AER was the only variable independently associated with CRP (p=0.030), whereas AER (p=0.0003), HDL-cholesterol (p=0.014) and duration of diabetes (p=0.018) were independently associated with IL-6.
Conclusion: Low-grade inflammatory markers are associated with diabetic nephropathy in type 1 diabetic patients. The predictive value of low-grade inflammatory markers needs to be assessed.

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Oxidized LDL and microangiopathy in Type 2 diabetes.
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Background and Aims: Oxidized LDL (Ox-LDL) has been found in atherosclerotic plaques and in glomerulosclerotic lesions. In both sites they act as chemoattractant, recruiting macrophages. Furthermore, Ox-LDL is a potent stimulant for the oxidative injury and for the fibronectin synthesis in mesangial cells inducing cell proliferation and expansion of extracellular matrix. Thus we tested the association between complications and Ox-LDL in DM2.
Materials and Methods: A total of 531 type 2 diabetics (age 61±8 years, age at diagnosis of diabetes 48±12 years, DD 13±10 years, BMI 28.7±5.3 kg/m2; HbA1c 8.7±1.8 %) were included in the study: 45% had normal AER, 34% microalbuminuria (mA) and 21% overt nephropathy (ON); 44% had no retinopathy (nR), 38% background (bR) and 18% proliferative or laser-treated retinopathy (pR). 80% of subjects had hypertension. Furthermore, 100 healthy subjects act as controls. Fasting blood samples were collected for measuring lipid profile. Triglycerides, total-C and HDL-C were measured by enzymatic methods. LDL-C was calculated using the Friedewald formula. Ox-LDL has been measured by a solid phase two-site enzyme immunoassay (Mercodia AB, Uppsala, Sweden). HbA1c was measured by HPLC and urinary albumin by an immunoturbidimetric method. Retinopathy was assessed by retinal photographs.
Results: Ox-LDL level was not different in controls (49.0±11.1 U/l) and DM2 (55.6±25.3 U/l, p=0.16). There was no correlation between Ox-LDL and HbA1c (r=0.08) and only a modest one with BMI (r=0.14, p=0.004). Ox-LDL correlated with total-C (r=0.43), triglycerides (r=0.30), LDL-C (r=0.41, all p<0.0001) and inversely with HDL-C (r=-0.11, p=0.044). Lipids as well Ox-LDL were not different in patients with or without retinopathy (Ox-LDL: nR 52±23, bR 55±28, pR 57±26 U/l). Triglycerides, however increased with greater AER (nA :134±72, mA: 155±94, ON 177±109 mg/dl, p<0.005) and Ox-LDL concentration was significantly higher (p<0.0001) in both subjects with mA (60±26 U/l) and ON (63±27 U/l) than in normoalbuminurics (44±20 U/l). Similar results were observed by splitting in males and females. The three nephropathy groups differed for age (60±8, 62±8, and 63±8 years, p=0.004), diabetes duration (11±9, 13±8 and 16±11 years, p=0.0002), blood pressure (144±19/82±10, 150±1983±9 and 159±20/86±13 mmHg, p=0.0001/0.009), and also for BMI (p=0.05) and HbA1c (p=0.05). In a multiple logistic regression analysis, HbA1c (p=0.04), systolic BP (0.0028) and Ox-LDL (<0.0001), but not other lipid parameters, are independent correlates of nephropathy. Inclusion of retinopathy in the model (p=0.0007), remove HbA1c (p=0.36), while both systolic BP (p=0.0016) and Ox-LDL (p<0.0001) are confirmed as independent correlates.
Conclusion: Ox-LDL levels are indipendent of HbA1c. As such they may contribute indipendently of glycemic control to the risk of diabetic nephropathy.

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Comparison of unadjusted *vs.* BSA-adjusted Cockroft-Gault for ranking patients according to GFR. Results from 5198 subjects with Type 2 diabetes.

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Background & Aims: Routine biological follow-up type 2 diabetic subjects requires frequent assessment of kidney function. Since absolute clearance measurement requires the inconvenience of timed urine collection, it is estimated using Cokcroft and Gault's (CG) serum creatinine formula as surrogate estimate of glomerular filtration rate (GFR). As creatinine concentration is influenced by sex, muscle mass and age, adjusting CG for body surface area (BSA) is increasingly advocated.
Material & Methods: To assess the prevalence of normal- or hyper-filtration (>90) and of mild (<90), moderate (<60) and of severe (<30 mL/min/1.73 m^2) loss of CG-estimated GFR in a large cohort of type 2 diabetes subjects using unadjusted- or BSA-adjusted-CG, and to compare it with CG-GFR for age and sex categories (NHANES III linear regression equations). The cohort consisted of 5198 adult subjects with type 2 diabetes whose mean age (±1 SD) was 66 (12) years, sex ratio (M/F) 50/50, weight 80 (15) kg, and BMI 28.6 (5.2) $kg.m^{-2}$.
Results: see Table
Conclusions: using BSA-adjusted Cockroft-Gault disclosed a much higher absolute prevalence (+23%) of subjects with moderate decrease in GFR compared with unadjusted CG, and use of the former should therefore be preferred

Prevalence of GFR categories according to CG

	Age-predicted CG	Unadjusted CG	BSA-adjusted CG
Normal or > GFR	18.3	28.8	18.0
Mild < GFR	71.0	38.6	43.0
Moderate < GFR	10.8	29.3	35.9
Severe < GFR/KF	0	3.3	3.2

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Relationship of diabetic nephropathy to plasma levels of IL-6, PAI-1, total homocysteine and LP(a) levels in Japanese Type 2 diabetics.

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Background and Aims: Increased plasma levels of plasminogen activator inhibitor type-1 (PAI-1) are considered as a risk factor of thrombosis associated with atherosclerosis, and it is demonstrated that interleukin-6 (IL-6) promotes the growth of renal mesangial cells in animal studies. Hyperhomocysteinemia and increased plasma levels of lipoprotein(a) (Lp(a)) have been recognized as strong risk factors of macro- and microangiopathy in type 1 diabetics, although a few studies had examined in type 2 diabetics. Identification of progression in diabetic nephropathy is important. We therefore investigated the relationship of diabetic nephropathy to plasma levels of PAI-1, IL-6, total homocysteine (tHcy) and Lp(a) in Japanese type 2 diabetics.
Materials and Methods: In this cross-sectional study, 483 type 2 diabetics; 258 subjects with diabetic nephropathy (DN group) and 225 without diabetic nephropathy (DM group) were investigated. None of them had histories of cardiovascular or cerebrovascular diseases. The criteria of DN group was as follows ; 30 mg/g creatinine ≤ urine albumin excretion (UAE) < 1000 mg/g creatinine, serum creatinine level < 1.0 mg/dl. Furthermore, 258 of DN group were divided into 2 subgroups ; 172 subjects with stage 2 diabetic nephropathy (30 ≤ UAE < 300 mg/g creatinine), and 86 with stage 3A diabetic nephropathy (300 ≤UAE < 1000 mg/g creatinine). We measured HbA1c and total- and LDL-cholesterol, and plasma levels of IL-6, PAI-1, tHcy and Lp(a) in these subjects.
Results: There were no significant differences in duration of diabetes, HbA1c, total- and LDL-cholesterol, and with/without hypertension between 2 groups. Plasma levels of IL-6 were significantly higher in DN group (DN: DM = 4.4 : 1.8 pg/ml, $p<0.05$), and the levels of which were significantly increased as stage of nephropathy progressed (DM : stage 2 DN : stage 3A DN = 1.8 : 3.4 : 6.5, $p<0.05$). Plasma levels of PAI-1 were significantly higher in DN group (DN : DM = 19.4 : 12.5 ng/ml, $p<0.05$), but no increment of PAI-1 levels were shown even the stage of nephropathy progressed. There was a tendency that plasma tHcy levels were slightly higher in DN group (DN : DM = 15.8 : 14.8 mmol/L), but, which was not significant. In contrast, there was no significant differences in plasma Lp(a) levels (DN : DM = 26.8 : 25.9 mg/dl).
Conclusion: Plasma levels of PAI-1 and IL-6 may be useful in evaluation of diabetic nephropathy in Japanese type 2 diabetics. However, in Japanese type 2 diabetics, differed from type 1 diabetics, neither tHcy nor Lp(a) levels were independent determinants of diabetic nephropathy.

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Different determinants of urinary albumin excretion rate in „salt-sensitive" and „salt-resistant" hypertensive Type 2 diabetic patients.

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Background and Aims: Experimental and clinical data indicate quite different mechanisms of blood pressure response to salt load, as well as of renal damage, in salt-sensitive vs. salt-resistant subjects. So, the aim of this study was to determine the influence of duration of diabetes and hypertension duration and severity parameters on urinary albumin excretion in these subjects.
Materials and Methods: The study included 66 (35 female/31male) hypertensive type 2 diabetic patients (mean age: 49.51±6.29 years; duration of diabetes: 11.29±5.02 years and duration of hypertension: 11.86±6.02 years). The increment of mean arterial pressure (Δ MAP) on high salt (300 mmol Na/day) diet vs. low salt (40 mmol Na/day) diet exceeding 10 mm Hg was used as the criterion for salt sensitivity (SS). According to the given definition 34 (51.51%) of studied hypertensive type 2 diabetic patients were salt-sensitive. The sodium sensitivity index (SSI) of blood pressure was calculated as the reciprocal of the slope of the pressure–natriuresis curve. It was considered as an indirect parameter of glomerular hypertension. Urinary albumin excretion (UAE) was correlated with MAP, SSI of blood pressure and the duration of diabetes i.e. hypertension in „salt-sensitive" and „salt-resistant" patients. Statistical analysis of covariance (ANCOVA) and the least-squares method were used.
Results: Both MAP (r=0.77657, P<0.00001) and SSI (r=0.64685, P=0.000036) determined UAE in salt-sensitive patients. However, influence of the duration of neither diabetes (P=0.859583), nor hypertension (P=0.223642) on UAE could not been proven in these patients. On the contrary, in salt-resistant patients, significant correlation existed between UAE and MAP (r=0.37968, P=0.032086), but not between UAE and SSI (r=0.27034, P=0.134539). At the same time, in these patients UAE increased gradually with the longer duration of illness (P=0.003329 for diabetes; P<0.00001 for hypertension).
Conclusion: These results demonstrated that in "salt-sensitive" patients urinary albumin excretion rate was determined by the glomerular and systemic hypertension, independently of diabetes and/or hypertension duration. In "salt-resistant" patients urinary albumin excretion rate was dominantly determined by the systemic hypertension – its duration as well as by its severity.

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Trends in pulmonary function in Type 1 diabetic patients with nephropathy.

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Background and Aims: The abnormalities in small blood vessels have also been described in the lungs of patients with diabetes mellitus. The aim of this study was to assess the presence of pulmonary function abnormalities in patients with diabetes mellitus and verify possible association with renal microangiopathy.
Materials and Methods: Forty type 1 diabetic patients (19 male and 21 female; age: 449±.09 years; diabetes duration: 16±12.03 years) without overt lung disease and with no history of smoking were studied. Stepwise regression method was used to analyze the influence of predictor variables: patient's age, diabetes duration, systolic (SBP) and diastolic blood pressure, protein excretion rate (PER), serum creatinine, creatinine clearance, lipid values, glycated haemoglobin, body mass index and C-peptide level on diffusion capacity for carbon monoxide (DLCO), forced expiratory flow in the first second (FEV1), and forced expiratory flow when 50% of the forced vital capacity had been exhaled (FEF50).

Results: DLCO correlated significantly with PER (p=0.0002), creatinine (p=0.011) and creatinine clearance (p=0.0009), but not with diabetes duration (p=0.146). After stepwise regression method PER was pointed out as the most important variable in the prediction of DLCO (R^2=0.55); PER, RRS, and creatinine in the prediction of FEV1 (R^2=0.38); and PER and diabetes duration in the prediction of FEF50 (R^2=0.31). Significant difference (Mann-Whitney U test) was found between lung parameters: DLCO, FEF50, and FEV1 in the groups of patients with and without significant proteinuria (p=0.001 vs. p=0.001 vs. p=0.039).
Conclusion: Pulmonary function abnormalities are common in patients with IDDM and signs of diabetic microangiopathy. This could be explained by impaired pulmonary microvasculature but also with pulmonary interstitial changes. PER was found to be the important variable in the prediction of DLCO, FEV1 and FEF50.

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Nephropathy: Markers of Progression

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Serum prorenin levels in Type 2 diabetic patients, measured by a novel antibody-activating direct enzyme kinetic assay method, predict the progression of early diabetic nephropathy.

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Background and Aims: It has been reported that the concentrations of plasma (total) prorenin increased in the presence of diabetic microangiopathies, and that diabetic nephropathy would be worsen more rapidly in the group with higher prorenin in type 1 diabetic patients. In this study, we tried to evaluate the clinical implications of serum prorenin in type 2 diabetic patients, measuring prorenin as AAD-PR (antibody-activating direct prorenin) by a novel antibody-activating direct enzyme kinetic assay.
Materials and Methods: The levels of serum prorenin, a precursor of renin, were measured in type 2 diabetic patients (n=673) and normal control subjects (n=105), using a newly developed antibody-activating direct enzyme kinetic assay. Briefly, AAD-PR was expressed as generated angiotensin I concentration catalyzed by immunoactivated prorenin using two kinds of specific anti-profragment peptide. antibodies (antibodies to profragment of human prorenin). All data were shown as mean±SD
Results: The circulating prorenin named as AAD-PR was about two times higher than that measured by a conventional method using trypsin (total renin-renin). The concentrations of AAD-PR in normal control subjects were higher in males than in females, namely, the sexual dimorphism (105±35 in males and 91±36 pg/dl in females, p=0.0572). Hence, the data were separately analyzed in males and females, thereafter. The levels of AAD-PR in type 2 diabetic patients were significantly higher than those of normal control subjects in males and females, respectively, also showing the positive results correlated to the severity of diabetic microangiopathies. The significant relationship was found between the levels of AAD-PR in sera and urinary albumin excretion index (U-AEI)(r-0.253 P<0.001 in males, and r-0.203 P=0.0019 in females). The levels of AAD-PR increased in the groups suffering from uncontrolled hypertension and/or hyperglycemia, even in the absence of any microangiopathies. More noticeably, among the patients with normoalbuminuria (U-AEI <30mg/gram creatinine) or microalbuminuria (30 ≤ U-AEI<300), U-AEI significantly worsened in the patients presenting hypertension and/or higher AAD-PR after 2 years of observation.
Conclusions: AAD-PR is a sensitive marker for the presence of diabetic microangiopathies, at least in part, predicting the progression of early diabetic nephropathy.

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Poor prognosis in proteinuric Type 2 diabetic patients with retinopathy: insights from the RENAAL study.

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Objective: Diabetic retinopathy is the clinical hallmark of generalized microangiopathy. We examined the relation of this abnormality to end stage renal disease (ESRD) and death in type 2 diabetes.
Research Design and Methods: A total of 1513 type 2 diabetic patients with nephropathy participated in the RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II antagonist Losartan) study, for a mean of 3.4 years. Identification of diabetic retinopathy was assessed at baseline by opthalmoscopy or fundus phogography in more than 96% of subjects. The primary RENAAL end point was the composite of a doubling of the baseline serum creatinine concentration, ESRD or death.

Results: 65% of the patients had diabetic retinopathy. Subjects with retinopathy had higher systolic blood pressure, albuminuria and lower GFR, hemoglobin and serum albumin values than patients without retinopathy. No differences in prevalence of smoking, serum cholesterol or glycemic control were observed. By univariate analyses the presence of retinopathy was associated with a 45% increase in the primary composite end point (HR, 1.45, 95% CI 1.23 – 1.71, p < 0.001). Patients with retinopathy had a 29% increase in risk of death (p = 0.038) and a 46% increased risk of ESRD (p = 0.001) compared to subjects without retinopathy. In multivariate analyses, the presence of retinopathy was associated with a 23% increase (p = 0.015) in the primary composite end point and a 22% increase in ESRD or death (p = 0.038).
Conclusion: The presence of diabetic retinopathy is associated with type 2 diabetic patients who have more proteinuria and a higher risk for ESRD and death.

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Risk factors for nephropathy in the Early Treatment Diabetic Retinopathy Study (ETDRS).
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Background: Diabetic retinopathy (DR) and nephropathy (DN) often coexist. Both complications are associated with duration of DM and glycemic control. Dyslipidemia and markers of inflammation are well-known risks for macrovascular disease, but their effect is less well-established for microvascular disease including DN. Furthermore, other metabolic abnormalities may be associated with increased risk for DN. We investigated the effects of dyslipidemia, fibrinogen, serum albumin (Salb), hematocrit (hct) and severity of DR on the risk for DN in participants from the Early Treatment Diabetic Retinopathy Study (ETDRS).
Methods: Baseline measures including demographic data, routine chemistry panels, hct, lipids, BP, and serum fibrinogen were analyzed for associations with the risk to develop severe renal disease (dialysis or renal transplantation. From among 3504 patients a subset of pts (n=2226) with all baseline variables was analyzed (group I). From among group I a further subset of pts (n=834) without hypertension or clinical atherosclerotic disease at baseline was analyzed (Group II).
Results: Table. Development of Severe Renal Disease

Severe Renal Disease	Group I Absent	Present	Group II Absent	Present
n	1949	277	758	76
Female (%)	45.1	44.4	39.8	42.1
Type 1 DM (%)	41.4	45.6	67.4	67.1
Creatinine (µ mol/L)	90 (21)	114 (45)**	86 (14)	89 (17)**
Proteinuria (baseline) %	26.3	60.3**	21.9	44.7**
HgbA1c (%)	9.6 (2.10)	10.4 (2.2)**	9.7 (2.0)	11.0 (2.1)**
Systolic BP mm Hg	137 (22)	142 (23)	120 (11)	120 (12)
TC (mmol/L)	5.8 (1.2)	6.4 (1.8)**	5.4 (1.2)	5.8 (1.3)*
HDL-C (mmol/L)	1.2 *0.4)	1.2 (0-.3)**	1.3 (0.3)	1.2 (0.3)
LDL-C (mmol/L)	3.6 (1.0)	3.9 (1.4)**	3.3 (1.0)	3.6 (1.2)
TG (mmol/L)	1.6 (1.2)	2.2 (1.6)**	1.4 (1.0)	1.8 (1.3)*
Fibrinogen (g/L)	2.9 (0.8)	3.3 (1.1)*	2.6 (0.6)	2.9 (0.9)*
Salb (g/L)	34.3 (4.1)	30.8 (4.9)**	34.7 (4.0)	32.1 (4.5) **
Hct (vol frac) male	0.46 (0.04)	0.43 (0.05)**	0.46 (0.04)	0.45 (0.04)
Hct (vol frac) female	0.42 (0.04)	0.40 (0.04)**	0.42 (0.04)	0.42 (0.04)
Developed PDR (%)	51.9	72.2**	60.0	81.6*

Data presented as: mean (S.D.); * P<0.01 **P<0.001

Logistic regression models to evaluate variables that were associated with risk for baseline proteinuria and Cox proportional hazards models for risk factors for developing severe renal disease were analyzed for each group. The results for the total group are shown in the table for OR or HR (99% CI).

	Odds ratio: Baseline proteinuria	Hazard Ratio: Severe Renal Disease
SBP (per 10 mm increment)	1.21 (1.14-1.27)	1.09 (1.02-1.16)
Serum Creatinine (per 88.4 mmol/L increment)	6.87 (4.46-10.58)	3.92 (3.14-4.88)
Cigarette Smoking (Current vs. Never)	1.40 (1.08-1.82)	1.62 (1.19-2.22)
Diabetic Retinopathy		
Severe NPDR vs. Mild/Mod NPDR	1.78 (1.34-2.36)	
PDR	1.55 (1.17-2.95)	2.04 (1.54-2.72)
Neuropathy Decreased Vibration	1.47 (1.17-1.86)	1.54 (1.16-2.06)
Amputation	1.86 (1.06-3.27)	1.66 (0.87-3.17)

Conclusions: Proteinurnuria and severe renal disease in ETDRS patients are associated with other complications of diabetes, and the known risk factors HgbA1c, hypertension and elevated creatinine. In addition, renal disease is associated with dyslipidemia, reduced Salb and hct as well as fibrinogen even in the absence of hypertension or a history of atherosclerotic vascular disease.

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Predictive factors of progression from normoalbuminuria to microalbuminuria in Type 1 diabetic patients.
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Background and Aims: The aim of this study was to establish risk factors associated with the progression of the urinary albumin excretion rate (UAE) in normoalbuminuric type 1 diabetic patients.
Materials and Methods: A total of 100 type 1 diabetic patients with minimum diabetes duration of 5 years were included. HbA1c, lipid profile, smoking status, 24h UAE, 24 h ambulatory blood pressure monitoring (AMBP) and a battery of cardiovascular reflex tests were assessed at the beginning of the study and every six months during the follow up period of 5 years. Urinary albumin concentration was determined by an immunoturbidimetric assay. Twenty four hour blood pressure monitoring was performed by an oscilometric method and patients classified according to night/day diastolic blood pressure (dBP) ratio > or < 0.9 as dippers and non-dippers. Tests of heart rate variation (HRV) included the coefficient of variation (CV) and the low-frequency (LF), midfrequency (MF) and high-frequency (HF) bands of spectral analysis at rest, HRV during deep breathing (CV, mean circular resultant), Valsalva ratio and maximum/minimum 30:15 ratio. Autonomic neuropathy was characterized as an abnormality of more than two tests.
Results: Patients who progressed to microalbuminuria showed higher HbA1c value (8.9 vs. 6.7%, p<0,05) and longer diabetes duration (10.8 vs. 7.0 years, p<0,05) at baseline compared to nonprogressors.There was an overrepresentation of non-deepers (74 vs. 24%, p< 0,05) in patients who progressed to microalbuminuria compared to those who did not. Autonomic neuropathy was more often seen in progressors than in nonprogressors at baseline (68 vs. 14%; p < 0.001). Age, gender, smoking status and lipid profile did not differ between progressors and nonprogressors. In a multiple logistic regression analysis, night/day dBP ratio (p= 0.001) and autonomic neuropathy (p = 0.001) were independent predictors of progression of UAE.
Conclusions: An impaired night dBP fall and autonomic neuropathy are independent risk factors for progression of UAE in normoalbuminuric type 1 diabetic patients.

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Prediction of mortality in haemodialysis patients by gene expression analysis on mRNA level.
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Background and Aims: Patients with end-stage renal failure and haemodialysis treatment have a high mortality mainly due to an acceleration of atherosclerosis and severe vascular events. Because development of atherosclerosis has been recently proposed as immune mediated disease we tested the hypothesis that the activity state of circulating leukocytes reflect and predict mortality. Therefore, gene

expression of pro- and anti-inflammatory cytokines in peripheral leukocytes was analysed on mRNA in comparison to serum protein levels.
Materials and Methods: Of a dialysis centre all current haemodialysis patients (n = 69) were analysed before and 2 hours after beginning of the haemodialysis session in comparison to healthy age-matched controls (n = 40). RNA was stabilised immediately after blood drawing by a new stabilising solution (PAX-gene™) and TNF-α and TGF-β mRNA was measured by quantitative PCR (TaqMan®). Protein concentrations of cytokines (TNF-α, TGF-β, IL-6), soluble cytokine receptors (sTNF-α, sIL-6) and acute phase proteins (CRP, SAA) in serum were determined by ELISA. During followed for 36 months 22 haemodialysis patients died mainly (68%) due to vascular events.
Results: mRNA gene expression of the proinflammatory cytokine TNF-α in haemodialysis patients was significantly elevated in comparison to controls (0.9 ± 0.1 versus 0.5 ± 0.1 molecules per 10^3 β-actin molecules; $p < 0.01$) and further increased after two hours of dialysis treatment (1.5 ± 0.2 molecules per 10^3 β-actin molecules; $p < 0.001$), while gene expression of the antinflammatory cytokine TGF-β was significantly decreased (54 ± 5 versus 173 ± 14 molecules per 10^3 β-actin molecules; $p < 0.001$). Protein analysis in serum revealed that the anti-inflammatory cytokine TGF-β (145 ± 7 versus 120 ± 10 pg/ml; $p < 0.001$) was significantly decreased, while pro-inflammatory cytokines or acute phase proteins as IL-6, soluble TNF-α-receptor or CRP were significantly ($p < 0.001$) increased in haemodialysis versus controls without changes during haemodialysis. TNF-α protein in serum could be detected only in a minority of patients or controls without differences between the two groups. Patient who died during the 36 months observation period were characterized by significantly increased mRNA levels of TNF-α (1.3 ± 0.3 versus 0.6 ± 0.1 molecules per 10^3 β-actin molecules; $p < 0.05$) and significantly decreased TGF- β mRNA expression (55 ± 12 versus 64 ± 8 molecules per 10^3 β-actin molecules; $p < 0.05$). Interestingly, survival analysis indicated a predictive value of increased TNF-α levels ($p < 0.02$) or TNF-α/TGF-β ratios ($p < 0.001$) for mortality.
Conclusion: This study shows for the first time that mRNA gene expression in peripheral leukocytes has a predictive value for mortality in hemodialysis patients. Gene expression patterns of circulating leukocytes may qualify as important new diagnostic tool predicting outcome in atherosclerosis patients.

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Importance of remnant lipoproteins in diabetic nephropathy - lesson from apolipoprotein E genotype study.
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Background and Aim: In 1994 we first demonstrated that the apolipoprotein (apo) E e2 allele was associated with the progression of diabetic nephropathy. It is well known that Apo E2 contributes to increase plasma levels of remnant lipoproteins. The aim of the present study is to further investigate the association between apo E genetic polymorphisms and remnant lipoproteins in type 2 diabetic patients, and diabetic nephropathy.
Subjects and Methods: Type 2 diabetic patients who had a diabetes duration over 10 years were divided into the 3 apo E groups; apo E2/- (n=22), E3/3 (n=102) and E4/- (n=34). Plasma levels of lipids and remnant lipoproteins were measured. The histological study was performed using renal biopsy specimens in apo E2 patient.
Results: The overt nephropathy was more frequent in apo E2 subjects (59.1%) and less frequent in apo E4 subjects (8.8%) compared with apo E3/3 subjects (34.3%). Plasma levels of triglyceride and remnant-like lipoprotein particles (RLP)-chol were significantly higher in apo E2 and lower in apo E4 compared with those in apo E3/3 patients. Logistic regression analysis showed that the odds ratio of high plasma remnant (RLP-C above 5.2 mg/dl) for the presence of overt nephropathy was 3.614 (p=0.0208). Histological findings of the kidney in apo E2 patient were almost identical with those in diabetic nephropathy. Immunohistochemical analysis demonstrated a positive staining of apo E and apo B in the glomeruli of apo E2 subjects.
Conclusion: Apo E2 increases the plasma level of remnant lipoproteins and may induce the progression of diabetic nephropathy, whereas apo E4 decreases the plasma level of remnant lipoproteins and may reduce the progression of nephropathy. Thus the apo E genotype may play an important role on the progression of diabetic nephropathy through regulating the plasma levels of remnant lipoproteins.

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Interleukin-6 polymorphism (-634C/G) in the promoter region and the progression of diabetic nephropathy in the Type 2 diabetes.
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Background and Aims: InterleukinIn-6 (IL-6) is a multifunctional cytokine produced by many different cell types including glomerular mesangial cells. Recently, a novel C/G polymorphism at position –634 in the promoter region of the IL-6 gene has been reported. The aim of this study was to investigate whether the –634C/G polymorphism is associated with an increased risk for progression to diabetic nephropathy as well as elevated levels of IL-6 secretion by peripheral blood mononuclear cells.
Materials and Methods: The frequency of the –634C/G polymorphism was determined in Japanese patients with type 2 diabetes and either normoalbuminuria (n=162), microalbuminuria (n=138) or macroalubuminuria (n=154) by polymerase chain reaction-restriction fragment length polymorphism analysis. The level of IL-6 secretion in relation to genotype was assessed in lipopolysaccharide or advanced glycation end products stimulated IL-6 secretion by peripheral mononuclear cells.
Results: The frequency of the –634G/G genotype and –634*G allele were significantly increased in the patients with macroalbuminuria compared with patients with normoalbuminuria (Genotype; c__6.787, Pc=0.0368, Allele; c__9.080, Pc=0.0104). The stepwise multiple regression analysis in these patients showed that hypertension (F=40.48) and IL-6-634 gene polymorphism (F=5.48) were the relevant variables for the progression of type 2 diabetic nephropathy. Analysis of the IL-6 secretion data revealed that individuals carrying the –634*G allele had a higher IL-6 secretion capacity than those without the *G allele ($P< 0.05$).
Conclusion: These results suggest that the IL-6 –634C/G polymorphism may be a possible genetic susceptibility factor for the progression of diabetic nephropathy.

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The C-106T polymorphism in promoter of aldose reductase gene is risk factor for diabetic nephropathy in Type 2 diabetes patients with bad glycaemic control.
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Background and Aims: Excessive flux through the polyol pathway has long been thought to be involved in the pathogenesis of diabetic microvascular complications. Aldose reductase (AR) is the first, and rate-limiting enzyme in the pathway that catalyses the reduction of glucose to sorbitol. The C-106T polymorphism in the promoter of the AR gene has been described, which may change the expression of the gene. The aim of the study was to examine if the C-106T polymorphism was associated with diabetic nephropathy in type 2 diabetes.
Materials and Methods: We collected 444 patients with type 2 diabetes and divided them into three groups according to the renal status: 162 patients with normoalbuminuria, 153 with microalbuminuria and 129 with persistent proteinuria. Each subject was genotyped for the C-106 polymorphism using the PCR-based RFLP protocol.
Results: When the whole study population was analysed, no distortion in the genotype frequency among the study groups was observed. When we stratified the study population by HbA_{1c} we found that in patients with $HbA_{1c} \geq 9\%$ (median) the CT and TT genotypes were more frequent in patients with diabetic nephropathy (proteinuria and microalbuminuria) than those with normoalbuminuria.
Conclusion: The C-106 T polymorphism in the AR gene is a risk factor for development of diabetic nephropathy in type 2 diabetes in patients with bad glycaemic control.

C-106T genotype in type 2 diabetes patients with HbA1c>=9%

Genotype	CC	CT/TT	OR (95% CI)
Normoalbuminuria	33 (46%)	39 (54%)	reference
Microalbuminuria	25 (30%)	60 (70%)	2.03 (1.05-3.92)
Proteinuria	11 (29%)	27 (71%)	2.07 (0.90-4.81)
Nephropathy (microalbuminuria + proteinuria)	36 (29%)	87 (71%)	2.04 (1.12-3.74)

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LPL gene and progression of nephropathy in hypercholesterolemic type 2 diabetic patients.

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Background and Aims: A more atherogenic plasma lipoprotein profile has been described in microalbuminuric type 2 diabetic (T2D) patients as compared to normoalbuminuric ones, and recent prospective studies have identified hyperlipidemia as an independent determinant of diabetic nephropathy. Lipoprotein lipase (LPL) is a key enzyme in the postprandial processing of triglycerides and VLDL. Among a number of common sequence variants, particular interest has been attributed to the HindIII, occurring in intron 8 of the gene encoding LPL, associated with CHD and, more recently, with the presence and severity of microalbuminuria in T2D. No studies have prospectively evaluated the progression of renal disease in hypercholesterolemic T2D patients, in relation to this specific polymorphism.
Materials and Methods: We consecutively enrolled 65 micro-macroalbuminuric T2D patients; 28 had hypercholesterolemia (Group A), whereas 37 (Group B) had normal cholesterol levels. Routine biochemical parameters, AER, GFR (by Cockroft-Gault formula) were measured. LPL gene polymorphism HindIII and LDL receptor gene polymorphisms NcoI and AvaII were determined on genomic DNA. After the baseline evaluation, patients were followed for four years, with repeated determinations every twelve months.
Results: Group A showed a faster AER increase (ΔAER: 45 µg/min in Group A vs. 15 µg/min in Group B, $p<0.03$) and a faster GFR decline (Group A: from 54±16 to 44±15; Group B: from 58±13 to 52±10 ml/min/1.73m^2, $p<0.02$). When examining the progression of AER and GFR according to the different genotypes of the three studied polymorphisms, we did not find differences in the rate of decline of kidney function by LDLR gene polymorphisms, whereas patients homozygous for the mutation +/+ in the HindIII genotype of the LPL gene showed a faster decline of GFR (from 56 to 45 ml/min/1.73m^2) and a higher increase of AER (from 76 to 118 µg/min) in comparison with +/- or -/- patients (GFR: from 56 to 52 and from 60 to 57 ml/min/1.73m^2; AER: from 85 to 97 and from 59 to 65 µg/min, respectively; both $p<0.05$). Regression analysis showed age, BMI and HindIII polymorphism as independent determinants of final GFR ($p<0.001$, $p=0.010$ and $p=0.004$, respectively), and HbA_{1c} as determinant of final AER ($p=0.034$).
Conclusion: Hypercholesterolemic T2D patients are prone to a more aggressive course of their renal complications, and this time-course is accelerated in subjects carrying the H^+/H^+ genotype of the HindIII polymorphism at the LPL locus. Our findings are consistent with a distinct genetic predisposition to develop diabetic nephropathy in these subjects.

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Analyses of genotype combinations in genes of the renin-angiotensin-aldosterone system and diabetic nephropathy in Type 1 diabetic patients in the FinnDiane Study.

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There is evidence for genetic involvement in the pathogenesis of diabetic nephropathy (DN). Genes in the RAAS include ACE and AT1, as well as AT2 and aldosterone synthase (CYP11B2) not yet studied in DN. There are so far no published data of combining more than 2 loci in DN. Therefore, the aim of this study was to test a novel computer program designed for multilocus SNP analysis by using a pattern discovery framework called the naive Bayes model, and to apply it in the analysis of 9 common polymorphisms in four genes of the RAAS and their association with DN. The algorithm attempts to predict the phenotype from the given genotype combinations. If it succeeds in it more often than it would by change only, all genotypes are tested to extrapolate genotype combinations responsible for the deviation. In this cross-sectional, case-control study we studied 996 patients with type 1 diabetes from 17 referral centres from the FinnDiane Study. The mean age of the entire cohort was 40±1 years, duration of diabetes 28±1 years, BMI 25.1±0.1 kg/m2, HbA1c 8.5±0.1%. Patients were classified based on their AER: NORMO (dur >15 yrs, n=321), MICRO (n=166), PROT (n=325), end stage renal disease (ESRD, n=184). The frequencies of rare genotypes of ACE I/D, 5 SNPs in the AT1, 2 SNPs in the AT2, and T-344C in the CYP11B2 genes did not differ between the groups in separate analyses. However, the naive Bayes model identified a significant deviation in NORMO and PROT when analysing all polymorphisms together. Further analyses showed that the combination of the homozygous mutant genotype of AT1 L191L, the heterozygous genotype of ACE I/D and the homozygous mutant genotype of the CYP11B2 T-344C polymorphism was more prevalent in NORMO than in PROT (Fisher exact, $p=0.02$). These preliminary results suggest that the naive Bayes method is a powerful novel approach in the search for genotype combinations in diabetic nephropathy and other complex diseases.

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Peroxisome proliferator-activated receptor-γ2 (PPARγ) polymorphism Pro12Ala and diabetic nephropathy in Type 1 diabetic patients in the FinnDiane Study.

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Background and Aims: Both genetic and environmental factors are thought to play a role in the pathogenesis of diabetic nephropathy. Diabetic nephropathy is accompanied by increased cardiovascular morbidity and mortality. Insulin resistance could be the common denominator between these disorders. PPARγ is a ligand-activated transcription factor, which is expressed in mesangial cells, smooth muscle cells and macrophages. It is involved in lipid and glucose metabolism and thiazolidinediones, drugs that act as “insulin sensitizers”, mediate their effects through PPARγ. The Ala allele of a Pro12Ala polymorphism of the PPARγ gene has been associated with less type 2 diabetes, lower BMI and AER in type 2 diabetic patients.Therefore, the aim was to assess whether the PPARγ gene (Pro12Ala polymorphism and 4 additional SNPs) is associated with diabetic nephropathy in type 1 diabetic patients.
Materials and Methods: We studied 1498 type 1 diabetic patients (532 normoalbuminuria, 297 microalbuminuria, 427 macroalbuminuria and 242 ESRD) participating in the ongoing FinnDiane Study. In addition to demographic measurements, blood and urine samples for determination of e.g. HbA_{1c}, lipids and AER, the phenotyping included data on diabetes

complications and cardiovascular disease. Genotyping was performed using Taqman technology.
Results: The prevalence of the mutant Ala/- genotype of the Pro12Ala polymorphism was 31.4% in NORMO, 29.7% in MICRO, 30.1% in MACRO and 40.7% in ESRD (p=0.021). Patients with ESRD were more often carriers of Ala/- than the remaining patients (40.7 vs. 30.6%; p=0.002). ESRD with and without the Ala/- genotype did not differ with regard to sex, duration to ESRD or prevalence of coronary heart disease, myocardial infarction or stroke. Within the ESRD group, there was a tendency towards higher frequency of Ala/- in the highest quartile of diabetes duration (>36.5 yrs) in comparison with patients with shorter duration (50.0 vs. 36.8%; p=0.067). In the total population, Ala/- had higher HDL-cholesterol (1.62±0.57 vs. 1.53±0.47 mmol/l; p=0.008) and a trend towards lower BMI (24.9±0.2 vs. 25.3±0.1 kg/m^2; p=0.081) than Pro/Pro. No effect of the polymorphism was observed for waist-to-hip ratio, blood pressure, or triglycerides. There was no difference in AER between patients with the mutant and the wild genotype even after stratification for duration of diabetes or HbA_{1c}. Additional SNP analyses are undergoing.
Conclusion: The PPARγ Ala12 allele is not associated with diabetic nephropathy. However, the allele might be associated with increased survival in patients with end-stage renal disease.

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Atrial natriuretic peptide gene and nephropathy in Type 1 diabetes.
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Background and Aims: Atrial Natriuretic Peptide (ANP) plays an important haemodynamic role (vasodilatation, natriuretic effect) and may be involved in development of diabetic nephropathy. Published results of cross-sectional studies are conflicting. We searched for an association between polymorphisms at ANP gene locus and diabetic nephropathy.
Materials and Methods: We tested the associations between 8 polymorphisms (direct sequencing method) : G663A, C708T, T2238C, G2311T, T2325C, T2332C, T2455C and one new insertion/deletion polymorphism of 11 bp inserting after nucleotide at position 2479 in ANP gene and renal involvment in 2 studies : GENEDIAB, a cross-sectional multicentric study of 489 type 1 subjects with proliferative retinopathy, and SURGENE, a prospective observational follow-up of 301 type 1 patients (median follow-up 6 years, range 2-10, 53 renal events). All subjects were scored for nephropathy as : absent (normoalbuminuria), incipient (microalbuminuria), established (macroalbuminuria) and advanced (renal insufficiency). In GENEDIAB, nephropathy severity was tested against genotypes using a chi2 test. In SURGENE, time-to-first-renal event (progession to further stage) curves were generated by Kaplan-Meyer method.
Results: There was no association between nephropathy and the studied polymorphisms in GENEDIAB (Table). In SURGENE there was no association between renal events and the studied polymorphisms except for T2238C polymorphism and renal progression. The C allele (frequency 16 %) was associated with a marginal worse prognosis : CC + TC genotypes vs TT : hazard ratio 1.69 (95% CI : 0.98-2.94) ; Kaplan-Meyer estimation Breslow-Gehan-Wilcoxon rank-test : p<0.04.
Conclusions: These results are not in agreement with previous works, which found no or a protective effect of the uncommon allele of T2238C polymorphism. The functional significance of this genetic variation remains to be elucidated.

Allelic frequency of the uncommon allele for each polymorphism (%) in GENEDIAB study

Stage of Nephropathy	G663A : Allele A	C708T : Allele T	T2238C : Allele C	T2332C : Allele C	2479 I/D : Allele D
Absence (n=155)	4.5	10.5	14.5	5.0	5.0
Incipient (n=103)	4.0	15.0	20.5	3.5	8.5
Established (n=125)	6.5	11.0	15.0	4.0	4.0
Advanced (n=106)	5.0	11.5	7.5	6.0	7.5
All patients (n=489)	5.0	12.0	16.5	4.5	6.0
p (chi^2 for genotype distribution)	0.82	0.73	0.39	0.40	0.27

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The plasminogen activator inhibitor-1 and the angiotensin II Type 1 receptor genes and their association with diabetic nephropathy among smokers.
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Background and Aims: We have previously shown that the A1166C polymorphism in the angiotensin II type 1 receptor gene (AGTR1) is associated with risk of diabetic nephropathy (DN) in type 1 diabetes patients and that the effect was stronger among smokers. In DN accumulation of extracellular matrix (ECM) will occur. Plasmin degrades ECM but plasminogen activator inhibitor-1 (PAI-1) prevents the formation of active plasmin. Nicotine and angiotensin II induces the expression of PAI-1. Previous studies have shown that the PAI-1 4G/5G polymorphism is associated with DN among type 2 diabetes patients. We therefore speculate that these two genes might interact with each other and perhaps also with smoking.
Materials and Methods: In this case-control study, 43 cases were included (patients with T1DM and albumin excretion rate of ≥200 µg/min, overt nephropathy). Patients with at least 20 years duration of T1DM, albumin excretion rate <20µg/min, were included as controls, n=149. Smokers and former smokers were considered as smokers in the statistical analyses.
Results: Patients hetero- or homozygous for the 4G allele of the PAI-1 4G/5G polymorphism had a slightly increased but non-significant risk for having nephropathy. Patients homozygous for the A allele of the A1166C polymorphism had a doubled risk of overt nephropathy compared to patients with AC or CC genotype. When comparing patients homozygous for the A allele and also hetero- or homozygous for the 4G allele with patients carrying none or only one of them, the risk was 2.7 times higher. Among smokers (smoking cases compared to smoking controls) the risk was five times higher (table 1).
Conclusion: Patients homozygous for the A allele (of the A1166C polymorphism) in combination with hetero- or homozygosity for the 4G allele (of the PAI-1 4G/5G polymorphism) seem to have a higher risk of developing nephropathy than patients carrying none or only one of them. This risk is further elevated if the patients are smokers.

Table 1: Data are given as crude OR (95% CI).

	AA1166	PAI-1 4G/4G or 4G/5G	PAI-1 4G/4G or 4G/5G in combination with AA1166
All patients	2.22 (1.07-4.61)	1.73 (0.67-4.47)	2.68 (1.31-5.47)
Non-smokers	1.34 (0.46-3.85)	0.83 (0.20-3.41)	1.50 (0.52-4.32)
*Smokers	3.89 (1.26-12.04)	2.86 (0.77-10.66)	5.11 (1.78-14.69)

*Smoking cases compared to smoking controls.

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Racial differences in antioxidant enzyme activity in patients with Type 2 diabetes.
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Background and Aims: An increase in oxidative stress is considered to promote the development of the long-term complications of diabetes mellitus. This may be a factor in the increased susceptibility to nephropathy in patients of African-Caribbean (AC) origin in the United Kingdom. We have previously reported an increase in lipid hydroperoxide concentration which was inversely associated with vitamin C levels in AC compared with Caucasian (CA) patients. In order to help determine if an environmental or inherent mechanism accounted for this observation, we investigated whether there were racial differences in antioxidant enzyme activity.
Materials and Methods: We studied 80 hospital outpatient attendees with type 2 diabetes of AC (n=34) and CA(n=46) origin. The mean [SD] age and duration of diabetes duration 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 30.0[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 7.7[1.6]%; p=0.33. Fasting venous blood was sampled for the measurement of α-tocopherol by HPLC and actity of the antioxidant enzymes, catalase, superoxide dismutase and glutathione peroxidase spectrophotometrically. Total antioxidant capacity (TAC) was measured using three versions of Analysis By Emitted Light (ABEL®) tests including quenching of superoxide anion (TAC-$O_2^{\bullet-}$), quenching of hypochlorous acid (TAC-HOCl) and lag-phase to peroxidation by peroxynitrite (TAC-$ONOO^-$).
Results: Superoxide dismutase activity was significantly higher, but glutathione peroxidase activity lower in group AC compared with group CA (721.4[600.4] vs 459.4[419.5] u/l; p=0.005 and 122.14[81.6] vs 181.5[97.6] u/l; p=0.02. There were no differences in catalase activity or the concentration of α -tocopherol (30.2[42.3] vs 30.45[33.8] KU/l;p=0.99 and 31.6[10.0] vs 38.3[14.3];p=μ mol/l;p=0.08) between the AC and CA groups. There was no differences in TAC-$O_2^{\bullet}$ or TAC-$ONOO^-$ but TAC-HOCl was diminished in the AC compared with the CA group as a result of a significant reduction in the quenching of HOCL (15.8[4.4] vs 20.20.0[6.5] counts;p=0.02)
Conclusion: These data suggest that racial differences exist in the activity of antioxidant enzymes in patients with type 2 diabetes and may contribute to the capacity to reduce oxidative stress.

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Interactions among ACE, AGT,TNF-α and ALR2 gene on the susceptibility to nephropathy in Chinese with Type 2 diabetes.
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Background and Aims: Previous studies have suggested that D allele of angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphsim, T allele of angiotensinogen (AGT) gene M235T polymorphism, z-2 allele of 5'-$(CA)_n$ and T allele of C-106T polymorphisms of aldose reductase (ALR2) gene were associated with diabetic nephropathy. The A allele of tumor necrosis factor alpha (TNF-α) gene G-308A polymorphism may also be associated with nephropathy through insulin resistance. We aimed to investigate the association of gene-gene interaction among these genetic markers with nephropathy in 711 Chinese Type 2 diabetic patients.
Materials and Methods: In this case-control study, patients who had duration of diabetes >10 years and plasma creatinine<100 μmol/l and spot urine albumin creatinine ratio<3.5 mg/mmol were considered as the control case. Patients who had either plasma creatinine≥150 μmol/l or spot urine albumin creatinine ratio ≥25 mg/mmol were considered to have nephropathy. The ALR2 $(CA)_n$ and ACE I/D polymorphisms were examined by PCR followed by capillary and agarose gel electrophoresis, respectively. The other 3 polymorphisms were examined by PCR-RFLP.
Results: Patients with nephropathy (N=323) were older, more obese, hypertensive and had worse glycemic and lipid control, higher percentage of retinopathy, neuropathy as well as macrovascular complications than those without nephropathy (N=388). There were no differences in genotype and allele frequencies of I/D, M235T and G-308A polymorphisms between patients with and without nephropathy when the polymorphisms were studied separately. For the ALR2 $(CA)_n$ polymorphisms, patients with nephropathy had higher frequency of the z-2 allele (24.1% vs. 18.6%, P=0.01) and lower frequency of the z+6 allele (3.4% vs. 5.6%, P=0.04). They also had higher frequency of the T allele of C-106T (25.8% vs. 21.4%, P=0.05) compared to those without nephropathy. By taking the DD/ID genotype of I/D, TT genotype of M235T, GG genotype of G-308A, x/z-2 or z-2/z-2 genotype and CT/TT genotype of ALR2 gene as plausible risk genotypes for nephropathy, the possibility of gene-gene interactions were studied. Of the 711 patients, 64 (9.0%) had 0 or 1 risk genotype, 176 (24.8%) had 2 risk genotypes, 290 (40.8%) had 3 risk genotypes and 181 (25.5%) had 4 or 5 risk genotypes. Compared to patients with ≤1 risk genotype, the odds ratio of having nephropathy increased from 1.4 (95% CI 0.8-2.4, P=0.3) to 1.8 (95% CI 1.1-1.3, P=0.03) and to 2.0 (95% CI 1.1-3.6, P=0.02) in patients with 2, 3 and ≥4 risk genotypes, respectively.(P=0.006 for trend).
Conclusions: There are gene-gene interactions among ACE I/D, AGT M235T, TNF-α G-308A, ALR2 5'-$(CA)_n$ and C-106T polymorphisms for development of nephropathy in Chinese patients with Type 2 diabetes.

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Relationship between genetic polymorphisms within the pro-oxidant/antioxidant systems and diabetic nephropathy – preliminary results.
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Background and Aims: Association of selected genetic polymorphisms in genes encoding for glyoxalase I (A111E GLYI), paraoxonase (R192Q and M55L PON), NAD(P)H: quinone oxidoreductase (P187S NQO1) and methylenetetrahydrofolate reductase (677C/T MTHFR) with diabetic nephropathy (DN) in patients with both type 1 and type 2 diabetes mellitus was studied. Further, interactions of studied polymorphisms with previously associated polymorphism 2184A/G in the gene for receptor of advanced glycation end products (2184A/G RAGE) were investigated.
Materials and Methods: A total of 318 Caucasian subjects was so far enrolled in the association study: diabetics with parallel DN (n=135, mean age 63.7 ± 11.7 yr.) and diabetics without DN (n=183, mean age 64.5 ± 11.7 yr.). DN group comprised patients with (i) persistent proteinuria, (ii) chronic renal failure and (iii) end-stage renal disease (ESRD) with regular hemodialysis. Allele frequencies of polymorphisms were determined by polymerase chain reaction based methodology.
Results: Significant difference in allele frequencies of the P187S NQO1 between DN and non-DN group was found (P=0.04, Fisher exact test); frequency of the P allele was higher in DN group (19.5% vs. 14.1%). Moreover, marginally significant association of allele T with DN was detected for the 677C/T MTHFR polymorphism (P=0.07); 36.7% in DN group vs. 32.1% in non-DN group. Nor PON Q192R neither M55L allele frequencies did not differed significantly (P>0.05), however, frequency of 192R was higher in DN than in non-DN group (28.9% vs. 25.7%). Frequencies of combined genotype combinations of MTHFR, NQO1 and PON polymorphisms were not significantly different between the DN and non-DN groups (P>0.05, chi-square test), however, proportion of a combination TT-SS-RR was apparently higher in the DN group. Similarly, frequencies of twofold genotype combinations of studied polymorphisms and 2184A/G RAGE did not differed significantly, which might indicate their independent asset to DN susceptibility.
Conclusion: The preliminary data indicate that certain polymorphisms in genes encoding pro-oxidant/antioxidant enzymes could be regarded as contributors to genetic risk factors for DN. Association of these polymorphisms with susceptibility to develop DN and rate of progression and severity of DN is a subject of larger ongoing study.

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Dinucleotide repeat polymorphism at upstream of matrix metalloproteinase-9 gene is associated with Type 2 diabetic nephropathy in Chinese.
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Background and Aims: Degradation of type IV collagen by matrix metalloproteinase-9 (MMP-9) in the glomerular mesangium may involved in the diabetic nephropathy. To investigate the association between the (AC)n dinucleotide repeat polymorphism of MMP-9 gene and diabetic nephropathy in Chinese type 2 diabetes mellitus.
Materials and Methods: A case-control study for 328 Chinese subjects including 245 type 2 diabetes mellitus with and without nephropathy and 83 non-diabetic control was performed. The type 2 diabetic patients were divided into three groups based on their urinary albumin excretion rate (AER) as follows: uncomplicated group (DN-0), normal albuminuria group with the duration of diabetes more than 10 years, n=78; microalbuminuria group (DN-1), n=129; overt proteinuria group (DN-2), n=38. The number of (AC)n dinucleotide of MMP-9 gene were determined by PCR-denaturing acrylamide gel electrophorosis.
Results: 9 alleles with 7 to 15 repeats of the dinucleotide repeat polymorphism were identified in Chinese, which is different from Japanese to be reported with 17 to 25 repeats. The frequency of allele containing 12 repeats i.e. $(AC)_{12}$ was most abundant (42.8% in control and 40.4% in diabetic subjects), followed by 10 repeats $(AC)_{10}$, 11 repeats $(AC)_{11}$ and 9 repeats $(AC)_9$ in order. The $(AC)_9$ allele was more frequent in DN-2 than DN-0 (11.8% vs 3.8%, Fisher's exact p=0.042) and the odds ratio for overt proteinuria nephropathy in carrier of $(AC)_9$ was 3.7 (95%CI 1.2-11.4). The $(AC)_{11}$ allele was less frequent in DN-1 than DN-0 (23.7% vs 13.9%,

Fisher's exact p=0.016) and the odds ratio for microalbuminuria nephropathy in non-carrier of $(AC)_{11}$ was 2.0 (95%CI 1.1-3.6).
Conclusion: Our results indicate that there is a diffrence in race between Chinese and Japanese in the number of dinucleotide repeat polymorphism in MMP-9 gene. Patients with $(AC)_9$ allele of the MMP-9 gene may have the tendency to develop into overt proteinuria nephropathy. Whereas the patient with $(AC)_{11}$ allele of the MMP-9 gene may be protected from the development of diabetic microalbuminuria nephropathy. Thus, the microsatellite polymorphism of the MMP-9 gene could be a genetic marker for Chinese type 2 diabetic nephropathy.

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Genetic basis for nephropathy phenotype in Asian Indians with Type 2 diabetes mellitus.
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Background and Aims: Nephropathy present in many Asian Indians with undiagnosed or newly diagnosed type 2 diabetes could be explained by population-specific gene expressions. Early markers for nephropathy phenotype will facilitate preventing or delaying morbidity in type 2 diabetes.
Materials and Methods: Microarray gene profiling of 13,474 sequence-verified, nonredundant human cDNAs was employed to compare leucocyte gene expression in Asian Indians with type 2 diabetes and nephropathy (DN: n=3) vs. (age, gender, duration of diabetes and glycosylated hemoglobin) matched diabetics without nephropathy (DM: n=3) and matched controls (n=3).
Results: Differentially expressed genes in function categories (%) were : enzyme (41), nucleic acid binding (30), ligand binding or carrier (10), cell adhesion (5), signal transducer (5), transporter (4), cell cycle regulator (1), chaperone (1), defense/immunity protein (1), transcription factor binding (1), tumor suppressor (1). Significant differential expression (and fold change <0.3 or >3) was noted for 897 genes in DM vs. controls and 813 genes in DN vs. controls. Of those genes, only 475 were common for both DM vs control and DN vs control. Genes (fold change) with potential roles in diabetic nephropathy included insulin-like growth factor binding protein 5 (6.4), cathepsin E (5.7), insulin-like growth factor 1 receptor (5.5), interferon, alpha-inducible protein (clone IFI-6-16) (5.3), monocyte to macrophage differentiation-associated (5.3), spectrin, alpha, erythrocytic 1 (elliptocytosis 2) (4.8), inositol polyphosphate-5-phosphatase, 40kDa (4.7), alkylglycerone phosphate synthase (4.4), integrin, beta 8 (4.2), CD37 antigen (4.0), hepatic leukemia factor (3.8), interferon regulatory factor 3 (3.7), pyruvate dehydrogenase (lipoamide) beta (3.5), alpha thalassemia/mental retardation syndrome X-linked (3.4), glycerol-3-phosphate dehydrogenase 1 (soluble) (3.3), uronyl-2-sulfotransferase (3.3), adenomatosis polyposis coli (3.2), creatine kinase, mitochondrial 2 (sarcomeric) (3.2), lactate dehydrogenase C (3.1), translocating chain-associating membrane protein (3.1), glucosamine-6-phosphate isomerase (3.1), thiosulfate sulfurtransferase (rhodanese) (3.1), deiodinase, iodothyronine, type II (3.1). Lipoprotein lipase (0.1), vitamin D (1,25-dihydroxyvitamin D3) receptor (0.1), IGF-II mRNA-binding protein 3 (0.2), sulfotransferase, estrogen-preferring (0.2), monoglyceride lipase (0.2), fibronectin 1 (0.2), protein kinase C, delta (0.2), bone morphogenetic protein 7 (osteogenic protein 1) (0.2), low density lipoprotein receptor-related protein 6 (0.3), uroporphyrinogen III synthase (congenital erythropoietic porphyria) (0.3), oxytocin receptor (0.3), protein tyrosine phosphatase, receptor type, C-associated protein (0.3) were also significantly differentially expressed.
Conclusion: Microarray gene profiling has revealed candidate genes, some of them novel, which may account for population-specific nephropathy phenotype in type 2 diabetes mellitus.

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The impact of blood pressure on renal resistance index in hypertensive Type 2 diabetic patients.
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Background and Aims: Changes in the vascular compartment in diabetic nephropathy result in the elevation of Doppler sonographic renal resistance index (RI). The aim of this study was to investigate the influence of blood pressure (BP) compared to other factors on RI changes.
Materials and Methods: Fifty-four type 2 diabetic patients with arterial hypertension (25 male and 29 female; age-range: 49±5.84 years; diabetes duration: 12±5.44 years) were studied. Twenty-nine patients were treated with ACE-inhibitor (group A) and 25 with ACE-inhibitor/Ca^{2+} antagonist combination (group B). Patients were followed during a 2-year period. RI was determined at the beginning and at the end of the study. At the beginning of the study, stepwise regression method was used to analyze the influence of predictor variables: patient's age, diabetes duration, systolic (SBP) and diastolic blood pressure, albumin excretion rate (AER), Tamm-Horsfall protein excretion rate (THPER), lipid values, glycated hemoglobin, serum creatinine and creatinine clearance on the elevation of the resistance index.
Results: Stepwise regression method showed that SBP, disease duration and AER together explained nearly 54% of RI variance. SBP explained almost 22%, disease duration nearly 24% and AER nearly 7.5% of RI variance. At the end of the study a significant reduction in BP was found (p=0.0001), but it was not accompanied by RI reduction. Significant RI elevation was found in group A (p=0.006) and in the group of patients with diabetes duration ≥ 10 years (p=0.001). Group A consisted of significantly more (19 of 27) patients with diabetes duration ≥ 10 years compared to the group B (10 of 22). Significant differences in predictive variables between group A and group B were not found. Significant difference was found between the groups of patients with diabetes duration <10 years and ≥ 10 years for SBP (p=0.033), AER (p=0.009), THPER (p=0.003), creatinine (p=0.005), creatinine clearance (p=0.001) and RI (p=0.002).
Conclusion: Although BP was found to be an important variable in the prediction of RI, reduction of BP at the end of the study was not accompanied by RI reduction. Diabetes duration seems to be an important variable in RI elevation. RI could be considered as an indicator of microangiopathy changes in type 2 diabetic patients.

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Long-term prevention of impairment in renal function in patients with insulin-treated diabetes mellitus.
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Aims/Methods: Population-based, in the city of Jena, Germany, in all patients with insulin-treated diabetes mellitus aged 16 to 60 years quality of metabolic control, renal function and serum concentrations of the AGE-products N-ε -carboxymethyllysine (CML) (ELISA) and pentosidine (HPLC) were measured. The follow-up in 44/55 patients with type 1/2 (aged 47.9± 12.2/58.3± 7.0, diabetes duration 22.3± 12.7/16.5± 7.4 years) were 5 years, 1994/95 to 1999/2000. Table 1 shows patients' characteristics.
Results: 1994/95 to 1999/2000 diabetes control improved substantially. Parallel in patients with type 1 the concentrations of CML and pentosidine, in patients with type 2 diabetes CML decreased (Table 1). In the mean in patients with type 1 and type 2 diabetes there was no impairment in renal function (Table 2).
Only in 15/44 patients (34%) with type 1 an increase of serum creatinine of more than 5% was observed 1999/2000 vs 1994/95. Patients with an increase had higher HbA1c-values in 1994/95 (9.15± 2.55% [n=15] vs 7.4± 1.3% [n=29], p=0,023), a higher systolic blood pressure in 1994/95 (138.3± 21.9 vs 125.9± 12.6, p=0,021) and in 1999/2000 (145.3± 17.3 vs 127.5± 17.1, p=0.003) and also higher diastolic blood pressure values in 1999/2000 (85.3± 9.8 vs 78.0± 8.3, p=0.021). Following multivariate analysis in patients with type 1 mean systolic blood pressure in 1994/95 (ß=0.349, p=0.002), in 1999/2000 (ß=0.499, p=0.001), mean HbA1c in 1994/95 (ß=0.289, p=0.017) and patients' age (ß=0.483, p=0.002) revealed an

association with an increase in creatinine (R-square=0.452). In patients with type 2 diabetes an increase in creatinine was overt in 13/55 (24%). Here there was an association (R-square=0.340) with pentosidine-concentrations in 1999/2000 (ß=0.552, p<0.001) and the HbA1c-levels in 1994/95 (ß=0.289, p=0.012).
Conclusions: Also over longer periods of time good quality of diabetes control can prevent impairment in renal function in both patients with type 1 and type 2 diabetes mellitus. The most important parameters associated with an impairment in renal function are HbA1c, blood pressure control and in patients with type 2 diabetes serum concentrations of the AGE-proteine pentosidine.

Table 1. Characteristics of the patients studied.

Type 1 (n=44)	1994/95	1999/2000	p-value
Body-mass index (kg/m²)	25.1± 3.7	26.2± 3.7	<0.001
HbA1c (%)	8.0± 2.0	7.45± 1.85	0.014
Systolic blood pressure (mmHg)	130.1± 17.2	133.5± 19.0	0.245
Diastolic blood pressure (mmHg)	81.5± 10.1	80.5± 9.4	0.321
Type 2 (n=55)			
Body-mass index (kg/m²)	28.5± 4.1	29.8± 5.0	0.001
HbA1c (%)	**8.6± 1.85**	7.7± 1.5	0.001
Systolic blood pressure (mmHg)	**138.2± 18.4**	137.4± 16.4	0.768
Diastolic blood pressure (mmHg)	**82.7± 9.3**	80.7± 10.6	0.234

Table 2. Changes in renal function

Type 1 (n=44)	1994/95	1999/2000	p-value
Serum creatinine (µmol/l)	80.0 (44.0-150.0)	72.5 (46.0-541.0)	0.345
Creatinine-clearance (ml/min)	109.8± 37.8	117.8± 42.3	0.135
Albuminuria (mg/l)	7.5 (3.0-1014.0)	8.8 (2.7-1364.3)	0.056
CML (ng/ml)	1122.0± 416.8	750.8± 361.6	<0.001
Pentosidine (pmol/ml)	210.3± 127.3	161.9± 112.7	0.054
Type 2 (n=55)			
Serum creatinine (µmol/l)	81.0 (45.0-137.0)	74 (45.0-455.0)	0.400
Creatinine-clearance (ml/min)	105.5± 27.8	113.8± 36.7	0.036
Albuminuria (mg/l)	13.0 (2.0-3157.0)	14.1 (2.6-4404.5)	0.671
CML (ng/ml)	1097.6± 347.5	739.8± 304.8	<0.001
Pentosidine (pmol/ml)	189.5± 75.3	155.0± 147.4	0.117

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Kidney function after withdrawal of long-term antihypertensive treatment in patients with Type 2 diabetes and microalbuminuria.
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Background and Aims: Irbesartan is renoprotective in patients with type 2 diabetes and microalbuminuria. Whether the reduction in microalbuminuria is reversible (haemodynamic) or persistent (structural/biochemical normalization) after prolonged antihypertensive treatment is unknown. Therefore, the present IRMA-2 sub-study investigated the reversibility of kidney function changes after withdrawal of two years antihypertensive treatment.
Materials and Methods: The sub-study included 133 type 2 diabetic patients from a total of 590 hypertensive type 2 diabetic patients with persistent microalbuminuria in IRMA-2, randomized to double-masked treatment with either placebo, Irbesartan 150 mg or Irbesartan 300 mg o.d. for two years, alone or in combination with conventional antihypertensive treatment if required to reach a blood pressure target < 135/85 mm Hg. Arterial blood pressure, overnight urinary albumin excretion rate (UAE) and glomerular filtration rate (GFR, $Cr^{51}EDTA$) were determined repeatedly.
Results: Baseline characteristics were similar in the placebo, Irbesartan 150 and 300 mg groups: Mean arterial blood pressure (MABP) were 112 (1), 111 (1) and 112 (2) mm Hg (mean (SEM)), UAE values 46 (21 to 159), 60 (19 to 243) and 51 (21 to 174) µg/min (median (range)) and GFR levels were 108 (4), 117 (3) and 113 (4) ml/min/1.73 m², respectively (NS between groups). At the end of the study, MABP were similarly lowered to 105 (2), 103 (2) and 102 (2) mm Hg, respectively (p<0.05 vs baseline) and UAE reduced by 8 % (-16 to 27) (NS), 34 % (8 to 53) and 60 % (46 to 70)(95 % CI) (p<0.05). Rates of decline in GFR were 1.3 (0.7), 1.2 (0.7) and 1.0 (0.8) ml/min/1.73 m² /month, respectively, during the initial three months of the study. The sustained GFR decline were 0.3 (0.1), 0.3 (0.1) and 0.4 (0.1) ml/min/1.73 m² /month in the remaining study period. One month after withdrawal of all antihypertensive medication, MABP remained unchanged in the placebo group, 105 (2), but increased significantly in the Irbesartan groups to 109 (2) and 108 (2) mm Hg, respectively. Compared to baseline, UAE was increased in the placebo group by 14 % (-17 to 54) and 11 % (-26 to 65) in the Irbesartan 150 mg group, but persistently reduced by 47 % (24 to 73) in the Irbesartan 300 mg group (p<0.05). GFR levels increased to baseline values, 109 (5) in the placebo group, but only approached initial levels in the Irbesartan groups, 107 (6) and 108 (6) ml/min/1.73 m², respectively.
Conclusion: Persistent reduction of microalbuminuria after withdrawal of all antihypertensive treatment suggests that high dose Irbesartan treatment confer long-term renoprotective effects, which may reflect reversal of renal structural and/or biochemical abnormalities.

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Antiproteinuric effects of losartan in normotensive patients with Type 2 diabetes mellitus and microalbuminuria.
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Background and Aims: ACE inhibitors have shown antiproteinuric effects in normotensive and hypertensive patients with diabetes mellitus. Angiotensin receptor antagonists reduce urinary albumin excretion and the risk of renal and cardiovascular complications in hypertensive patients with type 2 diabetes. The effect of angiotensin antagonists in normotensive diabetics with microalbuminuria has not been reported. This study assesses the antiproteinuric effect of losartan in normotensive patients with type 2 diabetes mellitus and microalbuminuria.
Materials and Methods: A multicentre, placebo-controlled randomised study of losartan in normotensive patients with type 2 diabetes mellitus and microalbuminuria was conducted. Patients (n=149) were randomised to treatment with losartan 50-100 mg or placebo for 10 weeks. Study endpoints were the change in urinary albumin excretion rate as the primary endpoint, and the change in creatinine clearance and blood pressure as secondary endpoints. Safety and tolerability of losartan were monitored.
Results: A generalized linear mixed model for longitudinal data was used to analyse the data. A significant 25% relative reduction in the albumin excretion rate was observed after 5 weeks of losartan 50 mg (p=0,0009), with a further improvement over the subsequent 5 weeks on losartan 100 mg (relative reduction 34%) (p=0,0001). Creatinine clearance did not improve and blood pressure was slightly reduced by losartan. No differences in side effects were observed as compared to placebo.
Conclusion: The angiotensin antagonist losartan reduces urinary albumin excretion in normotensive patients with type 2 diabetes and microalbuminuria. The antiproteinuric effect results from a direct renal effect rather than from a blood pressure lowering effect. Furthermore, losartan was safe and well tolerated in this normotensive patient population.

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Renoprotective effects of adding an angiotensin II receptor blocker to maximal recommended doses of ACE-inhibitor in patients with Type 2 diabetes and nephropathy.
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Background and Aims: We evaluated the renoprotective effects as reflected by short-term changes in albuminuria 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 once 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; albuminuria (turbidimetry), 24-h ambulatory blood pressure ((ABP), Takeda-TM2420) and glomerular filtration rate ((GFR), ^{51}Cr-EDTA plasma-clearance technique).
Results: During mono blockade of the RAS by ACEI treatment alone albuminuria was (geometric mean(IQR)) 706(349, 1219) mg/24-h, 24-h ABP(mean(SEM)) 138(3) / 72(2) mm Hg and GFR 77(6) ml/min/1.73m². During dual blockade of the RAS by addition of candesartan 16 mg daily, there was a mean reduction (95% CI) in albuminuria of 28(17, 38) %, as

compared to ACEI alone (p<0.001). There was a modest reduction in systolic/diastolic 24-h ABP of 3(-2, 8) / 2(-2, 5) mm Hg, as compared to ACEI alone (NS). Individual changes in albuminuria between treatment periods did not correlate to changes in 24-h ABP. Addition of candesartan 16 mg daily induced a small insignificant decline in GFR of 4(-1, 9) ml/min/1.73 m^2.
Conclusions: Dual blockade of the RAS provides superior short-term reduction in albuminuria independently of systemic blood-pressure changes as compared to mono blockade of the RAS with maximally recommended doses of ACEI in type 2 diabetic patients with nephropathy. Studies are needed to confirm the long-term renoprotective effects.

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Proteinuria reduction during antihypertensive therapy predicts renal and cardio-vascular protection in patients with Type 2 diabetic nephropathy.

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Background and Aims: Recently, proteinuria was established a risk marker for progressive renal function loss. Proteinuria can be effectively lowered with antihypertensive drugs that intervene in the renin-angiotensin-system. We questioned first, whether proteinuria is not only a marker of renal disease, but also a target to monitor therapeutic renoprotective efficacy. Second, we questioned whether proteinuria plays the same role in cardiac progression and protection.
Materials and Methods: To this end we analyzed the data from the RENAAL (Reduction in Endpoints in Non-insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan) study, a double-blind, randomized trial to examine the effects of losartan on the composite endpoint of doubling of serum creatinine, end-stage renal disease (ESRD) or death (primary endpoint) and on cardiovascular morbidity and mortality (secondary endpoint), in 1513 type 2 diabetic patients with nephropathy.
Results: Patients with high baseline albuminuria (>3000 mg/g creatinine) showed a 7.6-fold increased risk of progressing to ESRD (95% CI: 5.7-10.1), and a 2.6-fold higher risk (95% CI: 1.8-3.7) for hospitalisation for heart failure (HF) compared to the low albuminuria group (<1500 mg/g). Losartan reduced proteinuria by -28% (95% CI: -25 to -36%), placebo +4% (95% CI: +8 to -1%) in the first 6 months of therapy. Modelling of the initial 6 months change in different risk parameters for predicting the long-term renal and CV risk showed that initial proteinuria reduction is the strongest independent predictor of both renal and cardiovascular outcome: ESRD (Hazard Ratio: 1.59 [95% CI: 1.48-1.72], HF (Hazard Ratio: 1.21 [95% CI: 1.08-1.36]). Every 50% reduction in proteinuria in the first 6 months halved the risk for ESRD and also for HF during follow-up.
Conclusion: In conclusion, proteinuria is the predominant renal and cardiovascular risk marker in patients with type 2 diabetic nephropathy on conventional treatment: the higher proteinuria, the more risk. Reduction in proteinuria affords both renal and cardiovascular protection: the more reduction, the more protection. The specific renal- and cardio-protective effect of the AngII-antagonist losartan in this study appear to linked to its antiproteinuric effect. Proteinuria should be considered a risk marker for both renal and cardiac progression in type 2 diabetes with nephropathy, as well as a target for therapy.

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Dual blockade of the renin angiotensin system versus maximal recommended dose of ACE-inhibition in Type 1 patients with diabetic nephropathy.

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Background and Aims: Albuminuria and hypertension are predictors of poor renal and cardiovascular outcome in diabetic patients. We tested whether dual blockade of the RAS with both an ACE-inhibitor (ACE-I) and an Angiotensin-ll receptor blocker (ARB) is superior to maximal recommended dose of ACE-I in Type 1 patients with diabetic nephropathy (DN).
Materials and Methods: We performed a randomized double blind crossover trial with 8 weeks treatment with placebo and irbesartan 300 o.d. added on top of enalapril 40 mg o.d. We included 24 Type 1 patients with DN. At the end of each treatment period albuminuria (Alb), 24-hour blood pressure (BP) and glomerular filtration rate (GFR) were measured.
Results: Values on ACE-I + placebo were: Alb (mean (95% CI): 519 (342-789) mg/24 h, BP (mean (SEM)): 131 (3) / 74 (1) mm Hg and GFR (mean (SEM)): 65 (5) ml/min per 1.73m^2. Dual blockade of the RAS induced a reduction in Alb (mean (95% CI)) of 25 (15, 34) % (p<0.001), a reduction in systolic BP of 8 (4, 12) mm Hg (p=0.002) and a reduction of 4 (2, 7) mm Hg (p=0.003) in diastolic BP. GFR and plasma-potassium remained unchanged during both treatment regimes. Dual blockade was safe and well tolerated.
Conclusions: Dual blockade of the RAS is superior to maximal recommended dose of ACE-I with regard to lowering of albuminuria and blood pressure in Type 1 patients with DN. Long-term trials are needed to further establish the role of dual blockade of the RAS in renal and cardiovascular protection.

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Optimisation of the vascular risk profile by implementation of a disease management programme (DMP) for diabetes mellitus: a two year prospective analysis.

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Background and Aims: To analyse the effects of a defined DMP on vascular risk factors and the course of nephropathy.
Materials and Methods: The DMP consists of 3 modules: (1) basic module for patient education, lifestyle changes and multifactorial intervention of the vascular risks (2) module for early detection of secondary complications (3) module for management of high-risk patients (i.e. patients with micro-/macroalbuminuria or serum creatinine >1.3 mg/dl). Provision of a guideline-based handbook for intervention and interdisciplinary care to all physicians. Quarterly visits of patients with structured documentation of vascular risk factors and intervention. Central data aggregation and evaluation with a rule-based system (SAS version 8.02). Generation of an individual Care Card for both physician and patient containing follow-up data of 1 year and reminder functions. So far inclusion of 4056 patients from 202 primary care physicians. Analysis of 555 patients participating for at least 8 follow-up visits (age 63.7±11.4 years, 52.4% males, 47.6% females, type 1 diabetes 9.6%, type 2 diabetes 90.4%, diabetes duration 9.1±8.5 years, intervention period 24.7±5.5 months). Test for statistical significance of intraindividual differences by Wilcoxon signed-rank test for paired samples.
Results: At inclusion and after 25 months ( ): HbA1c 7.5±1.5 (7.2±1.3) %, p=0.0016; RRsyst 146±18 (141±16) mm Hg, p<0.0001; RRdiast 84±10 (80±9) mm Hg, p<0.0001); total cholesterol 222±45 (213±50) mg/dl, p<0.0001; triglycerides 205±160 (178±123) mg/dl, p<0.0001; normoalbuminuria 23% (48%); microalbuminuria 53% (28%); macroalbuminuria 9% (10%); serum creatinine >1,3 mg/dl 6% (6%); Modifiable risk according to a risk score: 0-5 points (pt.): low risk: 37% (52%); 6-10 pt. 53% (45%); 11-15 pt.: high risk: 10% (3%).
Conclusion: A DMP with regular, structured data feedback for each patient leads to highly significant improvements of the vascular risk profile and regression of incipient nephropathy.

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The relative impact of individual symptoms and signs on clinical global impression in patients with diabetic peripheral neuropathy treated with ruboxistaurin mesylate.

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Background and Aims: Clinical Global Impression (CGI) scale is a validated and extensively used tool for establishing clinical significance in a variety of trials including those of diabetic peripheral neuropathy (DPN). The relative impact of individual symptoms and signs of DPN on CGI is unknown.
Materials and Methods: Two hundred five patients were enrolled in a multinational clinical trial evaluating efficacy of 32 mg and 64 mg of ruboxistaurin (RBX; LY333531) mesylate, a selective protein kinase C (PKC) β inhibitor, in treatment of patients with DPN. The CGI in different treatment groups was correlated with the symptoms and signs of DPN.
Results: DPN was identified using a vibration detection threshold (VDT) above the 97th percentile, corrected for anthropometric measures and age. DPN positive sensory symptoms were assessed using Neuropathy Total Symptom Score (NTSS)-6. Clinical signs were evaluated using the Neuropathy Impairment Score of the lower limbs (NIS[LL]), and its subset (NIS legs), which is most relevant to DPN. One hundred seventy three patients completed the study. The CGI and the measured clinical parameters of DPN were correlated. Multiple regression analysis was used to determine which one of the clinical parameters had the largest impact on CGI.
Conclusion: Combined RBX-treated groups had a statistically significant trend for improved CGI score (p=0.044). For completers, CGI score correlated with changes in NTSS-6, NIS[LL], and NIS legs scores, as well as VDT. Multiple regression analysis indicated that NTSS-6 score is the main predictor of CGI (p<0.0001). Five of six individual symptoms evaluated by NTSS-6 correlated with CGI (p≤0.021). Prickling and burning sensations provided the strongest correlation.

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Nephropathy - Animal Models

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Nephropathy in nutritionally-induced model of Type 2 diabetes: early and late stage with changes in Na-K-ATPase activity and its gene expression.

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Background and Aims: *Psammomys obesus* develops hyperglycemia and hyperinsulinemia on a high energy diet. Our study was designed to examine the variation in renal functions in relation to the diabetic state in the kidney of the *Psammomys*, with emphasis on changes in Na-K-ATPase activity and its α and β subunits mRNA.
Materials and Methods: The following groups of *Psammomys* were studied: Control animals fed a low energy diet (LE) and hyperglycemic - hyperinsulinemic animals fed a high energy diet (HE) for 20 and 30 days. Regarding the creatinine clearance which represents the glomerular filtration rate (GFR), the diabetic animals were divided into two groups: HE-1 with high GFR, and HE-2 with lower GFR than in the LE group.
Results:

Group	Creatinine clearance ml/min	Urine protein excrtion 10^{-3} g/24 h	Na-K-ATPase activity μmol/Pi mg prot/h		α subunit Na-K-ATPase mRNA αNa-K-ATPase / β Actin	
			Cortex	Medulla	Cortex	Medulla
LE	0.52± 0.07	0.4± 0.04	35.9± 3	52.5± 5	1400± 228	1750± 102
20 day						
HE	0.96±	2.95±	72.5±	151±	1990±	30021±
HE-1	0.07*	0.35*	15*	16*	100	150*
HE-2	0.38± 0.03*	1.15± 0.1*	44± 10	75± 5	1238± 95	1820± 87
30 day						
HE	1.49±	3.81±	97±	189±	2500±	4000±
HE-1	0.08	0.96*	10*	14*	152*	275*
HE-2	0.27± 0.03*	1.48± 0.5*	39.8± 5	77.2± 14	1100± 100	1900± 98

n=10 of all groups; * p<0.001 vs. LE group

The table shows that *Psammomys* fed HE diet develop diabetes already after 20 and 30 days, and can be divided into two subgroups consistent with their GFR: group HE-1 with GFR higher than control (LE), and group HE-2 with GFR values below those in the LE group. Accordingly there was a linear correlation between GFR and Na-K-ATPase activity (r=0.798, p<0.05) and there was also linear correlation between α subunit mRNA of Na-K-ATPase and its activity (r=0.88, p<0.001).
Conclusions: These experiments represent a well defined animal model of type 2 diabetes with nephropathy. The *Psammomys* with diabetes and high GFR - HE-1 - represent the early stage of diabetic kidney lesion (high GFR and proteinuria) while reduced kidney function of the *Psammomys* of group HE-2 (GFR lower than LE and proteinuria higher than LE) represents the progression of diabetic nephropathy to chronic renal failure.

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Connective Tissue Growth Factor (CTGF) regulates matrix degradation through TIMP-1: possible role in matrix accumulation in diabetic nephropathy.

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Background and Aims: Matrix metalloproteinases (MMPs) are responsible for matrix degradation. They are secreted as pro-enzymes which are activated on the cell surface. Once activated they can be inhibited by specific Tissue Inhibitors of MMPs (TIMPs). Our previous studies have shown that high glucose can affect MMP activities, however the precise mechanism of these processes is not well understood. TGFβ is increased by high glucose concentration (HG) and is known to regulate MMP and TIMP expression. Recent studies have shown that the prosclerotic cytokine CTGF is a potent down-stream mediator of TGFβ actions, although its role in the

regulation of MMP activities in diabetes has not been studied. Therefore in this study we investigated the role of CTGF in regulation of MMP activities in mesangial cells (MC).
Methods: Confluent primary cultures of human MC were cultured in media containing 5mM or 25mM glucose (HG), or 5mM glucose and rhCTGF (500ng/ml). In some experiments, either CTGF Ab (30ug/ml), TIMP-1 Ab (20ug/ml) or control IgG alone, was added. After 72h, media was collected for measurement of degradative activity using a radiolabeled substrate, and RNA was extracted for determination of MMP and TIMP mRNA by real time RT-PCR.
Results and Conclusions: Similar to HG, addition of CTGF significantly increased MMP-2 and TIMP-1 gene expression, and had no effect on MT1-MMP or TIMP-2. Addition of CTGF Ab to HG prevented the increase in TIMP-1mRNA but had no effect on MMP-2.

	MT1-MMP mRNA	MMP-2 mRNA	TIMP-1 mRNA	TIMP-2 mRNA	DPM released
HG	48.2±6.8*	255.4±6.8*	187.4±7.2*	89.2±5.6	58.2±6.5*
rhCTGF	110.2±4.3	236.6±4.7*	206.5±8.5*	102.4±3.1	62.5±8.5*
HG+CTGF Ab	55.1±3.6*	278.5±2.5*	87.5±7.3	92.3±4.2	84.6±4.6*

*p<0.05 different from 5mM, ANOVA; Results are expressed as % change from 5mM glucose control (100%).

The HG-induced decrease in the matrix degradative ability of MC media was partially prevented by CTGF Ab. Further studies showed that addition of TIMP-1 Ab abolished the rhCTGF induced decrease in degradative ability (to 126.5+24.5% of control) and it partially prevented the HG induced change (to 82.5+4.7% of control). These results indicate that CTGF plays a role in the regulation of TIMP-1 expression, and by this action it may contribute to the decrease in MMP activities and reduced ECM degradation in diabetic nephropathy.

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The renal expression and its significance of TGF-β-induced Gene h3, βig-h3 in Otsuka Long-Evans Tokushima Fatty (OLETF) Rat.
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Background and Aims: Diabetic nephropathy is the leading cause of end-stage renal disease and is a major contributing cause of morbidity and mortality in patients with diabetes. Diabetic nephropathy seems to occur as a result of an interaction of metabolic and hemodynamic factors such as hyperglycemia, increased arterial pressure, and activation of various vasoactive hormone pathways. As the major pathologic feature of diabetic nephropathy is diffuse mesangial matrix expansion, transforming growth factor-β (TGF-β), a pro-sclerotic cytokine is a leading candidate to mediate the progression of the disease such as glomerulosclerosis and tubulointerstitial fibrosis. TGF-β-induced gene-h3 (βig-h3) is an extracellular matrix protein and adhesion molecule which is induced by TGF-β in many cells. As TGF-β plays an important role in diabetic complications and βig-h3 serves as a cell substrate of TGF-β, we hypothesized that diabetic condition might increase βig-h3 expression in kidney and body fluid and that it may subsequently contribute to the pathogenesis of diabetic nephropathy.
Material and Methods: Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of type 2 diabetes mellitus were used for this experiment. Long-Evans Tokushima Otsuka (LETO) rats were used as control. We measured urine and serum βig-h3 concentration by ELISA methods. We checked pathologic changes of kidney by periodic acid schiff (PAS). We also investigated localization and expression of βig-h3 in the kidney by immunoblotting analysis and immunohistochemistry.
Results: Urinary βig-h3 concentration was higher in OLETF than LETO rats from 16 weeks to 32 weeks according to increase of blood glucose levels. However, there was no difference of serum βig-h3 concentration between LETO and OLETF rats. PAS staining showed focal mesangial proliferation, glomerular hypertrophy, and tubular damage in OLETF rats (24 and 32 weeks). An immnohistochemical study showed that prominent labeling of the βig-h3 is seen at the proximal tubule cells (S3 segment) of inner cortex and outer medulla of LETO and OLETF rats (16, 24, and 32 weeks). In glomeruli, the βig-h3 labeling was weakly observed at the basal part of the parietal epithelial cells in the Bowman's capsule. βig-h3 protein levels were higher in the membrane fraction from renal cortex and outer strip outer medulla of OLETF rats especially, 32 week-aged rats compared to those of LETO rats.
Conclusion: These results suggest that βig-h3 may play an important role in diabetic nephropathy and could be a useful predictor of progression of diabetic nephropathy.

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Involvement of inflammatory process in the pathogenesis of diabetic nephropathy.
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Background and Aims: Infiltration of macrophage is prominent in renal tissues of diabetic patients and diabetic animals, suggesting that inflammatory processes are involved in the pathogenesis of diabetic nephropathy. We previously revealed that intercellular adhesion molecule-1 (ICAM-1) is up-regulated and mediates macrophage infiltration into diabetic kidney. In this study, to evaluate the roles of inflammatory process in the pathogenesis of diabetic nephropathy, we induced diabetes in ICAM-1 knockout (KO) mice and examined the gene expression profile in the renal tissues using DNA array technique.
Materials and Methods: Eight-week-old male ICAM-1 KO mice and wild type (C57BL/6J) mice were injected with streptozotocin to induce diabetes. Mice were divided into 4 groups: 1) Diabetic ICAM-1 KO mice (DM-KO), 2) diabetic wild type mice (DM-WT), 3) non-diabetic ICAM-1 KO mice (ND-KO) and 4) non-diabetic wild type mice (ND-WT). Mice were killed at 6 months after induction of diabetes and the kidneys were harvested. Blood glucose, HbA1c, systolic blood pressure, serum creatinine, urinary albumin excretion (UAE) and creatinine clearance(Ccr) were measured. Glomerular size and mesangial matrix area were measured by morphometry. We evaluated the number of macrophages, expression of TGF-β and type IV collagen in glomeruli by immunohistochemical study. To evaluate the gene expression profile, mice were killed at 2 weeks after induction of diabetes and total RNA was extracted from kidneys. DNA array system (Atlas™ Nylon cDNA Expression Array, Clontech, CA USA) was used to compare the expression of 1,176 genes related to inflammatory process, including cytokines, chemokines and signal transduction molecules.
Results: There was no significant difference in HbA1c, blood pressure and Ccr between DM-WT and DM-KO. UAE, glomerular hypertrophy, mesangial matrix expansion were significantly suppressed in DM-KO compared with DM-WT. Macrophage infiltration, expression of TGF-β and type IV collagen in glomeruli were decreased in DM-KO as compared with DM-WT. DNA array study revealed that expression of several genes including osteopontin and heat shock proteins were increased in DM-WT than in ND-WT and suppressed in DM-KO as compared with DM-WT. Signal intensity of osteopontin was increased in DM-WT than in ND-WT (ratio of DM-WT vs ND-WT; 5.2 : 1.0), and decreased in DM-KO compared with DM-WT (ratio of DM-WT vs DM-KO; 2.4 : 1.0).
Conclusion: The current results strongly suggest that inflammatory processes play an important role in the pathogenesis of diabetic naphropahy. ICAM-1 might be a target for the therapy of diabetic nephropathy. Osteopontin may be one of the key molecules in development of diabetic nephropathy.

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High ambient glucose stimulates reactive oxygen species generation through protein kinase C-dependent pathway in cultured podocytes.
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Background and Aims: The increased reactive oxygen species (ROS) production may be involved in the onset or development of diabetic vascular complications. The podocyte plays a crucial role in maintaining the permselectivity function of the glomerular capillary wall. The release of ROS from podocyte may play a role in the pathogenesis of glomerular damage and proteinuria. Although it is assumed that the podocyte injury play an important role in diabetic renal injury, the mechanism is still unknown. We examined whether high ambient glucose increased ROS in cultured podocyte, whether it was restored by various antioxidants, and whether the protein kinase C (PKC) pathway was involved in this process.
Materials and Methods: To examine the effect of high glucose on ROS generation by podocytes in vitro, differentiated murine podocytes were

stimulated for 5, 12, 24, 48, 72 hours with 30mM glucose (5.6mM glucose as a control). Dichlorofluorescein(DCF)-sensitive intracellular ROS was measured by a laser scanning confocal microscope.
Results: High glucose (30mM for 24 hr) raised ROS generation 3.8 fold than control (5.6mM) (p<0.05) in cultured podocytes. This high glucose-induced ROS generation were increased time-dependent manner at 5, 12, and 24 hours and the ROS level was higher in high glucose medium up to 72 hours than control. The increase of high glucose-induced ROS generation by podocyte was effectively inhibited by pretreatment with catalase (300 U/ml) (2.3 fold, p<0.05), superoxide dismutase (30 U/ml) (1.9 fold, p<0.05), 2- mercaptopropionyl glycine (5 mM) (1.8 fold, p<0.05), and glutathione (5mM) (2.0 fold, p<0.05). We also observed the increase of free radical production by high glucose medium which was completely restored by PKC depletion by pretreatment of the cells with phorbol myristate acetate (80mM).
Conclusion: In this study, we showed that cultured murine podocytes produce ROS in response to high glucose, and identified PKC was involved in this process. These results suggest that increased oxidative stress in podocytes may play a role in the pathogenesis of podocyte injury and proteinuria in diabetic nephropathy.
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High glucose triggers apoptotic and survival signals in human mesangial cells.
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Background and Aims: A decline in the glomerular filtration rate in diabetic kidney is associated with loss of glomerular cells, possibly due to impaired cell survival. However, intensive control of hyperglycemia has been shown to delay the onset and progression of kidney damage in diabetes. The objectives of the present study were: i.) to assess whether high glucose concentrations may activate apoptosis in human mesangial cells; and ii.) to define the intracellular signaling intermediates mediating the effects of high glucose.
Materials and Methods: Two independent human mesangial cell lines in primary culture were obtained from renal biopsies. Cells were grown to confluence and incubated for 4-72 h in the presence of normal glucose (5 mM D-glucose, NG), high glucose (25 mM D-glucose, HG), or osmotic controls such as mannitol (5 mM D-glucose + 20 mM mannitol, M) and L-glucose (5 mM D-glucose + 20 mM L-glucose, L). Apoptosis was determined by ELISA detection of oligosomes released in the cytoplasm, which represent an early marker of apoptosis. In addition, FITC-conjugated annexinV/propidium iodide staining was used to detect apoptotic or necrotic cells by immunofluorescence.
Results: Apoptotic rates were increased in the mesangial cell lines exposed to HG, with maximal effect following 72 h of incubation (170% of NG, p<0.05). By contrast, no changes in apoptotic rates were detected in cells incubated with M or L. To define the signaling molecules contributing to HG-induced apoptosis, the protein content and phosphorylation state of intracellular regulatory kinases, including p38 MAPK, Erk-1/2, Akt, and JNK, were next determined. Cells exposed to HG showed a two-fold increase in both p38 MAPK and Erk-1/2 phosphorylation (p<0.05 vs. NG). Treatment of cells with the p38 MAPK inhibitor SB203580 did not abrogate the pro-apoptotic effect of HG, whereas inhibition of Erk-1/2 with PD098059 resulted in enhanced apoptosis in response to HG. Both HG and M increased the level of Akt phosphorylation on Ser-473 at early time points; however, at later exposure times, Akt phosphorylation returned to control values in the presence of HG but remained elevated with M. Finally, both HG and M induced an increase in JNK phosphorylation.
Conclusions: i.) HG specifically increases apoptosis of human mesangial cells; ii.) p38 MAPK is activated by HG, but does not appear to mediate the pro-apoptotic effect of HG; iii.) the lack of sustained Akt activation following prolonged exposure to HG may contribute to increased apoptotic rates; and iv.) HG-mediated activation of Erk-1/2 appears to generate also pro-survival signals that may antagonize the apoptotic response.

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Interaction between non-enzymatic glycation and the polyol pathway on mesangial cell gene expression in an aldose reductase transgenic mouse model.
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Background and Aims: Diabetic nephropathy is a leading cause of end-stage renal disease. The aldose reductase (AR) gene, which codes for the first and rate-limiting enzyme in the polyol pathway, has been implicated in the etiology of diabetic nephropathy, based on genetic association studies and findings from animals treated with AR inhibitors. However, the exact role of AR in the development of diabetic nephropathy and its interaction with other pathogenetic pathways, such as non-enzymatic glycation, remain controversial. In this study, we investigated the effects of increased AR gene expression on pathogenetic changes that could lead to diabetic nephropathy, in a transgenic murine model.
Materials and Methods: Transgenic mouse lines expressing the human AR (hAR) gene in kidney mesangial cells were established, using a construct that contained the type A scavenger receptor promoter. The interaction between AR and advanced glycation end-products (AGEs) was examined in primary cultures of mesangial cells derived from hAR transgenic and wild type mice, with regard to changes in AR activity, transforming growth factor-β1 (TGF-β1) and type IV collagen gene expression, in response to incubation with AGE modified BSA (AGE-BSA).
Results: hAR mRNA expression could be detected by Northern blot analysis in the kidney glomeruli of transgenic mice. hAR mRNA and protein expression were also found in primary cultures of transgenic mesangial cells, as assessed by RT-PCR and Western blot analyses. Enhanced AR activity was seen in both transgenic and wild type mesangial cells when treated with AGE-BSA. This increase was significantly higher (p<0.05) in the transgenic mesangial cells. The rise in AR activity in the presence of AGE-BSA was accompanied by increases in TGF-β1 and type IV collagen transcripts in mesangial cells, which reached statistical significance only in transgenic mesangial cells (p<0.01) and was abolished by the addition of an AR inhibitor zopolrestat (p<0.05).
Conclusion: These results suggested that the AR gene was involved in the increased expression of TGF-β1 and type IV collagen observed in the transgenic mesangial cells in vitro. Increased AR gene expression might contribute to the mesangial cell proliferation and matrix protein production in diabetic nephropathy, in part through an interaction with AGEs.
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Effect of systemic arterial hypertension on urinary transforming-growth factor beta in streptozotocin-diabetic rats.
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Background and Aims: Experimental diabetic nephropathy presents with progressive extra-cellular matrix deposition in the mesangium, which is induced by local production of transforming-growth factor beta (TGF-β1). Key determinants of TGF-β1 synthesis are high glucose concentration and the stretching of mesangial cells induced by hypertension. We have previously demonstrated that urinary TGF-β1 is increased in streptozotocin (STZ)-diabetic rats, but these animals do not present hypertension, as is usual in human diabetic nephropathy. The aim of the present study to the present study to evaluate the effect of genetically determined hypertension, isolated and associated with diabetes on urinary TGF-β1.
Materials and Methods: We studied 40 spontaneously hypertensive rats (SHR) and 32 Wistar-Kyoto (K) rats (190-260g). Twenty-six SHR (DSHR) and 20 K rats (DK) were injected with STZ, 50mg/kg, IV, while 17 SHR and 12 K rats received citrate buffer. After 30 days, animals were put into metabolic cages for 24h urine collection for glucose, creatinine and TGF-β1 (ELISA, R&D; Systems). Catheters were implanted into the femoral artery

and vein (PE-10) to measure arterial pressure and for blood collection (to measure blood glucose). Rats were conscious during the experiments. Recorded data were analyzed on a beat-to-beat basis.
Results: Shown in the table.MAP= Mean arterial pressure. * p<0,05 vs K and DK; # p<0.05 vs K and SHR; ** p<0.05 vs DK (ANOVA; posthoc: Student Newman Keuls)
Conclusion: Isolated systemic hypertension does not have a great impact on urinary TGF-β1 in SHR; however, the association between hypertension and diabetes increases urinary TGF-β
1 excretion more than diabetes alone, suggesting a synergism that could contribute to the progression of diabetic nephropathy.

Table

Group	MAP (mmHg)	Blood Glucose (mg/dl)	Urinary Glucose (mg/24h)	Urinary TGF-β1/ Creatinine(pg/mg)
K (n=12)	113±2	113±5	16±2	63±20
DK (n=20)	117±3	448±21#	7053±813#	279±32#
SHR (=14)	155±9*	116±5	17±5	132±54
DSHR (n=26)	141±5*	408±18#	5552±300#**	390±49#**

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Mechanical stretch and cytokines upregulate vascular endothelial growth factor and its receptors in glomerular epithelial cells *in vitro*.
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Background and Aims: Glomerular haemodynamic perturbations are key to the pathogenesis of diabetic glomerulopathy. In diabetes Vascular endothelial growth factor (VEGF) is overexpressed in glomerular epithelial cells (GECs) that, together with the glomerular basement membrane, represent the main barrier to protein filtration. Blockade of VEGF ameliorates proteinuria in experimental models of diabetes. We studied whether mechanical stretch induced the VEGF/VEGF receptor system in GECs, and explored the molecular mechanisms of this effect.
Materials and Methods: Murine GECs were exposed to mechanical stretch (average 10% elongation) for 6, 12, 24, 48 hours in 7 mM glucose, and to either TGFβ1 (10 ng/ml) or TNFα (10 ng/ml) for 24 and 48 hours after serum deprivation. VEGF protein secretion in the supernatant was measured by ELISA, and VEGF receptors 1 and 2 (VEGFR-1, VEGFR-2) proteins by western immunoblotting.
Results: Mechanical stretch induced a 2-fold VEGF upregulation at 6 and 12 hours (p<0.05), which was not mediated by PKC or p38MAPK. Both VEGFR-1 and VEGFR-2 were expressed at baseline. Mechanical stretch determined a 2.5-fold upregulation of VEGFR-2, but not of VEGFR-1, at 24 and 48 hours (p<0.05). Both TGFβ1 and TNFα induced a 1.5-2 fold VEGFR-2 overexpression after 48 hours (p<0.05); VEGFR-1 was induced only by TGFβ1 by 2-fold after 24 hours (p<0.05).
Conclusion: Mechanical forces and cytokines mediated modulation of the VEGF/VEGF receptor system in GECs might represent one of the cellular mechanisms responsible for the altered permeability to protein that characterise diabetic glomerulopathy.

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Reactive oxygen species via mitochondria electron transport chain induce cyclooxygenase-2 gene expression in human mesangial cells: potential mechanism in diabetic nephropathy.
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Background and Aims: Increased oxidative stress is considered to be one of the common pathogenic factors in diabetic complications. Recently, we have shown that hyperglycemia increases the production of reactive oxygen species (ROS) from the mitochondrial electron transport chain in bovine endothelial cells (Nishikawa *et al.* 2000; *Nature* 404:787-90). Because several studies have postulated a role for prostaglandins (PGs) in the glomerular hyperfiltration seen in early diabetes, we evaluated the effect of mitochondrial ROS on expression of the inducible isoform of cyclooxygenase (COX-2) in cultured human mesangial cells (HMC).
Materials and Methods: HMC were incubated with 5.6 mM glucose, 30 mM glucose or 30 mM glucose with / without an inhibitor of electron transport chain complex II (TTFA), or uncoupler (CCCP), or overexpression of uncoupling protein-1 (UCP-1) or manganese superoxide dismutase (MnSOD) by adenovirus system. In these conditons, mitochondrial membrane potential, intracellular ROS, COX-1 and COX-2 mRMA expression, COX-2 protein expression and PGE2 synthesis were measured by fluorometer method, fluorescence microscopy, quantitative RT-PCR method, Western blot analysis and EIA method, respectively. Furthermore, to evaluate COX-2 gene promoter activity, transient DNA transfection experiments using luciferase as a reporter gene were performed.
Results: An increase in fluorescence of RedoxSensor Red CC-1 with MitoTracker Green FM induced by increased mitochondrial membrane potential was observed when the cells were incubated with 30 mM glucose compared with 5.6 mM glucose. Likewise, CM-H2DCFDA-associated fluorescence, representing intracellular ROS production, was significantly increased by incubation with 30 mM glucose (290.5 ± 15.0 % of 5.6 mM glucose). Incubation of HMC with 30 mM glucose significantly increased COX-2 mRNA (415.2 ± 35.8 % of 5.6 mM glucose) but not COX-1 mRNA, compared with 5.6 mM glucose. Similarly, incubation of HMC with 30 mM glucose significantly increased COX-2 protein expression and PGE_2 synthesis (456.5 ± 57.4 % of 5.6 mM glucose). Incunation with high glucose induced activation of the COX-2 gene promoter (400.3 ± 34.8% of 5.6 mM glucose), which was completely abrogated by mutation of two NF-κB (nuclear factor-κB) binding sites in the promoter region. Finally, these events including intracellular ROS production, COX-2 gene promoter activation, COX-2 mRNA and protein expression and PGE2 synthesis were completely suppressed by TTFA or CCCP, or by overexpression of UCP-1 or MnSOD.
Conclusion: Our results suggest that hyperglycemia increases mitochondrial ROS production, resulting in NF-κB activation, COX-2 mRNA induction, COX-2 protein production and finally PGE2 synthesis. This chain of events might contribute to the pathogenesis of the glomerular hyperfiltration observed in early diabetes.

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Low molecular weight age-peptides in diabetic nephropathy.
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Background and Aims: Incomplete degradation of proteins containing advanced glycation end products (AGEs) results in the formation of low molecular weight AGE-peptides. These reactive byproducts are able to form intramolecular cross-links (secondary glycation) and interact with AGE receptors at distant sites via the circulation.
Methods: To determine the role of AGE-peptides in diabetic nephropathy, a flow injection assay was developed to measure AGE-fluorescence (Ex370/Em440nm) in de-proteinated serum samples. AGE-peptide content was then serially measured in Sprague-Dawley rats with and without streptozotocin-induced diabetes.
Results: AGE- peptide increased with chronological age in all animals. A significant difference between diabetic (D) and control (C) animals was demonstrable after as little as three weeks of diabetes and increased with the duration of diabetes (32 weeks, C=13.8±0.6 arbitrary units (AU); D=23.7±0.5 AU, p<0.001). Treatment with insulin reduced AGE-peptide levels compared to untreated (U) animals (8 week, C=7.8±0.4 AU, D=12.7±0.3 AU, U=15.6±0.5 AU, p<0.001). Treatment with aminoguanidine or ramipril reduced AGE-peptide levels to non-diabetic levels. Sera form patients with diabetes was then examined for AGE-peptide content. GFR was strongly associated with AGE-peptide levels. However, at each level of GFR, patients with albuminuria or an elevated filtration fraction had significantly higher levels of AGE-peptides.
Conclusion: It is likely that impaired excretion and increased production both contribute to elevations in AGE-peptides seen in diabetes. It remains to be established whether these represent a manifestation or pathogenic mechanism of tubular injury in diabetes.

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Effects of nicotine on urinary albumin excretion and plasma total homocystein concentration in Streptozotocin-induced diabetic rats.

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Background and Aims: The cardiovascular system and kidney are important target organs of smoking-induced damage, and the risk is particularly high in diabetic patients. Also, high plasma total homocysteine (t-Hcy) concentration is associated with cardiovascular disease and increased urinary albumin excretion rate (UAE) in diabetic patients. Therefore, high t-Hcy concentration with increased UAE is a potential new link among microalbuminuria, diabetic nephropathy and cardiovascular disease. We investigated the effects of nicotine on UAE and plasma t-Hcy concentration in Streptozotocin(STZ)-induced diabetic rats.
Materials and Methods: 6 week-old male Sprague-Dawley rats were divided into 5 groups: A; normal untreated control (n=4), B; nicotine-treated normal rats (nicotine ditartrate salt 4 mg/kg/day in tap water, n=5), C; STZ- induced diabetic rats (STZ 100 mg/kg i.p. n=6), D; STZ diabetic rats treated with nicotine (STZ induction followed by nicotine in tap water, n=5), E; nicotine-pretreated STZ diabetic rats (4 week nicotine pretreatment before STZ induction, n=5). 24- hour UAE was compared between the 5 groups and within the same group before treatment and at 4 and 8 weeks of treatment. At 8 weeks, urinary nicotine concentrations were determined in the nicotine-treated groups B, D, E and normal control group A. At 8 weeks, HbA1c, creatinine clearance (Ccr), t-Hcy, vitamin B12, B6 and folate were evaluated in the 5 groups.
Results: HbA1c at 8 week was significantly (P<0.0001) higher in the three diabetic groups (C, D and E) than the non-diabetic groups (A and B). Urinary nicotine concentrations were significantly (P=0.0263) higher in the three nicotine-treated groups compared with normal control group A. Urinary albumin excretion was not significantly different before treatment and at 4 and 8 weeks of treatment within group, or between the 5 groups . Ccr (ml/min/100 g body weight) was significantly higher in diabetic groups than non-diabetic groups. Plasma t-Hcy concentrations (nmol/ml) were significantly (P<0.0001) higher in non-diabetic groups A and B (5.15±0.90 and 5.0±0.61,respectively) than the diabetic groups C, D and E (2.5±0.10, 2.1±0.32 and 2.3±0.22, respectively). Co-enzymes of the metabolite enzyme of homocysteine, vitamin B12, B6 and folate concentrations were not significantlyz different.
Conclusion: Nicotine treatment did not affect the t-Hcy levels or ameliorat the UAE in the rats. These results indicate that nicotine treatment is not linked to homocysteinemia and UAE in the diabetic and nondiabetic rats during the experimental period of 8 weeks .

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Pyridoxamine dihydrochloride reduces established diabetic nephropathy (DN) at the level of the glomerulus, not at the level of the mesangial stem (progenitor) cells in the bone marrow.

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Background and Aims: We have shown that bone marrow (BM)-derived mesangial cell progenitors carry both a disease genotype and phenotype to naive recipient glomeruli. We also found that pyridoxamine hydrochloride (Pyridorin™) reduced glomerular lesions and albuminuria in B6 db/db mice with established DN. The purpose of the current experiments was to determine if the genotypic and phenotypic changes in DN were transferred by BM-derived mesangial cell progenitors and if Pyridorin™ -treatment changed the phenotype of BM-derived mesangial cell progenitors.
Materials and Methods: Female B6 +/+ mice were lethally irradiated and then received 1 million BM cells from either vehicle or Pyridorin™ -treated B6 db/db mice which had established albuminuria DN for a period of 16 weeks. A separate group of donors had been sacrificed to document the presence of histologic evidence of DN. Recipients were followed for 4 months. Urine albumin/creatinine was measured weekly and at sacrifice. Glomeruli were analyzed by morphometry.
Results: Normoglycemic B6 +/+ recipients of BM transplants from B6 db/db mice with established nephropathy were found to have developed albuminuria at 4 months (p=<0.001), and glomerular changes manifest by both overall hypertrophy and mesangial expansion (p=<0.01). Normoglycemic B6 +/+ recipients of bone marrow from B6 db/db mice with established nephropathy that had been successfully treated with Pyridorin™ also developed albuminuria and histologic evidence of DN which was indistinguishable from that seen in recipients which received BM from vehicle-treated B6 db/db mice.
Conclusion: Pyridoxamine dihydrochloride (Pyridorin™) reduces glomerular lesions in B6 db/db mice with established DN. The BM of B6 db/db mice with established DN contains mesangial cell precursors which transmit both the genotype and phenotype of DN to the glomeruli of normoglycemic, naive mice. Since BM transplants from mice successfully treated with Pyridorin™ and the vehicle controls induced similar glomerular lesions, we conclude that the reduction in glomerular lesions in treated mice occurs at the level of the glomeruli and not at the level of BM progenitors.

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Rosiglitazone prevents stretch-induced monocyte recruitment by inhibiting the NFkB-MCP1 pathway in human mesangial cells.

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Background and Aims: Glomerular macrophage infiltration driven by the chemokine Monocyte Chemoattractant Protein-1 (MCP-1) has been implicated in the pathogenesis of diabetic glomerulopathy. The PPAR-gamma ligand rosiglitazone has anti-inflammatory properties in vitro and renoprotective effects in vivo in experimental diabetes. We studied in human mesangial cells (HMC) the effect of stretch and rosiglitazone on monocyte chemoattractant activity.
Materials and Methods: HMC were exposed to mechanical stretch (10% elongation) in the presence and in the absence of rosiglitazone (10microM). MCP-1 mRNA and protein were measured by real-time-PCR and ELISA. Monocyte chemoattractant activity of conditioned supernatants from both stretched and non-stretched mesangial cells was assessed on freshly isolated human blood monocytes in a micro-chemotaxis chamber with 5micron-pore-size polycarbonate filter. NFkB DNA binding activity was determined by electrophoretic mobility shift assay (EMSA) on nuclear proteins.
Results: Stretching of HMC significantly enhanced their monocyte chemoattractant activity (2.1-fold increase over control, p<0.01). This effect was paralleled by a significant rise in both MCP-1 mRNA and protein levels (1.7 and 2.1-fold increase, p<0.05), and it was completely abolished by MCP-1 blockade (stretch: 2.6, stretch + anti-MCP-1 antibody: 0.8-fold increase, p<0.01). Stretch activated NFkB (2-fold increase in DNA-binding activity) and NFkB inhibition using the specific inhibitor SN50 (1microM), significantly reduced (80%, p<0.05) stretch-induced MCP-1. The addition of rosiglitazone significantly diminished stretch-induced NFkB activation, MCP-1 production, and monocyte chemotaxis (80% inhibition for all, p<0.05).
Conclusion: Stretching of HMC stimulates their monocyte chemoattractant activity via an NFkB-mediated-MCP-1-dependent pathway. This effect is inhibited by rosiglitazone. Prevention of monocyte recruitment may be important in PPAR-gamma ligands renoprotective action.

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Effect of lithospermate B on experimentalrenal injury, Type 2 diabetic OLETF rat.

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Background and Aims: Magnesium lithospermate B (LAB) is an active component isolated from *Salvia miltiorrhizae* with renoprotective properties due to its antioxidative effects. We showed that LAB had renoprotective effect (2003, JASN in press) on type 1 diabetic animal (STZ-induced diabetic rat). The previous data in our study showed that LAB inhibited ROS generation leading to PKC activation and TGF-β1 and

fibronectin upregulation in kidney cortex in the mesangial cell under high glucose condition. Moreover, treatment with LAB was found to significantly suppress the progression of renal injury in STZ-induced diabetic rats.
Materials and Methods: Thus, in the present study we examined the effects of LAB on renal injury in OLETF rats, Otsuka-Long-Evans-Tokushima fatty (OLETF) rats derived from spontaneously diabetic Long-Evans rats, and characterized by mild obesity and late-onset hyperglycemia (after 18 weeks of age) with complications related to chronic diabetes. Treatment of 10 mg of LAB/kg/day was started in 12 weeks of age and continued for 40 weeks.
Results: Significantly it suppressed serum malondialdehyde (MDA) (LETO: 0.04±0.01, LETO+LAB: 0.05±0.01, OLETF: 0.12±0.02, OLETF+LAB: 0.08±0.04, pmol), proteinuria (LETO: 14.9±4.3, LETO+LAB: 18.1±4.1, OLETF: 170.6±28.2, OLETF+LAB: 103.0±36.4, mg/day), glomerular hypertrophy (LETO: 1009±16.4, LETO+LAB: 95.5±13.3, OLETF: 113.6±20.9, OLETF+LAB: 104.0±18.7, % of control), mesangial expansion (LETO: 15.2±4.3, LETO+LAB: 16.4±4.3, OLETF: 21.7±5.3, OLETF+LAB: 18.4±4.2, %), and the upregulation of renal collagen in OLETF rats (LETO: 4.78±2.23, LETO+LAB: 4.22±2.42, OLETF: 12.94±3.78, OLETF+LAB: 4.91±2.75, positive staining area,-% of control).
Conclusion: LAB may become a new therapeutic agent for the treatment of diabetic nephropathy.

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The vasopeptidase inhibitor AVE7688 reduces nephropathy in Zucker diabetic fatty rats.
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Background and Aims: Pharmacological inhibition of the renin angiotensin system has proven clinical efficacy in nephropathies of various origin, including diabetic nephropathy. We tested whether simultaneous inhibition of both angiotensin converting enzyme (ACE) and neutral endopeptidase, reduces nephropathy in a model of type II diabetes.
Materials and Methods: Fifty six obese Zucker diabetic fatty (ZDF/Gmi-fa/fa) rats aged 34 weeks were treated with either placebo (n=9) or the vasopeptidase inhibitor AVE7688 in four different doses (each n=9; 3, 10, 30, or 60 mg/kg/d in chow). Eleven heterozygotic (+/fa) rats received placebo and served as non-diabetic, lean controls. In obese rats, urinary albumin-to-creatinine ratio (ACR) was assessed as a marker of nephropathy at baseline (age 34 weeks) and after 10 weeks of chronic treatment, at age 44 weeks.
Results: In all obese animals, glycated hemoglobin was marked elevated at baseline (HbA1c 12.2±0.2 % vs. 4.9±0.01 % in lean controls), and AVE7688 did not influence glycated hemoglobin concentrations. AVE7688, but not placebo, significantly decreased ACR in a dose dependent manner (Placebo 2.0±4.4 vs. 11.9±1.8, 13.4±0.7, 13.6±2.8, and 19.8±2.8 mg/mg in the 3, 10, 30, and 60 mg/kg/d groups, respectively; all treatment groups $p < 0.05$ vs. Placebo).
Conclusion: In Zucker diabetic fatty rats with established diabetes mellitus and related kidney damage, AVE7688 dose dependently reduces proteinuria without affecting metabolic control. Vasopeptidase inhibition with AVE7688 represents an effective novel therapeutic principle for intervention in diabetic nephropathy.

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High dose thiamine therapy suppresses the activation of protein kinase C_β in the glomeruli of streptozotocin-induced diabetic rats on insulin maintenance therapy.
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Background and Aims: Activation of protein kinase C_β (PKC_β) in cells suffering abnormal high cytosolic glucose concentrations in diabetes has been linked to the development of vascular complications of diabetes. Triosephosphate accumulation and *de novo* synthesis of diacylglycerol (DAG) mediates this effect. The aim of this study was to determine if the activation of the reductive pentosephosphate pathway by high dose thiamine and Benfotiamine (Bft) therapy could suppress activation of PKC in renal glomeruli of streptozotocin (STZ) induced diabetic rats on insulin maintenance therapy.
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 (n = 8 in each study group). PKC activity *in situ* was assayed with an epidermal growth factor receptor peptide fragment substrate VRKRTLRRL, and in membrane and particulate fractions with exogenous DAG and maintenance of the diabetic state by assay of plasma glucose concentration and glycated hemoglobin HbA_1. Data given are for 24 weeks. Statistical analysis: P and P', is the significance with respect to normal controls and diabetic controls, respectively (*t*-test).
Results: Plasma glucose concentration was increased 3-fold and HbA_1 increased one-fold in STZ diabetic rats, respectively ($P<0.001$); neither were decreased significantly by thiamine or Bft. In the thiamine study, glomerular PKC activity *in situ* increased 38 ± 3% in diabetic controls ($P<0.001$). This increase was reversed 38% by 7 mg/kg thiamine ($P'<0.05$) and 62% by 70 mg/kg thiamine ($P'<0.01$). Cytosolic and membrane PKC activities were increased 101% and 118% in diabetic controls, respectively ($P<0.001$). The increase in cytosolic PKC activity were reversed 55% by 7 mg/kg thiamine ($P'<0.001$) and 66% by 70 mg/kg thiamine ($P'<0.01$); and the increase in membrane PKC activity was reversed 43% by 7 mg/kg thiamine ($P'<0.001$) and 66% by 70 mg/kg thiamine ($P'<0.01$). In the Bft study, glomerular PKC activity *in situ* increased 54 ± 6% in diabetic controls ($P<0.001$). This increase was reversed 55% by 7 mg/kg Bft ($P'<0.01$) and 70% by 70 mg/kg Bft ($P'<0.001$). Cytosolic and membrane fraction PKC activities were increased 114% ($P<0.001$) and 128% ($P<0.01$) in diabetic controls, respectively. The increase in cytosolic PKC activity were reversed 66% by 7 mg/kg Bft ($P'<0.001$) and 81% ($P'<0.001$) by 70 mg/kg Bft; and the increase in membrane PKC activity was reversed significantly by 70 mg/kg Bft only (55%, $P'<0.001$). Thiamine and Bft had similar potency, therefore, although thiamine suppressed membrane PKC activity more effectively than Bft. High dose thiamine therapy also prevented the development of incipient nephropathy, as judged by microalbuminuria, in this study.
Conclusions: High dose thiamine and Bft therapy suppressed the activation of glomerular PKC in experimental diabetes. This may contribute to the prevention of incipient nephropathy by high dose thiamine derivatives.

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Prevention of incipient nephropathy by high dose thiamine and Benfotiamine in streptozotocin-induced diabetic rats with maintenance insulin therapy.
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Background and Aims: Thiamine supplementation at high dose may increase levels of thiamine pyrophosphate and thereby counter biochemical dysfunction linked to the development of diabetic nephropathy. The aim of this study was to determine the effect of high dose thiamine and the thiamine derivative Benfotiamine (Bft) on the development of incipient nephropathy in streptozotocin (STZ) induced diabetic rats on insulin maintenance therapy.
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. The development of nephropathy in STZ rats was judged by measurement of albuminuria and maintenance of the diabetic state by assay of plasma glucose concentration and glycated hemoglobin HbA_1. Statistical analysis: P and P', is the significance with respect to normal controls and diabetic controls, respectively (*t*-test or Mann-Whitney U).
Results: Plasma glucose concentration was increased 3-fold and HbA_1 increased one-fold in STZ diabetic rats, respectively; neither were decreased significantly by thiamine or Bft. Urinary albumin excretion was increased 9-fold in STZ diabetic rats. This increase was reversed by 76% and 79% by 7 mg/kg and 70 mg/kg thiamine and 76% and 80% by 7 mg/kg and 70 mg/kg Bft. In the thiamine study, urinary albumin (mg/24 h) at 24 weeks was: controls 2.1 ± 0.7, control + 70 mg/kg thiamine 3.6 ± 1.3, diabetic 33.3 ± 22.2 ($P<0.01$), diabetic + 7 mg/kg thiamine 9.1 ± 1.0 ($P<0.001$, $P'<0.01$), and diabetic + 70 mg/kg thiamine 8.7 ± 1.8 ($P<0.001$, $P'<0.01$); n = 6 – 7. In the Bft study, urinary albumin (mg/24 h) at 24 weeks was: controls 1.9 ± 0.3, control + 70 mg/kg Bft 2.0 ± 0.3, diabetic 19.1 ± 8.1 ($P<0.01$), diabetic + 7 mg/kg Bft 6.1 ± 2.1 ($P<0.001$, $P'<0.01$), and diabetic + 70 mg/kg Bft 5.4 ± 1.6 ($P<0.001$, $P'<0.01$); n = 5 – 9. Thiamine and Bft at both doses had similar potency in the prevention of microalbuminuria. Inhibition of proteinuria by thiamine and Bft was dose-

dependent where the 70 mg/kg dose was more potent than the 7 mg/kg dose. Bft delayed the development of hyperfiltration in diabetic rats whereas thiamine did not. High dose thiamine increased the activity of transketolase and the reductive pentose phosphate pathway, leading to a decrease of triosephosphates and fructose-6-phosphate implicated in the mitochondrial dysfunction, protein kinase c_{β}, hexosamine and methylglyoxal-dependent glycation pathways linked to diabetic complications. High dose thiamine therapy, therefore, targets multiple pathways of biochemical dysfunction linked to diabetic nephropathy.
Conclusion: High dose thiamine and Benfotiamine prevented incipient nephropathy in experimental diabetes and are candidates for preventive therapy of clinical diabetic nephropathy.

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Delayed intervention with tranilast attenuates the progression of advanced experimental diabetic nephropathy.
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Background and Aims: The accumulation of extracellular matrix is a pathological hallmark of diabetic nephropathy and is directly related to declining renal function. Tranilast (n-[3,4-dimethoxycinnamoyl] anthranilic acid) an agent used for the treatment of keloid scars has been shown to allergic disease also suppresses matrix synthesis. with recent preliminary studies showing a beneficial effect in advanced diabetic nephropathy in humans.
Methods: Studies were conducted using a transgenic model, the diabetic (mRen-2)27 rat, which develops many of the structural and functional characteristics of human diabetic nephropathy when diabetes is induced with streptozotocin (STZ). An experimental design was chosen to mimic, in part, the clinical context with drug therapy (tranilast 400 mg/kg/day) initiated in established disease (8 weeks after streptozotocinSTZ) and in the presence of persistent hyperglycaemia and hypertension.
Results: At 16 weeks, diabetes was associated with progressive albuminuria, tubulointerstitial fibrosis, tubular atrophy. Without affecting blood pressure or blood glucose, tranilast attenuated both albuminuria, TGF-ß immunostaining expression and the tubulointerstitial pathology that developed in diabetic Ren-2 rats ($p < 0.01$). In addition, a trend towards a reduction in diabetes-induced glomerulosclerosis was also observed ($p = 0.057$). In vitro studies in primary cultures of human renal cortical fibroblasts demonstrated a reduction in transforming growth factor-ß (TGF-ß) induced hydroxyproline incorporation and fibronectin synthesis with tranilast 100 µM.
Conclusion: These findings indicate that despite persistent hyperglycaemia and hypertension, tranilast has anti-fibrotic actions in the Ren-2 model of experimental diabetic renal disease via mechanisms that might include blocking the actions of TGF-ß.

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Macrophage scavenger receptor- a knockout mice are protected against diabetic nephropathy.
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Background and Aims: Macrophage scavenger receptor A (MSR-A) is the multiligand and multifunctional receptor, which recognizes advanced glycation endproducts (AGEs) as well as modified LDL. Moreover, MSR-A plays a role in migration of macrophages. Although MSR-A is known to be involved in the process of atherosclerosis, its role in diabetic microvascular complications is not clarified. Infiltration of macrophages is one of the characteristic features of diabetic nephropathy, suggesting that MSR-A is involved in its development. In this study, we used MSR-A knockout mice to evaluate the role of MSR-A in the pathogenesis of diabetic nephropathy.
Material and Method: We induced diabetes in 8-week-old male MSR-A knockout mice and wild type (C57BL/6J) mice by streptozotocin injection. Mice were divided into 4 groups: 1) Diabetic MSR-A KO mice (DM-KO), 2) diabetic wild type (DM-WT) mice, 3) non-diabetic MSR-A KO mice (ND-KO) and 4) non-diabetic wild type mice (ND-WT). Mice were killed at 6 months after induction of diabetes and the kidneys were harvested. Blood pressure, HbA1c, total cholesterol, creatinine clearance (Ccr), urinary albumin excretion (UAE) were measured. Glomerular size and mesangial matrix area were measured by morphometry using NIH image and Photoshop (Adobe systems, CA, USA). The number of macrophages and expression of intercellular adhesion molecule-1 (ICAM-1) were examined by immunohistochemistry using specific antibodies. We measured serum AGEs level by ELISA.
Results: There was no significant difference in HbA1c, blood pressure and Ccr between DM-WT and DM-KO. DM-WT presented increased UAE, glomerular hypertrophy and mesangial matrix expansion ($p<0.05$). DM-KO mice revealed reduced glomerular size ($p<0.05$), and mesangial matrix area ($p<0.05$) as compared with DM-WT. In DM-KO, the number of infiltrated macrophages in glomeruli was remarkably reduced ($p<0.05$). On the other hands, serum AGEs levels were elevated in both diabetic groups than in non-diabetic groups. Expression of ICAM-1 in glomeruli was not changed in DM-WT and DM-KO.
Conclusion: Diabetic MSR-A knockout mice revealed ameriolated UAE, glomerular hypertrophy and mesangeal matrix expansion as compared with diabetic wild type mice, although the levels of serum AGEs and intraglomerular expression of ICAM-1 were not changed. These reno-protective effects seen in this model might be explained by reduced macrophage infiltration in renal tissues. The current results suggest that MSR-A-dependent migration of macrophages plays important roles in the pathogenesis of diabetic nephropathy.

PS 83
Hypertension

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Microalbuminuria and pulse pressure are powerful predictors of 25-year mortality in Type 1 diabetes.

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Background and Aims: The very long-term prognostic significance of microalbuminuria and blood pressure variables for all cause mortality in type 1 diabetes is not yet clarified.
Materials and Methods: In 1977, 272 patients were identified through outpatients' clinics and GP's. The cohort constituted a representative sample of adult type 1 diabetic patients in Aarhus County. Inclusion criteria were age 15 years or over (mean 33.9±12.8) and duration of diabetes 5 years or more (mean 18.0±10.5). Urinary albumin concentration (UAC), blood pressure, presence of macrovascular disease, degree of retinopathy, a composite measure for glycemic control, s-creatinine, s-cholesterol, and smoking habits (pack years) were determined.
Results: Three patients were lost to follow-up. All-cause mortality was 37% (99/269) over 25 years for the whole group. As indicated in the table baseline BP and pulse pressure increased significantly with increasing albuminuria levels as did total mortality over 25 y.
Cox multiple regression analysis (age-stratified) identified the following significant independent predictors of all cause mortality: microalbuminuria (RR 2.8; 95% CI [1.5-5.2]), duration of diabetes (1.6 pr 10 y [1.1-2.3]), macrovascular disease at baseline (1.9; [1.1-3.79]), smoking (1.9; [1.1-3.3]), glycemic control (2.3; [1.1-4.7]), and pulse pressure (2.8; [1.3-6.3]). Systolic, diastolic and mean BP was excluded from the model as was gender, s-cholesterol, s-creatinine and retinopathy Mortality in patients with UAC 20-200 mg/l was significantly increased compared with patients with UAC <20 mg/l (p<0.001) and similar in patients with short (<16 years) and long (>16 years) duration (p=0.9, age-corrected). For comparison age specific, sex specific, and calendar year specific mortality rates were obtained from the Danish Institute of Epidemiology. As indicated in the table relative risk (RR) for total mortality was significantly increased in the diabetic patients vs the background population.
Conclusion: This long-term study documents that abnormally increased urinary albumin concentration, pulse pressure, glycemic control and smoking, all potentially modifiable risk factors, are important predictors of increased mortality in type 1 diabetes.

u-Albumin	Baseline blood pressure	Baseline pulse pressure	25 y total mortality	RR vs background population
<20 mg/l	130/82	48	29% (58/202)	2.3
20-200 mg/l	136/86	50	52% (23/44)	5.8
>200 mg/l	154/98	57	78% (18/23)	13.3
p	<0.001/<0.001	0.02	<0.001	<0.001

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Differential impact of ambulatory blood pressure monitoring on microalbuminuria in diabetic and non-diabetic patients. The preferential role of systolic blood pressure and the night-time period.

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Background and Aims: Many studies confirm higher figures of BP in patients with Microalbuminuria (MA), but few have done so between the parameters of Ambulatory Blood Pressure monitoring (ABPM) and the degree of MA.
Aims are: 1. To evaluate the rate of MA in a population with cardiovascular risk with/without high blood pressure (HBP). 2. To correlate MA with ABPM parameters.
Materials and Methods: Study population: N=115 patients from a population with cardiovascular risk, 53 male, 62 female, aged 26 to 80 years (59 +/-12); 33 active smokers, 59 hypercholesterolemic (LDL > 160mg/dl), 83 hypertensive (WHO Criteria), 50 type 2 diabetics (WHO Criteria), 35 both diabetic and hypertensive.
Methods: MA: Albuminuria 30-300mg/24h (average of two determinations). Nephelometry. Beckman Array 360 Chemistry Analyzer. HbA1c assessed by HPLC ABPM: Spacelabs 90207. Three periods were evaluated:
1. Global (24-h) (GP),
2. Daytime (9:00 to 22:00 h) (DP) and 3) Sleep-time (22:00 to 9:00 h) (SP) periods.
3. Critical (6 a.m. to 9 a.m.) periods.
The following parameters were assessed: Average Systolic Blood Pressure (ASBP); Average Diastolic Blood Pressure (ADBP); Average Blood Pressure Mean (ABPM=(SBP-DBP)/3+DBP); Standard systolic and diastolic blood pressure Stress (Systolic > 125 and diastolic > 75 mmHg readings percentages ;SSBPS/SDBPS); Endothelial systolic and diastolic stress (Systolic > 110 mmHg and diastolic > 65 mmHg readings percentages; ESBPS/EDBPS) based on The Allied Irish Bank Study (J. Human Hypertens 1991), and Blood Pressure and heart rate variabilities (BPV and HRV).
STATISTICAL ANALYSIS: Chi-square test. Linear Regression
Results:
1. The 21.53% of patients with average blood pressure recordings > 125 (systolic) and/or 75 mmHg (diastolic) had MA vs 3.57 % of patients with average blood pressure recordings < 125/75 (p<0.025).
2. A statiscally significant association was found between MA and the following blood pressure Holter monitoring parameters: ASBP (r=0.35;p<0.001) and SSBPS (r=0.3;p<0.003) in GP; ASBP (r=0.020;p<0.0001), ABPM (r=0.20;p<0.04), SSBPS (r=0.28;p<0.04) and BPV (r=0.20;p<0.04) in DP; ASBP (r=0.31;p<0.02), SSBPS (r=0.27;p<0.01) and ESBPS (r=0.20;p<0.04) in SP.
3. In diabetic patients no correlation between HbA1c and MA was found.
Conclusion:
1. Systolic arterial blood pressure but not diastolic arterial blood pressure correlates with the rate of MA.
2. Both daytime and Sleep-time periods influence MA, but, in the night-time period, lower blood pressure recordings (110 mmHg) may be sufficient.
3. There is no influence of metabolic control on MA, in diabetic patients.

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Hypertension a major complication issue in normoalbuminuric Type 1 diabetic patients: a 4-year prospective study using ambulatory blood pressure monitoring.

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Background and Aims: Even in strictly normoalbuminuric, normotensive, type 1 diabetic patients strong correlations exist between cardiac autonomic function, urinary albumin excretion (UAE) and blood pressure (BP). In order to evaluate the time-dependent covariation in these parameters and to examine the predictive value of each of them for the subsequent development of hypertension and microalbuminuria, we conducted a 4-year prospective study including normoalbuminuric, normotensive type 1 diabetic patients.
Material and Methods: One hundred and thirty one patients entered the study. Once a year 24h ambulatory blood pressure measurement (AMBP), UAE measurements in 3 overnight urine collections, blood samples (HbA1c and serum creatinine), and heart rate variability tests (short term spectral analysis) were performed.
Results: Clinical characteristics at baseline visit: 72 males/59 females, age 37.5 ± 12.1 year, diabetes duration 18.6 ± 10.1 year, BMI 23.8 ± 2.7 kg/m^2, day-time AMBP 128/80 ± 11/6 mmHg, Geometric mean of 3 overnight UAE 5.2 x/% 2.7 µg/min, HbA1c 8.4 ± 1.1 % (non-diabetic range 4.4 - 6.4%).
Within the study period 19 patients were excluded from the study in a preplanned manner, 12 patients developed hypertension (both clinic BP≥140/90 and day-time AMBP≥135/85 at two consecutive visits), one person became microalbuminuric (geometric mean of three overnight UAE ≥ 15 µg/min),and two patients developed both hypertension and microalbuminuria. Four patients were excluded due to other reasons. At baseline, patients who subsequently developed hypertension and/or microalbuminuria were characterized by: older age (45 ± 8.8 vs. 37 ± 9.2 years, p =0.002), higher BP values (140/87 ± 7/6 vs. 127/79 ± 10/6 mmHg, p= 0.0001), higher UAE (6.2 x/% 1.9 vs. 4.2 x/% 1.7 µg/min p= 0.01), higher serum creatinine (97 ± 11 vs. 91 ± 10 µmol/l, p = 0.03), whereas they did not differ regarding smoking habits, duration of diabetes, HbA1c, BMI or heart rate variability parameters.
Improper handling of informative drop outs that are associated with the response can cause highly biased estimates in ordinary regression models.

We have used a dynamic regression model, where the covariates besides standard demographic variables also include the responses at the previous visit. The annual increase in day-time AMBP was 0.85 ± 0.17/0.35 ± 0.10 mmHg (p= 0.001).The annual increase in night-time AMBP was 0.65 ± 0.17/0.45 ± 0.11 mmHg (p= 0.001). The annual increase in UAE was 1.00 ± 1.01µg/min (NS).
Conclusion: This 4-year prospective study reveals hypertension as the major complication in normoalbuminuric type 1 diabetic patients as only three patients developed microalbuminuria. In this well characterized study population day-time AMBP increased annually with 0.85 / 0.35 mmHg.

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Hemodynamic parameters in diabetic and non-diabetic subjects; a case control study.
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Background and Aims: To assess pulse pressure (PP), mean arterial pressure (MAP), isolated systolic hypertension (ISH) in a type 2 diabetic population and compare the results with non-diabetic controls. To investigate associations between hemodynamic variables and diabetic complications.
Materials and Methods: 194 type 2 diabetic outpatients' files were cross sectionaly screened for sociodemographics, body mass index (BMI), serum levels of A1c, total cholesterol, systolic and diastolic blood pressure values (SBP, DBP), presence of hypertension, diabetic complications and smoking habits. Corresponding data of 142 non-diabetic controls, who, except for the presence of diabetes, were matched in terms of vascular risk factors with the diabetic group, were obtained from the records (total n=336, mean age 58.2±9.5 yrs, mean BMI 29.1±4.8 kg/m^2, mean total cholesterol 212.6±43 mg/dL, prevalence of hypertension 68% and smoking 18,5%). PP was calculated as SBP-DBP and MAP as DBP+1/3 PP (mean of the last 3 recorded measures being used). Hypertension was defined as receiving anti-hypertensive drug therapy and/or SBP >=140 mmHg and/or DBP>=90 mmHg. SBP >=140 mmHg and DBP <= 90 mmHg were accepted as ISH.
Results: Although matching for age, BMI, cholesterolemia, prevalence of hypertension and smoking, diabetic patients (m/f: 75/119, mean age 58.9±10 yrs, mean BMI 29.1±4.5 kg/m^2, mean diabetes duration 10.2±4.4 yrs, mean A1c 7.8±2.3%, 79.4% diabetic complications (at least one), 53.1% hypertensive, 23% ISH, mean PP 61.8±19.4 mmHg, mean MAP 106±15.9 mmHg, mean serum total cholesterol 211.6±46.5 mg/dL) had significantly higher PP (61.8±19.4 vs. 56.6±17.4 mmHg, p=0.012) and a higher prevalence of ISH (23.7% vs. 12.7%, p=0.008) compared to controls (m/f: 33/109, mean age 57.4±8.8 yrs, mean BMI 29.1±5.2 kg/m^2, 56.3% hypertensive, 12.7% ISH, mean PP 56.6±17.4 mmHg, mean MAP 108.8±15.5mmHg, mean serum total cholesterol 218.6±36.2 mg/dL). In diabetic patients rising PP was significantly correlated with age (r=0.26, p<0.0001), BMI (r=0.21, p=0.003), presence of diabetic complications (at least one) (r=0.33, p<0.0001) and diabetic retinopathy (r=0.2, p=0.007). Higher MAP in diabetic patients was related to BMI (r=0.28, p<0.0001) and diabetic complications (at least one) (r=0.38, p<0.0001). ISH was significantly associated with diabetic age (r= 0.18, p=0.009). In non-diabetic controls, PP and MAP were significantly correlated with age and BMI (r= 0.3, p=0.001 for both), whereas ISH correlated with age only (r=0.3, p=0.005).
Conclusions: Although PP was associated with the same cardiovascular risk factors (i.e. older age and overweight) in both groups; diabetic patients had higher PP than non-diabetic controls. In diabetic patients increased PP was associated with diabetic retinopathy, which is a micro vascular complication. These facts may hint at a different interpretation of PP as a clinical marker in terms of vascular risk in diabetic and non-diabetic patients.

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Evaluation of the blood pressure in women with gestational diabetes mellitus (GDM).
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Background: Several studies have shown that diabetes mellitus is often associated with hypertension, also in women with gestational diabetes mellitus (GDM).
Aim: The aim of the retrospective study was observation of blood pressure (BP) values in women with gestational diabetes mellitus. The case history of patients were examined basing oneself on the Gestational Diabetes Monitoring System (GeDiaMos).
Materials and Methods: We retrospectively studied 304 diabetic pregnant women consecutively recruited in Department of Gastroenterology and Metabolic Diseases University School of Medicine in Warsaw in the years 1989-2002. The study was performed on group of women with GDM type G1(n=266) and women with GDM type G2(n=38).
Results: Mean BP values in the third trimester have been found to be - 115.6(±12.5)/73.9(±8.9) mmHg. Mean blood pressure > 130/85 mmHg was found in 5.6% patients. Mean HbA1c value was 5.8(±0.5)%, mean body mass index (BMI) 28.2(±4,9) kg/m^2. Positive correlation was found between BP in III trimester of pregnancy and fasting glycaemia (systolic blood pressure r=0.186, P=0.0011; diastolic blood pressure r=0.182, P=0.0014). Linear regression analysis indicated statistically significant correlation between: BP and BMI (systolic blood pressure r=0.332, P<0.0001; diastolic blood pressure r=0.313, P<0.0001), between BP and age of women (systolic blood pressure r=0.117, P=0.041; diastolic blood pressure r=0.140, P=0.014). Analysis of the mean differences between BP measured in GDM women treated with insulin and treated with diet showed statistically significant increase of BP in group treated with insulin, resulting from worse metabolic control in this group (systolic blood pressure 120.5(±13.4) mmHg vs. 114.8(±12.3) mmHg P=0.0083, diastolic blood pressure (76,6±10) mmHg vs. 73.5(±8.7) mmHg, P=0.00466).There were no significantly differences between BP and infant's birth weight (systolic blood pressure r=0.046, P=0.42; diastolic blood pressure r=0.07, P<0.227) and no significantly differences between mean BP measured in group of GDM women having episodes of hypoglycemia and those without hypoglycemia (systolic blood pressure 114(±13.7) mmHg vs. 115.6(±12.5) mmHg, P=0.66; diastolic blood pressure 73(±7.8) mmHg vs. 74(±9) mmHg).
Conclusions: The increase in maternal blood pressure values in III trimester of pregnancy correlate with increase of fasting blood glucose, BMI, age of women. Higher values of BP have been found to be in group of women treated with insulin in comparison to women treated by diet, resulting from significantly worse metabolic control in the first mentioned group.

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Results of the prospective, population-based survey (JEVIN) of insulin treated patients with Type 1 and Type 2 diabetes mellitus: improvement of diabetes control and the management of arterial hypertension 1989/90 – 1999/2000.
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Background and Aims: In 1989/90 JEVIN started as prospective, 10 year follow-up, population-based survey of all insulin treated patients with type 1 and type 2 diabetes aged 16-60 years and living in the city of Jena (100,000 inhabitants), Germany. It aims to show the effect of decentralization of the health care system (1989/90-1994/95) and the implementation of structured patient education (structured treatment and teaching programs [TTP]). One further goal was the evaluation of changes in the management of arterial hypertension.
Materials and Methods: The quality of diabetes control and changes in hypertension treatment and control (defined as blood pressure >140/80 mmHg according to WHO) were examined in 190 (83% of the target population), 244 (90%) and 261 patients (90%) in 1989/90, 1994/95 and 1999/2000, respectively.
Results: Up to 1994/95 the HbA1c (HPLC, Diamat® , mean normal 5%) in patients with type 1 diabetes mellitus increased (1994/95 8.25± 1.75% vs 1989/90 7.60± 1.55%, p=0.002). In patients with type 2 diabetes HbA1c remained constant (8.75± 2.00% vs 8.90± 1.55, p=0.669). From 1994/95 up

to 1999/2000 there was a substantial improvement in HbA1c (type 1: 7.40± 1.50%, p<0.0001; type 2: 7.35± 1.25%, p<0.0001). Up to 1999/2000 87.7% of patients with type 1 (1989/90 0%, 1994/95 73.2%) and 96.6% of patients with type 2 diabetes (1989/90 0%, 1994/95 89.7%) participated in TTP's. Moreover, during the period from 1989/90 to 1994/95 both in type 1 and type 2 diabetes there was a substantial improvement in mean blood pressure control (p<0.05). Up to 1999/2000 the quality of blood pressure control remained stable.

	Type 1			Type 2		
	1989/90	1994/95	1999/2000	1989/90	1994/95	1999/2000
Number (n)	131	127	114	59	117	147
Systolic RR (mmHg	135,1±18,2	128,9±16,3	130,9±17,2	150,6±27,9	137,4±19,5	139,5± 18,4
Diastolic RR (mmHg)	84,9±9,1	79,6±13,1	80,0±8,9	91,4±13,2	82,1±12,5	84,1± 10,3

Of 131 type 1 and 59 type 2 diabetic patients, 114 (87.0%) and 52 (88.1%) had arterial hypertension at baseline. In 1999/2000 in 20/114 patients (17.5%, p<0.001) with type 1 and 62/147 patients (42.2%, p<0.001) with type 2 diabetes blood pressure values were at or above 140/80 mmHg. A greater proportion of hypertensive patients with type 1 (40.4% vs 21.4%, p<0.001) and type 2 (70.7% vs 45.8%, p<0.001) were treated with anti-hypertensive drugs at follow-up. The use of more than one anti-hypertensive drug increased (type 1: 1989/9 6.1% vs 1999/2000 14.0%, p=0,098/ type 2: 1989/90 22.0% vs 1999/2000 41.5%, p=0,004).
Conclusion: During the last decade there has been a substantial improvement in the quality of diabetes control and the management of hypertension. The broad implementation of structured patient education, intensified insulin therapy, blood glucose and blood pressure self-monitoring are major cornerstones for the improvement of quality of care.

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Addition of Irbesartan in Type 2 diabetic patients treated with ace inhibitors with persistent microalbuminuria and uncontrollEd hypertEnsion: the AIMEE Trial.
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Background and Aims: Most hypertensive patients with type 2 diabetes and incipient nephropathy remain uncontrolled in spite of full treatment with ACE inhibitors. The aim of this study was the assessment of the effectiveness and tolerance of the addition of an angiotensin receptor blocker (Irbesartan) to the previous treatment of these patients
Materials and Methods: 60 pacientes > 30 years old, with type 2 diabetes mellitus, uncontrolled hypertension (SBP ≥ 130 and/or DBP ≥ 85 mmHg) and persistent microalbuminuria (albumin excretion 20 - 200 µg/min.) in spite of treatment with full-dose ACE inhibitors (21.7% enalapril, 20.0% fosinopril, 13.3% trandolapril, 11.7% lisinopril, 10.0% ramipril, 10.0% captopril, 6.7% quinapril, 3.3% espirapril and 3.3% cilazapril) were recruited. Patients with plasma creatinine > 176.8 µmol/l (2 mg/dl), potassium > 5 meq/l or treated with angiotensin receptor blockers were excluded. 56.7% were women, their age was 60 ±15 years. 71.7% had additional antihypertensive drugs (41.7% diuretics; 13.3% calcium channel blockers, 16.7% adrenergic blockers). 70% had aspirin or clopidogrel and 66.7% had statins. Blood presure and heart rate, weight, weight and waist perimeter, fasting plasmatic glucose, HbA_{1C}, sodium, potassium, creatinine, lipid profile and albumin excretion (geometric mean of two or three consecutive overnight colections) were measured by standard procedures. Irbesartan 300 mg/day was added to the previous treatment of the patients without other changes in the previous antihypertensive treatments. After 2 and 6 months all measurements were repeated; compliance was assessed by pill counting, and tolerance by questionnaire
Results: 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 > 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); in DBP was 12.5 mmHg (11.2 - 13.9, p < 0.001). 20% of the patients reached adequate blood pressure control (p < 0.001). Weight, waist perimeter, heart rate, fasting glucose, creatinine, HbA1C, sodium and lipid profile did not change significantly. Potassium increased by 0.15 meq/l (p = 0.02) but no patients had clinical hyperkaliemia. Albumin excretion was reduced by 65.7% (61.7 - 69.7, p < 0.001); 36.7% of the patients reached normoalbuminuria. Linear regression analysis of percentual reduction in albumin excretion vs. reduction in mean blood pressure showed that there is a hipoalbuminuric effect of the addition of Irbesartán estimated in 29.4% (Pearson's R = 0.75, p < 0.001) independent of blood pressure reduction, plus and additional reduction of 2.5% for each mmHg of reduction in mean blood pressure.
Conclusion: Adding Irbesartan (300 mg daily) to the treatment of uncontrolled hypertensive patients with type 2 diabetes mellitus and microalbuminuria treated with ACE inhibitors was effective and well tolerated, achieving a significant reduction in blood pressure besides a remarkable fall in albumin excretion, which was in part independent of the fall in blood pressure

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A comparison of amlodipine and bendrofluazide as add-on therapy to ACE inihibition in hypertensive, Type 2 diabetic patients.
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on behalf of the Amlodipine (AML-UK-96-002) Investigators, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom.

Background and Aims: Effective blood pressure (BP) control is important in reducing macrovascular and microvascular risk in Type 2 diabetes. The majority of patients require more than one antihypertensive agent to achieve adequate BP control but there is little information on the most effective combination of drugs. The aim of this study was to compare the efficacy of amlodipine and bendrofluazide (BFZ) as add-on theray in hypertensive Type 2 diabetic patients already taking enalapril 20 mg daily.
Materials and Methods: 180 Type 2 diabetic patients with BP>140/90 (aged >60 years) or >130/80 mmHg (aged 45-60 years) despite enalapril 20 mg daily were randomised to receive amlodipine 5mg or BFZ 2.5 mg daily. The dose of both agents was doubled after 8 weeks if target BP was not reached [clinic BP<140/90 (aged >60 years) or <130/80 mmHg (aged 45-60 years)]. Doxazosin was added as third line agent if required. 24h blood pressure monitoring, urine albumin excretion (AER), glomerular filtration rate (GFR) and metabolic parameters were monitored for one year. Clinical characteristics were similar in the groups at baseline [all amlodipine vs BFZ; duration diabetes 8.1 (0.5-33.1) vs 7.1 (0.6 vs 24.2) years, clinic BP 158± 16/90±10 vs 158± 19/90± 8 mmHg, 24h SBP 144±16.5 vs 145±15.9 mmHg, 24h DBP 83± 8.9 vs 81± 7.8 mmHg, AER 7.2 (1.5-531.0) vs 7.7 (1.0-1470.3) µg/min, GFR 116± 34 vs 112± 38 ml/min, HbA_{1c} 7.4± 1.4 vs 7.5±1.4 %].
Results: The primary end-point, change in 24 h BP, was similar in the two groups (24h SBP -11.7±10.2 vs -13.9±11.2 mmHg, p=0.075; 24h DBP -6.9±6.1 vs -7.4±7.2 mmHg, P=0.775). There was no difference in any secondary end-point, including clinic BP(142±17/81± 8 vs 139±17/80± 8 mmHg), AER (7.7 (0.7-475.5) vs 5.3 (1.3-106.1 µg/min) and GFR (112± 33 vs 107± 37 ml/min). Metabolic parameters were unchanged throughout and similar in the 2 groups. In particular, fasting blood glucose (9.7±3.6 vs 9.5±3.8 mmol/l) and HbA_{1c} (7.2± 1.3 vs 7.5± 1.7 %) were similar. 74.5% of those on amlodipine and 74.4 % of those taking BFZ required uptitration to the higher dose. The proportion requiring doxazosin was similar in the two groups (20.3 vs 23.2 %). The number of withdrawals was also similar in the two groups.
Conclusion: Amlodipine and BFZ are similarly efficacious in reducing blood pressure and well-tolerated in Type 2 diabetic patients on ACE inihitors requiring second-line therapy. Doxazosin is effective as a third-line agent.

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Angiotensin converting enzyme polymorphism related to 24-h blood pressure in diabetic adolescents with Type 1 diabetes.
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Background and Aims: To assess the distribution of the insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene in type 1 diabetic children and adolescents and to evaluate possible association between ACE genotype and blood pressure (BP).
Materials and Methods: 124 normoalbuminuric [albumin excretion rate (AER)<20 µg/min], normotensive (normal clinic BP measurements) type 1 diabetic children and adolescents (male/female: 60/64, age: 14.2±2.5 years, diabetes duration: 5.2±1.8 years) were included in the study. ACE genotypes were assessed by polymerase chain reaction. Twenty-four hour ambulatory blood pressure monitoring were undertaken in all patients.
Results: The ACE genotypes were distributed as follows: 34 (27%) DD, 57 (46%) ID, 33 (27%) II. Age, gender, diabetes duration, HbA1c and body mass index did not differ in the three groups with different genotypes. Patients with DD genotype differed from ID and II patients in having higher mean 24-h diastolic BP (73.8±6.2 vs. 70.2±5.0 and 69.7±6.3 mmHg;

p=0.005) and lower diurnal variation in BP (11.8±4.6 vs. 14.2±4.2 and 14.8±4.3 %; p=0.011) as compared with ID and II genotype patients. AER related significantly with 24-h diastolic BP in patients with DD genotype (r=0.35; p=0.032) but not in ID and II patients.
Conclusion: DD genotype is associated with elevated 24-h diastolic BP and less diurnal variation in BP in normoalbuminuric diabetic children and adolescents. Diastolic BP rises concurrently with AER in patients with DD genotype.

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Raised erythrocyte sodium-lithium countertransport activity in Bangladeshi Type 2 diabetic subjects with family history of hypertension.
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Background and Aims: Raised Na^+/Li^+ countertransport (SLCT) activity in erythrocytes is claimed to be an early marker of nephropathy in type 1 diabetes mellitus. However, the role of the transport system in type 2 diabetes mellitus is still controversial. The present study aimed to explore the role of SLCT activity in RBC which is influenced by familial predisposition to hypertension in presence or absence of nephropathy.
Materials and Methods: 63 newly diagnosed type 2 Bangladeshi diabetic patients and 20 age- and BMI- matched control subjects were studied. The diabetic subjects were divided into 2 groups as diabetes with family history of hypertension (n=37) and diabetes without family history of hypertension (n=26). Diabetic subjects with familial predisposition to hypertension were further divided into normo- (n=16) and microalbuminuric (n=16) subgroups. Serum glucose was measured by glucose-oxidase; C-peptide by ELISA; lipid profile, blood urea, creatinine (serum and urinary) by enzymatic-colorimetric methods and albumin by immunoturbidimetry method. Lithium was measured by atomic absorption spectrophotometry.
Results: The mean age (yrs, M±SD) and BMI (kg/m^2) were 45±4 and 24.0±3.4 in patients and those were 47±9 and 22.4±3.8 in controls. SLCT activity was found to be significantly elevated in diabetic subjects with familial predisposition to hypertension when compared to Control [median (range), 0.067 (0.022-0.153) vs 0.046 (0.004-0.115) mmol Li/g RBC protein/h, p<0.006] and to diabetic subjects without familial predisposition to hypertension [0.072 ± 0.032 vs 0.039 ± 0.017 mmol Li/g RBC protein/h, p<0.001]. When SLCT activity was compared between normo- and microalbuminuric diabetic subjects with familial predisposition to hypertension, no significant difference was observed among them.
Conclusion: The study suggests that raised SLCT activity can be a marker of genetic predisposition to hypertension in diabetic population irrespective of presence of nephropathy.

PS 84
Lipids and Lipoproteins

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Is there a metabolic syndrome in Type 1 diabetes?
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Background and Aims: It is well known that the metabolic syndrome is an important reason for the poor prognosis of patients with type 2 diabetes. The aim of this study was to find out whether the features of the insulin resistance syndrome are also present in a subgroup of type 1 diabetes.
Materials and Methods: Type 1 diabetes was diagnosed according to WHO criteria and confirmed by low C-peptide levels and/or the presence of antibodies to islet cells, GAD or IA-2. Type 1 diabetic patients (n = 137) with age between 16 and 60 years, normoalbuminuria, HbA1c below 9.5 %, and without any other disease known to affect serum lipid concentrations were divided into two groups according to their fasting triglyceride level (cut-off point 1.1 mmol/l).
Results: Both groups did not differ with respect to age (37.0 ± 10.6 vs 35.8 ± 10.6 years), duration of diabetes (11.2 ± 11.2 vs 11.6 ± 10.2 years), sex distribution (54 vs 49 % men), HbA1c (7.5 ± 1.1 vs 7.7 ± 1.0 %), fasting C-peptide (0.15 ± 0.28 vs 0.11 ± 0.17 nmol/l), serum creatinine (79 ± 14 vs 80 ± 13 µmol/l), albuminuria (10.2 ± 4.9 vs 9.3 ± 5.4 mg/l), apolipoprotein AI (1.67 ± 0.37 vs 1.68 ± 0.33 g/l), and lipoprotein (a) (0.24 ± 0.28 vs 0.25 ± 0.29 g/l). Type 1 diabetic patients with triglycerides of at least 1.1 mmol/l (n = 57) had lower levels of HDL cholesterol (1.59 ± 0.46 vs 1.76 ± 0.49 mmol/l; p<0.025), higher levels of LDL cholesterol (3.38 ± 1.00 vs 2.91 ± 1.00 mmol/l; p<0.005), apolipoprotein B (1.04 ± 0.27 vs 0.88 ± 0.24 g/l; p<0.0005), uric acid (267 ± 89 vs 240 ± 66 µmol/l; p<0.025), and fibrinogen (3.3 ± 1.5 vs 2.9 ± 0.7 g/l; p<0.05), showed higher systolic (126 ± 18 vs 120 ± 14 mm Hg; p<0.025) and diastolic (77 ± 11 vs 73 ± 8 mm Hg; p<0.025) blood pressure, a higher body weight (72.4 ± 11.3 vs 69.4 ± 8.8 kg; p<0.05) and body mass index (25.0 ± 2.8 vs 23.8 ± 2.5 kg/m^2; p<0.005), needed more insulin (0.64 ± 0.27 vs 0.53 ± 0.17 U/kg body weight; p<0.005), and suffered more often from hypertension (21.1 vs 5 %; p<0.0025) and coronary heart disease (3.5 vs 0 %; p<0.05).
Conclusion: A subgroup of type 1 diabetes shows the typical cluster of the insulin resistance syndrome when compared with the remaining patients. Hence especially this subgroup may be at high risk for the development of life-shortening complications.

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Reduced paraoxonase mass in Type 1 diabetes and its clinical significance.
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Background and Aims: Paraoxonase (PON) activity and mass have been reported to be reduced in type 1 diabetes in some studies, but unchanged in others. The clinical significance of any reduction in PON mass or activity is also unclear as paradoxically genotypes associated with high PON activity are associated with increased vascular complications in some studies. The aim of this study was to establish whether PON mass and/or activity are altered in type 1 DM taking account of the strong genetic determination of PON. The clinical significance of any differences was examined by examining the relationship of PON1 genotypes that determine mass and activity with aspects of vascular disease.
Methods: In 400 men and women (50% with Type 1 DM) serum paraoxonase activity was measured by spectrophotometry as the increase in extinction at 412nm in a mixture of 0.1M Tris-HCl pH 8.0, 1mM CaCl2 and 1.2mM paraoxon. After addition of 10-20µl of serum per ml, the initial increase in extinction was followed at 25°C. Results are expressed in Units/L. PON mass (n=364) was measured using a competitive ELISA (µg/mL). Genotypes at the LEU55MET(rs854560) and GLN192ARG (rs662) polymorphisms were determined by restriction isotyping. Coronary artery calcification, a measure of atheroma burden was measured by electron beam CT.
Results: The GLN coding allele at rs662 and the MET coding allele at rs854560 were both associated with lower PON activity and mass (p<0.001 for allthese associations). Type 1 DM patients had substantially lower PON mass than controls with and without controlling for genotype but

differences in PON activity were of borderline significance (Table 1). PON genotypes were not associated with albumin excretion rate, or coronary artery calcification levels. However both higher PON mass and the higher mass associated genotype (LEU/LEU) at rs854560 were associated with higher systolic blood pressure (p=0.003 and p=0.001 respectively). **Conclusion:** Type 1 diabetes is associated with reduced PON mass but the clinical significance of this remains unclear.

Table 1 Difference in PON mass and activity between diabetic and non diabetic subjects

PON activity (units/L x1000)	mean difference between diabetic and non diabetic subjects(95% CI)	P-value
adjusted for		
age and sex	-1.7 (-17, 21)	ns
age, sex and genotype	-8.3 (-17, 0.5)	0.07
age, sex, genotype and HDL-C	-10 (-19, 1.4)	0.02
PON mass µg/mL		
adjusted for		
age and sex	-9 (-13, -4)	<0.001
age, sex and genotype	10.7 (-15, -6)	<0.001
age, sex, genotype and HDL-C	-12 (-16, -8)	<0.001

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Phospholipid transfer protein mass is elevated in Type 1 diabetes mellitus but does not explain the elevation in phospholipid transfer protein activity.

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Background and Aims: Type 1 diabetes is associated with substantial elevation in PLTP activity that may have important consequences for atherosclerosis risk. Plasma PLTP circulates as both inactive and active forms. Whether the elevated PLTP activity in type 1 diabetes is because of an increase in PLTP mass or an increase in the proportion of circulating PLTP that is in the active form is unknown as assays to measure PLTP mass have not yet been used in diabetes. We have compared PLTP mass between type 1 diabetic subjects and controls and have examined whether PLTP mass accounts for the increased PLTP activity in diabetes.
Methods: Fasting samples from 199 type 1 diabetic patients (mean diabetes duration 23 ± 7.5 years) aged 30-55 years and 200 age and sex matched controls were used. Plasma PLTP activity was measured with labeled liposome vesicles as the phospholipid donor and excess pooled human HDL as the phospholipids acceptor, expressed as the percentage of the activity in a reference pooled plasma in arbitrary units (AU). Plasma PLTP mass (µg/mL) was measured by a sandwich ELISA using two specific monoclonal antibodies to human plasma PLTP.
Results: PLTP mass was elevated in both men and women with DM (Table 1). Adjusting for triglycerides, LDL-C and HDL-C or ApoA1 and ApoAII the diabetic difference in PLTP mass remained apparent (a difference of 1.7 µg/mL in men, p<0.001 and 0.6 µg/mL in women, p=0.2). PLTP mass and activity were only weakly related in diabetic and non-diabetic subjects (r=0.23, p=0.001 and r=0.15, p=0.03, respectively). Even adjusted for PLTP mass, diabetes remained associated with elevated PLTP activity in men (a difference of 12 AU in men, p<0.001) and women (a difference of 14 AU p<0.001) and this was independent of lipids.
Conclusion: Although PLTP mass is elevated in type 1 diabetes this is to a lesser extent than PLTP activity and does not explain the elevation in PLTP activity. Thus in type 1 DM a greater proportion of PLTP may be in the active form or the specific activity of all forms may be increased. Neither the elevation in mass nor specific activity is explained by concomitant lipid or lipoprotein differences associated with diabetes.

Table 1 PLTP mass (µg/mL) by Diabetes and Sex

	Non-diabetic Mean (95% CI)	Diabetic Mean (95% CI)	p-value for diabetic difference
Men	9.7 (9.0-10.3)	12.2 (11.5-12.9)	<0.0001
Women	10.8 (10.1-11.5)	12.2 (11.4-12.9)	0.01
All	10.3 (9.8-10.8)	12.2 (11.7-12.7)	<0.0001

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Plasma lipoproteins and severity of coronary atherosclerosis in diabetic and non diabetic patients.

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Background: Most studies have related lipoprotein levels only to the presence or absence of coronary artery disease (CAD) but not to its angiographic extent. Whether CAD patients (pts), with or without noninsulin-dependent diabetes mellitus (NIDDM), have similar severity of CAD is in dispute. Furthermore, the determinants of the extent of CAD in pts with NIDDM are not well known. The aim of this study was to evaluate the relationships between risk factors (RF) and the extent of CAD in 282 NIDDM (68 +/- 10 years ; 70%, men) and 283 non diabetic pts (66+/- years; 65% men).
Methods: All the 565 consecutive pts underwent coronary angiography and none of them had a previous revascularization. The pt's characteristics, RF, treatment before hospitalization were recorded for each pt. Blood samples were drawn upon admission to the hospital. The extent of CAD atherosclerosis was assessed by 2 different scores, the gensini score and the Atherosclerotic score, calculated as the average severity [grades 0 (95% stenosis)] of all 15 coronary segments.
Results: NIDDM pts had a higher prevalence of hypertension (63 vs 42%) and a higher body mass index (29 vs 26 kg/m2) than non diabetic pts. HDL cholesterol (0.43 vs 0.48 g/L), LDL cholesterol (1.22 vs 1.30 g/L), and Lp (a)(0.34 vs 0.41 g/L) levels were significantly lower and triglycerides levels (1.66 vs 1.41 g/L) were significantly higher in NIDDM than in non diabetic pts. The Gensini (35 vs 27) and Atherosclerotic (0.50 vs 0.43) scores were significantly higher in NIDDM than in non diabetic pts. In backward stepwise multiple regression analyses, age, sex, admission with an acute coronary syndrome, a previous history of stroke or peripheral arterial disease, a diagnosis of CAD older than 1 month, and hypertension (for NIDDM only) were significantly ($p < 0.05$) associated with the 2 scores in NIDDM and non diabetic pts. In the same models, LDL cholesterol ($p < 0.05$) levels were associated with the Gensini score in NIDDM and non diabetic pts whereas HDL cholesterol (p<0.02), LDL cholesterol (p<0.02) and Lp(a)(p<0.006) levels were associated with the Atherosclerotic score only in non diabetis pts.
Conclusions: Lipoproteins are more strongly associated with the severity of coronary atherosclerosis in non diabetic than in NIDDM pts. Further studies are needed to study the role of lipoprotein particles as determinants of coronary atherosclerosis in NIDDM pts.

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Association of plasma lipids and oxidized LDL with apolipoprotein polymorphism in Type 2 diabetes.

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Background and Aims: Variation in the ApoE gene, coding for the three common isoforms E2, E3 and E4 is known to have a strong and consistent influence on plasma lipid levels and although their effects on myocardial infarction risk remain uncertain. We evaluated the ApoE genotypes distribution and allele frequency (E2, E3 and E4) in 531 type 2 diabetic subjects and the contribution of ApoE polymorphism on plasma lipid profile, including oxidized LDL (ox-LDL) concentration.
Materials and Methods: Patients age was 61 ±8 years, age at diagnosis of diabetes 48 ±12 years, duration of diabetes (DD) 13 ±10 years, BMI 28.7 ±5.3 kg/m2 and HbA1c 8.7 ±1.8 %. A fragment of 244 bp (exon 4) was amplified by PCR. Alleles were determined by CfoI digestion (RFLP) of the fragment spanning the polymorphic sites. Ox-LDL level was measured by a solid phase two-site enzyme immunoassay (Mercodia AB, Uppsala, Sweden).
Results: Genotype distribution E2E2-E2E3-E2E4-E3E3-E3E4-E4E4 was 0.5/11.9/0.8/76.8/9.7/0.3% and allele frequency was 0.876, 0.068 and 0.056 for E3,E2 and E4, respectively. Patients features, lipid profile and ox-LDL were compared in E3E3 (E3c, n. 415) versus E2 carriers (E2c = E2E3 and E2E2, n.59) and E4 carriers (E4c = E4E3, E4E4 and E2E4, n.57). The three groups did not differ as far as age, DD, BMI, HbA1c and systolic and diastolic blood pressure. E2c patients showed lower total cholesterol (E2c: 190 ±42, E3c: 205 ±42 and E4c: 200 ±43 mg/dL, p<0.04) and LDL cholesterol levels (E2c: 111 ±32, E3c: 127 ±35 and E4c: 128 ±34 mg/dL, p<0.005), while E4c showed the lowest HDL cholesterol concentration

(E2c: 48 ±12, E3c: 47 ±14 and E4c: 42 ±10 mg/dL, p<0.05). No differences were observed in triglyceride levels (E2c: 154 ±91, E3c: 152 ±86 and E4c: 158 ±90 mg/dL, p=0.92). Finally the three groups significantly differed for ox-LDL (E2c: 46 ±22, E3c: 55 ±25 and E4c: 60 ±27 U/L, p=0.006). This result is consistent by sex but differences are particularly relevant in females (E2c: 44 ±20, E3c: 56 ±25 and E4c: 69 ±25 U/L, p=0.004). Nevertheless, prevalence of cardiovascular disease was not different between E2c, E3c and E4c (around 25% in the three groups).
Conclusions: In type 2 diabetes, patients carrying the E2 allele showed a favourable lipid profile and lower levels of ox-LDL, but this associations did not translate into significant difference in risk of cardiovascular disease.

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Post-prandial triglycerides and their nutritional determinants in a population based sample of Type 2 diabetic patients.
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Background and Aims: Postprandial lipemia is an emerging risk factor for cardiovascular disease. Little information exists on the daily triglyceride profile and their determinants in free leaving diabetic patients. Aim of this work is to study the daily triglyceride profile in a population based sample of type 2 diabetic patients and evaluate the impact thereon of dietary habits.
Materials and Methods: One hundred forty five patients (66 males / 79 females, age range 45-65) residents of a health district in the province of Naples (Italy) were studied. Triglycerides were measured on capillary blood by dry chemistry (Accutrend, Roche, a previously validated method) on four different days at fasting; before, 2 and 3 hours after lunch; before, 2 and 3 hours after dinner. Average values were used in the analyses. Dietary habits were assessed by a dietitian with a semi-quantitative standardised, validated questionnaire.
Results: Triglyceride values (mmol/L, M±SD) were 2.22±0.93 at fasting, decreased before lunch (2.03±0.81), reached the peak three hours after lunch (2.73±1.11) and remained substantially high before dinner (2.47±1.0), that is 6-8 hours after lunch (all p<0.001 vs fasting). Fasting triglycerides correlated strongly with values three hours after lunch (r=0.66), however among those with optimal fasting values (less than 1.69 mmol/L) , 30% had triglycerides 3 hours after lunch between 1.69 and 2.25 mmol/L and 31% levels higher than 2.25 mmol/L. Plasma triglyceride increments (three hours after lunch minus before lunch concentrations) significantly correlated with amount (g/day) of total (r= 0.21, p<0.01), saturated (r= 0.19, p<0.01), monounsaturated (r= 0.17, p<0.01) and polyunsaturated (r= 0.19, p<0.01) fat intake. No correlation was observed with amount of carbohydrate intake (g/day).
Conclusions: The majority of type 2 diabetic patients have triglyceride levels above optimal (>1.69 mmol/L) for several hours after a meal. Elevated triglycerides in the postprandial state are observed in a fairly large proportion of patients with optimal fasting triglyceride levels. Fat intake is the major nutritional determinant of postprandial triglyceride increments.

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Serum levels of lipoprotein(a) are low and not predictive for survival and vascular events in patients with diabetes mellitus Type 2 referred to coronary angiography.
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Background and Aims: The little prospective data on lipoprotein(a) as a cardiovascular risk factor in patients with diabetes are contradictory. We therefore investigated the impact of this lipoprotein on the risk of death and vascular events in a prospective cohort study enrolling patients with and without diabetes mellitus type 2.
Materials and Methods: We measured lipoprotein(a) in 593 consecutive patients referred to coronary angiography. After a mean follow-up period of 2.29 ± 0.37 years the incidence of death and vascular endpoints was recorded.
Results: Lipoprotein(a) was significantly lower in patients with type 2 diabetes (n = 136) than in nondiabetic patients (n = 451; 11 mg/dl [0-30] vs. 16 mg/dl [0-51]; p = 0.025). At baseline lipoprotein(a) was predictive for the presence of significant stenoses of 50% or more in the total cohort (p = 0.001) and in nondiabetic patients (p = 0.001), but not in diabetic patients (p = 0.437). Prospectively, lipoprotein(a) proved independently predictive for overall death and cardiac death in the total cohort (p = 0.014 and p = 0.021) and in nondiabetic patients (p = 0.001 and p = 0.004), but not in patients with type 2 diabetes. Only among nondiabetic patients it was predictive for nonfatal vascular events (p = 0.035).
Conclusion: Among patients referred to coronary angiography, lipoprotein(a) is a predictor of death and vascular events in nondiabetic patients, but not in patients with type 2 diabetes. Therefore, in this population measurement of lipoprotein(a) provides useful information in nondiabetic patients but not in patients with type 2 diabetes.

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Low LDL cholesterol in diabetic patients with coronary atherosclerosis.
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Background and Aims: Diabetes mellitus (DM) is strongly atherogenic and induces a typical dyslipidemia with elevated triglycerides, and decreased HDL cholesterol. However, the serum levels of LDL cholesterol in DM are at dispute.
Materials and Methods: We enrolled 756 consecutive patients undergoing coronary angiography from October 1999 through October 2000. Comparisons were made between patients with DM (either established or newly diagnosed by means of oral glucose tolerance tests) and nondiabetic patients. LDL cholesterol was measured directly with QuantolipLDL (Roche, Switzerland), and the LDL peak particle diameter was estimated by polyacrylamide gradient gel electrophoresis in a subset of 337 patients. Patients taking lipid lowering drugs (n = 221) were excluded from the analyses.
Results: Patients with DM (n = 103) exhibited higher triglycerides (191.75 ± 125.26 mg/dL vs. 146.91 ± 85.31 mg/dL; p = 0.001), and lower HDL cholesterol (43.94 ± 13.01 mg/dL vs. 50.49 ± 14.52; p <0.001). Interestingly, significantly lower LDL cholesterol (123.05 ± 37.29 mg/dL vs. 135.27 ± 137.41 ± 33.62 mg/dL; p = 0.001) was found in patients with DM. ApoB was similar in patients with and without diabetes (113.50 ± 28.59 mg/dl vs. 115.57 ± 24.33; p = 0.419), whereas the ApoB/LDL cholesterol ratio was significantly lower in patients with diabetes (1.08 ± 0.20 vs. 1.19 vs. 0.17; p <0.001). Patients with diabetes had a significantly lower LDL peak particle diameter (257.53 ± 7.88 nm vs. 259.62 ± 7.34 nm; p = 0.021) than nondiabetic patients.
Conclusion: Coronary patients with diabetes mellitus exhibit low LDL cholesterol in addition to the low HDL/high triglyceride pattern. The lower LDL cholesterol is at least in part attributable to a lower LDL peak particle diameter.

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Acute elevation of plasma free fatty acids (FFA) increases the macrophage migration inhibitory factor (MIF) in healthy subjects.
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Macrophage migration inhibitory factor (MIF) is a proinflammatory pituitary and immune cell cytokine, secreted from the pituitary in response to stress or inflammatory stimuli. Elevated levels of MIF has been reported in patients with type 2 diabetes and in vitro studies have shown that MIF induces an increase TNF-α expression in adipocytes. The activation of the inflammatory pathways has been proposed to be one of mechanisms by which FFAs induce insulin resistance. We have previously shown that acute elevation of FFAs causes activation of NF-κB and reactive oxygen species generation. To further characterize the acute pro-inflammatory effects of fatty acids, we induced an increase in plasma FFA concentrations with a lipid and heparin infusion for 4 h in 10 healthy subjects (7 males and 3 females, age; 28.7± 5.6 yrs, BMI 24.5 ± 5.6 kg/m^2). Infusions were stopped at 4 h and blood samples were collected at 0, 2, 4 and 6 h. Fifty ml of normal saline was infused for 4 h in the same subjects on another day to provide control samples. Plasma FFA concentrations increased from 0.37 ± 0.15 mM to 0.73 ± 0.14 mM and 1.26 ± 0.59 mM at 2 and 4 h declining to 0.34 ± 0.05 mM at 6 h. Plasma MIF concentrations increased from a median of 1217 [770-1619] pg/ml at baseline to a median of 2144 [1353-2519] pg/ml ($P < 0.01$) at 2 h and 2660 [2024-4109] pg/ml ($P < 0.001$) at 4 h and declined to a median of 1477 [1015-4795] pg/ml (P = ns) at 6h. Saline or 5% dextrose infusion for 4 h did not alter the plasma MIF concentration. We conclude that FFAs, induce a pro inflammatory process which is in parallel with our previous observations of FFA on activation of NF-κB and reactive oxygen species generation.

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Effect of D-glucose on human serum paraoxonase (PON1) gene transcription in cultured HepG2 cells.

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Background and Aims: Human serum paraoxonase (PON1) is associated with high-density lipoprotein, and inhibits oxidative modification of low-density lipoprotein. The liver plays a key role in the PON1 synthesis, and to date, the PON1 gene expression has been observed only in the liver. We have previously demonstrated that Sp1 is a positive regulator of PON1 transcription, and that an interaction between Sp1 and protein kinase C (PKC) is a cruicial mechanism for the effect of Sp1 on PON1 transcription in cultured HepG2 cells. Since several PKC isoforms are known to be activated under hyperglycemic conditions, we examined an effect of D-glucose, which can activate diacylglycerol-PKC pathway, on the PON1 promoter activity.
Materials and Methods: For a reporter gene assay, a DNA fragment of the promoter region of PON1 gene (-1230/-6) was amplified using a PCR method, and the DNA fragment was introduced into the firefly luciferase expression vector. Transient transfection into HepG2 cells was performed using a cationic lipid method, and luciferase activity was determined at 48 hrs after transfection. Comparisons between 2 groups were made using the unpaired Student's *t* test. *P* values less than 0.05 were considered significant.
Results: D-glucose enhanced PON1 promoter activity in dose- and time-dependent manners (the promoter activity after a treatment with 25 mM D-glucose for 48 hrs was 2~3-fold higher as compared to that with 5 mM D-glucose). However, L-glucose or mannitol has no effect on PON1 promoter activity. Both the PKC inhibitor bisindolylmaleimide (10 μM) and Sp1 inhibitor mythramycin (100 nM) significantly inhibited the D-glucose-induced PON1 promoter activation.
Conclusion: It has been reported that Sp1 activation by PKC is one of key mechanisms in regulation of several gene expression such as vascular endothelial growth factor, platelet-derived growth factor, and insulin-like growth factor II. Our data suggest that D-glucose enhances PON1 promoter activity through Sp1 activation by PKC.

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Fibrates disrupt mitochondrial respiration in isolated rat skeletal muscle.

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Background and Aims: Fibrates are applied for the treatment of lipid disorders as frequently associated with type 2 diabetes. Muscle damage (rhabdomyolysis) is a rare side effect of fibrate treatment, in particular when fibrates are combined with statins. Rhabdomyolysis can occur as a consequence of mitochondrial dysfunction or of defective glycogen storage. In this context it is of note that fibrates have been described to affect mitochondrial bioenergetics in vitro. We used isolated rat skeletal muscle to investigate the direct effects of fenofibrate and clofibrate on mitochondrial respiration and glycogen storage.
Materials and Methods: The influences of fenofibrate and clofibrate on glycogen storage as well as on aerobic and anaerobic energy metabolism were examined in isolated strips of soleus muscle from male Sprague-Dawley rats. Muscle specimens were incubated for 24 h with or without the fibrates, and the rates of insulin-stimulated (100 nmol/l) lactate release and CO2 production from palmitate were measured during the subsequent hour. Glycogen content was measured at the end of the experiment. To analyze the activity of enzyme complex I of the respiratory chain, specimens of red gastrocnemius muscle were sampled, homogenized, and sonicated to disrupt the plasma and mitochondrial membranes. Complex I activity was measured in the homogenates in the absence or presence of fenofibrate and clofibrate using a spectrophotometric assay. Specificity of the assay was controlled with 1 μmol/l rotenone, a specific inhibitor of complex I, which reduced enzyme activity by -96±2%.
Results: In isolated specimens of soleus muscle, both fibrates (100 μmol/l) significantly increased lactate release (fenofibrate, +17±6%; clofibrate, +27±8%; $p<0.01$ each) and reduced glycogen content (fenofibrate, -20±5%; clofibrate, -17±4%; $p<0.002$ each). A decrease in CO2 production from palmitate was seen only with fenofibrate (fenofibrate, -22±7%, $p<0.005$; clofibrate, -4±6%, ns). Furthermore, both fenofibrate and clofibrate had an immediate inhibitory action on the activity of respiratory complex I in muscle homogenates. Fenofibrate had a higher inhibitory effect (% reduction in complex 1 activity; 10 μmol/l, -41±7%, 30 μmol/l, -70±2%, 100 μmol/l, -78±4%, $p<0.001$ each) when compared to clofibrate (10 μmol/l, +4±7%, 30 μmol/l, -2±7%, 100 μmol/l, -27±7%, $p<0.005$ at 100 μmol/l).
Conclusion: Our findings show that fenofibrate and clofibrate directly inhibit enzyme activity of the respiratory complex I, which could be the cause of impaired mitochondrial respiration and of reduced glycogen content observed in fibrate-exposed skeletal muscle in vitro. Reduced mitochondrial function resulting from inhibition of complex I, therefore, could be responsible for myopathies that have been reported in association with fibrate treatment.

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Assessment of LDL-C and triglycerides efficacy of atorvastatin at different starting doses in patients with Type 2 diabetes.

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Background and Aims: Patients with diabetes are at high risk for cardiovascular disease and diabetes is considered a CHD risk equivalent. Dyslipidemia is one of the most important risk factors for the development of cardiovascular disease. The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) recognizes LDL-C as the primary target in patients with diabetes with an LDL-C goal <100 mg/dL and considers TG<150 mg/dL as optimal. HMG CoA reductase inhibitors (statins) are the drugs of choice for patients with elevated LDL-C and TG. Many dyslipidemic patients with diabetes, however are still not reaching their LDL-C goal and have elevated TG levels. This may be because they were not started at the appropriate statin dose or the dose was not titrated properly. The recommended start dose for atorvastatin is 10 or 20 mg daily and for patients requiring LDL-C reduction >45%, 40 mg once daily.

Materials and Methods: We describe the recent results of The New Atorvastatin Starting Doses: A Comparison (NASDAC) study comparing the LDL-C and TG efficacy and safety of atorvastatin at starting doses of 10 mg, 20 mg, 40 mg, and 80 mg/day in patients with type 2 diabetes. NASDAC is an 8-week, multicenter, double-blinded, randomized, comparative, parallel-arm trial. Following an 8-week placebo washout period, a total of 919 patients, were randomized to atorvastatin 10 mg, 20 mg, 40 mg, or 80 mg daily for 8 weeks, irrespective of their baseline LDL-C. 147 subjects (16%) had type 2 diabetes (85 males, 62 females, mean age 64 yr, mean weight 203lbs, 83.4% white). The primary efficacy parameter was the mean percent reduction from baseline in LDL-C and TG at Week 8.
Results: At baseline mean LDL-C levels were >160 mg/dL and mean TG >190 mg/dL in all 4 dose groups. In the patients with diabetes, atorvastatin significantly reduced LDL-C (42.1%–54.6%, P<0.001) and TG (21-45%, P<0.001) levels across the 10–80 mg dose range. The 20, 40 and 80 mg doses each provided significantly greater decreases in LDL-C and TG than all lower doses (p<0.001). All doses also significantly reduced total cholesterol, LDL-C/HDL-C ratio and apoB and raised HDL-C from baseline. The proportions of all subjects with diabetes that achieved the LDL-C goal <100 mg/dL on 10, 20, 40 and 80 mg atorvastatin were 58%, 63%, 83% and 90%, respectively. Similar trends were observed in the other risk category groups with a larger proportion of subjects receiving higher starting doses of atorvastatin achieving goal than subjects receiving lower starting doses. Atorvastatin was well tolerated at all dose levels.
Conclusion: This study showed that atorvastatin initiated at starting doses of 10, 20, 40 and 80 mg was effective and safe for lowering LDL-C and TG levels in patients with diabetes. Patients with diabetes at high risk of CHD can benefit from starting the appropriate doses of atorvastatin to achieve their LDL-C goal and optimal TG levels.

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The effect of pravastatin on LDL oxidation and myocardial blood flow in young adults with Type 1 diabetes.

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Background and Aims: Type 1 diabetes has been associated with increased oxidative stress and impairment in vascular function. Since statins may attenuate oxidative susceptibility of LDL, we hypothesized that pravastatin may decrease LDL oxidation and improve myocardial blood flow capacity in patients with type 1 diabetes with normal LDL cholesterol levels.
Materials and Methods: In this randomized, double-blind study LDL oxidation and myocardial blood flow was measured in 42 normocholesterolemic patients (age 30 ± 6 years, LDL cholesterol 2.48 ± 0.57 mmol/l) with non-complicated type 1 diabetes before and after 4 month treatment with pravastatin 40 mg/day or placebo. Oxidized LDL was measured by determining the level of baseline diene conjugation in lipids extracted from LDL. Blood flow was measured with positron emission tomography (PET) and $[^{15}O]H_2O$ using dipyridamole as a vasodilating agent during euglycemic clamp.
Results: The level of LDL oxidation was similar in the pravastatin and placebo groups before treatment (23.9 ± 4.6 vs 25.6 ± 9.5 µmol/l, respectively). Also, no difference in the level of either resting or dipyridamole-induced myocardial blood flow was seen between the pravastatin and placebo groups at baseline (1.06 ± 0.27 vs 1.03 ± 0.24 ml/min/g and 4.42 ± 1.32 vs 3.99 ± 1.19 ml/min/g, respectively). Oxidized LDL decreased significantly during pravastatin treatment to 19.5 ± 5.0 µmol/l (p<0.005). Nevertheless, no change in either resting or dipyridamole-induced myocardial blood flow was seen following treatment. Glycemic control and whole-body glucose uptake remained unchanged during treatment.
Conclusion: In conclusion, four month treatment with pravastatin decreases baseline diene conjugation as a measure of LDL oxidation in young normocholesterolemic patients with non-complicated type 1 diabetes. However, no improvement in myocardial blood flow capacity could be demonstrated suggesting that mechanisms other than oxidative modification of LDL cholesterol are responsible for the impairment in myocardial vasodilatory function in type 1 diabetes.

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Effects of ezetimibe added to on-going statin therapy on LDL-C goal attainment in high risk hypercholesterolemic patients with or without Type 2 diabetes mellitus.

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Background and Aims: Larger LDL-C reductions can be achieved when statins are coadministered with the cholesterol absorption inhibitor, ezetimibe (EZE).
Materials and Methods: To assess the efficacy of statin+EZE on LDL-C goal attainment and overall lipid responses in high risk hypercholesterolemic (HC) patients with and without type 2 diabetes (DM2), post-hoc analyses were performed on data from a study of 769 HC patients already on statin monotherapy and then randomized to placebo (Pbo) or 10 mg EZE for 8 weeks. For the present analysis, high risk patients with coronary heart disease (CHD) and/or DM2 (LDL-C goal ≤ 2.6 mmol/L) were divided into those with (n=191) and without (n=330) DM2. Statistical adjustments were made for potential imbalances in baseline parameters (eg. gender, age, CHD status, lipid levels).
Results: Baseline LDL-C, HDL-C, non-HDL-C, and triglyceride (TG) levels, respectively, were 3.1, 1.2, 3.9 and 1.9 mmol/L in the DM2 group, and 3.3, 1.3, 4.1 and 1.7 mmol/L in the non-DM2 group. Forty nine % of the DM2 patients also had CHD. Of patients with baseline LDL-C >2.6 mmol/L, more DM2 than non-DM2 patients reached LDL-C goal on statin + EZE (table); however, the adjusted odds of reaching goal on statin + EZE versus statin + Pbo [odds ratio (95% CI) = 26.4 (13.7, 50.7)] were not significantly different for the 2 subgroups. The effects of EZE on lipids were consistent between patients with and without DM2 (table). HbA1c levels remained stable for all DM2 patients (~7.0%) during the 8 week treatment. Statin+EZE was well tolerated and had a safety profile similar to statin+Pbo.
Conclusion: Thus, for patients who fail to reach LDL-C goal with statin monotherapy, adding EZE provides significant LDL-C lowering and facilitates LDL-C goal attainment in high risk HC patients with or without DM2.

	DM2		non-DM2	
	Statin + Pbo	Statin + EZE	Statin + Pbo	Statin + EZE
% of patients attaining LDL-C goal	17	84	20	67
LDL-C % change	-2	-27	-1	-24
TG % change	-5	-16	-3	-12
non-HDL-C % change	-2	-25	-1	-22
HDL-C % change	2	2	2	4

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Pioglitazone in combination with sulfonylurea results in changes in lipid subspecies and subparticle profiles in patients with Type 2 diabetes.

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Background and Aims: Dyslipidemia in type 2 diabetes is commonly associated with increased triglycerides, decreased HDL, and increased small LDL subparticles. A higher proportion of small, dense LDL particles tends to be linked directly to a higher risk for cardiovascular events. Two relevant LDL phenotypes have been determined: pattern B with small dense particles and pattern A with large, more buoyant particles. We evaluated whether treatment with pioglitazone (PIO) in combination with sulfonylurea (SU) resulted in changes from baseline in the lipid subspecies and subparticle profiles.
Materials and Methods: Blood samples were obtained from patients with type 2 diabetes mellitus who participated in a randomized double-blind clinical study to examine the effects of PIO in combination with SU. Samples were obtained from patients at Baseline, Week 12, and Week 24. A total of 95 subject sets (45 at 30 mg PIO, 50 at 45 mg PIO) were randomly chosen from a blinded subject list. In this LOCF analysis, missing values at Week 24 were replaced by corresponding values from Week 12. Missing values at Week 12 were not replaced. Samples for each subject set were analyzed for LDL subparticle profile.
Results: There were statistically significant increases in average and peak LDL particle sizes compared to baseline. The overall shifts in LDL particles subclasses (large, intermediate, small) from baseline were also statistically

significant. The percentage of large (A) LDL particles increased, while the percentage of small (B) LDL particles correspondingly decreased. These changes were statistically significant.

Lipid Subspecies Parameters	Baseline	Δ from baseline Week 12	Week 24
	(n = 95)	(n = 72)	(n = 95)
Average LDL particle size (Å)	255.3	3.9*	2.9*
Peak LDL particle size (Å)	254.0	5.5*	4.0*
LDL Large A (%)	30.11	13.37*	9.64*
LDL Small B (%)	51.20	-14.56*	-10.80*

*$P < .0001$ when compared to baseline.

Conclusions: The results of this analysis demonstrate that treatment with PIO in combination with SU significantly increases average and peak LDL particle size and significantly shifts LDL subclass (large, intermediate, small) category distribution. This shift is primarily observed in significant increases in large (A) LDL particle percentages and significant decreases in small (B) LDL particle percentages.

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The long-term effects of pioglitazone (PIO) and glibenclamide (GLB) on plasma lipids in patients with Type 2 diabetes (T2D).

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Background and Aims: Previous studies with PIO have investigated its short-term (≤6 months) anti-hyperglycemic and lipid-altering effects compared with placebo. We studied the long-term (1 year) effects of PIO and micronized GLB on insulin sensitivity (primary endpoint) and plasma lipids (secondary endpoint) in T2D patients.
Materials and Methods: In this randomized, double-blind, multicenter study in Scandinavian countries, patients with T2DM (HbA1c >7.5% and ≤11% for those not on oral anti-hyperglycemic medication (OAM), or HbA1c >7.5% and ≤9.5% for patients on 1 OAM [sub-maximal dose]) were randomized to either PIO (initially 30 mg QD, n = 91) or GLB (initially 1.75 mg QD, n= 109) as monotherapy. Doses were titrated to achieve glycemic targets during the next 12 weeks; it was recommended that this dose be maintained for the remainder of the study. Fasting plasma TG, cholesterol (total and HDL), glucose, insulin and HbA1c were determined at various times during the study. HOMA-S, atherogenic index of plasma (AIP = log TG /HDL-C) and LDL-C were calculated. Data were analyzed by an analysis of covariance using intention to treat patients (ITT) (last observation carried forward).
Results: In each group, approximately 30% of the patients were OAM-naive at baseline. Body weight increased 1.1 ± 0.4 kg and 3.0 ± 0.50 kg for GLB and PIO, respectively (p=0.003).

Variable	Group(n)	Baseline mean±SD	LS Change mean±SEM	p-value vs baseline	p-value vs GLB
TG mM	GLB (96)	2.3 ± 1.9	-0.03 ± 0.10	0.752	0.019
	PIO (82)	2.0 ± 1.1	-0.36 ± 0.11	0.001	< 0.001
HDL-C mM	GLB (92)	1.1 ± 0.3	0.03 ± 0.03	0.325	
	PIO (82)	1.2 ± 0.3	0.21 ± 0.03	< 0.001	0.141
LDL-C mM	GLB (84)	3.5 ± 0.8	-0.03 ± 0.08	0.744	
	PIO (79)	3.6 ± 0.9	0.14 ± 0.09	0.123	
Total-C /HDL-C	GLB (92)	5.3 ± 1.6	- 0.07 ± 0.13	0.603	0.004
	PIO (82)	5.1 ± 1.6	- 0.59 ± 0.14	< 0.001	
AIP	GLB (92)	0.2 ± 0.3	-0.02 ± 0.02	0.497	0.001
	PIO (82)	0.2 ± 0.3	-0.12 ± 0.03	<0.0001	
HOMA S (%)	GLB (87)	99 ± 64	-13.0 ± 5.5	0.020	0.001
	PIO (74)	84 ± 51	17.0 ± 6.0	0.006	
HbA1c (%)	GLB (96)	8.5 ± 0.8	-0.4 ± 0.14	0.002	0.789
	PIO (83)	8.4 ± 0.7	-0.5 ± 0.15	0.001	

Conclusion: PIO and GLB produced similar reductions in HbA1c. Neither drug treatment produced significant changes in LDL-C. PIO decreased TG, AIP, and Total-C/HDL-C compared with GLB. In addition, PIO increased HDL-C and HOMA-S compared with GLB. These data suggest that PIO may have long-term beneficial effects on lipids in patients with T2D.

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Effects of pioglitazone, gliclazide and metformin on high density lipoprotein subfractions in early Type 2 diabetes.

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Background and Aims: Reduced high density lipoprotein (HDL) cholesterol is associated with macrovascular disease. Increasing HDL may reduce this risk. HDL particles can be separated into subclasses by density (HDL2 and HDL3 - HDL2 being more cardioprotective) and by apolipoprotein (apo) content (apoA1 only and both apoA1 and A2 - apoA1 being more cardioprotective). Thiazolidinediones may have beneficial effects on total HDL (HDLT). Effects on subfractions and apos are less clear, with little comparative data to other oral hypoglycaemics. We have studied effects of pioglitazone, metformin and gliclazide on HDLT, HDL subfractions and apoA1 and A2.
Materials and Methods: 60 overweight type 2 diabetes patients on diet (HbA1c > 7%) or oral monotherapy (HbA1c < 7.5%) without lipid treatment were recruited. After 3 months dietary run-in off drug, patients were randomised to metformin, pioglitazone or gliclazide. Over 3 months drug doses were uptitrated 4 weekly aiming for a fasting glucose <7mmol/l. Doses were then fixed for 3 months with blood drawn for HDL and HDL subfractions at 0 and 6 months. HDLT and HDL3 were prepared in a two-step precipitation process. Cholesterol, free cholesterol, triglycerides, apoA1 and apoA2 were measured in HDLT and HDL3. HDL2 values were calculated.
Results: HbA1c, cholesterol and triglycerides were comparable across groups at baseline and over time. On pioglitazone HDLT cholesterol increased (1.28:1.36mmol/l; p=0.02), unlike with metformin (1.26: 1.18mmol/l; p=0.16) or gliclazide (1.39:1.37mmol/l; p=0.31). An overall significant difference in HDLT between treatment groups (p=0.001) was largely due to difference between pioglitazone and metformin (p=0.026). The apoA1/apoA2 ratio was reduced on pioglitazone (-5% p=0.03) in contrast to a rise on metformin (+5.2% p=0.18) and no change on gliclazide. HDL3 cholesterol was reduced (0.9 to 0.85mmol/l p=0.005) only on metformin. There were significant falls in HDL3 apoA1/AII ratio on gliclazide (7.7%; p=0.01) and pioglitazone (7.7%; p=0.03) not seen on metformin. There were no changes in HDL2 apo AI/AII.
Conclusion: Oral hypoglycaemics vary in their effects on HDL. The net metformin effect seems to be anti-atherogenic with a shift from HDL3 and a relative apoA1 increase. This may partly explain the positive effect of metformin and not gliclazide in UKPDS. The significance of the pioglitazone changes is unclear. The HDLT reduction is potentially anti-atherogenic but the importance of the proportional decrease in apoA1 overall and particularly in HDL3 is uncertain. Ongoing outcome studies may tell us how HDL changes on pioglitazone impact long term outcomes.

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Oxidative stress influenced by simvastatin or fenofibrate treatment in Type 2 diabetic patients with dyslipidemia.

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Background and Aims: A decrease in serum lipid concentrations may influence oxidative stress which plays an important role in the pathogenesis of atherosclerosis. The aim of this study was to compare the effect of two groups of hypolipidemic agents represented by simvastatin (S) and fenofibrate (F) on selected parameters of oxidative stress in Type 2 diabetic patients with dyslipidemia.
Materials and Methods: Twenty Type 2 diabetic patients (12 men, age 57±4 yrs, duration of diabetes 7±2 yrs, BMI 30.5±3 $kg.m^{-2}$) with dyslipidemia were examined. After randomization of this cross-over study 10 patients were treated with 20 mg of simvastatin and 10 patients with 200 mg of micronized fenofibrate in one daily dose for 3 months. After 2 months of wash-out period the therapy was crossed and treatment continued for another 3 months. Laboratory samples for determination of serum total, HDL-, and LDL- cholesterol (TC, HDL-C, LDL-C), triglycerides (TG), ascorbic acid (AA), alpha-tocopherol (AT) and plasma malondialdehyde (MDA) concentrations and of superoxide dismutase (SOD) activity in erythrocytes were collected before and after treatment by each drug. AT concentrations were related to the sum of TC and TG. Results were statistically evaluated by paired t-test and Pearson's correlation.
Results: Diabetes control did not change significantly during study (HbA_{1C} 9.0±2.1% vs 9.0±2.3% before and after simvastatin, 9.0±2.4% vs 9.0±2.2% before and after fenofibrate). The results of laboratory variables are shown in the Table.

	TC (mmol/l)	TG (mmol/l)	MDA (μ mol/l)	SOD (IU/ml)	AA (μ mol/l)	AT/(TC+TG) (mg/mmol)
S0	6.62± 0.83	3.61± 2.09	2.56± 0.45	0.88± 0.30	56± 23	1.79± 0.26
S3	5.19± 0.75^{y}	3.25±1.98	2.39±0.50	1.01±0.37	75± 21^{y}	1.79± 0.32
F0	6.52± 0.65	4.14± 2.96	2.78± 0.40	0.92± 0.39	54± 21	1.81± 0.37
F3	5.97± 0.92^{x,b}	2.46± 1.42^{x,a}	2.36± 0.37^{x}	0.93± 0.40	50± 18^{b}	1.76± 0.33

Table 1: Biochemical variables before and after simvastatin (S0, S3) or fenofibrate (F0, F3) treatment. Statistical significance of differences between treatment $^{a}p<0.05$, $^{b}p<0.001$, and between samples of the same treatment $^{x}p<0.01$, $^{y}p<0.001$.

A significant decrease of TC and LDL-C but not TG caused by simvastatin was accompanied by increase of SOD activity and AA concentration without changes of MDA concentration. On the contrary, fenofibrate caused a decrease of TG but less of TC concentrations which were accompanied by significant decrease of plasma MDA without changes of SOD activity or AA values. No changes were found in AT concentrations related to TC and TG. Significant relationship was found between MDA and TG concentrations before and after treatment ($p<0.01$).

Conclusions: Simvastatin and fenofibrate have different mechanisms of action and different power to decrease either serum cholesterol or triglyceride concentration. Our results support a suspicion that fenofibrate diminishes oxidative stress whereas changes caused by simvastatin may be due to the activation of antioxidative mechanisms together with decreased elimination of ascorbic acid.

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A lipoprotein lipase activator, NO-1886 prevents impaired endothelium-dependent relaxation of aorta caused by exercise in old-aged rats.

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Background and Aims: Exercise decreases plasma total cholesterol and triglycerides, and simultaneously, increases high density lipoprotein (HDL) cholesterol. As a result, exercise is believed to aid in preventing atherosclerosis. However, we do not know whether exercise protects against the development of atherosclerosis in the elderly. The aim of this study was to ascertain whether the lipoprotein lipase activator NO-1886 had an effect on the prevention of atherosclerosis in aged rats which undergo exercise.

Materials and Methods: Agent NO-1886, diethl 4-[(4-bromo-2-cyanophenyl) carbamoyl] benzylphonate was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory, Inc. Two years old male SD rats were divided into three groups. The control group was fed chow, the exercise group performed exercise for 3 months, and the NO-1886 group was fed chow containing NO-1886, which was equivalent to 50mg/kg body weight of NO-1886, for 3 months in rats which underwent exercise. Exercise was performed once a day. Following an overnight fasting after the final dose, the animals were killed. Blood samples were collected for lipid measurement. The thoracic aorta was removed for measurement of vasorelaxation response. Superoxide dismutase and Lipid peroxide were determined. Sections of the thoracic aorta between the aortic arch and the diaphragm were dissected from the same animal following exsanguinations. These were mounted as ring preparations and normalized for the measurement of isometric tension. The arteries were contracted with phenylephrine. Sodium Nitropurusside were added cumulatively. Relaxation responses were measured with an isometric transducer and expressed as percentages of the phenylephrine-induced contraction.

Results: Exercise for 3 months did not affect plasma lipids but decreased the accumulation of visceral fat in 2-year-old rats (aged rat). Exercise also resulted in an elevation of plasma lipid peroxide (LPO) levels and impaired the endothelium-dependent relaxation of the thoracic aorta caused by acetylcholine in aged rats. On the other hand, NO-1886 decreased plasma triglycerides and increased HDL cholesterol and suppressed the elevation of plasma LPO levels caused by exercise. Furthermore, NO-1886 prevented impaired endothelium-dependent relaxation caused by exercise.

Conclusion: In summary, the results of our study indicate that exercise may cause impaired endothelium-dependent relaxation by elevation of LPO in aged rats, and that NO-1886 prevents this impaired endothelium-dependent relaxation of aorta by reducing plasma triglycerides, elevating HDL cholesterol, and suppressing the elevation of plasma LPO caused by exercise.

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Silent Ischaemia - Cardiac Function in Diabetes

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The relation of the silent ischemia in asymptomatic 2nd Type diabetics to diabetes compensation and to some biochemical laboratory parameters connected with risk of ischemic heart disease.

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Backrounds and Aims: The aim of our study was to evaluate the relation between the presence of silent ischemia in asymptomatic 2nd type diabetics to diabetes compensation, fibrinogen plasma level, serum uric acid, homocystein, hsCRP (high sensitivity C-reactive protein), TAC (total antioxidant capacity) and to the presence of micro or macroalbuminuria.
Material and Methods: In 108 asymptomatic 2nd type diabetics with no history of ischemic heart disease (IHD) and with the presence of 2 other risk factors of IHD at least (lipid abnormalities, hypertension, smoking, positive family history and positive micro or macroalbuminuria) we have examined myocardial perfusion SPECT after exercise and at rest in order to find the silent ischemia. At the same time we have drawn the blood samples for assesment of fasting blood sugar, glycated hemoglobin (HbAlc), C-peptid, fibrinogen, homocystein, uric acid, hsCRP and TAC. The micro or macroalbuminuria was detected by 12 hours urine collection in the night hours. The measured biochemical values were related to the diabetic patients according to the presence or absence of silent ischemia For statistical analysis we used unpaired T test and $\chi 2$ test.
Results: We have examined 108 2nd type diabetics (44 women). In 35 (32%) we have found the silent ischemia during SPECT. The average age of the patients with the silent ischemia was 61,2 ± 6,9 years to 59,1 ± 6,9 years in the group with negative SPECT(N.S.). The results of the examined biochemical parameters and its relation to the silent ischemia are in the table.

	Silen ischemia (n=35)	No ischemia (n=73)	P
Blood sugar mmol/l	9,6 ± 2,0	9,1 ± 2,3	N.S.
HbA1c (%)	7,3 ± 1,3	7,1 ± 1,6	N.S.
C-peptid pmol/l	1316 ± 588	1341 ± 622	N.S.
Fibrinogen g/l	3,54 ± 0,8	3,18 ± 0,64	0.05.
Homocystein umol/l	12,05 ± 3,47	10,5 ± 2,67	0.05
Uric acid umol/l	343 ± 62	356 ± 54	N.S.
hsCRP mg/l	3,7 ± 1,1	3,0 ± 0,9	0.01.
TAC mmol/l	1,54 ± 0,13	1,49 ± 0,13	N.S.
Albuminuria (number)	22 (63%)	20 (27%)	0.05

Conclusion: In asymptomatic 2nd type diabetics with the silent ischemia we have found the significantly more patients with micro or macroalbuminuria. HsCRP, fibrinogen level and homocystein are significantly higher in the group with silent ischemia, however compensation of diabetes was not related to the presence of the silent ischemia.

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The effect of diabetes mellitus on ischemic preconditioning and myocardial damage in myocardial infarction.

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Background and Aims: Having a prodromal angina a short while before acute myocardial infarction may possess some positive effects by the preconditioning mechanism. However, little is known about the effect of ischemic preconditioning on diabetic patients. The aim of this study was to evaluate the effect of ischemic preconditioning and coronary artery disease on mortality of type II diabetic patients presenting with myocardial infarction compared to non-diabetics.
Materials and Methods: Two hundred seventy two patients who had an acute myocardial infarction for the first time between 1999-2001 were included in the study. After admittance to coronary unit, detailed history of angina was obtained personally by the investigator and serum creatine kinase (CK) levels were determined. Having pain with anginal character during the last 48 hours prior to infarction was defined as preconditioning. Coronary angiograms were performed a mean of ten days after the myocardial infarction and having ≥50% stenosis in coronary angiography was defined as coronary artery disease. The mortality rate of the patients was recorded during hospitalization and statistical analysis was performed by Mann-Whitney U test.
Results: One hundred seventy two (63%) of the patients had type 2 diabetes mellitus. The mean age of the diabetic patients was 62±11 years, whereas it was 56±12 years in non-diabetic patients. Male sex was predominant among non-diabetics compared to diabetics (91% vs 55.2%, p<0.001). Ninety two (70.2%) diabetic patients had preconditioning while only 54% of non-diabetic patients had preconditioning (p<0.001). Peak CK level was lower in diabetic patients (1393±1570 IU/L) compared to non-diabetics (2277 ± 2368 IU/L, p<0.01). The coronary angiographic examinations of the diabetics revealed 31.3% one, 23.9% two, 44.8% three or more coronary arteries while 22% of non-diabetics had multivessel disease (p=0.03). During the hospitalization, 17 diabetic patients (9.8%) and two non-diabetic patients (2%) died. Hospital mortality rates in diabetic patients were higher than non diabetic patients (p=0.01).
Conclusion: In conclusion, even though ischemic preconditioning is more common and myocardial damage is lower in type II diabetic patients compared to non-diabetic patients, hospital mortality is higher in diabetic patients.

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The relation of the silent ischemia in patients with diabetes mellitus 2nd Type to the presence of diastolic dysfunction, some changes in carotid artery and endothelial function.

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Backrounds and Aims: The incidence of ischemic heart disease (IHD) is known to be significantly higher in diabetic than nondiabetic population including the presence of the silent ischemia (SI). In our study we compared the relation of the some changes in carotid artery, diastolic dysfunction assesed by echocardiography and endothelial function to the presence of SI discovered with myocardial perfusion spect.
Material and Methods: In 125 patients with diabetes mellitus 2nd type (DM), no history of IHD, no ECG specific changes for ischemia, two other risk factors at least (hypertension, lipid abnormalities, smoking, family history, micro or macroalbuminuria) and no clinical signs of peripheral atherosclerosis we examined myocardial perfusion spect after exercise and at rest, transthoracal 2-dimension and Doppler echocardiography. Diastolic dysfunction (DD) was evaluated according to mitral valve flow (when A>E), by carotid sonography we measured intima- media thikcness (IMT), the presence and size of atheromas (more than 1,5 mm) and distensibility (D) of carotid artery calculated by Reneman formula (mm/100 mg Hg). The endothelial function was evaluated by sonography method - flow mediated dilatation (FMD). FMD was calculated as percentage change to the basal value. We compared number of the patients with DD, presence of carotid atheromas larger than 1,5 mm in carotid artery, mean IMT, D and FMD in patients with positive respectively negative spect. For statistical analysis we used unpaired T-test and $\chi 2$ test.
Results: 39 patients (31%) had the positive spect, while 86 (69%) negative. The other results are in the table.

	Positive spect n=39	Negative spect n=86	p
Age (years)	60,1±6,5	59,8±6,8	N.S.
A>E	31 (79,5%)	45 (52%)	0.01
Atheromas> 1,5mm	29 (75%)	18 (21%)	0.01
IMT mm	0,71±0,07	0,66±0,09	0.01
D	0,31±0,083	0,32±0,075	N.S.
FMD	12,1±4,1	14,3±4,1	0.01

Conclusion: DD and atheromas larger than 1,5 mm in carotid artery are common in the diabetic patients with silent ischemia, while absence of these changes is connected with negative spect. These simple tests may be used in stratification risk before more complex examination

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Long term clinical outcome of ST segment elevation myocardial infarction patients with and without diabetes mellitus in the Zwolle Trial.

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Background and Aims: Diabetes mellitus (DM) is an adverse prognostic factor after ST segment elevation myocardial infarction (STEMI). However, there is limited information about long term clinical outcome in STEMI patients with DM treated with PCI or thrombolysis. We sought to compare long term survival after STEMI in patients with and without DM treated with primary percutaneous coronary intervention (PCI) or thrombolytic therapy.
Materials and Methods: Patients with STEMI (n = 395) were randomized to treatment either with intravenous streptokinase or PCI. Mean follow up was 8 ± 2 years. We studied long term mortality of patients with DM (n = 32) and without DM (n = 363) and the interaction with treatment regimen.
Results: After 8 years, a total of 17 patients with DM (53%) died compared to 88 (24%) patients without DM (OR 3.5, p < 0.001). Reduced left ventricular ejection fraction (LVEF) after STEMI was more often present in patients with DM compared to patients without DM (33% vs. 16%, p = 0.01). Multivariate analysis revealed that DM (OR 2.6; 95%CI: 1.4 – 4.7, p = 0.002), reduced LVEF (OR 2.4; 95%CI: 1.5 – 3.8, p < 0.001) and age >= 60 years (OR 2.4; 95%CI: 1.5 – 3.8, p < 0.001) were independent risk factors for long term mortality. Patients with DM treated with PCI had less reduced LVEF (13% vs. 53%, p = 0.01) and lower long term mortality rates (38% vs. 69%, p = 0.08) compared to treatment with thrombolysis.
Conclusion: DM patients with STEMI are a high risk group with higher long term mortality rates compared to patients without DM. PCI is the treatment of choice, particularly in DM patients.

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Tissue doppler imaging for the early detection of myocardial dysfunction in patients with Type 2 diabetes mellitus.

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Background and Aims: The prevalence of type 2 diabetes mellitus is rapidly expanding, which can cause the incidence and prevalence of heart failure to increase. Myocardial dysfunction may be a consequence of diabetic cardiomyopathy and is an important factor for the poor prognosis in patients with diabetes mellitus. Accordingly early detection of myocardial dysfunction in diabetic patients is extremely important for implementing secondary and tertiary prevention in order to improve the prognosis. This case-control study tested if quantitative Tissue Doppler Imaging (TDI) may be a suitable tool for the detection of myocardial dysfunction in diabetic patients.
Materials and Methods: A total of 43 diabetic patients and 34 non-diabetic control subjects without any clinical signs of heart failure and with normal global LV-function by standard 2-D echocardiography were investigated with TDI at rest and during dipyridamol and/or dobutamin stress. Global myocardial function was calculated as mean value from six basal myocardial segments for the peak velocity at systole (Vs), early diastole (Vd) and atrial contraction (Va). The diabetic and control groups were well balanced in respect to demographics and case histories including coronary artery disease.
Results: The rate-pressure product and pulse pressure were significantly higher in the diabetic patients at rest (9363 vs.7840; p<0.01 and 63.1 vs. 49.5;p<0.001)and during Dipyridamol stress (rate pressure product: 11035 vs. 9833; p=0.04 and pulse pressure: 63 vs. 50.8; p=0.001). Compared to controls the diabetic patients had a compromised Vd at rest (8.6 vs. 9.6 cm/sec; p=0.02) and during Dobutamin stress (10.0 vs. 13.1 cm/sec; p<0.01). Their resting Va was higher (10.1 vs. 8.9 cm/sec; p=0.01) and the Vd/Va ratio at rest (0.9 vs. 1.1; p<0.01) and during Dipyridamol and Dobutamin stress significantly lower than in the control group (Dipyridamol: 0.9 vs.1.1; p<0.01 and Dobutamin: 0.8 vs. 1.1; p<0.01). Compared to controls Vs was not reduced at rest in the diabetic patients (p=0.09). It was, however, lower during Dobutamin stress (10.7 vs. 13.6 cm/sec; p<0.05).
Conclusion: Patients with diabetes mellitus have early signs of predominant diastolic and also systolic myocardial dysfunction. This can be identified by quantitative TDI before any clinical signs of heart failure are apparent. Accordingly, TDI may be used for screening diabetic patients for myocardial dysfunction and for monitoring therapeutic strategies.

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Positive single-photon emission computed tomography myocardial perfusion imaging and coronary artery disease are associated with different clinical/biological profiles in asymptomatic Type 2 diabetic men and women.

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Background and Aims: Stress radionuclide myocardial perfusion imaging (MPI) is an appropriate mean of evaluating diabetic patients for silent coronary artery disease (CAD), but screening all patients is not practical. We studied whether a clinical/biological profile could be helpful to identify asymptomatic type 2 diabetic men and women who should benefit from screening.
Materials and Methods: 439 consecutive asymptomatic type 2 diabetic patients (174 women/265 men) were evaluated with stress MPI. 39 men and 114 women had an unequivocally positive test (UPT) and were compared to 83 men and 103 women with an unequivocally negative test (UNT). 81 men and 27 women with UPT underwent coronarography; 43 and 17 of them showed significant CAD and were compared by sex with patients with UNT.
Results: Factors associated with UPT were elevated triglycerides (TG, p=0.009), body mass index (BMI, p=0.003) and peripheral arterial disease (PAD, p=0.002) in men and HbA1c (p=0.020), fibrinogen level (fg, p=0.005), nephropathy (p=0.042), PAD (p=0.037) and hypertension (p=0.049) in women. Logistic regression analysis showed that BMI (RR 1.10; 95%CI 1.02-1.18), PAD (RR 3.21; 95%CI 1.45-7.10) and elevated TG (RR 1.98; 95%CI 1.05-3.75) in men and fg (RR 1.10; 95%CI 1.01-1.20) and smoking (RR 6.74; 95%CI 1.09-41.77) in women were independent predictors for UPT. Factors associated with CAD were HDL-cholesterol (p=0.030), PAD (p<0.0001), nephropathy (p=0.007) and smoking (p=0.001) in men and HbA1c (p=0.001), fg (p=0.012), nephropathy (p=0.042), PAD (p=0.041) and retinopathy (p=0.031) in women. Logistic regression analysis showed that smoking (RR 4.52; 95%CI 1.28-16.13) and PAD (RR 4.42; 95%CI 1.52-12.82) in men and HbA1c (RR 1.91; 95%CI 1.22-2.99), hypertension (RR16.04; 95%CI 1.48-174.34), fg (RR 1.13; 95%CI 1.01-1.26), retinopathy (RR 6.22; 95%CI 1.18-32.25) and PAD (RR 7.19; 95%CI 1.19-43.48) in women were independent predictors for CAD.
Conclusion: This study shows that the clinical/biological profiles associated with positive stress MPI and with CAD are different. These profiles differ also between men and women.

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Subendocardial longitudinal function of left ventricle in patients with diabetes mellitus and "pure" diastolic dysfunction.

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Aims: Patients with diabetes mellitus usually have diastolic dysfunction, as assessed by Doppler transmitral flow, with preserved systolic function, as assessed by ejection fraction. The velocities measured at the mitral annulus by pulsed tissue Doppler imaging (pTDI) are likely to be indexes of global longitudinal function of the left ventricle. The aim of the study was to evaluate in such patients the longitudinal subendocardial systolic function, by means of pTDI. We also want to see if at this patients the systolic function was correlated with the metabolic control .
Material and Methods: From 100 patients with type 2 diabetes mellitus we studied 28 patients, 57.4 ± 7.4 years, 65% men, who had normal ejection fraction, calculated from B-mode images according to Simpson's rule and diastolic dysfunction (E/A ratio < 1) , calculated from transmitral flow. Systolic (Sa) and diastolic velocities (Ea, Aa) were measured by pTDI at the mitral annulus at six sites (lateral, septal, anterior, inferior, posterior and antero-septal), from three apical views (4-chamber, 2-chamber, and long-axis view), and were averaged. Ea/Aa ratio was calculated for each site and averaged .We done blood analyses and we measured hemoglobin A1c (HbA1c) , cholesterol and triglyceride. We done statistical analysis and we achieved correlation between Ea/Aa and Sa. We also correlated Sa

and HbA1c , cholesterol and triglyceride. We analysed the correlation between our characteristics using Student's test for a significance limit p = 0.02 .
Results: The average Sa was 6.26 ± 1.82 cm/s. The average Ea/Aa ratio was 0.82 ± 1.1. Sa demonstrated a good correlation with Ea/Aa (r = 0.695). The average HbA1c was 9.1 ± 2.4 % , and we found a good correlation with Sa (r = -0.622). The average of cholesterol values was 270 ± 110 mg/dl , and result worst correlation with Sa (r = -0.301). Using the Student's test , the value of correlation index considered significant was 0.313 , and result no correlation between these two characteristics. The average of triglyceride was 320 ±200 mg/dl and result a good correlation with Sa (r = -0.613).
Conclusions: Patients with diabetes mellitus who seem to have pure diastolic dysfunction, might have also systolic sudendocardial dysfunction, as assessed in longitudinal axis, by measuring mitral annulus velocities with pulsed TDI. The systolic function might depends on metabolic control (HbA1c and triglyceride).

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The role of BNP in the diagnosis of cardiac dysfunction in subjects with diabetes mellitus.
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Background & Aims: Patients with diabetes mellitus (DM) have a high incidence of coronary heart disease and congestive heart failure (CHF) with high rates of cardiac mortality and morbidity. Brain natriuretic peptide (BNP) has been reported to be useful in the diagnosis of CHF, diastolic dysfunction and with risk stratification in patients with acute coronary syndromes. Subjects with DM have a high prevalence of diastolic and/or systolic dysfunction on echocardiographic examination (TTE). The aim of this study was to assess the correlation between BNP and cardiac dysfunction on TTE in subjects with DM.
Methods: 201 consecutive subjects attending a diabetes clinic underwent TTE examination to assess cardiac function with collection of serum and urine for BNP, Creatinine (Cr) and albuminuria analysis.
Results: The TTE findings were classified into normal (N=63), left ventricular hypertrophy alone (LVH) (n= 16), systolic (n=30) and diastolic dysfunction (n=92) groupings. Subjects with diastolic dysfunction alone had higher BNP levels compared to subjects with a normal TTE (160±24 pg/ml vs 58±11 pg/ml, p<0.001). BNP levels were highest in subjects with systolic dysfunction (299±55 pg/ml, p< 0.001). BNP levels were higher in subjects with renal impairment (serum Cr <0.1mmol/l, 104±12 pg/ml vs serum Cr≥ 0.1mmol/l, 325±62 pg/ml, p<0.001), those with macroalbuminuria ((normoalbuminria 98±14 pg/ml vs macroalbuminuria 318± 75 pg/ml, p<0.001) and with ageing (age <60 yrs, 70± 11 pg/ml vs age ≥ 60 yrs, 235±35 pg/ml, p<0.001). There was no statistical difference detected in BNP levels according to gender.
Conclusions: In conclusion, diabetic subjects have a high prevalence of cardiac dysfunction on TTE examination, which can be diagnosed by serum BNP levels.

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Effect of acute hyperglycemia on coronary flow in healthy subjects.
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Background and Aims: Acute hyperglycemia has been demonstrated to reduce endothelial function in brachial artery in normal subjects. It is unknown whether hyperglycemia is also able to affect coronary circulation.
Materials and Methods: Blood flow velocity in the 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 ecocardiography in nine young healthy men (age 30 ± 2 years, BMI = 26±1 Kg/m^2). Coronary flow reserve (CFR) was defined as the ratio of dipyridamole-induced coronary peak diastolic flow velocity to resting peak diastolic flow velocity. CFR was measured both in normoglycemia (N) and after 3-hours hyperglycemia (H) (≈ 14mmo/l) maintained by a variable glucose infusion. Octreotide (0.4 mg/h) and low insulin dose (0.15 mU/Kg/min) were coinfused to keep insulin at its basal value.
Results: Mean blood pressure at rest was 88±3 in N and 87±4 mmHg in H and remained substantially unchanged during dipyridamole. Resting coronary diastolic flow velocity was 18.3±0.9 cm/sec during N and increased to 20.2±0.9 cm/sec in H (p=0.008). Average CFR was 2.67±0.13 in N and slightly decreased to 2.47±0.12 in H (p=0.07).
Conclusion: Acute hyperglycemia is associated with a significant increase in resting coronary diastolic flow, possibly due to increased myocardial glucose utilization. No significant change in CFR is observed in healthy subjects.

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Cardiac expression of natriuretic substances in experimental diabetes mellitus combined with either angiotensin II induced cardiac hypertrophy or insulin treatment.
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Background and Aims: In order to gain insight into the cardiac adaptive mechanisms in diabetes we studied if insulin or angiotensin (AII) treatment alters atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) gene expressions in the left ventricle of the diabetic rat heart.
Materials and Methods: Diabetes was induced by streptozotocine (STZ; 60 mg/kg b.w., iv). AII (33 μg/kg/hour) was administered for the last 24 hours via osmotic minipumps 2,5 or 7 weeks after treatment with STZ or vehicle in male Wistar rats. Ultralente insulin (I) was used subcutaneously. Left ventricular weight to body weight ratio (LV/BW) - as an index of left ventricular hypertrophy - and left ventricular expression of ANP, BNP, skeletal and cardiac α actin was measured (total RNA isolation by guanidine isothyocianate-CsCl method and Northern-blotting). Six groups of the animals (n=9-13) were investigated at both time points (2,5wks and 7wks): control (C), AII treated control (AII), diabetic (DM), insulin treated diabetic (DMI), AII treated diabetic (AIIDM) and insulin and AII treated diabetic (AIIDMI).
Results: All groups of diabetic animals had significantly higher blood glucose and fructosamine values compared to controls and AII treated groups (p<0,001). Insulin significantly lowered blood glucose and fructosamine levels (p<0,001). Insulin elevated LV/BW ratio more than AII or diabetes did alone, both at 7 and 2,5 week (p<0,001), however at 7 week, diabetes and AII together elevated this ratio (p<0,05). Left ventricular ANP expression was elevated by AII and diabetes together at 2,5 week (p<0,001). At 7 week diabetes elevated the ANP mRNA levels (p<0,0049), diabetes and AII together further elevated this value (p<0,01). Interestingly insulin did not elevated further this level in diabetic AII treated animals (p<0,001). Changes in left ventricular BNP expression were similar but smaller, it was elevated by AII and diabetes together at 2,5 week (p<0,05) and insulin did not elevated further this level in diabetic AII treated animals (p<0,001). At 7 week same changes occurred, however AII treatment did not elevated this value compared to insulin treated diabetic animals. The changes in α actin levels could be described by the ratio of skeletal actin pro cardiac actin. At 2,5 week only AII treatment in diabetic animals elevated this ratio (p<0,001). At 7 week AII treatment elevated the ratio in controls and diabetic animals, and further elevated by insulin treatment in diabetic animals.
Conclusion: Long-term insulin treatment administered once daily lead to cardiac hypertrophy due to its growth hormone effect. However in insulin treated animals the elevation of the natriuretic peptides was smaller or missed than compared to diabetic or AII treated diabetic animals. Partial reversion of hypertension-induced changes in cardiac protein expression by insulin treatment may reflect beneficial effects contributing to enhancing readaptation of the heart to overload.

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Ethnic differences in diabetic with multivessel coronary disease: Indian Asians versus White Europeans - results from a prospective registry from 1998 to 2001.

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Background and Aims: The incidence of diabetes in Asian-Americans, Indian Asians and Pacific Islanders is growing at an alarming rate, with 90-95% being type 2 diabetes. Studies in the United Kingdom revealed a five-fold difference in the prevalence of diabetes in Asians living in West London compared to an age-matched European population. Therefore we planned a study to compare the outcomes of White European and Indian Asian diabetics known to have multivessel coronary artery disease.
Materials and Methods: From January 1998 to December 2001 we recruited all diabetic patients with multivessel coronary artery disease (MVD), underwent to catheterisation at the Cardiology Division of Hammersmith Hospital in London, and compared outcomes (inhospital and 1-year mortality, repeat revascularisation at 1 year) between White Europeans and Indian Asians whose ethnic origin is in the Indian subcontinent, but who are now living in the United Kingdom. The MVD was defined as narrowings of at least 50% in two or more major epicardial vessels.The analysis was performed by SPSS statistical program
Results: Of 9586 patients 1714 were found to be diabetic and 970 (56.6%) to have multivessel disease. The proportion of patients in the community in West London which refer to our hospital who are Indian Asian is known to be 12% in the 30 to 85 year age range. However over the course of the 4 years of the study 28% of the patients undergoing coronary angiography were Indian Asians compared to 58% who were White Europeans. The rate of diagnosis of coronary artery disease at coronary angiography is similar but significantly Indian Asians accounted for a disproportionately large number of the diabetics undergoing coronary angiography 41.0% versus 46.7%. Furthermore Indian Asians who were diabetic were then more likely to have multivessel disease. Of the 970 patients with this condition Indian Asians accounted for 49.0 % versus 39.3% in White Europeans. However these two groups were equally likely to receive the same mode of therapy ie PCI, CABG or medical treatment. Follow up data is complete in 98.1% of patients in this study and preliminary analysis indicates that outcomes do not differ significantly between Indian Asians and White Europeans with diabetic multivessel disease.
Conclusion: Our registry data show that Indian Asians in the United Kingdom have a higher risk of developing coronary artery disease than White Europeans and that they account for a disproportionately large number of patients who have diabetes, in particular diabetic multivessel disease. These results according to international studies suggest that the high prevalence of diabetes in Asian immigrants may be a result of westernization and urbanization with an increase in consumption of animal fat and sedentary lifestyle, superimposed upon a predisposed genetic background

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The Italian DAI study on macrovascular complications in patients with Type 2 diabetes.

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Background and Aims: Diabetic patients are at increased risk of cardiovascular disease, and a vast quantity of data has been accumulated on the determinant role played by risk factors associated with type 2 diabetes (i.e., dyslipidemia, hypertension, visceral obesity) in the onset of macrovascular diseases.
The DAI study is a multicenter cohort study promoted in September 1998 by the Diabetes and Informatics study group (**D**IAINF) along with the Italian Association of Clinical Diabetologists (**A**MD) and the Italian National Institute of Health (**I**SS).
The study aimed to: estimate the prevalence and incidence of macrovascular complications among type 2 diabetic patients followed by the Italian diabetic care units; evaluate the association between cardiovascular risk factors (classic and diabetes related) and fatal and non-fatal events. Study events were: myocardial infarction, ischemic heart disease, coronary artery bypass, coronary angioplasty, cerebral thromboembolism, and amputations.
Materials and Methods: The reference population consists of all patients with type 2 diabetes visited at the participating units between September 1998 and June 1999. Patients were randomly chosen to create a representative sample of the diabetic population visited at the units. 100 diabetic care units participated to the incidence study. During each visit, for each patient included in the cohort, a standard questionnaire was used to collect all of the information relative to prognostic variables and study events. All the study events needed to be documented by hospital admission or by a specialist.
Results: A cohort of 9006 patients were followed up for one year. The mean age was 66 years (±9) and 51% were females. The mean duration of diabetes was10 years. At the enrolment visit 20% of patients had evidence of macrovascular complications (prevalent cohort). At the first annual follow-up 538 patients (6.0%) had a cardiovascular events. Patients in the prevalent cohort had an increased risk of cardiovascular diseases (13.8% - men: 14.6%; women: 12.7%) than patients free from events at baseline (4.0% - men: 4.1%; women: 3.9%). Ischemic heart disease was the most frequent event both in the prevalent cohort (7.9%) and in patients without previous events (2.7%). Presence of microvascular complications resulted as an important predictor for cardiovascular events. Age-sex adjusted all cause mortality rate was 1.1%, cardiovascular events accounted for 41% of all deaths. Patients in the prevalent cohort had a twofold higher risk of death from all causes than patients with no previous events.
Conclusions: This large cohort is representative of diabetic patients cared for by Italian diabetic care units but not of all Italian diabetics. Nevertheless, incidence rates are similar to those estimated in other studies, and the role of microvascular complications as risk factor for cardiovascular disease is confirmed.

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Macrovascular events after kidney-pancreas transplantation in Type 1 diabetic patients.

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Background and Aims: Kidney-pancreas transplantation (KPTx) is the therapy of choice in patients with type 1 diabetes who have end-stage renal disease, but there are very few studies concerning the effect of KPTx on the progression of macrovascular disease and contradictory results have been reported. The aim of our study was to retrospectively evaluate the incidence of macrovascular events after functioning KPTx.
Materials and Methods: We studied 146 patients (96 males/50 females) who had undergone KPTx from February 1983 to September 2001, with more than one year of evolution and with both grafts normofunctioning. The mean follow-up of the patients after KPTx was 5 ± 3 years. For statistical analysis of the data the SPSS program (Statistical Package for Social Sciences) for Windows was applied. The chi-square test was applied with significance established at $P < 0.05$.
Results: Before KPTx 29 patients presented 42 macrovascular events. During the follow-up after transplantation, intermittent claudication remained present in 25 patients (86.2%) with 11 new macrovascular events appearing (1 stroke, 1 angina pectoris, 1 myocardial infarction and 8 minor amputations) in 10 (34%). Among the 117 patients without antecedents of macrovascular events prior to KPTx 38 (32.5%) presented a total of 63 macrovascular events (26 intermittent claudication, 4 stroke, 8 angina pectoris, 7 myocardial infarction, 11 minor and 7 major amputations). Seventy-nine patients (54% of the whole group) did not have macrovascular history prior to transplantation and remained asymptomatic during the follow-up. Before transplantation, 88.4% of the patients presented hypertension, 42.5% smoked and 14.4% had been treated for dislipemia. After transplantation we observed an important reduction in the percentage of patients with hypertension (48.6%) and smokers (25.5%), without relevant variations in the prevalence of dyslipemia (19.9%). Hypertension after transplantation was clearly associated with the appearance or persistence of macrovascular events.

Conclusion: In our experience the incidence of macrovascular disease after KPTx is relevant because a 43% of the transplanted patients presented macrovascular events. It is important to note the elevated prevalence of cardiovascular risk factors presented in the patients with KPTx, since this may play a relevant role in the progression of cardiovascular disease. Therefore, it is very important to treat these patients according to the current recommendations for diabetic patients in order to reduce cardiovascular risk.

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Vascular aging in young Type 1 diabetic individuals – the Eurodiab Prospective Complications study.

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Background and Aims: Type 1 diabetic individuals are thought to have increased arterial stiffness, and are at risk for cardiovascular disease. Pulse pressure is a marker of arterial stiffness above 50 years of age in non-diabetic individuals, but whether this is also the case in (relatively young) type 1 diabetic individuals is not known. If it is, one would expect a) an association of age with pulse pressure; and b) an association of pulse pressure with cardiovascular disease. The present study investigated the association of age and pulse pressure and the influence of the presence of microvascular complications on this association. In addition, we determined the association of pulse pressure and mean arterial pressure with cardiovascular disease in type 1 diabetic individuals.
Materials and Methods: We studied a cohort of 3250 type 1 diabetic individuals of the EURODIAB Prospective Complications Study. Mean age at baseline and median follow-up were 33 (standard deviation, 10) and 7.4 years (interquartile range, 0.0 to 9.1). Two-hundred and thirty seven individuals developed cardiovascular disease. Linear regression analyses was used to determine the association of age with pulse pressure. Relative risks of cardiovascular disease were estimated by Cox regression analyses adjusted for age, sex and mean arterial or pulse pressure.
Results: Age was associated with pulse pressure in individuals without and more so in those with microvascular complications (crude regression coefficient 0.50; $p<0.001$; adjusted regression coefficient with interaction of age with microalbuminuria 0.24, $p<0.0001$; adjusted regression coefficient with interaction of age with retinopathy 0.18, $p<0.0001$; p-interaction <0.001). In other words, the association between age and pulse pressure was stronger in the presence of micro- or macroalbuminuria or retinopathy. In prospective analyses, both mean arterial pressure and pulse pressure were associated with incident cardiovascular disease (adjusted relative risks and [95% confidence interval] per 10 mmHg increase were 1.17 [1.05 – 1.31] and 1.06 [0.98 – 1.16]).
Conclusions: This study shows an association of age with pulse pressure in young type 1 diabetic individuals. This association is influenced by the presence of microvascular complications. In addition, mean arterial pressure and pulse pressure are associated with the presence of cardiovascular disease. These findings support the concept of accelerated vascular aging in type 1 diabetes, especially in the presence of microvascular complications.
**** and the Eurodiab Prospective Complications Study Group***

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Gender differences in cardiovascular outcomes among persons with diabetes.

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Background and Aims: Cardiovascular disease (CVD) is the leading cause of death among both men and women with diabetes mellitus (DM). We used a population-based approach to evaluate gender differences in cardiac outcomes among persons with DM in Ontario, Canada.
Materials and Methods: A validated algorithm based on hospitalization and physicians' billing records was used to identify all adults with diabetes in Ontario between 1994 and 1999 (N=514,755). Age-adjusted rates of admission for acute myocardial infarction (AMI) and cardiac procedures were compared between men and women with and without DM (total population N=10,453,815). The impact of gender on the risk of AMI was evaluated using a Cox proportional hazards model to adjust for other baseline differences.
Results: Compared to the non-diabetic population, individuals with DM had dramatically higher AMI rates (1339.5 vs. 179.5 per 100,000 in 1999). The disparity in rates was more marked among women than men. Diabetic women were over 9 times more likely to suffer from an AMI then their non-diabetic counterparts (age-adjusted OR 3.65; 95% CI: 3.47-3.83), compared to a 6-fold gradient between men with and without DM (age-adjusted OR 2.68; 95% CI: 2.57-2.78). Moreover, AMI rates in women with DM far exceeded those in men without the disease. Within each population, men were more likely to suffer from CVD then women, but the difference between genders was vastly reduced by the presence of DM. Among the non-diabetic population, AMI rates were two-fold higher among men than women. In contrast, diabetic men had AMI rates that were only one-third higher than women with DM. On multivariate analysis, gender remained an important predictor of AMI among those with DM, but its impact was reduced after accounting for differences in age, comorbidity, socioeconomic status and health care utilization (OR: 1.22; 95% CI: 1.18-1.25). Despite modest differences in AMI rates, men with DM were nearly twice as likely to undergo revascularization (coronary artery bypass surgery or percutaneous coronary interventions) than women with this disease.
Conclusion: Diabetes substantially reduces the gap in CVD rates normally observed between men and women. Larger gender differences in cardiac procedure use may reflect variations in the severity or pattern of coronary artery disease among men and women with DM and/or reduced access to these services for women.

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Cardiovascular outcomes in patients with Type 2 diabetes mellitus after the first acute myocardial infarction.

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Background and Aims: Diabetes increases the risk of both short and long-term cardiovascular complications, particularly in patients after the first cardiovascular event. The long–term prognosis in patients with type 2 diabetes mellitus (DM2) who survived over the 30 days after the acute myocardial infarction (MI) was assessed in the study. The primary objective was to assess CV risk by collecting information on the composite CV endpoint (new MI, stroke, death, and hospitalization due to acute coronary syndrome [HACS] whichever occurred first). The secondary objectives were to assess frequency of each individual CV end point event and risk factors that have independent impact on the risk.
Materials and Methods: This retrospective observational study was conducted in 5 sites in Poland (four geographically defined areas). Cardiovascular outcomes in 521 patients (201 women and 320 men, age 62.4 ±10.1y) analysis of the 521 patients with DM2 who survived over 30 days after acute MI, were retrospectively followed for up to 6 years. Data on smoking, obesity, hypertension, dyslipidemia and pre-existing coronary artery disease (CAD) were collected.were hospitalized in the Cardiological Care Unit (CCU) due to AMI between 1996-2000 and survived under 30 days. The study was done in Poland. The primary objective was the evaluation of the composite outcomes : new myocardial infarction (MI), stroke, death, and hospitalization due to acute coronary syndrome (HACS), whichever occurred first. The second objective the evaluation of the above end points as a singular event.
Results: 269 patients (52%) suffered one of the outcomes from the composite CV endpoint. HACS was the first event in 164 cases, MI in 59, death in 32, and stroke in 14 patients. Analyzing prevalence of individual cardiovascular events we have found: HACS in 184 patients (35%), next MI in 79 patients (15%), death in 59 patients (11%), stroke in 30 patients (6%).During the maximum of 6 years observation 52% patients experienced cardiovascular events evaluated as composite outcomes.Taking into consideration singular cardiovascular events; 35% patients experienced HACS, 15% next MI, 11% death, and 6% stroke. Only dDyslipidemia, arterial hypertension, and CADcoronary artery diseases were independent risk factors with an impact on composite CV endpoint (RR 1.5; 95% CI [1.14;1.97]; p=0.003, RR 1.55; 95% CI [1.12;2.15]; p=0.008; RR 1.49; 95% CI [1.1;2.0], p=0.01, respectively]. Other analyzed risk factors like smoking and obesity did not have independent effect on the CV risk in this subgroup of people with diabetes.

Conclusions: In this retrospective follow up we determined that HACS is the most frequent cardiovascular event in individuals with DM2 after acute MI. The CV risk in patients with DM2 who suffer at least one MI is further increased in those with coexisting dyslipidemia, arterial hypertension or coronary artery disease.

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Recovery from stroke in patients with diabetes mellitus.

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Background and Aims: The diabetes mellitus (DM) is recognized as an important risk factor for stroke; moreover it can influence the post-stroke clinical evolution, especially in the initial phase, increasing the extension of the cerebral injured area. Few studies are aimed to the infuence of the diabetes on the functional recovery after stroke and their results are contradictory. In order to better understand the impact of DM on the functional and motor recovery, we performed a perspective observation on a group of patients in the Rehabilitation Department of Prato Hospital (Tuscany - Italy). The aim of the present study is to estimate the correlation between DM, recovery of the autonomy and motor recovery.
Materials and Methods: In a perspective study n. 311 acute stroke patients were selected in an intensive rehabilitation department and divided in 2 groups on the basis of the presence of diabetes mellitus type 2 (DM+ and DM-). Outcomes were assessed on the basis of the Barthel Index (BI), the Fugl-Meyer Assessment scale (FM) and the mobility part of the motor assessment chart according to Lindmark (MA). Measurements were performed at admission on department (T1), at discharge (T2) and at follow up (T3) in a whole period of 3 months from stroke. The t-Test has been used to compare the differences among time and ANOVA was performed to evaluate differences among groups at each assessment session.
Results: The DM+ group and the DM- group included respectively n.70 patients (age 74.9 ± 5.3) and n. 241 (age 71.3 ± 6.9). Both groups showed a significant and progressive improvement in all outcome measures (BI, FM, MA), with no statistical difference between groups (table 1). The level of statistical significance was set at 0.001.
Conclusion: Our results show similar outcomes in patients with and without diabetes, therefore the diabetes seems not to influence the functional prognosis within three months after stroke.

Table 1: Scores of outcome measures of DM+ and DM- groups at three assessment sessions

	DM +	DM-
BI T1	32.2±14.5	29.9±15.1
BI T2	51.4±11.9	52.9±12.2
BI T3	67.9±15.0	66.9±14.6
FM T1	141.3±32.2	140.7±31.9
FM T2	161.8±34.1	161.9±33.7
FM T3	167.1±35.6	168.1±36.4
MA T1	9.7±2.1	10.1±1.6
MA T2	16.3±3.0	17.8±2.4
MA T3	19.8±2.9	20.3±3.3

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Differences in mortality from stroke by sex and five year's periods of a quarter century follow-up of patients with Type 2 diabetes in relation to cardiovascular risk.

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Aims: A loss by females of the otherwise increased resistance against atherosclerosis-related diseases in non-diabetic women as compared to men holds also for the risk of death from stroke. The aim of the study was to check whether there were differences in the rate of change of the risk of fatal stroke between the sexes in different periods of follow-up observation of a long-lasting cohort of patients with type 2 diabetes.
Materials and Methods: In a cohort of 4323 patients with type 2 diabetes (1945 males and 2378 females) aged 30-68, with the disease of up to 10 years' duration, the base-line measurements of arterial blood pressure were taken, the prevalence of ecg-based myocardil ischaemia established, and the smoking status and the rate of intermittent claudication assessed based on the standardized interview. During 26 years' time span of follow-up 513 deaths from cerebrovascular diseases (ICD Ixth Revision Nos 430-438) were ascertained , more numerous among women than men. The deaths therefrom were related to the aforementioned risk factors using multiple Cox regression models, each for 5 year cumulated intervals of observation. The outcomes were presented as sex-specific rate ratios, and staticstical significance assessed using standard procedures.
Results: The ever-present risk of death from stroke ascribed to high systolic hypertension (≥180 mm Hg) in both sexes was in the initial 5-year follow-up classes much higher for females than males, equalising thereafter. Much higher initially for females was also the risk of lethal stroke for ecg coronary signs, which subsided thereafter, then disapeared as a predictor of death, still present among males. There was also a high risk of death from stroke among females in the initial 5-year period, ascribed for intermittent claudication, but no sex-period differences in mortality from stroke could be detected for smoking of cigarettes.
Conclusions: The high relative risk of death from stroke for arterial hypertension and cardiac ischaemia among women diabetic patients followed by fading of predictive properties of the two risk factors thereafter faster than in men, may be interpreted as less tolerance of these risk factors by diabetic women than men. No differentiating role of smoking among sex-specific incidence of death from stroke could however be detected, and the role for intermittent claudication in this respect is uncertain.

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Advanced therapeutic approaches reduce mortality in diabetic patients with acute myocardial infarction: the Munich registry.

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Background and Aims: The myocardial infarction registry of the Academic Schwabing Hospital, Munich, assesses hospital mortality in diabetic (D) and non-diabetic (ND) patients. In 1999, it was demonstrated that hospital mortality is nearly doubled in diabetic patients compared to non-diabetic patients. The aim of the present study was to study the effects of new therapeutic approaches (early PCI, GPIIb/IIIa-Receptor-antagonists (RA), glucose-(GI)-Infusion) on hospital mortality in the patients.
Materials and Methods: Data of the registry of 1999 and 2001 were compared. In 1999, 96 (38%) D and 204 (62%) ND were treated. In 2001, 91 (31%) D and 205 (59%) ND were admitted. The registry was analyzed with regard to mortality and therapeutic approaches.
Results: Clinical characteristics of patients were comparable between 1999 and 2001: D age 73±11 (X±SD) vs. 71±13 yrs, ND 65±14 vs. 67±14 yrs, D HbA1c 7,3±1,9 vs. HbA1c 7,7±1,6 % (n.s. respectively). In 1999, none of the patients received GI-infusion, less than 10% had early PCI and 32 % obtained GPIIb/IIIa-RA. In 2001, GI-infusion was applied in 46% of D at 1,6±2,2 IU/h for 33±13 h. Early PCI was performed in 50% D and 52% ND, 52% D and 57% ND obtained GPIIb/IIIa-RA (p<0.0001 vs 1999, respectively).
In 1999, mortality within 24h after admission was 14% in D compared to 5% in ND (p=0.01). No significant differences were observed during subsequent hospitalization (16% vs.11%). Total hospital mortality was 29% and 16% (p<0.01). In 2001, mortality D and ND was both 4% within 24h after admission, mortality of subsequent hospitalization was 13% and 10% (n.s.) and total hospital mortality was 17% and 14% (n.s.). In D, mortality within 24h after admission decreased from 1999 to 2001 by 67% (p=0.027) and total hospital mortality by 44 % respectively (p=0.03). In ND, changes in mortality were not significant.
Conclusion: The analysis demonstrates that new therapeutic approaches are significantly beneficial in diabetic patients with acute myocardial infarction. The hospital registry for myocardial infarction enables close monitoring of hospital outcome in diabetic and non-diabetic patients.

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Glucose-insulin-potassium infusion in primary angioplasty for acute myocardial infarction: clinical results in all patients and patients with diabetes mellitus.

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Background and Aims: High-dose glucose-insulin-potassium (GIK) infusion can be beneficial in acute myocardial infarction (MI) as is the combination of glucose-insulin infusion in combination with strict metabolic regulation thereafter in patients with diabetes mellitus (DM). We aimed to investigate the effect of infusion of glucose, insulin ànd potassium on short-term and long-term mortality in patients with and without diabetes mellitus (DM).
Materials and Methods: We performed a randomized trial of GIK (80 mmol potassium in 500 mL glucose 20%, at a rate of 3 mL/kg body weight/h, and short-acting insulin for 8-12 hours) in patients with acute MI, treated with primary angioplasty. To obtain blood-glucose levels between 7.0 mmol/L and 11.0 mmol/L, the rate of insulin infusion was set according to a nomogram.
Results: From April 1998 to September 2001, 940 patients were randomized to receive GIK (n-=476) or no infusion (n=464). After 30-days 23 patients in the GIK group compared to 27 patients in the control group had died (4.8% vs. 5.8%, p=0.50) at 1-year 31 patients in the GIK group compared to 38 patients in the control had died (6.5% vs. 8.2%, p=0.32). In the 856 patients (91.1%) without signs of heart failure (Killip class I) 11 patients died in the GIK group compared to 28 patients in the control group (2.6% vs. 6.5%, p<0.01). In the 84 patients (8.9%) with signs of heart failure (Killip class ≥II) 20 patients in the GIK group versus 10 patients in the control died (40% vs. 29.4%, p=0.32). In patients with DM 4 patients in the GIK group compared to 6 patients in the control group died (8.0% vs. 12.2%, p=0.48).
Conclusion: In all patients the infusion of GIK did not result in a significant beneficial effect on short-term and long-term mortality. Infusion of GIK in patients with DM is favourable, although with 99 patients our study was not powered to detect a statistical difference. A significant reduction in mortality is demonstrated in patients without heart failure treated with high-dose GIK in combination with primary angioplasty for acute MI. In this predefined subgroup, 26 patients have to be treated for up to 12 hours with GIK to save one after 1 year. There is a non-significant higher mortality of GIK in patients with heart failure on admission, possibly caused by volume load of GIK.

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Effects of isoproterenol and noradrenaline on heart hypertrophy in diabetes.

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Background and Aims: Effects of isoproterenol (ISO) and noradrenaline (NA) exerted on heart hypertrophy were investigated in metabolically healthy /control/ (H), untreated (D) and insulin-treated (DI) diabetic Wistar rats (n=3-8).
Materials and Methods: The compounds were administered for 24 h (ISO: 100 µg/kg/h, NA: 300 µg/kg/h) and the observed effects were characterised by the hypertrophic index (RV/BW, LV/BW) and by the left ventricle gene expression of A- and B-type natriuretic peptides, determined by Northern blot. The yielded results were evaluated compared to the sham-operated (S) animals.
Results: Right ventricle index was elevated only in DI group after ISO-administration (0.6864) in comparison to S animals (0.5497, p<0.001) and the value was the highest among all the groups also here (HIso: 0.5892, HIso-DIIso: p<0.03; DIso: 0.5357, DIso-DIIso: p<0.01). Left ventricle index was considerably enhanced in H (HS: 2.2293, HIso: 2.7709, p<0.0001) and DI (DIS: 2.3549, DIIso: 2.9038, p<0.001) rats, although the diabetic state itself did not influence it. After the treatment, LV/BW was lower in D animals compared to the other two groups (DIso: 2.5881; HIso-DIso: p<0.03, DIso-DIIso: p<0.003). ANP gene expression was significantly increased by diabetes itself (HS: 1.0, DS: 4.6092, p<0.04), but it was reduced to similar level than in the control group by insulin-treatment (DIS: 1.0636, DS-DIS: p<0.008). ISO-administration enhanced considerably ANP gene expression both in H and DI groups (HIso: 2.4236, p<0.02; DIIso: 3,0196, p<0.0001). Despite this fact, however, untreated diabetes generated significantly higher level compared to insulin-treatment (DIso: 6.007, DIso-DIIso: p<0.05). BNP gene expression was changed neither in D, nor in DI sham-operated groups (HS: 1.0, DS: 1.4209, DIS: 1.2116), but after ISO-administration it was elevated in all cases (HIso: 3.278, HS-HIso: p<0.002; DIso: 2.8897, DS-DIso: p<0.03; DIIso: 4.0964, DIS-DIIso: p<0.0001). After NA-treatment, right ventricle index was unchanged in contrast with ISO. However, left ventricle index was elevated both in D and DI groups compared to sham-operation (DS: 2.0833, DNa: 2.5211, DS-DNa: p<0.009; DIS: 2.0571, DINa: 2.5795, DIS-DINa: p<0.003). ANP gene expression increased in H and DI groups after NA (HS: 1.0, HNa: 3.6029, p<0.05; DIS: 1.6063, DINa: 3.342, p<0.05). BNP gene expression was significantly elevated only in the control group (HS: 1.0, HNa: 3.5096, p<0.04).
Conclusion: According to the results, the observed compounds exert stronger hypertrophic effect during insulin-treatment, but ANP gene expression – elevated ab ovo in diabetes – is reduced. BNP gene expression is less higher in diabetes, but the considerable enhancement during ISO-effect is remained.

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Risk of congestive heart failure after the initiation of anti-hyperglycemic therapies.

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Background and Aims: We recently demonstrated that insulin and oral anti-hyperglycemic agents were independent predictors of congestive heart failure (CHF) after controlling for other risk factors. The objective of this study was to estimate the specific incidence rate of CHF associated with initiating sulphonylurea, metformin or insulin in mono and combination antidiabetic regimens.
Materials and Methods: The study population consisted of all diabetes patients in the Kaiser Permanente Northwest (KPNW) diabetes registry as of January 1, 1998. Patients with a prior history of CHF were excluded. We defined the start of the subject study period as the date when their drug regimen changed, either through switching to or adding another antidiabetic drug. We defined the new therapy as the index therapy, and the date of initiating the new therapy as the index date. Patients were then followed until the index therapy was discontinued or changed, or until June 30, 2002. We calculated the incidence rate of CHF in patients on various therapeutic regimens adjusting for age, gender, diabetes duration, existing ischemic heart disease and glycemic control (HbA1c).
Results: We observed 195 incident cases of CHF in 4356 patients who initiated a new therapy over a mean follow-up of 22 months; an unadjusted incident rate of 2.50 cases per 100 person-years. After adjustment for CHF risk factors, patients initiating insulin monotherapy were significantly more likely to develop CHF compared to patients receiving sulphonylurea monotherapy (RR=1.68, p=.010) or metformin monotherapy (RR=2.10, p=.022). Patients receiving triple therapy were twice as likely to develop CHF compared to patients initiating sulphonylurea/metformin combination therapy (RR=2.00, p=.009).
Conclusion: Before and after adjustment for other risk factors, diabetic patients using insulin, alone or in combination with sulphonylureas or metformin, were more likely to develop CHF. Combination therapy with sulphonylurea and metformin did not increase the risk of CHF compared to either agent used as monotherapy. Age, duration of diabetes, prior history of ischemic heart disease and poorer glycemic control were significantly associated with CHF, but gender was not.

Therapy Category	N	Adjusted Incident Rate per 100 Person Years (95% CI)
Sulphonylurea Monotherapy	1,596	1.94 (1.64-2.29)
Metformin Monotherapy	320	1.55 (0.02-2.62)
Insulin Monotherapy	158	3.27 (2.27-4.69)
Sulphonylurea/metformin combination	1,713	1.68 (1.41-2.00)
Insulin/sulphonylurea combination	165	2.63 (1.65-4.18)
Insulin/metformin combination	262	2.88 (2.03-4.08)
Insulin/sulphonylurea/metformin	142	3.36 (2.06-5.50)

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Glipizide (Glucotrol) does not inhibit ischemic preconditioning in anesthetized rabbits at a clinically relevant and efficacious dose.

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Background and Aims: Pre-clinical data suggest that K_{ATP} channel blockers can inhibit cardioprotection from ischemic preconditioning (IP). This has raised some concern that diabetic patients treated with sulfonylureas may be more susceptible to cardiac ischemic injury. However, not all sulfonylureas necessarily share the same pharmacological profile, and pre-clinical studies have often used supra-pharmacological doses of these drugs. Therefore, we examined the effects of a clinically relevant and efficacious dose of glipizide (Glucotrol) on IP in anesthetized rabbits and compared them to those of glyburide, a sulfonylurea reported to block IP.
Materials and Methods: All studies were conducted in open-chest anesthetized NZW rabbits. Initially, a steady-state i.v. infusion dose of glipizide (0.17 mg/kg loading dose; 0.12 mg/kg/h) was selected that achieved a clinically relevant plasma concentration (1-2 ug/ml) and significantly ($p<0.05$, n=8) increased plasma insulin by 51 ± 17% from baseline values. The i.v. infusion dose of glyburide (0.05 mg/kg loading dose; 0.03 mg/kg/h) was selected to obtain equivalent increases ($p<0.05$, n=6) in plasma insulin (57 ± 17%). Both doses of glipizide and glyburide significantly lowered ($p<0.05$) plasma glucose from baseline values. In a subsequent study, myocardial injury (30 min of coronary artery occlusion (CAO) followed by 2 h of reperfusion, R) was induced midway during a 2 h infusion of equal volumes of vehicle (cyclodextrin), glipizide or glyburide at the aforementioned doses. The cardioprotective effects of IP (a 5 min CAO, 10 min R before the 30 CAO) on myocardial injury were also evaluated during each treatment. Myocardial injury was quantified histochemically and expressed as infarct area vs. area at risk (%IA/AAR). Data presented are mean ± SEM (all groups: n=10).
Results: In the vehicle group (no IP), the %IA/AAR was 61 ± 4% and neither glipizide nor glyburide alone (no IP) altered %IA/AAR when compared to the vehicle group. In vehicle- or glipizide-treated rabbits, IP significantly reduced %IA/AAR to 39 ± 5% and 45 ± 4%, respectively ($p<0.05$ vs. vehicle group) and there was no significant difference in %IA/AAR between the two groups (glipizide+IP vs. vehicle+IP). However, in glyburide-treated rabbits, IP failed to significantly reduce %IA/AAR (53 ± 4%; p=0.2 vs. vehicle group). Furthermore, the %IA/AAR in the glyburide+IP group was significantly ($p<0.05$) greater than in either the glipizide+IP or vehicle+IP groups. There were no significant differences in the AAR, mean arterial pressure or rate pressure product among the groups.
Conclusions: At doses that were both clinically relevant and demonstrated equivalent elevations in plasma insulin, glipizide, unlike glyburide, did not significantly limit the cardioprotective effects of IP in anesthetized rabbits. Thus, these data suggest that glipizide treatment is unlikely to increase the risk of cardiac ischemic injury.

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Effects of simvastatin treatment on cardiac performance in streptozotocin-induced diabetic rats.

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Background and Aims: Simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is widely used in the treatment of cardiovascular disease due not only to decrease in concentration of cholesterol, but also to non-lipid-involving mechanisms. In the present study, we investigated the mechanisms of the effects of simvastatin treatment on cardiac performance and some biochemical parameters in ventricular tissue.
Materials and Methods: The Wistar rats were grouped as control and diabetic. Diabetes was induced by single intraperitoneal injection of streptozotocin (45mg/kg). After 8 weeks induction of diabetes, some of the control and diabetic rats were treated with simvastatin (10 mg/kg rat/ day; orally) for 4 weeks. Cardiac performance was evaluated in isolated right atria and papillary muscle. Nitric oxide, glutathione, malondialdehyde, hydoxyproline and protein levels were measured on ventricular homogenates.
Results: In the diabetic myocardium, as a marker of lipid peroxidation, malondialdehyde level, was significantly elevated and non-enzymatic antioxidant, glutathione level, was decreased and simvastatin restored these alterations in diabetics. Simvastatin also reduced the increased nitric oxide levels in diabetic heart. Either diabetes or the treatment did not affect the myocardial hyrdoxypyroline and total protein levels. In basal conditions, heart rate of atria was markedly decreased in spite of increased contractility in papillary muscle in diabetics ($p<0.001$). In diabetic atria, whereas noradrenaline-induced chronotropic response decreased, isoprenaline-induced response did not significantly change compared with controls ($p>0.5$). The inotropic effects of both noradrenaline and isoprenaline significantly increased in diabetic heart ($p<0.001$). Simvastatin treatment restored the noradrenaline-induced both inotropic and chronotropic responses, and isoprenaline-induced inotropic response.
Conclusion: Our results suggest that simvastatin treatment may have clinical importance in the prevention of diabetes induced cardiovascular complications via decreasing oxidative stress and regulating cardiac performance in myocardium.

Some biochemical parameters of myocardium of experimental groups

Parameters	Control (n=8)	Diabetic (n=8)	Simvastatin treated Diabetic (n=10)
Malondialdehyde (nmol/mg protein)	0.054±0.001#	0.71±0.001*	0.059±0.01#
Glutathione (µmol/g)	6.34±0.20	5.84±0.03¶	6.60±0.13‡
Nitric Oxide (µM)	85±2.50#	164.42±3.42*	106.08±1.19#
Hydroxypyroline (µg/mg tissue)	0.76±0.07	0.69±0.03	0.76±0.08
Total Protein (µprotein/mg tissue)	25.67±0.61	25.14±1.06	27.52±1.02

*P<0.001, ¶P<0.05 *vs.* control; #P<0.001, ‡P<0.01, †P<0.05 *vs.* diabetic.

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Insulin suppresses Vascular Endothelial Growth Factor (VEGF) and Matrix Metalloproteinase-9 (MMP-9).

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We have recently demonstrated a potent anti-inflammatory and thus a potential anti-atherogenic effect of insulin in human aortic endothelial cells *in vitro*, and mononuclear cells (MNC), *in vivo*, at physiologically relevant concentrations. We have now further investigated the anti-inflammatory action of insulin on vascular endothelial growth factor (VEGF). VEGF plays a central regulatory role in angiogenesis and it contributes to the pathogenesis of proliferative diabetic retinopathy and may also accelerate atherosclerosis. Matrix metalloproteinases (MMP) are induced by VEGF and may also contribute to angiogenesis. Insulin was infused (2 IU/h) in 5% dextrose (100 mL/h) and KCL (8 mmol/h) into ten fasting obese non diabetic subjects for 4 hours. Blood samples were obtained at 0, 2, 4 and 6 hours. Plasma insulin concentrations increased from a basal level of 12.5 ± 2.2 m U/mL to 28.2 ± 3.3m U/mL at 2 h and 24.4 ± 3.7 m U/mL at 4 h after insulin infusion. VEGF concentration decreased significantly ($P< 0.001$) to 73.5 ± 20.9 % (mean ± S.D.) of the basal level (307 ± 164 pg/mL) at 2 h, 67.1 ± 23.2 % at 4 h and 81.9 ± 18.5% at 6 h. Plasma MMP-9 concentrations decreased to 83 ± 22% of the basal level (468 ± 223 ng/mL) at 2 h and to 82 ± 21 % of the basal level at 4 h ($P<0.05$) and returned to the baseline level at 6 h. Dextrose infusion (5%, 100 mL/h) alone did not change plasma VEGF or MMP-9 concentrations. We conclude that insulin suppresses plasma VEGF acutely. These data are consistent with an anti-inflammatory and a potential neo-vascularization suppressive effect of insulin. These observations may have implications for 1) a potential anti-retinopathic and anti-atherosclerotic effect of insulin in the long-term; and 2) the pathogenesis of retinopathy in diabetes mellitus.

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Modulation of macrophage scavenger receptor, CD36, in macrophage-derived-monocytes by troglitazone.

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Background and Aims: Atherosclerosis, an important complication of diabetes mellitus, is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries. Macrophage foam cells, one of the principal cell types involved in atheroma formation, are considered to be crucial players in the initiation processes and progression of atherosclerosis by their ability to take up oxidised LDL through the scavenger receptor, CD36. The role of PPARs in the differentiation of monocytes into macrophages, induced by pro-inflammatory cytokines in vitro, is unclear. Our aim, therefore, was to investigate the potential beneficial effects of the PPARγ agonist (troglitazone, TROG), on cytokine-induced monocyte differentiation into macrophages.
Materials and Methods: Cells of the human monocyte-like cell line (THP-1) were exposed for 48h to physiological (5mM) or pathophysiological (25mM) concentrations of glucose ± TROG (10μM). Differentiation of monocytes into macrophages was achieved by culturing cells in a cytokine cocktail containing IL-1β [10 ng/ml], TNFα [50 ng/ml], and IFNγ [1000 IU/ml]. Expression of macrophage specific markers and scavenger receptor CD36 were assessed by flow cytometry. PPARα, γ, and δ gene expression were examined by RT-PCR. Binding of the transcription factors NF-kB and AP-1 was assessed using EMSA.
Results: In normal (NG, 5mM) and high (HG, 25mM) glucose conditions, the cytokine cocktail was able to induce a significant increase in expression of CD11b (2 and 4 fold, respectively), CD14 (42 and 132 fold, respectively), CD68 (63 and 24 fold, respectively), and CD36 (12 and 70 fold, respectively); TROG reduced this effect by 36 to 99%. PPARγ expression was significantly down-regulated by cytokines or by HG conditions (15.5%), and in the presence of HG this was partially reversed by TROG. Cytokines had no effect on PPARα and δ expression. The cytokine cocktail markedly increased NF-kB (x12) and AP-1(x2) nuclear binding (in both NG and HG); TROG reduced NF-kB binding by 13 and 27%, and AP-1 binding by 21 and 9% in NG and HG conditions, respectively.
Conclusion: We have demonstrated that macrophage scavenger receptor CD36 expression is up-regulated during cytokine-induced monocyte differentiation into macrophages. This up-regulation involves the PPARγ pathway and activation of the transcription factors NF-kB and AP-1.

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Classical Risk Markers of Atherosclerosis in Diabetes (I)

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To correlate risk factors like atherogenic index in plasma (AIP), TG/HDL ratio, obesity in Type 2 diabetes mellitus with IHD in India.

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Background and Aim: To correlate risk factors like Atherogenic index in plasma (AIP), TG/HDL Ratio, Obesity in TYPE - 2 DM with IHD in India:
Material and Methods: 946 Type 2 diabetic patients registered with Om Diabetes Clinic and Research Centre were selected at random for this retrospective study. All these patients history, findings, duration of diabetes, risk factors like Wt . BMI, W/H ratio, lipid profile and ECG were noted Patients were divided in two groups; One with history of myocardial infarction in recent past (patients biochemical parameters and other risk factors values prior infarct considered) other group without myocardial infarction (patients with non conclusive ECG, Patients on lipid lowering agents were excluded). All these patients Triglyceride /HDL ratios and AIP = Log (TG/HDL)calculated.
The standardization of normal values of risk factors in Indian patients was as follows.
BMI in Males normal < 23and in females normal < 21.
W/H ratio in male normal < 0.95 and in females normal< 0.85
TG/ HDL ratio normal values < 4.0 ,Atherogenic index in plasma normal values < 0.50.
Results: Out of 500 males 65 males (13%) had positive history of MI .where as out of 446 females 40 females (8.9 %) had positive history of MI.

IHD POSITIVE

	BMI	W/H	TG/HDL	LOG(TG/HDL)	All Four Risk Factors present
MALE (N=65)	63.0.%	64.%	52.0%	60.0%	49.0%
FEMALE (N=40)	87.0%	77.0%	55.0%	72.0%	52.0%

IHD NEGATIVE:

	BMI	W/H	TG/HDL	LOG(TG/HDL)	All Four Risk Factors present
MALE (n=435)	64.5%	53.3%	36.7%	48.5%	14.2%
FEMALE(n=406)	92.0%	76.0%	27.0%	1.0%	22.0%

Analysis: Out of 946 patients 105 Type 2 DM Patients had positive history of MI. Of Which 53 patients had all four risk factors for MI above normal values , compared to 52 patients with MI Who had less than four risk factors positive this observation was statistically significant ($p<0.016$) . There was statistically no significant difference between male and female MI patients. I n IHD negative group out of 841 patients 91 female and 62 males also had all these four risk factors positive this observation was not statistically significant .
Conclusion: Thus presence of AIP, BMI, W/H. TG/HDL RATIO all Four risk factors above normal has strong correlation for IHD in type 2 DM patients. This observation can be used to predict future MI with current normal ECG and other investigations like stress testing ,angiography should be advocated in Type 2 DM patients with all four risk factor positive.

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The North Catalonia Diabetes Study (NCDS). Characteristics of Type 2 diabetic subjects with and without cardiovascular disease.

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Background and Aims: Cardiovascular disease (CVD) is the most frequent cause of morbidity and mortality in subjects with diabetes mellitus type 2 (T2DM). The aim of this study was to evaluate the prevalence of cardiovascular disease (CVD) and classical cardiovascular risk factors (CHD) in T2DM subjects of North Catalonia Region.

Materials and Methods: The study was performed in three different regions (92.912 inhabitants). The random sample selected was 307 patients with T2DM (61,6% men), age: 59,63 ±7,87 years; diabetic evolution: 8,6 ±7; HbA1c: 7,0% ±1,44; BMI 30,01 k/cm^2 ±4,7. The reference group selected was 307 subjects without diabetes mellitus matched for sex and age. Classical risk factors (CHD)[diabetes, high blood pressure (HBP), dyslipidemia, smoking, age, gender, obesity , waist circunference and CVD family events] were measured. The CHD risk level was performed with estimate of 10-Years Risk (Framingham Point Scores) according to ATP III criteria.
Results: The prevalence of CVD among the T2DM was 21,97% versus 11,92% in reference group (p<0.001). Peripheral ischemia was observed in 4,6% vs.1,0% (p<0,01).The prevalence of CHD were: High Blood Pressure (HBP) in T2DM: 64,3% vs.32,9% in reference population (p<0.001); dyslipidemia: 56,4% vs.43% (p=0.001); smoking: 15% vs.18,6% (p=0,28); no differences in age, obesity: 45% vs.28,3% (p<0.001); family CVD events: 38,5% vs.17,4% (p<0.001). The presence of 3 or more CHD risk in T2DM subjects was 91,39% vs.76,9% in non diabetics. CVD in the total group was correlated to the number of CHD risk factors, HBP, dyslipidemia, obesity, family antecedents of CVD and diabetes evolution (p<0.001).
In the total, group CVD and CHD were more frequent in men than women (p<0.001). The CVD and CHD risk was higher in diabetic women than in the reference population (p<0.001). In table 1, it was represented the different characteristics of diabetic and non diabetic populations with CVD. A significative differences were observed in T2 DM with CVD, related to age (p=0,043), CVD family events (p=0.001), waist circunference, diabetic evolution (years) and CHD risk (p=0,053). It was found a significative differences in non-diabetic group patients with CVD: a high CHD risk level, worse kidney function and only a tendency related to age and waist circunference.
Conclusion: A high average of diabetic and non diabetic populations manifested 3 or more classical factors of CHD. High CVD events and high CHD risk level were found in the diabetic group. It is suggest an aggressive intervention specially in the control and prevention of major classical cardiovascular risk factors.

Characteristics	T2DM SAMPLE With and Without CVD CVD:21,97%	Non-CVD	P	REFERENCE Group With and Without CVD CVD:11,92%	Non-CVD	P
BHP 140/90	65,7%	63,6%	P=0.775	30,56%	35,2%	p=0.851
Dyslipaemia	59,7%	55,1%	p=0.577	58,3%	41,7%	p=0.073
Obesity (BMI=>30)	49,3%	44,1%	p=0.489	30,6%	28,2%	p=0.844
Current smoker	11,9%	16,0%	p=0.562	13,9%	19,2%	p=0,647
Age	94,0%	84,0%	p=0,043	97,2%	85,3%	p=0,063
CVD Family Events	57,6%	33,1%	p=0,001	13,9%	17,8%	p=0,647
Waist circumference cms.	105,03 ±10,86	100,78	p<0,01	99,91 ± 11,95	96,28 ±12,15	p=0,093
Non HDL cholesterol	146,08 ± 37,96	158,07 ±40,97	p=0,033	153,17 ± 44,83	157,92 ±35,78	p=0,545
Creatinine	1,25 ±1,13	0,99 ±0,25	p=0,065	1,07 ±0,19	1,01 ±0,16	p=0,05
10-Years CHD Risk	11,58% ±7,11	9,56% ±7,63	p=0.053	11,44% ±5,99	7,87% ±6,26	p<0,001

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The evaluation of risk factors influencing the extent of the first myocardial infraction (MI) in Type 2 diabetes patients.
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Background and Aims: Type 2 diabetes mellitus (DM2) is a well-recognized risk factor of MI. Both ambulatory and early and late mortality in this patient group is 2-3 times higher than that in general population. The aim of the study was to evaluate the risk factors influencing the extent of first MI in patients with DM2.
Materials and Methods: Retrospective, epidemiological questionnaire study including patients hospitalized in Szczecin due to recent MI in the years of 1996-2000. Study group: 144 patients (70F, 74M) aged 41-80.2 yrs. (x=62.6±9.4); DM2 duration 0-30.7 yrs. (x=7.5±6.7); BMI 20.5-38.4 kg/m^2 (x=29.9±3.6). Arterial hypertension (HT) was found in 58.3%, previously diagnosed coronary heart disease (CHD) in 40.3%, dyslipidaemia in 52.1%. Study group was divided according to the electrocardiographic extent of MI. Extensive MI was defined as that of anterior or antero-lateral location and of 2 locations, non-extensive MI was defined as that of either_inferior or lateral or posterior location. The following factors influencing the extent of MI were assessed: duration, mode of treatment of DM2, fasting glucose (FG) before MI, dyslipidaemia, HT, CHD, cigarette smoking, sex.
Results: Extensive MI was more frequent in patients with a longer DM2 duration (> 10 yrs.) (RR 1.92; 95% CI [0.91; 4.08]; p=0.089). The prevalence of extensive MI increased in patients with FG > 6.1 mmol/L (RR 1.15; 95% CI [0.83; 3.24]; p< 0.027). In patients treated with oral sulphonylureas (SU) the prevalence of extensive MI was higher than that in other groups (RR 1.65; 95% CI [0.83; 3.24]; p=0.15).
Conclusion: The risk of extensive MI in DM2 patients was increases along with diabetes duration and FG value. Only nearly euglycaemic FG values decreased the risk of extensive MI. Treatment with SU was related to the extent of MI. High prevalence of MI as a first manifestation of CHD is worth mentioning. The other risk factors did not increase the risk of extensive MI.

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Blood glucose at admission is a predictor of long-term outcome in patients with a first manifestation of ischemic heart disease.
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Background: Hyperglycaemia has been associated with increased morbimortality in critically ill patients which is reduced with the normalization of blood glucose levels. Thus, the identification of high risk patients may be important to improve the survival rates.
Aim: To determine the role of glycaemia at admission as a predictor of total mortality or serious cardiovascular events in patients with a first manifestation of coronary heart disease.
Patients and Methods: We studied 504 patients (including diabetic and non diabetic subjects) consecutively attended in our hospital from the 1997 to 2000 for a first ischemic cardiac event . During a mean follow-up of 700 ± 424 days , 70 subjects died and 434 subjects remained alive. Plasma glucose concentration was measured at admission. Other prognostic variables considered were age, sex, hypertension, smoking habit and lipid profile.
Results: Patients who died showed higher age (72.5 ± 11.5 years vs. 62.5 ± 12.2 years;p < 0.01) and glycaemia (10.1 ± 4.9 mmol/l vs 8.7 ± 4.7 mmol/l; p< 0.05). When we consider the whole group of patients, age and admission glycaemia were the only variables related to serious cardiovascular events (including cardiac death and/or myocardial reinfarction and/or stroke) in logistic regression analyses. Patients with plasma glucose concentrations greater than or equal to 6.1 mmol/l had a higher rate of serious cardiovascular events (24.5% vs 11.0%; p< 0.01), cardiac events (22.6% vs 9.7%; p< 0.01) and cardiac mortality(13.1% vs 6.2%; p<0.05). When we divide the group of patients according to different cut-off points of glycaemia at admission (6.1, 7.0 and 11.1 mmol/l), those with concentrations above these points have significantly lower cumulative survival (Kaplan-Meier method).
Conclusion: Admission glycaemia is an independent prognostic factor of serious cardiovascular events in patients with a first manifestation of coronary heart disease. Detection and correction of this alteration may be relevant to improve the prognosis of these high risk patients.

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HbA1c is an independent predictor of total mortality in non-diabetic but not in diabetic patients with acute myocardial infarction and heart failure: a substudy to the OPTIMAAL trial.
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Background and Aims: Glycated haemoglobin (HbA1c) is a measure of average blood glucose concentration over three months. Thus, HbA1c is not affected by stress hyperglycaemia during an acute myocardial infarction (MI). In a large population of patients with MI complicated with heart failure, we examined the prognostic importance of HbA1c in diabetic and non-diabetic patients.
Material and Methods: 2841 of the 5477 patients in the OPTIMAAL trial had HbA1c measured at baseline, and were included in this study. Of these, 495 had diabetes by history. Patients without known diabetes were stratified into three categories according to the HbA1c levels: HbA1c < 4.9 % (n = 1642), HbA1c: 4.9-5.1 % (n = 432), and HbA1c ≥ 5.2 % (n = 272). HbA1c 5.2 % is upper limit of normal range of the present HbA1c assay. The

patients were followed for a mean of 2.5 years. Differences in mortality were compared against the group of non-diabetic patients with HbA1c < 4.9 %, and the value of baseline HbA1c to predict total mortality was tested in multivariate analysis using a Cox regression model with adjustment for age and sex.
Results: During follow-up there were 435 (15.3 %) deaths from any cause in this cohort. Mortality rates were 13 % in patients with HbA1c < 4.9 % (RR=1.0), 17 % in patients with HbA1c: 4.9-5.1 % (RR = 1.19, P =0.20), 22 % in non-diabetic patients with high HbA1c ≥ 5.2 % (RR =1.30, P=0.080) and 18 % (RR =1.18, P=0.20) in patients with known diabetes, adjusted for age and sex. An increase of 1 % in HbA1c was in non-diabetic patients associated with 28 % increased risk of mortality (HR: 1.276, 95 % CI: 1.045-1.559, P = 0.017). Among patients with known diabetes however, there were no increase in risk with increasing HbA1c (HR: 1.024, 95 % CI: 0.878-1.194, P = 0.76).
Conclusion: In a high-risk MI population, HbA1c level was an independent predictor of total mortality in non-diabetic patients, but interestingly not in patients with diabetes.
Furthermore, a history of diabetes was not associated with an increased risk of mortality in this population. The new diagnostic criteria of diabetes mellitus, including patients with less severe abnormalities of the glucose metabolism, could partially explain this unexpected finding.

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Mean HbA1c during 18 years predicts autonomic cardiac function, coronary atheromatosis and physical fitness in Type 1 diabetes.
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Background and Aims: The long term effects of glycemic control on cardiovascular function are not established in type 1 diabetes. We studied the association between 18 years mean HbA1c, cardiac autonomic tests ,coronary atheromatosis and physical fitness (maximum heart rate) to characterize the role of chronic hyperglycaemia on cardiac function.
Materials and Methods: At 18 years follow-up of 39 patients (23 men,16 women) with type 1 diabetes, mean age 43 (range:35-48)years mean HbA1c during 18 years was calculated and cardiac autonomic tests were performed. 29 of these patients were examined with intracoronary ultrasound. Heart rate variation (HRV:RR variation on the ECG) was examined during normal and deep breathing, a Valsalva maneuver and a tilt test. Maximum heart rate during exercise ECG was registered.
Results: Mean HbA1c during 18 years was 8.2 (range:6.6-11.3)%. The patients in the highest HbA1c tertile (HbA1c ≥ 8.4 %) were compared with the two lower tertiles (HbA1c<8.4). Each cardiac autonomic test was significantly different in the two groups (table). Duration of disease was associated to HRV during deep respiration and the Valsalva test. When corrected for duration of disease, HRV during deep breathing was still significantly associated to mean HbA1c during 18 years (p=0.012) but the Valsalva test was not. Age, gender, smoking, total cholesterol, systolic -, diastolic blood pressure, urine albumin and BMI were not significantly associated with any autonomic tests. HbA1c predicted coronary atheromatosis investigated as mean % vessel area stenosis. Furthermore, mean heart rate during bicycle exercise test was significantly higher in the group with HbA1c< 8.4 % (p=0.009) .
Conclusion: Long- term glycemic control has a major impact on cardiovascular function in type 1 diabetes. Patients with HbA1c < 8.4% during 18 years had significantly less cardiac autonomic neuropathy, less coronary atheromatosis and were in better physical condition than those with HbA1c ≥ 8.4%.

The test results for patients with mean HbA1c <8,4% compared to those with HbA1c ≥8,4%

Test Mean	HbA1c < 8.4%	Mean HbA1c ≥ 8.4%	p-values
Normal breathing HRV	15.3 (SD 8.0)%	9.9 (SD 7.0)%	p=0.038
Deep breathing HRV	40.0 (SD 21.5)%	19.9 (SD 14.5)%	p=0.005
Valsalva ratio	1.6 (SD 0.4)	1.3 (SD 0.3)	p=0.016
Tilt test ratio	1.2 (SD 0.1)	1.0(SD 0.1)	p=0.001
Intra coronary examination (mean %vessel area stenosis)	26 (SD 21)	41 (SD 17)	P=0.044
Max heart rate during exercise test	174 (SD 14)	157 (SD 23)	P=0.009

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The combination of renal dysfunction and impaired glucose tolerance is associated with a poor prognosis after acute myocardial infarction.
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Background and Aims: It is becoming more evident that non-diabetic patients with impaired glucose tolerance carry a worse clinical outcome after acute myocardial infarction (AMI). The detrimental influence of renal dysfunction on survival after AMI is also well recognised. To what degree these factors interact and if this has effect on long term prognosis is not known. We sought out to investigate if there was a relation between blood glucose levels and renal function and long term prognosis in non-diabetic patients with AMI.
Materials and Methods: 349 non-diabetic patients with AMI were consecutively included in our study. They were all treated with reperfusion therapy, either with angioplasty or thrombolysis. Patient data were recorded in a dedicated database and patients were followed for up to 8± 2 years. Patients were divided into three groups depending on their admission glucose level and presence of renal dysfunction. Chi-square tests were used in the analysis of the different groups. See Table below.
Results: Mortality rates during the follow up period were significantly different between the three patient groups. Lowest mortality (16%) was found in the group with neither impaired glucose tolerance nor renal dysfunction. A higher mortality (26%) was found in the group with the presence of one of them, and highest mortality (39%) was seen in the group with both of these disturbances present.
Conclusion: We conclude that both impaired glucose tolerance and renal dysfunction are associated with a higher long-term mortality in non-diabetic patients after AMI. However, it seems that especially the patient group with the combination of both have a poor prognosis after acute myocardial infarction. Recognition of this high risk patient group offers the ability to institute more aggressive and intensive treatment regimens for these patients, which are likely to positively influence survival.

Patients with AMI: groups divided depending on admission glucose (mmol/L) and renal function

	Group I Glucose<7,8 and normal renal function	**Group II** Glucose>= 7,8 or renal dysfunction	**Group III** Glucose >= 7,8 and renal dysfunction	**P value**
N=	137	81	31	
Age > 60 yrs.	60(44%)	99(55%)	27(87%)	<0,001
Male	123 (90%)	146 (81%)	17(55%)	<0,001
Death	22 (16%)	47 (26%)	12 (39%)	0,012 *

Renal dysfunction defined as: women: creatinine > 90 µ mol/L, male: (=< 50 years) creatinine >110 and (> 50 years) creatinine > 127 µ mol/L (upper limits of the normal laboratory values at our institution)

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Additive vascular effects of microalbuminuria and hypertension in patients with Type 2 diabetes mellitus.
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Background and Aims: Diabetes mellitus (DM) is a strong risk factor for the progression of cardiovascular disease. Microalbuminuria (MA) and hypertension (HTN) are predictors of poor cardiovascular outcome in diabetic patients. However, the link between DM, MA and HTN and vascular damage was not fully established. The aim of our study was to assess the association between DM and endothelial function and aortic distensibility in patients with or without MA or HTN.
Materials and Methods: Our cross-sectional analysis included 193 type 2 DM patients (pt), mean age 69 +/- 7 yrs, evaluated during a 2 yrs period. MA was defined as urinary albumin excretion of 20-200mg/24h in 3 non-consecutive samples. Endothelial function was evaluated with flow-mediated vasodilatation (FMD) and the endothelium-independent Nitroglycerine vasodilatation. FMD was defined as percent change in brachial artery diameter at 1 min. after 5 min. of upper arm blood pressure cuff occlusion. Aortic distensibilty (AD, 10^{-3} $mmHg^{-1}$) was defined as difference between end-systolic and end-diastolic aortic area divided by the product of brachial pulse pressure (PP) and end-diastolic aortic area. Aortic

area was determined by echocardiography at 4 cm above aortic valves. Association of DM, HTN and MA with both FMD and AD was assessed by means of multivariate logistic regression (odds ratios-OD) using diabetics without MA and HTN as comparison.
Results: Presence of both MA and HTN in DM pt was significantly associated with both decrease in FMD (OR: 2.74, 95%CI: 1.32-5.36) and decrease in AD (OR: 2.65, 95%CI: 1.46-5.03). Presence of only MA was also associated with decreased FMD (OR:1.97, 95%CI: 1.20-2.53) and decreased AD (OR: 1.87, 95%CI: 1.17-3.10). A weaker association was noted between diabetics with HTN and the decreased FMD (OD: 1.47, 95%CI: 0.90-2.10). No significant association was detected between hypertensive diabetics and decreased AD.
Conclusion: Association of MA or HTN in older diabetic patients has negative impact on the endothelial function and arterial distensibility. In these pt MA and HTN have additive deleterious vascular effects and could reflect a diffuse vascular disease.

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Homocysteine and glutathione metabolism in diabetes mellitus Type 2 patients with cardiovascular atherosclerosis.
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Background and Aims: The increased cardiovascular risk in subjects with diabetes mellitus type 2 (DM2) is partly explained by an association with established risk factors. Elevated homocysteine is by some autors regarded as an independent risk factor for cardiovascular disease (CVD). The etiological role of homocysteine in atherogenesis is far from clear. The purpose of this study was to assess its role in DM2 with CVD compared with control group of patients with CVD without DM2.
Materials and Methods: Fasting plasma homocysteine, its metabolites – cysteinylglyceine, cysteine and glutathione and cofactors of its metabolism - pyridoxine, vitamine B12 and folic acid concentration in plasma and erytrocytes, HbA1c, fasting plasma glucose, serum lipids (total, and HDL cholesterol and triglycerides), blood urea nitrogen, creatinine were measured in DM2 patients (n=50, BMI= 30,58±3,888, mean age =57±5,76y) and in a matched control group without diabetes (n=189, BMI=28,26±3,876, mean age=54,9±6,64y). Both groups have by coronarography proved CVD. The sulfur containing metabolites were determined fasting and 6 hour after the methionine test (100 mg/kg) also.
Results:see table
The diferrencis were evaluated by use of the Mann-Whitney test, multiple regression analysis was used to determine relationships between variables of interest. There were no differences in plasma or blood vitamine levels between two groups.
Conclusion: DM2 patients have lower fasting homocysteine and fasting and postmethionine glutathione levels in plasma. There were no differences in homocysteine, its metabolites and glutathione levels after methionine test in blood. Our findings suggest that homocysteine metabolism in DM2 with CVD is comparable with patients with CVD without DM2. Homocysteine and other metabolites levels do not show a correlation with therapy of diabetes or hyperlipidaemia. The role of homocysteine in pathogenegis of macrovascular complication in DM2 is unknown but our findings may suggest that factors other than the homocysteine could have a stronger impact on atherogenesis.

	Plasma (mediane) (micromol/l)		Blood (mediane) (micromol/l)	
	T2DM (n=50)	NonDM (n=189)	T2DM	NonDM
Homocysteine0	10,8*	11,7*	1,5	1,6
Homocysteine6	36,4	38,4	6,6	6,7
Cysteinylglycine 0	38,8	40,0	16,5	17,1
Cysteinyglycine 6	33,8	34,5	17,6	17,9
Cysteine 0	335,7	337,8	86,1	83,8
Cysteine 6	317,0	319,7	93,7	92,7
Glutathione 0	9,7**	11,1**	1002	1050
Glutathione 6	9,3	10,1**	1037	1096

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Urinary orosomucoid excretion independently predicts mortality in Type 2 diabetes but not in Type 1 diabetes at 5 years of follow-up.
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Background and Aims: Patients with diabetes mellitus have increased risk of cardiovascular and all-cause mortality compared to the background population. Microalbuminuria is a well-known predictor of increased mortality in patients with Type 1 and Type 2 diabetes. The excess mortality is mainly due to cardiovascular and kidney diseases. We have previously shown that urinary orosomucoid excretion rate (UOER) independently predicts increased over-all and cardiovascular mortality in patients with Type 2 diabetes in a short-time follow-up study. The aims of the present study were to determine whether increased UOER predicts mortality in patients with Type 2 diabetes in a longer follow-up period and in patients with Type 1 diabetes.
Materials and Methods: 430 patients with Type 2 diabetes and 148 patients with Type 1 diabetes were followed for a mean period of respectively 4.9 years and 5.3 years. Urinary orosomucoid excretion rate (UOER) and urinary albumin excretion rate (UAER) were measured by immunoturbidimetry in timed overnight urine samples. Detection limits and intra-series imprecision (CV%) were: U-Orosomucoid: 1.2 mg/l (5.6); U-Albumin: 3.3 mg/l (3.0). Cut-off levels were: UOER > 0.88 µg/min and UAER > 20 µg/min.
Results: Patients with Type 2 diabetes (273 M/157 F) had a mean (SD): age 59 (11) years, HbA1c 8.4 (1.9) %, and median (range) known duration of diabetes 3.0 (0.02-37) years. In Type 1 diabetes patients (91 M/57 F) the same figures were: age 40 (16) years, HbA1c 8.4 (1.7) %, and duration of diabetes 7.0 (0.02-66) years. Type 2 diabetes patients with increased UOER had a preponderance of hypertension, heart failure, longer duration of diabetes, higher age, HbA1c, P-Creatinine and UAER compared to patients with normal UOER. Type 1 diabetes patients with increased UOER had higher P-Creatinine and UAER compared to patients with normal UOER. During the study period 82 Type 2 diabetes patients died versus 17 Type 1 diabetes patients. There was a significant difference in survival between Type 2 diabetes patients with normal UOER compared to patients with elevated UOER (Log-Rank Test: p<0.0004). In Type 1 diabetes patients the difference in survival between the 2 subgroups was significant as well (p<0.04). In multivariate analysis of Type 2 diabetes patients: age, P-Creatinine, increased UOER, the presence of coronary heart disease, and stroke were independently associated with increased over-all mortality. Type 2 diabetes patients with increased UOER at baseline had 1.75 (95% CI: 1.04-2.95, p<0.04) times greater risk of over-all mortality compared to patients with normal UOER at baseline. In Type 1 diabetes: age and UAER were independently associated with increased over-all mortality. UOER was not an independent predictor of over-all mortality in Type 1 diabetes patients.
Conclusion: Increased UOER independently predicts over-all mortality in Type 2 diabetes patients but not in Type 1 diabetes patients at 5 years of follow-up. The results probably reflect differences in cardiovascular morbidity and pathophysiology between patients with Type 1 diabetes and Type 2 diabetes.

PS 90

Classical Risk Markers of Atherosclerosis in Diabetes (II)

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Influence of tobacco smoking on coronary atherosclerosis in diabetic and non-diabetic patients: results of the „Diabetics“ study.

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Background: A great number of studies have shown the difference in the coronary disease evolution and prognosis in smoker and non-smoker patients with sometimes paradoxical results. Smoker patients were often prone to better prognosis.
Aim: The aim of this study was to compare coronary angiography scores in current, former and non-smoker coronary patients in two cohorts of diabetic and non-diabetic.
Methods: Angiographic results of consecutive patients, diabetic and non-diabetic, were recorded. Patients with a previous history of PTCA or coronary bypass were excluded. Definition: current smoker : at least one cigarette per day or smoking cessation < 3 months ; former smoker : smoking cessation > 3 months ; non-smoker : never smoked. Five scores were considered to study the extent and severity of coronary atherosclerosis (Tab. 1).
Results: Coronary angiographies of 362 diabetic patients (175 non smokers, 133 former smokers, 54 current smokers) and of 321 non-diabetic patients (165 non smokers, 193 former smokers, 63 current smokers) were examined. The results are given in table 1. Former and current tobacco smoking was closely linked to coronary atherosclerosis progression in diabetic patients (4 out of 5 scores). Conversely, among the non-diabetic patients this relationship was found only for one score. The progression of the coronary disease was much more significant in former smokers and less remarkable in non-smokers. These results were confirmed after adjustment in a multivariate analysis (backward logistic regression).
Conclusion: The progression of atherosclerosis was more significant in smoker and in former smokers than in non smokers. This relationship was more particularly remarkable in the diabetic population.

	Diabetic patients				Non-diabetic patients			
	Non smokers	Former smokers	Current smokers	P	Non smokers	Former smokers	Current smokers	P
Gensini Score (mean)	31.4	40.2	30.7	0.03	24.6	31	6 25.1	NS
At least one stenosis > 50% (%)	71.4	82.7	79.6	NS	63.1	71.0	65.1	NS
At least one stenosis > 70% (%)	61.4	77.4	77.8	0.01	57.6	65.6	60.3	NS
At least one stenosis > 95% (%)	25.1	41.4	33.3	0.01	15.8	31.2	27	0.01
>=2 segments with stenosis > 70% (%)	32.6	44.4	27.8	0.01	23	34	27	NS

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Lipid but not glucose control predicts the incidence of cardiovascular disease in Type 2 diabetes over a 6 year period.

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Background and Aim: Cardiovascular disease (CVD) is the main cause of morbidity and mortality in patients with diabetes and modifiable risk factors for it are extensively sought. To determine the incidence of CVD in patients with type 2 diabetes and investigate the risk factors possibly related with it.
Materials and Methods: A total of 373 patients with type 2 diabetes without CVD at baseline were followed for 6 years. Mean age at baseline: 55.4 years, mean duration of diabetes: 6.7 years. CVD was defined as angina pectoris, myocardial infarction, stroke, PTCA, or bypass surgery. All patients were examined at baseline and 2-4 times yearly thereafter. HbA1c, fasting and postprandial glucose were measured at every visit and lipids once per year.
Results: During the 6 years of follow up, 25 patients experienced one or more CVD events (Group A), the incidence of CVD being thus 6.7%/6years. At baseline Group A compared to normals (Group B), after adjustment for age and duration of diabetes, had higher systolic blood pressure (SBP): 141.0vs133.7mmHg p<0.05, higher triglycerides: 214.5vs159.9mg/dl p<0.05, and lower HDL cholesterol, 43.0vs50.3 mg/dl p<0.05. On the other hand no difference was found in BMI: 26.5vs26.4Kg/m^2, smoking 28.0vs22.5%, fasting blood glucose (FBG), 186.3vs193.8mg/dl, 2h postprandial blood glucose (2hBG), 177.6vs192.9mg/dl, HbA1c, 8.1vs8.8%, total cholesterol, 237.6vs246.8mg/dl, LDL, 154.1vs160.4mg/dl or non-HDL, 187.7vs200.0mg/dl. During the follow up period glucose control improved, but was not different between Groups, A vs B, FBG, 168vs170.4mg/dl, 2hBG, 176.4vs172.0mg/dl and HbA1c 6.7vs7.0%, p>0.05. However, during the follow-up, the prevalence of hypertension and dyslipidemia was higher in group A than in group B: 68.0vs47.1% p<0.05 and 76.0vs52.6% p<0.05, respectively. In a logistic regression analysis model, SBP and HDL cholesterol were independent risk factors for CVD incidence. SBP was positively associated, B=0.031, p<0.03, while HDL cholesterol was negatively associated with CVD, B= -0.033, p<0.03, while no association was found with blood glucose, fasting or postprandial.
Conclusions: These data support the view that, apart from blood glucose control, indispensable for the prevention of diabetic microangiopathy, a multifactorial therapeutic approach, comprising blood pressure and lipid control, is needed, to prevent diabetic macroangiopathy. The role of post-prandial glycemia in the development of diabetic macroangiopathy has been probably overestimated.

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Apolipoprotein E polymorphism is related to macro – and microangiopathy in Type 2 diabetic patients.

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Background and Aims: Apolipoprotein E (apo E) is an important structural constituent of serum chylomicron, very low density lipoproteins and high density lipoproteins. By isoelectric focusing and immunoblotting, three common alleles were demonstrated. The relative frequency of the E 3 allele were higher than that in Caucasians, whereas the frequency of E 4 allele was lower in the Chinese population. To study the relationship between apo E phenotypes and diabetic complications in patients with type 2 diabetes**.**
Materials and Methods: We compared the distribution of apo E phenotypes in type 2 diabetic patients (n = 200) and age, sex-matched nondiabetic controls (n = 200) who participated in a case-control study. A total of 200 type 2 diabetic patients were divided into the three apo E groups; apo E 2 group (apo E 2/3 and apo E 2/4), apo E 3 group (apo E 3/3), and apo E 4 groups (apo E 4/3 and apo E 4/4). Coronary angiographic disease was assessed by angiography and defined as ≥ 1 lesion leading to minimum 50% lumen narrowing of any these 4 coronary arterial segments. Cerebrovascular disease was defined as a previously documented stroke or presence of hemiparesis. The diabetic retinopathy (DR) status was divided into normal, background, preproliferative and proliferative DR. Diabetic nephropathy was defined as a positive result on a dipstick test. Neuropathy was confirmed if the motor nerves showed abnormal conduction velocity.
Results: The distribution of apo E phenotypes in type 2 diabetic patients was similar to that of the normal control subjects. Apo E 2 protects from macrovascular complications in patients with type 2 diabetes. Apo E 2 also tends to protect from microvascular complications. In contrast, apo E 4 tend to increase the risk for macroangiopathy in type 2 diabetic patients. The lower prevalence of macroangiopathy in the subjects with apo E 2 was associated with lower serum LDL cholesterol levels.
Conclusions: Apo E phenotypes modulate the risk for diabetic complications in patients with type 2 diabetes. The confirmation of the association of apo E polymorphism with diabetic complications need long-tern follow up studies.

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Impact of overweight on cardiovascular risk factors of African American children.

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Background and Aims: Obesity is associated with increased risk for CVD. However, African Americans (AA) have higher CVD morbidity and mortality at any BMI level. We evaluated the effect of obesity on inflammatory markers, exercise capacity, lipid profile and fasting insulin to characterize cardiovascular risks of AA children.
Materials and Methods: Fifty healthy non-smoking, overweight, AA children (age=13.1± 0.4 yr, BMI =36±1.3, 70% F) referred for overweight management completed a physical examination; fasting levels of insulin (FI), fibrinogen (FIB), C-reactive protein (CRP), and lipids were measured. Additionally, subjects completed a maximal cardiopulmonary exercise test and wore a 24-hr Holter monitor for heart rate variability (HRV) analysis. Relative BMI (RBMI) was used to estimate percentage of overweight (BMI/50th percentile BMI on CDC chart for gender and age*100) with subjects stratified into 4 RBMI classes (125<150%, 150<175%, 175<200%, >200%).
Results: 50% of subjects had elevated resting BP (values>95th percentile based upon gender, age, and height). Exaggerated BP response to exercise occurred in 18% while 12% exhibited a rise in BP during passive recovery. Based on NCEP criteria, 57% had dyslipidemia (elevated cholesterol: 48% , LDL: 33%, triglycerides: 13%, low HDL: 24%). Elevations in FIB (>400) and CRP (>0.5) occurred in 32% and 40% respectively, while 100% had low exercise capacity (values below 85% of age-predicted VO_2). In addition to obesity, 60% of subjects have high CVR (≥3 risk factors) including low exercise capacity, dyslipidemia, hypertension, elevated CRP or FIB. Higher RBMI correlated with higher CRP (r =0.50, and FIB (r =0.49), measured and estimated VO_2 based on treadmill speed and incline (r = -0.51, r = -0.66), and increased number of CV risk factors (r=0.50, p<0.001 for all variables), but not HRV measures, lipid levels or FI. Groups with higher level of RBMI exhibited higher CVR (p≤0.01). A gender effect was present at the highest RBMI level (>200%) with males exhibiting greater CVR (p<0.02).

Relative BMI (n)	VO_2 (ml/kg/min)	% Age predicted VO_2	FI (µU/ml)	Cholesterol (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)	CRP (mg/L)	FIB (mg/dl)	High CVR (%)
Total Group (50)	18.8±0.6	40.5±1.2	22.1±5.2	170±4.2	103±3.9	95± 8	0.8±0.2	372±11	56
>125<150% (5)	26.2±1.9	55.5±3.3	9.1±12.7	164±13	100±13	86±23	0.2±0.6	303±31	0
>150<175% (13)	19.5±1.0*	42.8±1.8*	23.2±9.5	161± 9	91± 8	80±15	0.5±0.4	340±18	31
>175<200% (13)	18.7±1.0*	39.5±1.8*	12.3±9.5	181± 8	110± 7	114±15	0.7±0.4	384±18*	77
>200% (19)	16.7±0.9*	36.5±1.6*	35.1±9.1*	170± 6	106± 6	93±12	1.1±0.3	403±16*	74

Values reported as means±SE unless otherwise indicated. *p≤0.05 from the >125<150% group.

Conclusion: Overweight AA children exhibit increased number of CVR factors. In our study cohort, the proportion and severity of these CVR factors were driven primarily by the percentage overweight except for FI and lipid values. Low exercise capacity was an independent CV risk factor affecting AA children regardless of the severity of overweight. Implementation of programs to increase physical activity and early screening of risk factors may decrease the progression of obesity, cardiovascular morbidity and mortality in this ethnic group.

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Waist to hip ratio is a strong independent predictor for short term cardiovascular mortality, particularly in women.

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Background and Aims: Diabetes mellitus (DM) is an important risk factor for coronary artery disease (CAD), and it is strongly associated with obesity as measured by an increased body mass index (BMI) or waist to hip ratio (WHR). However, the roles of BMI and WHR as independent predictors for vascular events are at dispute.
Materials and Methods: We enrolled 756 consecutive patients undergoing coronary angiography from October 1999 through October 2000. These patients underwent follow-up after an average period of 2.27 ± 0.43 years. Significant CAD at baseline was defined as the presence of stenoses greater than 50%; extent of CAD as the number of such stenoses in a patient. The incidence of vascular events and total/cardiovascular mortality were recorded.
Results: At baseline, both WHR and BMI were increased among patients with DM over non-diabetic patients (n = 170; 28.37 ± 4.58 kg/m^2 vs. 26.80 ± 3.75 kg/m^2; p <0.001 and 0.97 ± 0.08 vs. 0.94 ± 0.09; p=0.04). WHR also was increased among patients with CAD as compared to those without (n = 461; 0.96 ± 0.08 vs. 0.93 ± 0.10; p = 0.001), and significantly correlated with the extent of CAD (r = 0.145; p <0.001), whereas BMI was not significantly different between patients with and without CAD. The incidence of vascular events (n = 97) was significantly lower in the first than in the 2nd through 4th quartiles of WHR (p = 0.012, p = 0.014, and p = 0.001) and in Cox regression analysis adjusting for conventional risk factors including diabetes status WHR proved independently predictive of total mortality (exp(B) = 1.510 (1.051-2.169); p = 0.026), cardiac mortality (exp(B) = 1.836 (1.098-3.068); p =0.020), and of total vascular events (exp(B) = 1.408 (1.058-1.875); p = 0.019). In contrast, a low (rather than a high) BMI was predictive of cardiac mortality (exp(B) = 0.410 (0.230-0.731); p = 0.003) and of total vascular events (exp(B) = 0.724 (0.549-0.946); p = 0.023). The impact of WHR was particularly evident among women.
Conclusion: WHR is elevated in patients with DM. It is an important independent predictor of total and cardiovascular mortality and of vascular events in patients undergoing coronary angiography, particularly among women.

1075

Prediction of hard coronary events by electron beam computed tomography in asymptomatic diabetic patients.

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Background and Aims: Prediction of hard cardiac events remains difficult in spite of the identification of several relevant risk factors for the development of coronary artery disease. Electron beam computed tomography (EBCT) permits the noninvasive quantification of coronary calcification as a marker of atherosclerosis. This report evaluated changes in coronary calcification score (CCS) in patients with type 2 diabetes to develop prediction models for hard cardiac events.
Materials and Methods: We conducted a prospective study of 107 asymptomatic diabetic men aged 61.4 +-9.5 years with a mean duration of 16.3+-11.2 years. We evaluated coronary artery calcification using EBCT twice during follow up period. Average follow up was 23+-11 month, and mean laboratory data during this study were evaluated.
Results: At baseline, the median CCS was 91 (range, 0-3980), and was 137(0-4834) at the end. Annual change in CCS was 17.5 (-240 – 2571). In the 107 asymptomatic diabetic patients, 6 patients had coronary hard events (angina or infarction). Multivariate logistic regression analyses revealed that only CCS at baseline could predict the event (odds ratio 10.4(95% confidence intervals: 1.14, 95.3, p=0.04) including age, smoking, BMI, blood pressure, HDL-cholesterol, HbA1C, and creatinine. Kaplan-Meier analysis showed that patients with CCS over 400 had more coronary hard events (4 events in 29 patients) than patients with CCS under 400 (2 events in 78 patients) by Logrank test (p=0.016). .
Conclusion: In conclusion, CCS could predict coronary events in asymptomatic diabetic patients

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The combined effects of diabetes and coronary atherosclerosis on the short term risk of vascular events.

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Background and Aims: Recent epidemiological data suggest that diabetes mellitus (DM) in patients without coronary atherosclerosis (CA) infers the same amount of risk for mortality from coronary heart disease as does preexisting coronary atherosclerosis (CA) in nondiabetic patients. However, absence of CA usually is not proven angiographically. Therefore we investigated in angiographically defined patients the single and combined effects of diabetes and CA on future cardiovascular events.
Materials and Methods: From October 1999 through October 2000 we recruited 756 consecutive patients undergoing coronary angiography. With respect to CA and DM, 4 groups of patients were built. Group 1: patients with neither CA nor DM (n = 197), group 2: patients without CA, but with

DM (n = 51), group 3 : patients with CA but without DM (n = 275), and group 4, patients with both CA and DM (n = 119). After an average period of 2.27 ± 0.43 years, the incidence of vascular end points (cardiac death, non coronary vascular death, non fatal myocardial infarction, ischemic cerebrovascular stroke, need for coronary bypass grafting, percutaneous coronary intervention, and vascular surgery at the carotid or peripheral arteries) was recorded.
Results: Event free survival was similar in groups 1 and 2. However, it was lower in groups 3 ($p < 0.001$) and 4 ($p < 0.001$) than in group 1. It also was lower in group 4 than in groups 2 ($p = 0.0016$) and 3 ($p = 0.0063$). Cox regression analyses adjusting for age, gender, smoking, hypertension, triglycerides, LDL cholesterol, and HDL cholesterol revealed odds ratios of 2.803 (1.398-5.618; $p = 0.004$) for group 3, and 4.320 (1.946-9.589; $p < 0.001$) for group 4 as compared to group 1. Adjusted odds ratios were 4.351 (1.238-15.045; $p = 0.022$) for group 2 and 1.636 (1.007-2.656; $p = 0.047$) for group 3 as compared to group 4.
Conclusion: In angiographically defined coronary patients, the presence of CA in angiography increases the risk of short term vascular events both among patients with and without DM. However, in contrast to the current opinion, in our diabetic patients without CA the risk of vascular events is not significantly increased. Patients with both DM and CA are at the highest risk.

PS 91
New Risk Markers of Atherosclerosis (I)

1077

Leptin, but not adiponectin, is associated with both risk for and time to a first–ever stroke in men.
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Background and Aims: Adipocyte-derived hormones like leptin and adiponectin may be important links between obesity and cardiovascular disease. In this prospective nested case-referent study, we tested whether leptin and adiponectin are risk markers for a first-ever stroke.
Materials and Methods: Two hundred seventy six cases with first-ever stroke (234 cases with ischemic and 42 with haemorrhagic stroke) were identified who, prior to the stroke, had participated in population based health surveys in northern Sweden. Referents were matched for sex, age, date and type of health survey and geographic region. Blood pressure, body mass index (BMI), presence of smoking, diabetes and hypertension were recorded and cholesterol was analysed. Leptin and adiponectin were analysed in stored samples. Risk markers for first-ever stroke were analysed by conditional logistic regression analysis.
Results: The stroke event occurred on average 4.9 years after the initial survey. Subjects with a future stroke were more obese and had higher levels of cholesterol and fasting glucose and had a higher frequency of diabetes mellitus and hypertension. Leptin levels were higher in male subjects with a future stroke ($p=0.004$) whereas adiponectin did not differ between cases and referents. Leptin correlated independently to diastolic blood pressure ($p=0.003$) and cholesterol ($p=0.01$) in men, and to postload glucose ($p=0.01$) in women whereas adiponectin correlated inversely with postload glucose in both men ($p=0.04$) and women ($p<0.001$). Leptin did not associate with adiponectin once adjusted for obesity. A high leptin level predicted stroke independently in men (ORQ4=3.16; 95%CI: 1.32-7.61) but not in women. The increased risk was similar for both ischemic and hemorrhagic stroke. Adiponectin did not associate with stroke. Males with high leptin developed their stroke faster than males with low levels ($p=0.0009$) and the time-related effect of leptin was independent of traditional risk factors. An interaction analysis indicated a positive interaction between high blood pressure and high leptin in men whereas high leptin levels and adiponectin were antagonists.
Conclusion: A high leptin level is independently associated with both risk for and time to a first-ever stroke in men but not in women whereas adiponectin does not associate with a future stroke. Leptin may be a key link in the development of cardiovascular disease in obesity.

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Adiponectin concentration in patients with newly diagnosed Type 2 diabetes mellitus and impaired glucose tolerance and coronary artery disease.
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Background and Aims: Adiponectin is a fat-derived hormone that enhances insulin sensitivity and controls body weight. The decreased adiponectin concentration was observed in obesity and type 2 diabetic patients. In experimental studies adiponectin was shown to have antiatherogenic properties by suppressing endothelial expression of adhesion molecules. The aim of the present study was to evaluate adiponectin concentration in men with coronary artery disease depending on disturbances of glucose metabolism.
Materials and Methods: The study was carried out in 62 men with stable coronary artery disease referred for coronary angiography. In the studied group the OGTT with glucose and insulin estimation was performed and insulin resistance index (HOMA) was calculated. In the fasting plasma adiponectin, HbA1c, soluble form of E-selectin and lipid parameters were estimated.
Results: Adiponectin concentration was not different in patients with normal glucose tolerance (n=26) in comparison to the group with type 2 diabetes mellitus and impaired glucose tolerance (n=36). There was no also significant difference in adiponectin concentration in relation to atherosclerosis progression (1-, 2-, 3- vessel disease). There was no significant correlation between adiponectin and calculated insulin resistance

index , while there was a marked inverse correlation between adiponectin and BMI (r=-0.30; p.=0.018), body weight (r=-0.33,p.=0.08), E-selectin (r=-0.263; p=0.039), TG (r=-0.27, p.=0.036), duration of coronary heart disease (r=-0.33;p.=0.009), and a borderline significance with ejection fraction (r=-0.268; p=0.06) was observed.
Conclusion: The obtained results suggest that adiponectin could be recognised as a protective protein for development of atherosclerosis and plasma adiponectin concentration is not different in patients with coronary artery disease in respect to disturbances of glucose metabolism.

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Serum adiponectin level inversely correlates with the heart-rate corrected QT interval in health examinees. A suggestion for a direct cardioprotective effect of the peptide in apparently healthy population.
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Background and Aims: It was recently reported that hypoadiponectinaemia is associated with coronary artery disease (CAD) suggesting the peptide has a cardiovascular effect. However, adiponectin is an insulin sensitizing peptide, so that an interpretation of the finding would be hypoadiponetinaemia was a causal factor for increased insulin resistance (IR), and then increased IR, not hypoadiponectinaemia *per se*, promoted development of CAD. To know if adiponectin possesses direct cardiovascular effect(s) besides its insulin sensitizing action, we analyzed relationship between serum adiponectin, the heart-rate corrected QT interval (QTc), and the established risk factors of atherosclerosis in Japanese health examinees. Most importantly, measurement of serum specific insulin (IRI) was included so that we could quantify the degree of IR. QTc was employed as a marker of subclinical atherosclerosis because it predicts future cardiac mortality and correlates well with the carotid intimal thickning in apparently healthy population.
Materials and Methods: In 102 consecutive male health examinees, serum level of adiponectin in fasting blood sample was measured by specific RIA, which was 8.1 ± 4.4 (mean ± SD) (range, 0.7-28.9) μg/ml. QTc of this population was 392 ± 17 (range, 356-454) msec. Anthropometric and other fasting data were, age 54 ± 11 yrs, body mass index 24.3 ± 2.8 kg/m^2, %adiposity in body composition 23.1 ± 4.4%, waist to hip ratio .895 ± .047, blood pressure 125 ± 16/75 ± 10 mmHg, triglycerides (TG) 143 ± 75 mg/dl, total cholesterol 208 ± 38 mg/dl, HDL-cholesterol 53 ± 12 mg/dl, plasma glucose 102 ± 18 mg/dl, IRI 6.8 ± 3.9 μU/ml, 1/IRI .20 ± .13 ml/μU and 'quantitative insulin sensitivity check index (QUICKI)' .36 ± .03 [log (μU/ml) + log (mg/dl)]$^{-1}$.
Results: When the multiple regression analysis was performed by taking QTc as a dependent variable, it significantly correlated with adiponection (β= -.272, *p*= .0048), %adiposity (β= .228, *p*= .033), and age (β= .216, *p*= .047) but with none of other variables including the indices of IR. Thus, it was evident that adiponectin was most strongly and significantly correlated with QTc, and the correlation is unrelated to adiponectin's insulin sensitizing action. On the other hand, when adiponectin was treated as a dependent variable, the multiple regression analysis revealed that it was significantly correlated with the indices of IR (1/IRI and QUICKI) and TG. Thus, an insulin sensitizing effect of the peptide was clearly discernible in this population.
Conclusion: For the fist time, we demonstrated a clear-cut inverse correlation between QTc and adiponectin in general population, indicating that hypoadiponectinaemia precedes development of CAD, if any. In this population, statistically significant correlation between QTc and IR was absent most likely due to low degree of IR as a group. An unequivocal, highly significant inverse correlation between QTc and adiponectin under such a circumstance strongly suggests that adiponectin possesses a direct cardioprotective effect in man. Further study is apparently needed to prove this hypothesis.

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Markers of inflammation in patients with Type 2 diabetes in the time of acute coronary event.
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Background and Aims: C-reactive protein (CRP) and other markers of inflammation, fibrinogen and albumin, have been proposed as a new coronary risk factor. Chronic inflammation may also be a risk factor for developing type 2 diabetes.This study was design to assess the relation between markers of inflammation and diabetes in the time of rupture of vulnerable atherosclerotic plaque.
Materials and Methods: To assess the relation of inflammatory markers and diabetes we studied 101 patients with acute coronary syndrome (ACS) on admission to Coronary Care Unit and in 30 days following up. All traditional risk factors were marked. Blood were sampled on admission for analyses all markers and again after 30 days.
Results: Patients were 61 ± 10.85 years old (65% men). Among patients with ACS 42% had type 2 diabetes. Both acute phase proteins CRP and fibrinogen were significantly higher in acute event comparing to control examination (p<0.001). In subgroup with diabetes levels of CRP were significantly higher then in non diabetic patients (8.97+6.5 vs 7.52+3.2 mg/l in unstable angina, 20.7+5.74vs 15.32+3.87 mg/l in non q wave MI; 28.21+15.4vs 20+12.54 mg/l in MI on admission and after following up. Also fibrinogen was significantly higher in diabetic patients (p=0.05). Levels of albumin decreased in acute coronary event , comparing to control examination (p<0.001) and patients with diabetes had significantly lower values then non diabetic patients (p=0.02).Also we found out positive correlation with lipid status and other traditional risk factors (smoking status, hypertension and obesity).
Conclusion: Inflammatory markers are highly involved in acute coronary event together with traditional risk factors. Type 2 diabetes together with ongoing atherosclerosis might be considered as the part of same process, inflammation.

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Prolonged postprandial proinsulin elevation in Type 2 diabetic patients with coronary artery disease, after an oral fat tolerance test.
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Background and Aims: Elevated proinsulin has been implicated in the pathogenesis of coronary artery disease (CAD) both in diabetic and non-diabetic individuals. However the postprandial performance of proinsulin after an oral fat tolerance test has not been extensively examined.
Materials and Methods: Forty-two male, non-insulin treated, type 2 diabetic patients were recruited. According to the presence of CAD were divided into two groups: CAD 1: n=22 with CAD and CAD 0: n=20 free from CAD. After an overnight fast blood samples were drawn for the determination of glucose, lipid profile (enzymatic method) and insulin. In addition, proinsulin levels were determined by RIA (Linco R.I.-HPI-15K, 95% cross-reactivity with des 31-32 proinsulin). Subjects underwent an oral fat tolerance test of 350 gr per 2m^2 of body surface area with 83.5% fat. Subsequently, blood samples were drawn at 2,4,6 and 8 hours after the meal. The area under the incremental curve (AUIC) was calculated by plotting the concentration of each parameter over the 8 h.
Results: The two groups were comparable according to age, BMI and WHR. Fasting proinsulin levels were similar between the two groups (15.8±12.3 pmol/L vs. 19.4±17.0 pmol/L, p:ns, for CAD1 vs. CAD0, respectively). Postprandially, proinsulin increased in both groups, with maximum levels observed at four and two hours, respectively (Friedman test: p<0.001 and p=0.008). However, CAD1 patients showed prolonged hyperproinsulinemia, with proinsulin levels at 4, 6 and 8 h significantly higher from baseline (p<0,005). Furthermore the AUIC at 4-6h and 6-8h was significantly higher in CAD1 patients compared with CAD0 (p=0.029 and p=0.024, respectively). In addition proinsulin response over the eight-hour period tended to be higher in CAD1 patients compared with CAD0 (p=0.062).
Conclusion: Diabetic patients with CAD display significantly prolonged proinsulin responses after a lipid load, abnormality that might contribute to the development of CAD.

1082

Serum Laminin level is associated with plaque progression in carotid atherosclerosis of Type 2 diabetic patients.
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Background and Aims: The thickening of basement membranes(BM) in is considered to be a characteristic histologic finding of diabetic macrovascular complication. Laminin which is the main non-collagenous constituent, can be changed by alteration of metabolism and distribution of this protein in diabetic patients. Because serum levels of laminin can reflect

these changes, its usefulness as an index of diabetic macrovascular complication has been postulated. The aim of our study is to measure serum concentration of laminin in a large group of type 2 diabetic patients in order to assess the relation of carotid atherosclerosis and the influence of several variables.
Materials and Methods: Subject patients were 109 type 2 diabetics (aged from 40yrs to 70 yrs). We evaluated the intima-media thickness (IMT) and plaques in the each segment of the both carotid arteries by duplex scan. The mean of the total IMT values (7 points on each side), the each mean value of CCA, bulb and ICA, the maximal IMT, plaque count and score were measured. Plaque score was defined by the sum of longitudinal diameter of each plaques. Serum laminin level was measured by Quantimatrix™ human laminin ELISA kit (Chemicon®, USA).
Results: The correlation with serum laminin level and measured IMT values was statistically nonsignificant ($r<0.15$, $p>0.05$). But, there are significant positive correlations between serum laminin and plaque count or plaque score ($r=0.24$, $p<0.05$). Stepwise multiple regression analysis, including waist hip ratio, mean value of bulb, apo B and presence of neuropathy revealed serum laminin as the predictor for plaque score ($p=0.20$).
Conclusion: These results suggest that elevated plasma laminin level may be a risk factor for carotid plaque progression rather than initiation of plaque formation or IMT thickening in carotid atherosclerosis in type 2 diabetic patients. Also, there is need to approach in discriminating the risk factors for plaque formation or progression and IMT thickening.

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Lower antioxidant enzyme activity is associated with insulin resistance in Type 2 diabetes patients with angiographically verified coronary heart disease.
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Background and Aims: It has been previously shown that impairments in systemic antioxidant enzyme activity in Type 2 diabetes patients could significantly contribute to appearance of coronary heart disease (CHD). In this study we analyzed: (a) insulin sensitivity levels and (b) lipid peroxide levels, being important oxidative agents, and three different types of antioxidant enzyme activities, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and glutation reductase (GR), in the following groups of patients: Type 2 diabetes and angiographically verified CHD (group A, N=20), Type 2 diabetes without angiographically significant changes in coronary arteries (without CHD) (group B, N=20), nondiabetics with CHD (group C, N=30) and nondiabetics without CHD (group D, N=30).
Materials and Methods: Insulin sensitivity levels were determined by the frequently sampled intravenous glucose tolerance (FSIGT) test with minimal model analysis (Si index). Lipid peroxide levels were tested in thiobarbituric acid-reacting substance (TBARS) assay and GSH-Px, SOD and GR activity were detected by spectrophotometry.
Results: We found that Si levels were significantly lower in group A compared to group B (1.16+/-0.22 vs 2.33+/-0.90 min-1/mU/lx104; $p<0.05$) and in group C compared to group D (3.55+/-0.76 vs 6.56+/-0.92 min-1/mU/lx104; $p<0.001$). Simultaneously, TBARS levels did not differ between group A and group B and were slightly but not significantly higher in group C vs group D. In contrast, GSH-Px, SOD and GR activity were significantly lower in group A vs group B and in group C vs group D (GSH-Px: A: 21.7+/-2.1, B: 24.9+/-4.2, C: 28.1+/-4.0, D: 32.3+/-3.9 U/gHb; SOD: A: 7.1+/-2.8, B: 8.9+/-2.3, C: 7.4+/-2.6, D: 9.1+/-2.3 U/mgHb; GR: A: 46.4+/-2.3, B: 53.9+/-3.1, C: 54.2+/-3.6, D: 87.5+/-5.5 U/gHb; A vs B and C vs D $p<0.05$, respectively). The changes in Si correlated significantly with GSH-Px, SOD and GR activity levels in diabetics (GSH-Px: r= 0.523, SOD: r=0.504; GR: r=0.499, $p<0.05$) and nondiabetics (GSH-Px: r=0.503, SOD: r=0.611, GR: r=0.456; $p<0.05$), while the changes in the TBARS levels did not correlate with Si in neither of the groups.
Conclusion: Our results have shown that insulin resistance was associated with decreases in all three types of antioxidant enzyme activity both in Type 2 diabetes and nondiabetic patients with CHD. Thus, the results imply that atherogenic influence of insulin resistance in those patients with CHD might be exerted through a significant impairment of antioxidant enzyme activity.

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Soluble Fas in diabetic patients with peripheral vascular disease.
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Background and Aims: Patients with diabetes mellitus (DM) are at increased risk of atherosclerotic vascular disease. The development of premature atherosclerosis is multifactorial, and not entirely explain by classical cardiovascular risk factors (CVRF). Fas/Fas ligand system is a key regulating system responsible for the activation of apoptosis in various cell types, including vessel wall. The soluble form of Fas (sFas) has been found elevated in haemodalysis patients with peripheral vascular disease (PVD). The aim of the study was to evaluate sFas and other CVRF in diabetic patients with and without PVD and analyze its utility as a novel marker of atherosclerosis.
Material and Methods:117 type 2 diabetic patients (57 with PVD and 60 without PVD matched for age and gender) were evaluated. PVD was defined by the presence of ankle-braquial index (ABI, measured by eco-doppler) less than 1 in at least one side of the body. Glucose, HbA1c, lipids levels, and ACE were evaluated by authomated methods. Immunonephelometry methods were used to evaluate excretion urinary of albumin, Lp (a) and CRP. Fibrinogen was quantified in a Sysmex CA-6000 (Dade Diagnostics). Homocysteine levels were evaluated by HPLC. Serum sFas levels were determined using a commercial ELISA (R&D Systems, Oxon, United Kingdom). Comparisons between the two groups were done with Student t'test or Mann-Whitney test depending from variable distribution. Correlations were done with Pearson or Spearman test in the same way.
Results: Pertinent clinical and biochemical characteristics of patients are shown below. No differences in SBP, DBP, glucose, HbA1c, total cholesterol, HDL, triglycerides, cLDL, urinary albumin excretion, fibrinogen, ACE, Lp(a), homocysteine and CRP were found (results no shown).

	PVD (n=57)	Non-PVD (n=60)	
Age	70±9	69± 8	p=0.8
Creatinine (mg/dl)	1.1±0.2	1.07±0.2	p=0.08
Right ABI	0.89± 0.26	1.12±0.08	p<0.001
Left ABI	0.89±0.24	1.12±0.07	p<0.001
sFas	10249±3675	8865±2583	p<0.02

sFas levels correlated significantly with creatinine and homocysteine in both groups and with urinary albumin excretion in the PVD patients.
Conclusion: In type 2 diabetic patients, classical and emergents CVRF not differentiate between patients with or without PVD. However, sFas is significantly elevated in presence of PVD. sFas may represent a novel marker of atherosclerosis in these patients.

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Clinical significance of hepatocyte growth factor for diabetic complications, including carotid atherosclerosis in Type 2 diabetic patients.
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Background and Aims: Hepatocyte growth factor (HGF) is a pleiotropic cytokine involved in tissue protection and repair in the endothelium and various organ systems. We measured serum HGF to determine the relationships of several complications, including carotid atherosclerosis in type 2 diabetes mellitus (DM).
Materials and Methods: We studied 90 patients aged 40 to 83 years, included 49 men and 41 women with type 2 DM in our hospitals, by measuring serum HGF using enzyme-linked immunosorbent assay (ELISA), intimal-medial thickness (IMT), plaque score (PS) of the common carotid artery by high-resolution B-mode ultrasonography with a 7.5 MHz probe, serum advanced glycation end products (AGEs) using ELISA, and by evaluating several parameters of DM, the stages of long-term complications (peripheral neuropathy, autonomic neuropathy, retinopathy, nephropathy, lacuna infarction, ischemic heart disease, arteriosclerosis obliterans, hypertension, hyperlipidemia).
Results: Significantly, serum HGF was positively correlated with IMT ($r = 0.265$, $P < 0.05$), PS ($r = 0.212$, $P < 0.05$), AGEs ($r = 0.270$, $P < 0.05$), age

($r = 0.294$, $P < 0.005$) and number of diabetic comlications ($r = 0.279$, $P < 0.01$). In addition, serum HGF was not associated with sex, body mass index (BMI), HbA1c, systolic blood pressure (SBP), Brinkman index, alcohol intake, total cholesterol (T-Chol), fibrinogen and duration of DM in univariate analysis. Multiple regression analysis showed that IMT ($r = 0.309$, $P < 0.05$) was independently associated with serum HGF in addition to HbA1c ($r = - 0.249$, $P < 0.05$) ($R = 0.487$, $P < 0.05$). The other independent varients inclusive of sex, BMI, HbA1c, SBP, Brinkman index, alcohol intake, T-Chol, fibrinogen and duration of DM were not significantly associated with serum HGF.

Conclusion: These results indicate that the serum concentration of HGF increases in relation to carotid atherosclerosis. As serum HGF was positively correlated with AGEs which is responsible for several complications of DM, these findings further support a possible role of HGF in the diabetic complications. The relationships between HGF level and number of diabetic comlications suggest that this cytokine might be a marker of a process that has a major impact in the development of the macroangiopathy and microangiopathy.

PS 92

New Risk Markers of Atherosclerosis (II)

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Decreased levels of matrix metalloproteinases –2 and –3 in patients with diabetes mellitus and chronic heart failure.

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Background and Aims: Extracellular matrix metabolism plays a significant role in the pathophysiology of left ventricular remodeling characterizing heart failure. Degredation of collagen depends on the equilibrium between matrx metalloproteinases (MMPs) and their inhibitors (TIMPs). Furthermore hyperglycaemia characteristic of diabetes mellitus (DM) leads to non enzymatic glycation of matrix macromolecules. It has been demonstrated in cell culture studies as well as in animal studies that MMP levels are decreased whereas TIMP levels are increased. With the present study we investigated the effects of diabetes mellitus on the fluctuation of the levels of MMP-1,-2 and -3 in patients with chronic heart failure.

Materials and Methods: Our study group consisted of 40 patients with chronic heart failure (NYHA III-IV). Group A consisted of 16 patients (6 men, of mean age 72±5 years) with DM and EF 42± 7 % (6 patients under treatment with insulin , 10 under treatment with antidiabetic tablets) with HbA1c > 6,5%, while group B consisted of 24 patients (12 men , of mean age 75±7 years) without DM and EF 41±5 %. Our control group consisted of 22 healthy subjects (mean age of 54±5 years). Using sandwich enzyme immunoassay serum levels of MMP-1,-2,-3 were measured. The comparison between the control and the two patients groups was performed using the non parametric Mann-Whitney U test. Values are expressed as medians and interquartitile ranges.

Results: We observed that MMP-2 and MMP-3 levels in both patient groups were significantly higher compared to control group (p=0.05). Moreover we observed significantly reduced levels of MMP-2 (298 ng/ml, 237-353 ng/ml, p=0.006) and MMP-3 (18.7 ng/ml, 13.6-25.3 ng/ml, p=0.027) in patients with heart failure and DM compared to patients without (MMP-2: 395 ng/ml, 316-485 ng/ml, MMP-3 : 26.5 ng/ml, 16.8-43.4 ng/ml). In contrast we did not observe significant difference in the levels of MMP-1 between patients with DM (6.5 ng/ml, 3.8-10.1 ng/ml) and patients without DM(4.2 ng/ml, 3.4-6.7 ng/ml, p=0.141).

Conclusion: The presence of diabetes mellitus in heart failure patients affects significantly circulating levels of metalloproteinases. The findings of the present study suggests that deranged collagen meatabolism characterizing DM patients, plays a significant role in heart failure prevelance in DM patients.

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Pathophysiological role of plasma EN-RAGE (S100A12) protein in patients with Type 2 diabetes.

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Background and Aims: EN-RAGE, also called S100A12 or CAAF1, is a newly identified ligand for the receptor for advanced glycation end products (RAGE). It has been shown that EN-RAGE induces adhesion molecules such as VCAM-1 and ICAM-1 in the vascular endothelial cell and mediates migration and activation of monocytes/macrophages through RAGE binding and that infusion of lipopolysaccharide (LPS) into mice causes time-dependent increase of EN-RAGE in the plasma. Therefore, circulating EN-RAGE protein may be involved in chronic inflammation in the atherosclerotic lesion. In this study, we developed ELISA system with the use of specific monoclonal antibodies against recombinant human EN-RAGE in order to investigate pathophysiological roles of plasma EN-RAGE protein in patients with type 2 diabetes.

Materials and Methods: On using EN-RAGE ELISA system we developed, the CV of intra- and inter-assay was less than 4% and 9%, respectively. The analytical lower detection limit was 0.1 ng/mL of recombinant EN-RAGE. High-sensitivity C-reactive protein (hsCRP) in plasma was measured by N Latex High Sensitivity CRP Kit (Dade Behring Inc.).
Results: Plasma EN-RAGE levels and other clinical parameters of the patients are shown in **Table** below as mean±SEM. Plasma EN-RAGE levels were more than twice as high in the patients with diabetes (DM group) compared to those without diabetes (Non-DM group). HbA1c, fasting glucose, triglyceride (TG) and hsCRP levels were also higher in the DM group. No differences were observed in Age, body mass index (BMI), LDL-C, HDL-C and white blood cell count (WBC). On univariate analysis in all patients, plasma EN-RAGE concentrations correlated with HbA1c (p<.001, R=.455), fasting glucose (p=.019, R=.281), hsCRP (p=.003, R=.333) and WBC (p=.007, R=.325). Multiple regression analyses revealed that only HbA1c (p=.0001, R=.467) and white blood cell count (p<.0001, R=.316) remained significant independent determinants of plasma EN-RAGE concentration.
Conclusion: These results suggest that the plasma EN-RAGE protein level was regulated by factors related to glucose control and subclinical inflammation and may contribute to the complications and accelerated atherogenesis in patients with type 2 diabetes.

Plasma EN-RAGE levels and clinical parameters in patients with and without diabetes

	Non-DM group	DM group	p-value
Age (yrs)	58.3±2.8	56.3±1.6	.5132
Gender (M/F)	21/14	19/21	.393
BMI (kg/m^2)	23.1±0.63	24.9±0.94	.1245
Plasma EN-RAGE (ng/mL)	8.1±0.81	19.6±5.26	.0007
HbA1c (%)	4.7±0.06	8.6±0.27	<.0001
Glucose (mg/dL)	105±3.3	224±13.9	<.0001
TG (mg/dL)	129±14.7	192±19.4	.0217
LDL-C (mg/dL)	121±6.0	124±5.3	.6635
HDL-C (mg/dL)	55±2.8	48±3.0	.1167
hsCRP (mg/L)	1.09±0.31	1.92±0.56	.0394
WBC (/mm^3)	5954±317	6203±348	.6100

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Levels of Pro- BNP-NT in relation to diastolic function and left ventricular geometry in normoalbuminuric patients with Type 2 diabetes.
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Background and Aim: Diabetes and hypertension is closely related to development of decreased left ventricular performance and finally congestive heart failure. Pro-BNP-NT is a sensitive marker of left ventricular wall stress and has previously been found increased in microalbuminuric patients with type 2 diabetes. The purpose of this study was to examine levels of Pro-BNP-NT and the relation to the left ventricular diastolic performance and left ventricular geometry in normoalbuminuric patients with type 2 diabetes.
Materials and Methods: The study comprised 60 patients with albuminuria within normal limits. Thirty patients without hypertension and 30 hypertensive patients, all in equal dosage ACE-inhibitor treatment. Exclusion criteria's were angina pectoris, prior myocardial infarction, arrhythmia, ejection fraction > 55 %. Thirty age and sex matched normal subjects served as controls. All included were examined with conventional echocardiography and color M-mode propagation of the mitral inflow to assess pseudonormalisation. Patient serum was collected and kept at -80 degrees Celsius and analyzed en block in triplicate with the Pro-BNP-NT, ROCHE assay.
Results: In the diabetes group the average age was 55 years with a diabetes duration of 6 years (± 4 years). Pro-BNP-NT was significantly elevated in type 2 diabetes patients compared to the control group (81 pg/ml (5-643) vs. 44 pg/ml (5-98)). Pro-BNP-NT was significantly higher among hypertensive patients compared to both normotensive patients and controls. There was a non-significant difference between normotensive patients and controls.
Pro-BNP-NT was significantly correlated to age (r= 0.41), left ventricular mass (r= 0.63), left ventricular wall diameter (r = 0.53) and left ventricular diastolic diameter (r = 0.41), but uncorrelated to traditional measures of diastolic function of the left ventricle, blood pressure, heart rate and body mass index.
Conclusion: Pro-BNP-NT is significantly increased in hypertensive and normoalbuminuric patients with type 2 diabetes. Pro-BNP-NT correlates well to left ventricular geometry, but is independent of measures of diastolic dysfunction.

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Polymorphisms of glucose transporter 1 gene and chronic diabetic complications.
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Background and Aims: It has been considered that there exists variability in the incidence of diabetes-derived chronic complications, which suggests the existence of genetic susceptibility to the development of those complications. Glucose transporter 1 (GLUT1) is widely expressed and facilitates basal glucose transport into cells, and GLUT1 gene has been widely analyzed as a possible contributor to the genetic susceptibility to type 2 diabetes. Recent studies have reported the possibility that the polymorphisms of GLUT1 gene may be involved in the pathophysiological mechanisms responsible for the development of diabetic nephropathy. However, consistent results have not been obtained and relationship with diabetic complications other than nephropathy has not been well investigated. Therefore, the present study was conducted to evaluate whether genetic variations of GLUT1 may determine susceptibility to various diabetic complications in Japanese type 2 diabetic patients.
Methods: A total of 130 type 2 diabetic patients (78 men and 52 women, diabetes duration more than 5 years) were enrolled in this study. Amplimers flanking the Xba-I polymorphic site in the second intron were employed to amplify DNA from subjects. The amplified DNA was restricted with endonuclease Xba-I, separated by gel electrophoresis, and visualized. In the absence of the Xba-I site, a fragment of 1.1 kilobase was seen, whereas fragments of 0.9 and 0.2 were generated if the Xba-I site was present. Results obtained from the subgroups of subjects were compared using χ^2 test and Student-t test.
Results: The frequencies of 0.9/0.9 genotype were 68.5% (n=89), 0.9/1.1 genotype were 25.4% (n=33) and 1.1/1.1 were 6.2% (n=8) (0.9 allele: 81%, 1.1 allele: 19%). There were no significant differences in genotypic or allelic distribution among patients with or without microangiopathy such as retinopathy, nephropathy and neuropathy. Concerning to macroangiopathy, there was a significant increase in the frequency of the 1.1 allele (0.9/1.1 and 1.1/1.1) in the patients with the episodes of ischemic heart diseases (p=0.0443). And the patients who have the episodes of ischemic heart diseases have treatments of hypertension (p<0.01). Therefore, it can be considered that the onset of ischemic heart diseases and hypertension are closely related with the polymorphisms of GLUT1 gene. There was no relationship between other macroangiopathy such as cerebrovascular diseases and arteriosclerosis obliterans, and the genetic variations of GLUT1.
Conclusion: Our results suggest that the 1.1 allele of the GLUT1 gene might be a genetic marker of susceptibility to ischemic heart diseases among Japanese type 2 diabetic patients.

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Recurrence of myocardial infarction: an association with decreased insulin sensitivity and increased plasminogen activator inhibitor 1 levels but not with lipoprotein subfraction impairments both in Type 2 diabetes and nondiabetic patients.
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Background and Aims: It has been previously demonstrated that insulin resistance and hypofibrinolysis marked by an increased plasminogen activator inhibitor (PAI)-1 activity, together with impaired lipoprotein subfraction especially non-HDL cholesterol (Ch) levels, represent important risk factors for coronary atherogenesis. However, the relationship between the recurrence of myocardial infarction versus insulin sensitivity, PAI-1 and lipoprotein subfraction levels have not yet been clarified neither in Type 2 diabetes nor in nondiabetic patients. Therefore, this study was aimed to compare insulin sensitivity, plasma insulin, plasminogen activator inhibitor

1 (PAI-1) and and lipoprotein subfraction (total cholesterol (Ch), HDL-Ch, non-HDL-Ch, LDL-Ch, and triglyceride (Tg)) levels in the following groups of patients: (a) Type 2 diabetics with a recurrent myocardial infarction (group A; N=22), (b) Type 2 diabetics with a single myocardial infarction (group B; N=25), (c) nondiabetics with a recurrent myocardial infarction (group C, N=20) and (d) nondiabetics with a single myocardial infarction (group D, N=30).
Materials and Methods: CHD was angiographically verified and groups were matched for duration of diabetes and CHD. Insulin sensitivity levels were determined by the frequently sampled intravenous glucose tolerance (FSIGT) test with minimal model analysis (Si index), plasma insulin (PI) levels were determined by RIA, PAI-1 levels were measured by plasminogen chromogenic plasmin substrate assay and lipoprotein subfraction levels by enzymatic method.
Results: We found that Si levels were significantly lower in group A compared to group B (A:1.03+/-0.21 vs B:2.02+/-0.30 min-1/mU/lx104; p<0.05) and in group C compared to group D (C:2.11+/-0.85 vs D:3.94+/-0.77 min-1/mU/lx104; p<0.01). Simultaneously, plasma insulin and PAI-1 levels were significantly higher in group A vs group B (PI: A: 28.9+/-3.1 vs B: 21.1+/-3.6 mU/l, p<0.05; PAI-1: A: 6.9+/-0.7 vs B: 5.1+/-0.4 U/ml, p<0.05), and in group C vs group D (PI: C: 14.7+/-1.1 vs D: 10.7+/-1.1 mU/l, p<0.05; PAI-1: C: 6.1+/-0.3 vs D: 4.2+/-0.6 U/ml, p<0.05). In contrast, we could not detect significant differences in total Ch, neither of its subfraction and Tg levels between the groups.
Conclusion: The results have shown that recurrence of myocardial infarction was strongly dependent on the level of insulin resistance and hypofibrinolysis, but not on lipoprotein subfraction impairments, both in Type 2 diabetes and nondiabetic patients.

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Prediction of impaired glucose tolerance and manifest diabetes in patients with acute myocardial infarction and no previous diagnosis of diabetes mellitus.

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Background and Aims: Patients with manifest diabetes mellitus have an increased morbidity and mortality from cardiovascular disease. In patients with acute myocardial infarction (AMI) the prevalence of diabetes may be as high as 40- 45 % if the diagnosis is based on an oral glucose tolerance test. The first aim of the present study was to characterise the metabolic profile of patients with AMI and no previous diagnosis of diabetes, during the hospital stay and three months thereafter. The second aim was to determine whether measurements of compounds related to the metabolic syndrome during the hospital stay add any information to HbA1c, fasting blood glucose and classification according to oral glucose tolerance test (OGTT) at discharge to predict a diagnosis three months thereafter of impaired glucose tolerance or diabetes mellitus according to WHO criteria.
Materials and Methods: The study population consisted of 145 patients with AMI, and without previous diabetes who were subjected to an OGTT at hospital discharge and three months thereafter. Based on three month's OGTT they were defined as normal (n=50), impaired glucose tolerance (n=59) or diabetes mellitus (n=36). Components related to the metabolic syndrome (BMI, blood glucose, insulin, pro-insulin, PAI-1, triglycerides and HDL-cholesterol) were recorded during hospital stay and 3 months thereafter. Insulin resistance was calculated with HOMA-IR according to WHO definition.
Results: Patients with AMI and either normal glucose tolerance, impaired glucose tolerance or newly detected diabetes had a high prevalence of insulin resistance (52%, 65% and 86%, respectively) at the three months follow up. Variables obtained during the hospital phase were entered into a logistic regression analysis. Fasting blood glucose, HbA1c and OGTT were significant predictors to outcome of the OGTT at three months while age, BMI, insulin, pro-insulin, HOMA-IR, PAI-1 and lipids did not add to the predictive power.
Conclusion: Patients with AMI and no previous diagnosis of diabetes have a high prevalence of insulin resistance. Commonly available factors as HbA1c, fasting blood glucose and an OGTT during acute myocardial infarction predict the diagnosis of impaired glucose tolerance or diabetes mellitus three months thereafter while more complex parameters of the metabolic profile do not contribute to this prediction. We recommend improved screening for dysglycemia in patients with AMI.

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Changes of left ventricular structure and function in Type 2 diabetic patients without hypertension - 3-year follow-up study.

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Background and Aims: The excess mortality in type 2 diabetic patients is mainly due to cardiovascular events. Left ventricular (LV) hypertrophy is an independent risk factor for ischemic heart disease, cardiac arrhythmia, and heart failure. The aim of our 3-year follow-up study is to know the changes of LV structure and function in type 2 diabetic patients without hypertension.
Materials and Methods: M-mode and Doppler echocardiography were performed by one experienced blinded examiner during 1998-1999 and 3 years after in 75 type 2 diabetic patients (48 men, age (mean(SD)) 59(9) years, known duration of diabetes was 14 (7) years, body mass index (BMI) 23 (3) kg/m^2) with blood pressure <140/90 mmHg and without diabetic nephropathy. M-mode parameters were measured LV end-diastolic diameter (LVDd), end-systolic diameter (LVDs), and ventricular septum thickness (SVTd) and posterior wall thickness (PWTd) in diastole, then caliculated LV mass index (LVMI) according to Penn's formula.
Results: After 3-year follow-up, HbA_{1C} and albumin creatinine ratio (ACR) increased as compared with baseline (7.2(1.0) to 7.6(1.0)%(p=0.001), 12.6(min-max: 2.3-120.3) to 13.9 (2.2-126.4) mg/g Cr(p=0.008), respectively). BMI and serum lipid level were about the same during 3 years. There was not significant changes in systolic blood pressure (122(10) to 121 (11) mmHg), but slightly decreased in diastolic blood pressure (76(7) to 74(10) mmHg (p=0.022). During 3 years, no one had hypertension. LVMI increased (102(23) to 106(28) g/m^2) with increased LV wall thickness (SVTd (8.7(1.3) to 9.0(1.3) mm), PWTd (8.7(1.3) to 9.0(1.2)mm), whether LVDd did not change. LV diastolic function, assessed by the ratio between the peak diastolic velocity and the peak atrial systolic velocity (E/A), significantly decreased during 3-year follow-up (1.09(0.31) to 0.98(0.25)). A multiple linear regression analysis revealed that elevated diastolic blood pressure was an independent risk factor for increased LVMI (relative risk(95%CI): 2.499(1.364-3.409)), (p<0.05), after adjustment age, changes of systolic blood pressure, BMI, $HbA_{1C,}$ and ACR during follow-up.
Conclusion: Our 3-years follow-up study shows that LVMI increased and LV diastolic function decreases in type 2 diabetic patients without hypertension. We suggest that diastolic blood pressure control prevents LV hypertrophy in type 2 diabetic patients even though blood pressure is normal range.

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Autoimmune thyroid disease in diabetes Type 1 and cardiovascular risk.

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Background and Aims: Intima media-thickness (IMT) of the carotid artery has been used as a subclinical index of early atherosclerosis. IMT appears to be increased in patients with type 1 diabetes mellitus. Many factors have been involved in the pathogenesis of macrovascular damage in diabetes type 1. In particular recent reports have showed that inflammation can be involved in the development and progression of atherosclerosis, but this factor has not been fully examined. Aim of our study was to evaluate IMT in a group of diabetics type 1 also affected by autoimmune thyroid disease and to compare them with a group of diabetics without thyroid autoimmunity.
Materials and Methods: We analyzed data from our young population of diabetics type 1 (227 patients aged 15-35 years) and we found 32 patients affected by thyroid disease. Considering that 58 didn't undergo thyroid examination, the prevalence of thyroid disease in our young population of diabetics type 1 was 18,9%. At the moment we have collected data of 12 patients affected by autoimmune thyroid disease (10 autoimmune hypothyroidism in replacement therapy and 2 euthyroid autoimmune thyroiditis) (group 1: mean age 32,2±4,3 years, duration of diabetes 22,2±7,7 years) and we compared them with data of a similar group of 15 diabetics type 1 without thyroid autoimmunity (group 2: mean age 31,1±5,1 years, duration of diabetes 19,1±3,8 years). We evaluated common carotid IMT and other clinical parameters such as HbA1c, lipid profile (total cholesterol, HDL, LDL, tryglicerides), smoking, blood pressure, urinary albumin excretion and eye examination. IMT values were registered in the common carotid arteries, 1-2 cm proximal to the bulb, at the far wall in both

right and left sides, using high risolution B-mode ultrasonography (linear probe 7.5 MHz, ATL HDI 3000).
Results: In patients affected by thyroid autoimmune disease IMT was significantly higher than in subjects without thyroid autoimmunity. In fact in group 1 IMT was 0,82±0,13 mm and in group 2 it was 0,66±0,12 mm (p=0,004).
Regarding all the other parameters considered we didn't observe any significant difference between the two groups, as showed in table 1.
Conclusions: It is well known that diabetics type 1 present increased IMT, marker of macrovascular damage. Our study demonstrates that concomitant autoimmune thyroid disease seems to increase cardiovascular risk. These findings needs to be confirmed in a wider group, bearing in mind that autoimmune mechanism have also been implicated in the pathogenesis of atherosclerosis.

	Group 1	Group 2	p
IMT (mm)	**0,82±0,13**	**0,66±0,12**	**0,004**
Total cholesterol (mg/dl)	198,4±19,7	201,3±33	ns
HDL (mg/dl)	62,8±15,3	58,5±17,6	ns
Tryglicerides (mg/dl)	87,6±36,6	102,3±49,2	ns
LDL (mg/dl)	117,8±19,2	126,2±31,9	ns
HbA1c (%)	9±1,3	8,8±1,5	ns
Presence of retinopathy	8/12 (67%)	12/15 (80%)	ns
Microalbuminuria ≥ 20 $\mu^{g/min}$	2/12 (16%)	3/15 (20%)	ns
Systolic blood pressure (mmHg)	119,5±11	125,4±11	ns
Diastolic blood pressure (mmHg)	78,2±11	81,2±5,6	ns
Smoking (< 10 cigarettes/die)	3/12 (25%)	5/15 (33%)	ns

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Effect of eicosapentaenoic acid on arterial stiffness and nitric oxide production in Type 2 diabetes.
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Background and Aims: Cardiovascular disease is one of the major complication of type 2 diabetes. Arterial stiffness is established as an important independent risk factor for cardiovascular disease. Vasoactive compounds like nitric oxide (NO) are formed in the endothelial cells to control the vascular tone. And NO production, an important indicator of endothelial function, is disturbed in atherosclerosis, hyperlipidemia and hypertension. Oral supplementation of eicosapentaenoic acid ethyl ester (EPA) improves the abnormalities of serum lipid profiles and may have beneficial effects in patients with atherosclerosis and cardiovascular disease. To determine whether EPA could also improve the vascular dysfunctions in type 2 diabetes, we investigated the effect of this drug on arterial stiffness and nitric oxide production.
Materials and Methods: The brachio-ankle pulse wave velocity (baPWV) was screened as an index of arterial stiffness using the recently developed apparatus, form ABI/PWV (Nihon Colin Co., Aichi, Japan) with 107 diabetic patients and with twelve normal subjects. Informed consent on this trial was achieved from forty diabetic patients whose baPWV were more than 1450cm/s. Half of them was assigned to EPA supplementation (1800mg/day), and the other half was treated for diabetes without EPA. After 6-month treatment with and without EPA, forty patients were evaluated by the baPWV again. And the whole body NO production were estimated by serum nitrate/nitrite (NO_3^-/NO_2^-) concentration.
Results: The baPWV increased in accordance with aging and systolic blood pressure. Glycemic control (ex. HbA1c) did not correlate with the baPWV. Matching with age, the baPWV showed significantly higher in diabetics than in normal subjects. After 6-month therapy, the baPWV increased slightly, but not significantly (1732±124 vs. 1758±138cm/s, p=0.08) in the patients without EPA. And, although systolic and diastolic blood pressure did not change during this trial, the administration of the agent significantly reduced the baPWV approximately by -14% (1829±229 vs. 1570±160cm/s, p=0.02). And serum NO_3^-/NO_2^- level was significantly increased by 1.6 times in this treatment (41.8±17.5 vs. 67.8±38.2 μmol/l, p=0.04). In addition, there were significant correlations between the decline of baPWV and the increment of serum NO_3^-/NO_2^- level.
Conclusions: These findings suggest that EPA administration can ameliorate endothelial dysfunction , at least in part, by increasing the NO production, and reduces arterial stiffness in type 2 diabetes.

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Lipid-lowering therapy for secondary prevention of macrovascular disease in a UK diabetic population: comparison of practice with guidelines.
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Background and Aims: UK guidelines for the secondary prevention of coronary heart disease dictate that total cholesterol (TC) concentrations should be less than 5 mmol/L in individuals aged under 75 years with a previous vascular event. We wished to determine population based data on the implications of these lipid lowering guidelines in type 1 (DM1) and type 2 (DM2) diabetes with established macrovascular disease (MVD).
Materials and Methods: We applied the lipid-lowering guidelines of the Joint British Recommendations on secondary prevention of coronary heart disease to the DARTS/MEMO database (2000-2001) of all diabetic patients in Tayside, Scotland (total population 385 500; DM2 n=8 686; DM1 n=1 128). Data on previous vascular disease (myocardial infarction, angina, coronary revacularisation, peripheral vascular disease, cerebrovascular disease), age, gender and TC were obtained. Prescription of statin and fibrate drugs was also recorded.
Results: No macrovascular events were recorded in age group <16 years. We identified 7330 patients aged 16-74 years, DM1 n = 1004, DM2 n = 6326. 70 (12.2%) males (M) and 54 (12.6%) females (F) with DM1 had a previous macrovascular event. Of these, 31 (44.3%) M and 23 (42.6%) F were receiving lipid-lowering therapy (LLT) with a statin or fibrate. 340

(33.9%) patients with DM1 recorded TC > 5mmol/L. 38 (11.2%) of these had MVD, although only 18 (47.4%) were receiving LLT. In DM2 patients, 1304 (37.1%) M and 868 (30.9%) F had MVD. Of these, 435 (33.4%) M and 291 (33.5%) F received LLT. 2633 (41.6%) of DM2 patients had TC > 5mmol/L and 755 (28.7%) of these had MVD. Only 237 (31.4%) of these received LLT.
Conclusion: These population data demonstrate that the prevalence of MVD and LLT is greater in DM2 than DM1 patients, aged <75 years. Of patients with MVD and TC > 5mmol/L, approximately half with DM1 and two- thirds with DM2 did not receive LLT. This indicates a considerable unmet need for LLT in high-risk individuals, out of keeping with current guidelines.

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The effects of atorvastatin on the endothelial function in diabetic patients and subjects at risk for diabetes.
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Background and Aims: We have investigated the effect of 20 mg qd atorvastatin on the endothelial function of patients with DM and subjects at risk of T2DM in a 12-week, prospective, randomized, placebo-controlled, double-blinded clinical trial.
Materials and Methods: Group A consisted of 30 healthy subjects at risk of T2DM and Group B of 37 patients with type 1 or type 2 diabetes with no serious complications. Both groups were matched for age, BMI and gender. Total cholesterol, LDL and HDL levels were similar to the average general population in both groups. 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. Laser Doppler perfusion imaging was employed to measure the vascular reactivity in the forearm skin.
Results: Atorvastatin-treated subjects had a 23% reduction in TC and 34% in LDL. The FMD improved in the atorvastatin-treated subjects in group A [6.9 ± 3.8 vs 6.3 ± 3.6, p<0.05, (exit visit vs baseline, % of increase in brachial artery diameter, mean ± sd)]. A similar improvement of the FMD that just failed to reach significance was found in atorvastatin-treated patients in group B (6.4 ± 3.5 vs 5.9 ± 4.1, p= 0.07). No changes were observed in the NID and the microcirculation reactivity measurements in either group. In group A, there was a decrease in the CRP (0.22 ± 0.22 vs 0.28 ± 0.24, p <0.05) and TNF-a (5.6 ± 10.3 vs 9.6 ± 15.6, p <0.05) in the atorvastatin-treated patients while in group B a decrease in endothelin-1(0.97 ± 0.29 vs 1.19 ± 0.42, p <0.05), PAI-1 (21 ± 18 vs 25 ± 18 , p <0.05), and tPA (6.3 ± 3.4 vs 7.3 ± 3.5 , p <0.05) was observed. No correlations were observed between the changes in FMD and changes in TC, LDL, HDL or triglycerides. No changes were observed in placebo treated subjects in either groups.
Conclusion: Atorvastatin improves the endothelial function of the macrocirculation in subjects at risk of T2MD and possibly in diabetic patients. Decreased levels of various markers of endothelial activation occurred in both diabetic patients and at risk subjects.

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WITHDRAWN

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Glimepiride shows an athroprotective effect by stimulating NO production in coronary artery endothelial cells.
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Background and Aims: Diabetic macroangiopathy, such as coronary artery disease and cerebral vascular disease is a serious problem determining the prognosis of the diabetic patients. A recent work demonstrated that glimepiride, a new sulfonylurea of the third generation, inhibited the formation of atheromatous plaques of thoracic aortae in high-cholesterol fed rabbits. However, the mechanism by which glimepiride induces its atheroprotective effect remains to be determined. Reviewing the reported mechanism of action of this drug, we hypothesized that glimepiride stimulates NO production via a PI3-kinase dependent pathway in arterial endothelial cells. In the present study, we tested this hypothesis using cultured human coronary endothelial cells (HCAECs).
Materials and Methods: HCACs were treated with glimepiride, glibenclamide, or vehicle, and then released NO was measured using a NOx analyzing high-performance liquid chromatography system. Activation of Akt/PKB was evaluated by Western blot and the effect of LY294002, a specific PI3-kinase inhibitor, on glimepiride-induced NO production was also examined.
Results: Glimepiride (0.1-10 μM) significantly stimulated NO production by HCAECs within 1 min after stimulation and increased 1.8-fold at 30 min (n=6, p<0.05), but glibenclamide did not. Akt/PKB was rapidly phosphorylated by glimepiride and LY294002 significantly inhibited glimepiride-induced NO production by 68% (n=3, p<0.05).
Conclusions: These data suggest that glimepiride at the concentration of 0.1 μM or more stimulates NO production in HCAECs via a PI3-kinase-Akt/PKB-eNOS pathway. Because blood concentration of glimepiride used in a clinical setting is reported to reach 0.1 μM~0.2μM, glimepiride may be a promising agent to prevent coronary artery disease in addition to lowering the glucose levels in type 2 diabetes.

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Acarbose treatment reduces the incidence of cardiovascular complications in patients with Type 2 diabetes: a metaanalysis.
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Background and Aims: Excessive postprandial (pp) hyperglycemia in type 2 diabetes is associated with increased incidence of cardiovascular complications. So far no information from placebo controlled trials is available whether a treatment that specifically acts on reduction of pp hyperglycemia lowers the incidene of cardiovascular events in patients with clinical type 2 diabetes. Acarbose, a worldwide used α glucosidase inhibitor delays the release of glucose from complex carbohydreates leading primarily to a reduction of pp hyperglycemia.
Materials and Methods: To get new information on the significance of control of pp hyperglycemia we performed a metaanalysis of all longterm placebo-controlled trials with acarbose. From the data pool of the Bayer AG 7 studies were available with a minimum duration of 52 weeks which fulfilled GCP criteria. The analysis included all patients of the valid for safety analysis because for these cardiovascular events were standardised monitored as “adverse events”. All together 1248 patients with acarbose and 932 with placebo could be analysed. In two studies the randomisation was 2:1 in favour of acarbose.
Results: The following events were registered: myocardial infarction, angina pectoris, cardiovascular death, stroke, peripheral vascular disease and cardiac failure. The studies were performed between 1987 and 99. The baseline characteristics between placebo and acarbose were well balanced. The primary analysis was cox regression analysis. A covariance analysis was performed for weight, blood pressure and triglycerides. Compared to placebo the aggregated cardiovascular events were reduced by 41% (p=0.0017). The strongest reduction of a prespecified cardiovascular event was observed with myocardial infarction: RR 0.32 (95% CI 0.14-0.71, p=0.0047. Furthermore the fasting and pp plasma glucose, HbA1C, body-weight, triglycerides and systolic blood pressure were significantly reduced. The significance of acarbose treatment persisted when body weight, triglycerides and systolic blood pressure were introduced as covariate.
Conclusion: In conclusion our metaanalysis proves that acarbose significantly reduces the incidence of myocardial infarction and of any cardiovascular events together with comorbidities of the metabolic syndrome. This findings emphasize that control of postprandial hyperglycemia is not only an essential part of diabetes control but also of benefit in the prevention of macroangiopathy.

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Favourable effects of pioglitazone mono or combination therapy on the atherogenic index of plasma - a surrogate marker of LDL particle size.
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Background and Aims: The tendency toward small, dense LDL particles - a profile called pattern B - is a common trait in 40-50 percent of heart disease patients and is also more prevalent in Type 2 diabetes. Small dense LDL is considered to be potentially more proatherogenic than larger buoyant LDL particles. Some studies have suggested that pioglitazone may

alter the qualitative distribution of LDL to the less proatherogenic pattern. Lipid profiles were assessed to determine this potentially import effect of pioglitazone.
Materials and Methods: A programme of four long-term studies-the Quartet studies involved more than 3,700 patients from 28 countries across Europe, ca. 1,850 of whom were treated with pioglitazone either in monotherapy or in combination with metformin or gliclazide. These studies were designed as head-to-head trials with the established oral antidiabetic agents metformin and gliclazide comparing the magnitude, as well as the durability, of the anti-hyperglycaemic effect over one year, both in monotherapy and as add-on therapy to metformin or SU. To assess the effect of oral hypoglycaemic agents on LDL particle size, we used the mean atherogenic index of plasma (AiP; the logarithmic transformation of the triglyceride:HDL cholesterol ratio) for each treatment group which correlates inversely with the LDL particle size to estimate mean changes with pioglitazone or comparator drugs as monotherapy or combination therapy.
Results: In both monotherapy and combination therapy trials pioglitazone caused larger mean reductions in AiP than gliclazide or metformin. Beneficial effects on lipids are apparent from the improvement in the AiP, which integrates HDL cholesterol and triglycerides and is useful as a predictor of macrovascular risk.
Conclusion: These data suggest a favourable change in the size of LDL particles with piogltiazone, which may reduce its atherogenicity.

Mean Change from baseline in atherogenic index of plasma (AiP)

Treatment Regimen	Patient Group	Mean Change (±SEM) from Baseline	Between Treatment p-value
Monotherapy	PIO	-0.34 (±SEM)	
	MET	-0.17 (±SEM)	<0.001
Monotherapy	PIO	-0.37 (±SEM)	
	GLIC	-0.2 (±SEM)	<0.001
Combination Therapy	PIO+SU	-0.31 (±SEM)	
	MET+SU	-0.17 (±SEM)	<0.001
Combination Therapy	PIO+MET	-0.36 (±SEM)	
	GLIC+MET	-0.07 (±SEM)	<0.001

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Rosiglitazone reduces synthesis and secretion of CD40L by primary human macrophages *in vitro*.
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Background and Aims: Increasing evidence supports a central role for CD40-CD40L interactions in the pathogenesis of inflammation and atherosclerosis, a process that is accelerated in type 2 diabetes mellitus. CD40-CD40L is highly expressed in multiple cell types associated with human atherosclerotic lesions, particularly in advanced or rupture-prone lesions. Since human macrophages express PPARγ and activation of this receptor by thiazolidinediones (TZD) suppresses production of a number of inflammatory mediators and markers of plaque stability, we assessed whether CD40L was also TZD-sensitive.
Materials and Methods: Macrophages were prepared *in vitro* by serum stimulation of primary cultures of peripheral mononuclear cells obtained from non-diabetic human volunteers. CD40L mRNA levels were measured using quantitative PCR (Taqman) and CD40L protein was determined by ELISA.
Results: Exposure of macrophages to the PPARγ agonist rosiglitazone (RSG: 1 nM to 10 μM) for 96 h produced a concentration-dependent suppression of CD40L mRNA expression of up to 80% ($P \leq 0.05$). RSG-mediated inhibition of CD40L expression was accompanied by parallel reductions in release of CD40L protein from macrophages into the incubation medium. Other markers of macrophage differentiation and function such as CD14 and osteopontin were unaffected by RSG treatment.
Conclusions: In summary, these data confirm that PPARγ activation by RSG selectively inhibits CD40L expression and secretion by macrophages suggest that in addition to its anti-inflammatory properties, RSG may have potential in preventing atherosclerotic plaque destabilisation.

PS 94
Predictors of Arterial Wall Stiffness in Diabetes

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Carotid artery intima media thickness and macrovascular disease in Japanese Type 2 diabetes.
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Background and Aims: Measurement of carotid artery intima media thickness (IMT) is a useful method for evaluating early stage of atherosclerosis. The purpose of this study is to clarify if IMT is commonly associatrd with differnt macrovascular diseases such as coronary heart disease (CHD), cerebrovascular disease (CVD) and peripheral artery disease (PAD), and if risk factors are different among these diseases in Japanese type 2 diabetes.
Materials and Methods: 1311 patients with Japanese type 2 diabetes, who were undergoing measurement of IMT in April 2000 to December 2002, at the out-patients department of Juntendo University Hospital, were enrolled in this study. Clinical data were collected from medical records cross-sectionally. Macrovascular disease was defined as CHD, CVD and PAD. Noninvasive measurement of IMT was made by high-resolution B-mode ultrasonography. Logistic regression analysis was used to calculate odds ratio for macrovascular disease, at least one of CHD, CVD and PAD. Separate analyses of CHD, CVD and PAD were also done.
Results: 304 patients have one or more macrovascular disease. Patients with macrovascular disease showed greater IMT than no macrovascular disease subjects.(1.39 ±0.44mm versus 1.11 ±0.32mm, p<0.001) Age (66.0 ±9.0 versus 60.1 ±10.9 years, p<0.001), duration of diabetes (13.6 ±10.2 versus 9.3 ±8.6 years, p<0.001), rate of hypertension (66% versus 50%, p<0.001), hyperlipidemia (63% versus 57%, p=0.04) and smoking habit (43% versus 36%, p=0.03) were significantly higher in patients with macrovascular disease. The association between IMT and macrovascular disease remained significant after adjustment for traditional risk factors. There was an increasing odds ratio for each quartile of IMT, from the second quartile (odds ratio, 1.68; 95% confidence interval, 1.01 to 2.78), to the third (odds ratio, 2.50; 95% confidence interval, 1.54 to 4.02) and fourth (odds ratio, 4.14; 95% confidence interval, 2.56 to 6.70). Age, duration of diabetes, hyperlipidemia and smoking habit were also significantly associated with macrovascular disease after adjustment in logistic regression. In separate analyses, CVD was significantly associated with age and IMT. CHD was significantly associated with age, duration of diabetes, hyperlipidemia and IMT. PAD was significantly associated with smoking habit, hypertension and IMT.
Conclusion: Greater IMT is commonly associated with different macrovascular diseases in Japanese type 2 diabetes. This result suggest that IMT might be a useful marker for these diseases in Japanese type 2 diabetes. The other risk factors might be different one another among CHD, CVD and PAD in Japanese type 2 diabetes.

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Intima-media thickness of common carotid artery is regressed by control of blood glucose, especially postprandial glucose than controls of lipid metabolism or blood pressure in Korean Type 2 diabetic patients.
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Background and Aims: To evaluate the role of metabolic abnormalities to the change of carotid intima-media thickness (IMT) in type 2 diabetic Korean patients without clinically demonstrated cardiovascular diseases.
Materials and Methods: Carotid IMTs of 198 subjects with type 2 diabetes (mean age 63.7 years) and without cardiovascular diseases were examined at beseline and after a mean follow-up of 21.1 months.
Results: Stepwise multivariate analysis demonstrated that the independent risk factors for the change of mean IMT in diabetic patients were average 2h-postplandial plasma glucose (2h-PPG) (beta=0.243, P=0.001) and

average LDL-C (beta=0.177, P=0.012). The change of mean IMT in well controlled group of average 2h-PPG (< 11.1 mmol/L, n; 73) was –55 ± 131 microns/year, and the rate in poorly controlled group (≥ 11.1 mmol/L, n; 125) was 3 ± 102 microns/year (P=0.018) after adjustment of other average parameters. That in well comtrolled group of average HbA1c (< 7%, n=58) was –70 ± 135 microns/year, and that in poorly controlled group (≥ 7%, n; 140) was 3 ± 116 microns/year after adjustment (P=0.018). Between well controlled and poorly controlled groups of average fasting glucose, total cholesterol, triglyceride, HDL-C, LDL-C, or blood pressure, there were no significant differences in the change of mean IMT each other.
Conclusion: Intensive control of blood glucose, especially that of postprandial glucose is useful method to prevent accerelated atherosclerosis in Korean type 2 diabetic patients.

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Associations of blood pressure with carotid intima-media thickness in elderly Finns with diabetes mellitus or impaired glucose tolerance.
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Background and Aims: We carried out a population-based study on the determinants of ultrasonographic manifestations of carotid atherosclerosis in northern Finland. Recently, we reported that the maximal intima-media thickness (IMT) of the common carotid artery (CCA) measured by ultrasound correlated inversely with insulin sensitivity measured by a novel insulin sensitivity check index (QUICKI), and that subjects in the two lowest QUICKI tertiles had a 5-fold risk for severe intima-media thickening (maximal IMT of the CCA ≥ 1.2 mm), compared to those in the highest QUICKI tertile. In the present paper, we highlight the associations of carotid atherosclerosis with systolic (SBP) and diastolic blood pressure (DBP) and pulse pressure (PP) in 65-year-old Finns.
Materials and Methods: Carotid ultrasonographic measurements were performed on 54 diabetic subjects, 97 subjects with impaired glucose tolerance (IGT) and 57 normoglycemic subjects (NGT). The subjects were classified into four quartiles of SBP, DBP and PP.
Results: SBP, DBP, PP and the use of antihypertensive drugs increased along with the deterioration of glucose status. The mean intima-media thickness (IMT) of the common carotid artery (CCA) from the lowest to the highest quartiles of SBP was 0.86 mm ± 0.25, 0.91 mm ± 0.25, 0.94 mm ± 0.22 and 1.01 mm ± 0.31(P = 0.043) and the maximal IMT CCA 0.98 mm ± 0.34, 1.00 mm ± 0.35, 1.03 mm ± 0.29, 1.18 mm ± 0.52 (P = 0.038), respectively. The differences in the mean IMT CCA and the maximal IMT CCA defined according to the DBP and PP quartiles were not statistically significant. The prevalence of severe intima-media thickening (maximal IMT CCA ≥ 1.2 mm) was 39 % in the subjects in the highest SBP quartile (≥ 170 mmHg) and 20 % in the subjects with lower SBP (P = 0.008). In multiple regression analysis, the adjusted OR for severe intima-media thickening was 2.9 (95 % CI 1.1 – 7.9) in the subjects in the highest SBP quartile compared to the subjects with lower SBP. The odds ratio for male gender was 3.6 (95 % CI 1.6 – 8.0), long-lasting smoking 2.8 (95 % CI 1.0 – 7.7) and the subjects in the two lowest QUICKI tertiles, compared to those in the highest tertile, was 5.5 (95 % CI 2.1 –14.4).
Conclusion: In the present study, high SBP was, but neither DBP nor PP were associated with severe carotid intima-media thickening. We suggest that the results can be generalized to apply to elderly Finnish subjects with DM and IGT, but not to normoglycemic subjects, on the basis of this study.

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Short acting insulin: a risk factor of atherosclerosis in patients with Type 1 diabetes.
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Background and Aims: Individuals with type I diabetes have increased rates of all cardiovascular disorders compared to subjects without diabetes. Hyperinsulinaemia has been associated with increased cardiovascular risk. Several observational studies have shown that higher endogenous insulin levels are related to an increased risk of cardiovascular disease (CVD). When high endogenous insulin levels indeed are a CVD risk factor one might expect an increased risk of CVD in patients treated with insulin, as this leads to high circulating insulin levels. Such risk elevation may counteract the benefits of tight glucose control. As appears from the Diabetes Control and Complications Trial and the UK Prospective Diabetes Study, intensive blood-glucose control has been more effective in decreasing the risk of microvascular complications than in decreasing the risk of macrovascular complications. The role of insulin treatment as a risk factor therein, is of major interest.
The aim of the present study was to evaluate, whether insulin treatment (insulin levels in plasma, cumulative insulin use and actual insulin dose) is a risk factor of atherosclerosis in patients with type I diabetes.
Materials and Methods: We performed a cross-sectional study in 214 subjects with type 1 diabetes. Clinical parameters, including blood pressure, ankle-brachial index, body mass index, medication use, smoking habits, alcohol consumption and chemical parameters as HbA1c, plasma lipids and micro-albumin were determined. The cumulative amount of insulin used was calculated from medical records since the diagnosis of diabetes. Cumulative insulin dose was converted to Z-scores [Z= (x - mean) / SD] for the different types of insulin, to standardize the different values. Atherosclerosis was assessed by measurement of carotid intima-media thickness (CIMT). The relation between CIMT and the different parameters was evaluated using univariate and multivariate linear regression analysis.
Results: The mean age of the patients was 43.5 (12.3 SD) years, and the mean duration of diabetes was 21.4 (11.1 SD) years. The range of cumulative insulin use was as follows: insulin analogs: 747 U - 80244 U (median 25267 U); regular insulin: 3469 U – 652346 U (median 151294 U); intermediate acting insulin: 836 U – 915265 U (median 110940 U). The cumulative dose of short-acting insulin (insulin analogs and regular insulin) showed a positive and significant relation with thickening of the intima media (increase of 16 µm in CIMT per standard deviation increase of insulin use; 95% CI 1 to 32) corrected for gender, age and duration of diabetes. This constitutes a 2.4% difference in CIMT. After adjustment for pulse pressure, total cholesterol, HDL cholesterol, triglycerides and HbA1c the relation remained significant. Adjustment for current BMI did not materially change the magnitude of the relation. The cumulative dose of intermediate acting insulin showed no relation with CIMT.
Conclusion: This study in type 1 diabetes suggests, that cumulative use of short-acting insulin is a risk factor of atherosclerosis.

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Post prandial lipaemia and carotid intimal medial thickness in patients with Type 2 diabetes mellitus.
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Background and Aims: Diabetic dyslipidaemia is believed to be an important risk factor for atherosclerotic vascular disease. Since atherosclerosis is being increasingly recognized now as a post prandial (PP) phenomenon, lipid abnormalities in the PPstate particularly PPhypertriglyceridimia may contribute significantly to the high macrovascular risk of diabetes. The present study therefore investigates the association of PPlipaemia in type 2 diabetic subjects with carotid intimal medial thickness (IMT), a reliable marker of early atherosclerosis.
Materials and Methods: A standard oral fat tolerance test providing 729K cal /m^2, 62.5 g fat, 24.75 g carbohydrates & 5.2g protein was given to fifty type 2 diabetic subjects after an overnight fast. Plasma glucose and serum total cholesterol, triglycerides(TG) and HDL-cholesterol were measured at 0, 2, 4, 6 and 8 h after the fat challenge. PPlipid responses were expressed as area under the curve (AUC), incremental area under the curve (iAUC) and peak lipid response during the 8h of the study. Carotid IMT measurements were made by high resolution B mode ultrasonography. Linear regression analysis was performed to determine the relation between carotid IMT and various PPlipid parameters.
Results: Study subjects (46% males and 54% females), had a mean (±SD) age of 51.18 ± 11.05 y (range: 32 -74y) with an average duration of diabetes of 3.80 ± 3.23 y (range: 0.75-12y). Their mean body mass index and waist to hip ratio were 23.17 ± 4.40 Kg/m^2 and 0.93± 0.07 respectively. Mean fasting blood glucose was 194 ± 53mg/dl, mean PPblood glucose 285± 61mg/dl and mean glycated hemoglobin levels were 8.9 ± 0.9 %. Fasting lipid profile revealed a serum TG level of 166± 47 mg/dl, HDL-C of 37±10 mg/dl and LDL-C of 124± 36mg/dl. In response to fat challenge, TG levels increased to a peak of 408± 247 mg/dl. Mean TG -AUC was 2440±755mg/dl.h for the 8h duration of the study while mean TG-iAUC was 1036±518 mg/dl.h.Mean carotid IMT in the diabetic patients was 0.73±0.31mm(range:0.35-2.10mm). No significant correlation was obtained between carotid IMT and any of the fasting (p>0.05)or PPlipid

parameters(p>0.05) including PPhypertriglyceridaemia (p=0.923 for TG-AUC; p=0.841 forTG-iAUC; p=0.645 for peak TG response).The lack of association between carotid IMT and PPlipaemic indices persisted even when male and female subjects were analyzed separately. Comparison of carotid IMT values between the uppermost and lowermost quartiles of PPTG levels also did not show a significant difference(p>0.05).
Conclusion: This study could not find a significant association between PP hyperglyceridaemia or any other measure of PPlipaemia with early carotid atherosclerosis in patients with type2 diabetes mellitus. These results do not support a clear role for PPlipid abnormalities in early diabetes related atherosclerosis.

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Microalbuminuria as a predictor for the severity of aortic and carotid atherosclerosis in Type 2 diabetes.
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Background and Aims: Microalbuminuria is considered a marker of systemic atherosclerosis as well as incipient diabetic nephropathy. Aortic pulse wave conduction velocity (PWV) and carotid intima-media thickness (IMT) assessed non-invasively by ultrasound have been established to be useful for diagnosis of arteriosclerosis. The aim of this study was to investigate the relation of micoralbuminuria with systemic arteriosclerosis in type 2 diabetic patients in terms of carotid plaque formation.
Methods: We studied cross-sectionally 97 type 2 diabetic outpatients of 50 to 70 years of age, of whom prevalence of carotid plaque formation was about 50% from preliminary study in patients with type 2 diabetes. These patients were with or without simple retinopathy, diabetic nephropathy less than microalbuminuria, and no evidence of cerebral, cardio- or peripheral vascular disease, and values of haemoglobin A1c (HbA1c) less than 8.0 %. PWV was measured, and IMT was bilaterally determined on common carotid artery far wall and carotid plaque formation was evaluated; 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. At over night fast, blood samples were measured: plasma glucose, HbA1c, serum lipids and thrombomodulin (TM), a marker of endothelial cell damage. Urinary albumin excretion ratio (AER) was determined in morning spot urine sample. Subjects were divided into microalbuminuric (30mg/gcr< or = AER<300mg/gcr) and normoalbuminuric (AER<30mg/gcr) groups.
Results: Thirty-six patients were microalbuminuria and 61 were normoalbuminuria. No difference between microalbuminuric and normoalbuminuric groups was found for age. PWV in microalbuminuric group was greater than that in normoalbuminuric group (mean+/-SD; 9.2+/-1.3 vs. 8.3+/-0.8m/sec, p<0.01). No difference between microalbuminuric and normoalbuminuric groups was observed for bilateral IMT. However, the prevalence of patients with carotid plaque formation was significantly higher (61.1 vs. 39.3%, p<0.05) in microalbuminuric group than in normoalbuminuric group. PS in microalbuminuric group was greater than that in normoalbuminuric group (2.9+/-3.2 vs. 1.2+/-1.8mm, p<0.01). Log AER in all patients was positively correlated with PWV (r=0.462, p<0.01) and PS (r=0.324, p<0.01), respectively. Microalbuminuric patients had higher TM (24.7+/-6.6 vs. 19.3+/-4.7U/ml, p<0.01) compared with normoalbuminuric patients.
Conclusion: These results suggest that microalbuminuria is a diagnostic predictor for aortic and carotid subclinical athrosclerosis, and is associated simultaneously with the severity of atherosclerosis in type 2 diabetes without advanced micro and macroangiopathy.

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Alterations in arterial rigidity in subjects with dismetabolic syndrome.
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Background and Aims: Increased arterial stiffness is an independent predictor of cardiovascular risk, and specially of coronary risk. A transversal study was carried out to determine the degree of arterial damage in subjects with dismetabolic syndrome (DMS) and related factors.
Material and Methods: DMS was defined as having at least 3 of the 5 following abnormalities: waist circumference >102 cm in men and >88 cm in women; serum triglycerides ≥ 150 mg/dL; high-density lipoprotein cholesterol (HDL-C) of < 40 in men and < 50 mg/dL in women; blood pressure (BP) of at least 130/85 mmHg; or serum glucose level of at least 110 mg/dL. Arterial rigidity was measured with the carotid femoral pulse wave velocity (PWV), using automatic equipment. Subjects had no previous cardiovascular complications.
Results: The study involved 405 subjects (78% female). The findings are presented in the table.
Subjects with DMS presented a higher level of arterial stiffness. In the linear regression analysis this difference persisted after adjusting for age, gender, systolic BP, creatinine levels, tobacco use, heart rate and the presence of hypertension and diabetes.
In the stepwise regression analysis the variables with the greatest effect on PWV in subjects with DMS were: age (R^2 = 0.40); and systolic BP (R^2= 0.41).
Conclusion: Subjects with DMS present a greater level of arterial damage, reflected in the PWV increase.

Variables	No DMS (n = 178) Mean (SD)	with DMS (n= 227) Mean(SD)
Age (years)	50.5 ± 11	61.2 ± 10 **
Waist (cm)	92.6 ± 12	96.1 ± 13 *
Systolic BP (mmHg)	129.8 ± 35	145.9 ± 32 *
Pulse pressure	48.4 ± 35	62.6 ± 29 *
Glucose (mg/dL)	136.8 ± 45	153.6 ± 59
HDL-C (mg/dL)	52.4 ± 11	48.8 ± 24 *
Triglycerides (mg/dL)	143.9 ± 26	160.8 ± 37
Age adjusted PWV (m/s)	9.2 ± 1.3	14.2 ± 4.3 **

*P< 0.01, **P<0.001

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Pulse wave velocity is elevated in diabetic patients with incipient nephropathy.
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Background and Aims: To examine whether brachial-ankle pulse wave velocity (baPWV), a possibly early marker for identification of atherosclerotic vascular damage, is associated with slight elevation of albuminuria in patients with type 2 diabetes.
Materials and Methods: baPWV was measured by automatic oscillometric method in 346 type 2 diabetic patients with normoalbuminuria (a mean level of 3 times measurements of albumin-to-creatinine (ACR) <30 mg/gcr; n=200), incipient nephropathy (a mean level of ACR ≥30 and <300 mg/gcr; n=119), and clinical nephropathy (a mean level of ACR ≥300 mg/gcr; n=27,).
Results: baPWV was significantly higher in patients with incipient nephropathy and clinical nephropathy than in patients with normoalbuminuria (p<0.0001, respectively). By univariate analysis it correlated significantly with age (r=0.44, p<0.0001), systolic blood pressure (r=0.55, p<0.0001), diastolic blood pressure (r=0.42, p<0.0001), albuminuria (r=0.24, p<0.0001) and HbA1C (r=0.11, p<0.05). Albuminuria revealed an independent significant association with baPWV (p<0.01) after adjustment for age, sex, smoking, BMI, HbA1C, hyperlipidemia, and hypertension. Multiple regression analysis showed age, diastolic blood pressure and albuminuria were independently associated with baPWV (adjusted R2=0.42, p<0.0001).
Conclusion: The results suggest that baPWV is likely a useful, valuable, and early marker for identification of atherosclerosis in type 2 diabetic patients.

1110

Prevalence of the metabolic syndrome and its association with arterial stiffness in Greek industrial workers.
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Background and Aims: The objective of this study was to assess the prevalence of the metabolic syndrome (MS) and its association with cardiovascular risk factors in Greek industrial workers.
Materials and Methods: Body mass index (BMI), waist/hip ratio (W/H ratio), lipid profile, fasting plasma glucose (FPG), fasting insulin, insulin resistance (homeostasis model assessment [HOMA-IR]), pulse wave

velocity (PWV), blood pressure (BP) and pulse pressure (PP) were assessed in 424 apparently healthy Greek industrial workers [mean age 42.3 ± 15.5 years, 298 (70.3%) males]. The above variables were considered in a logistic regression analysis using the diagnosis of MS as end point. MS was defined according to the guidelines of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) as the presence of at least three of the following factors: FPG ≥ 110 mg/dl, triglycerides ≥ 150 mg/dl, BP ≥ 130/85 mmHg (systolic/diastolic), waist circumference>100 cm (males)/88 cm (females) and HDL< 40 mg/dl (males)/50 mg/dl (females).
Results: The overall prevalence of the MS was 14.6% (62/424), being similar in both genders (15% in men versus 14.2% in women). Age ($p<0.05$), BMI ($p<0.01$), W/H ratio ($p<0.01$), mean BP ($p<0.01$), PP ($p<0.001$), PWV ($p<0.001$), total cholesterol ($p<0.001$), LDL ($p<0.01$) and HOMA-IR ($p<0.001$) were significantly associated with the presence of MS. When the above variables were entered in the multivariate model using clinically and statistically appropriate cutoff points, total cholesterol>240 mg/dl ($p<0.01$, OR 4.9, 95% CI 1.5, 9.7), PWV>8 m/s ($p<0.01$, OR 5.2, 95% CI 1.7, 13.9) and HOMA-IR ($p<0.001$, OR 8.1, 95% CI 2.1, 18.6) emerged as independent predictors of the presence of MS.
Conclusion: The prevalence of the MS was considerable in an apparently healthy non-diabetic population, suggesting that a more aggressive primary prevention is warranted. Apart from adiposity and insulin resistance evaluated by total cholesterol and HOMA-IR, respectively, arterial stiffness assessed by PWV appears to represent a novel predictor of the presence of MS.

1111

Examination of correlation of ASI and other indexes to evaluate arteriosclerosis in patients with Type 2 diabetes.
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Background and Aims: Because cardiovascular disease is the most important cause of morbidity and mortality in patients with type 2 diabetes, special attention should be given to the presence or development of arteriosclerosis. Though many procedures, invasive or non-invasive, have proposed, it is difficult even now, that only single method can evaluate to arteriosclerosis precisely. Recently, new device which can evaluate arterial stiffness by computerized oscillometry, is developed (Cardio Vision MS-2000, IMDP Corporation, USA). This device displays an index of arterial stiffness called ASI (Arterial Stiffness Index). The purpose of this study was to estimate whether there is the correlation between ASI and other arteriosclerosis evaluation methods.
Materials and Methods: 282 subjects with type 2 diabetes (male/female; 173/109, average age; 62 +/- 11 years old, average duration; 9.1 +/- 7.9 years, average body mass index (BMI); 23.3 +/- 3.3 kg/m^2, and average glycohemoglobin; 7.7 +/- 2.0 %) underwent evaluation of arteriosclerosis by means of ASI, pulse wave velocity (PWV), carotid artery echo and second derivation of photoplethysmogram (SDPG). ASI was repeated three times in one time of examination in order to restrain dispersion. The hb(heart-brachial) - PWV and ba(brachial-ankle) - PWV were used as index of PWV, and max-IMT(intima-media thickness) and plaque score (PS; total summation of height of plaque in observation range) were used as index of carotid artery echo, and b/a and d/a were used as index of SDPTG. We carried out each measurement inspection in the as much as possible one day, or in the day that is possibly close each other.
Results: Compared with ASI and PWV, ASI was significantly correlated with ba-PWV ($r=0.422$; $p<0.001$), but significant correlation between ASI and hb-PWV, was not found. ASI was significantly correlated with max-IMT ($r=0.221$; $p<0.001$) and PS ($r=0.232$; $p<0.001$). Compared with ASI and SDPG, ASI was significantly correlated with b/a ($r=0.196$; $p<0.001$), but no significant correlation was observed between ASI and d/a.
Conclusion: ASI measured by Cardio Vision was significantly correlated with other arterial sclerosis evaluation indexes. These findings suggest that ASI is associated with not only morphologic, but also functional changes caused by arteriosclerosis, in patients with type 2 diabetes.

PS 95
Inflammatory Markers in Diabetes

1112

Markers of vascular inflammation are more strongly associated with toe brachial index than with ankle brachial index in patients with Type 2 diabetes.
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Background and Aims: Ankle brachial index (ABI) is a simple and useful method for assessing peripheral vascular disease (PVD). Furthermore, an ABI value of less than 0.9 is an independent risk factor for incident cardiovascular disease (CVD), recurrent CVD, and mortality in elderly adults. However, the application of this index to the diabetic patients is still in some doubt due to the presence of medial artery calcification, giving a falsely high ABI. Therefore, in patients with diabetes, measurements of toe brachial systolic pressure (TBI) are recommended in clinical situation. In the present study, we investigated whether ABI or TBI is strongly correlated with the presence of CVD or systemic inflammation, as measured serum high-sensitivity C reactive protein (hs-CRP), in patients with type 2 diabetes.
Materials and Methods: We studied 101 patients with type 2 diabetes, including 23 patients with coronary artery disease and /or with stroke. Patients with PVD were excluded. TBI was measured at both great toes using a plethysmography. Serum hs-CRP was determined by an immunonephelometric assay. Plasma interleukin (IL)-6 and fibrinogen were also measured as markers of systemic inflammation. Data are expressed as mean±SD.
Results: Both the ABI and TBI were lower in the diabetic patients with CVD than in those without CVD (1.05±0.19 vs.1.14±0.09, $P = 0.0036$; 0.76±0.21 vs. 0.95±0.20, $P = 0.0003$, respectively). By linear regression analysis, TBI, but not ABI, was significantly inversely correlated with serum hs-CRP ($r = -0.37$, $P = 0.0012$), or fibrinogen ($r = -0.22$, $P = 0.0488$). Multivariate analysis showed that hs-CRP was an independent determinant of the TBI in type 2 diabetic patients.
Conclusion: TBI is more strongly associated with the prevalence of CVD or with vascular inflammation as estimated by hs-CRP in patients with type 2 diabetes.

1113

The relationship of inflammatory markers with the clinical and biochemical features in Type 1 diabetes patients.
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Background: Diabetic patients are characterised by increased risk of early development of macrovascular complications. One of the factors that can influence this phenomenon is a process of sub-clinical systemic inflammation.
Aim: To determine the association between selected inflammation markers with clinical and biochemical features in type 1 diabetes mellitus (T1DM).
Material and Methods: We included into this study 80 individuals: 51 T1DM patients (30 women and 21 men, mean age 30.9±9.9 years) and 29 controls. They underwent basic clinical examination. The parameters of the metabolic control (glucose concentrations in daily profile, HbA1c, lipids) were measured and the presence of late diabetic complications was determined. In addition, we measured the following inflammation markers in this group: CRP-protein by immunonephelometry, interleukin (IL6) and soluble P-selectin (sPS) by ELISA, Fb by modified Clauss method. The differences between the groups were analysed by t-Student test and U Mann-Whitney test. The correlation analysis was performed by Pearson and Spearman method.
Results: Concentration of sPS was significantly higher among diabetic men than in diabetic women (226.37±142.95 vs 146.94±61.56 ng/ml, $p<0.05$) and in smokers than in non-smokers in diabetic group (243.26±174.68 vs 158.91±61.81 ng/ml, $p<0.05$). T1DM patients with family history positive of coronary heart disease had higher Fb concentrations than those with negative family history (3.01±0.86 vs 2.5±0.58 g/l, $p<0,05$). In addition, there was a significant correlation between IL6 concentration and the following parameters: fasting glycaemia ($r=0.35$, $p<0.01$), HbA1c ($r=0.31$, $p<0.05$), leukocyte count ($r=0.26$, $p<0.05$), and BMI ($r=0.36$, $p<0.05$). Similarly, a positive correlation was detected between sPS concentration

and two parameters: waist circumference (r=0.57, p<0.05) and WHR (r=0.62, p< 0.05). There was also a negative correlation between sPS and HDL-cholesterol concentration (r=-0.32, p<0.05). Fb concentration correlated positively with duration of diabetes (r=0.52, p<0.01) and BMI (r=0.44, p<0.01).
Conclusions: The level of inflammation markers seems to correlate with the parameters of both metabolic control and metabolic syndrome in T1DM patients. Thus, the intensity of inflammation process may influence the development of macrovascular complication in T1DM group.

1114

White blood cell count, fibrinogen, and CRP in coronary patients with diabetes mellitus.
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Background and Aims: Diabetes mellitus (DM) infers a high risk of coronary artery disease (CAD), and CAD bears characteristics of an inflammatory process. Therefore, interest has focused on markers of inflammation in CAD and DM.
Materials and Methods: We enrolled 756 consecutive patients undergoing coronary angiography from October 1999 through October 2000. These patients underwent follow-up after an average period of 2.27 ± 0.43 years. Patients with DM (established or newly diagnosed by means of oral glucose tolerance tests) and nondiabetic patients were compared both at baseline and at follow-up. Vascular death (cardiac death or non coronary vascular death) was recorded.
Results: In patients with DM (n = 170), WBC (8.04 ± 2.66 vs. 7.22 ± 2.38 G/L; p <0.001) Fibrinogen (377.88 ± 97.66 vs. 338.01 ± 80.70 mg/dL; p <0.001) and CRP (0.86 ± 1.27 vs. 0.70 ± 1.60 mg/dL; p <0.001) were elevated at baseline. Also, at follow-up WBC (7.58 ± 2.03 vs. 6.53 ± 1.94 G/l; p <0.001), Fibrinogen (413.19 ± 83.80 vs. 378.49 ± 69.78 mg/dl; p <0.001), and CRP (0.45 ± 0.67 vs. 0.35 ± 0.65; p = 0.030) were significantly elevated in patients with DM. Overall, 29 patients suffered vascular death (cardiac death or non coronary vascular death). After adjustment for conventional risk factors including diabetes status, both fibrinogen (exp(B) = 2.083 (1.296-3.346); p = 0.002) and WBC (exp(B) = 1.508 (1.171-1.943); p = 0.001) proved independently predictive of vascular death. Estimation of leukocyte subtypes at follow-up revealed significant elevations of neutrophils (p = 0.021), eosinophils (p = 0.002), and monocytes (p = 0.019) in patients with CAD diagnosed at baseline. In patients with DM neutrophils (p <0.001), eosinophils (p = 0.001), basophils (p = 0.017), monocytes (p = 0.026), and lymphocytes (p = 0.036) were significantly elevated.
Conclusion: WBC, fibrinogen, and CRP are elevated in patients with DM as well as in patients with CAD. Both WBC and fibrinogen are independent predictors of vascular death among patients undergoing coronary angiography. Increased levels of inflammatory markers therefore might account for the worse prognosis of coronary patients with DM.

1115

High sensitive C-reactive protein and carotid intima-media thickness in Koreans - the Korean metabolic syndrome study.
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Background and Aims: Chronic inflammatory response is an important component in the development and progression of atherosclerosis. Since high-sensitive C-reactive protein (hs-CRP) assay has been developed, the association between increase of hs-CRP concentration and the development of atherosclerosis has been recently reported. In the present study, we investigated the relationship between hs-CRP, conventional cardiovascular risk factors and carotid intima-media thickness(IMT) in apparently healthy subjects.
Materials and Methods: This study was conducted as a branch of the Korean Metabolic Syndrome Study. Of total 1,230 individuals who had undergone routine check-up, 849 subjects without past history of cardiovascular diseases were selected. Hs-CRP was measured by an ELISA with human anti-CRP (CRP II Latex X2, Denka Seiken, Japan). Carotid IMT was measured by high resolution B-mode ultrasonography (SSA-270A, Toshiba, Tokyo, Japan).
Results: Hs-CRP levels were ranged from 0.10 up to 43.7 mg/l (mean 2.06, median 0.77 mg/l). There were significant positive correlations between hs-CRP and age, BMI, waist, BP, HOMA-IR, TC/HDL-C ratio. On multiple regression analysis, there were independent relationships between hs-CRP and obesity, hypertension, age(≥ 60 years), current smoking, male and HOMA-IR. There were positive correlations between carotid IMT and age, BMI, waist circumference, SBP, DBP, TC, TG, LDL-C, fasting blood glucose, HOMA-IR, and hs-CRP, and negative correlation between carotid IMT and HDL-C. On multiple regression analysis, independent relationships persisted between carotid IMT and age, SBP, TC/HDLc, HOMA-IR, waist circumference, and DBP. After adjusting conventional risk factors into multiple regression, there was no longer significant relationship between hs-CRP and carotid IMT.
Conclusion: There was strong correlation between hs-CRP and conventional cardiovascular risk factors, especially obesity. Also we found a highly significant association between hs-CRP and carotid IMT. However, hs-CRP *per se* is not a major independent risk factor of early subclinical atherosclerosis in Koreans.

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Relationships of plasma interleukin -18 concentrations to hyperhomocysteinemia and carotid intimal-medial wall thickness in patients with Type 2 diabetes.
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Background and Aims: We compared plasma interleukin (IL)-18 concentrations in patients with type 2 diabetes to those in age-matched control subjects, and investigated whether plasma IL-18 was associated with plasma total homocysteine (tHcy) concentration or carotid intima-media wall thickness (IMT), an early marker of atherosclerosis, in these patients.
Materials and Methods: We measured plasma IL-18 in 103 type 2 diabetic patients and 45 age-matched control subjects. In patients we also measured plasma total homocysteine and serum high-sensitivity C-reactive protein (hs-CRP). IMT was evaluated for both common carotid arteries.
Results: Plasma IL-18 was significantly higher in diabetic patients than in control subjects (203 ±153 vs.118±37 pg/mL, P<0.001). High IL-18 was defined as equaling or exceeding the mean+2SD of plasma IL-18 in control subjects (192 pg/mL). Patients with high IL-18 showed a greater carotid IMT than those with normal IL-18. Carotid plaques were more numerous in diabetic patients with high IL-18 than in those with normal IL-18. Plasma tHcy concentrations were significantly higher in patients with high IL-18 than in those with normal IL-18. Urivariate and multivariate analyses showed a strong independent association between tHcy and IL-18. Plasma IL-18 also correlated positively with serum hs-CRP.
Conclusion: In patients with type 2 diabetes, plasma IL-18 concentrations are greater than in healthy subjects. Plasma IL-18 is an independent determinant of plasma tHcy, which is linked independently with atherosclerotic carotid wall thickening.

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High plasma homocysteine concentrations are associated with endothelial dysfunction in patients with Type 2 diabetes and link diabetic nephropathy to macroangiopathy.
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Background and Aims: In type 2 diabetic patients with or without nephropathy, we examined relationships between plasma concentrations of total homocysteine (tHcy) and clinical macroangiopathy as well as endothelial dysfunction indicated by plasma thrombomodulin (TM) concentrations.
Materials and Methods: We studied 103 type 2 diabetic patients including 27 with macroangiopathy (14 patients with coronary artery disease, 10 with stroke, and 4 with peripheral vascular disease). Plasma tHcy was measured by high-performance liquid chromatography. Plasma TM was determined by enzyme immunoassay. As an index of glomerular filtration rate (GFR), creatinine clearance (Ccr) also was determined in a 24-hr urine collection.
Results: Considering all diabetic patients, plasma tHcy concentrations were significantly higher in those with macroangiopathy than in those without (10.4±3.7 vs. 8.5±2.8 mmol/L, P= 0.0077). By univariate and multivariate analyses, plasma tHcy was correlated inversely with Ccr. Plasma tHcy concentrations were significantly higher in the patients with overt

albuminuria than in those with normoalbuminuria or microalbuminuria. After exclusion of patients with renal insufficiency (Ccr of less than 60 mL/min), differences in plasma tHcy concentrations between patients with and without macroangiopathy were abolished. By multivariate analysis, total cholesterol, urinary albumin, Ccr, C peptide, and tHcy retained significant influence on the plasma TM. Even in patients with normal renal function (Ccr at least 80 mL/min), plasma tHcy was correlated positively with plasma TM.
Conclusion: Diabetic nephropathy is a main determinant of plasma tHcy elevation in type 2 diabetic patients. Since plasma TM is independently associated with plasma tHcy, in diabetic patients with overt nephropathy, elevation of tHcy reflecting reduced clearance is a likely cause of endothelial dysfunction, resulting in the atherosclerosis underlying development of cardiovascular disease.

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Determinants of plasma total homocysteine concentrations in Type 2 diabetes.

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Background and Aims: The association of mild elevations in total homocysteine (tHcy) plasma levels with diabetes and its long-term complications is still controversial, partly due to the lack of a complete information on the impact of common determinants of tHcy concentrations in diabetic subjects. Furthermore, diabetes-related factors may also play a peculiar role in modulating tHcy plasma concentrations, or in favoring its thrombotic action. Thus, we systematically investigated genetic, nutritional, lifestyle and metabolic suspected predictors of fasting tHcy plasma levels in a cohort of 383 consecutive type 2 diabetic subjects.
Materials and Methods: Methylene tetrahydrofolate reductase (MTHFR) C677T genotype, fasting tHcy, vitamin B12 and folate plasma levels were measured in diabetic patients and in 100 healthy controls. In diabetic subjects, fasting glucose, HbA1c, creatinine, and creatinine clearance measurements, together with BMI, blood pressure (BP) measures, information on alcohol and smoking habits, duration of diabetes and evidences of micro- and macroangiopathy were also available.
Results: Age- and sex-adjusted geometric mean tHcy was 11.7 (95% CI: 11.2-12.3) mmol/L in type 2 diabetic subject and 12.0 (10.9-13.4) mmol/L in controls, P=0.584. In diabetic subjects, after multivariable adjustment for age, sex, folate, vitamin B12, and creatinine levels, geometric mean tHcy was 14,9% higher in men than in women (P=0.02). Multivariable adjusted tHcy levels were significantly higher in diabetic subjects in the highest quartiles of age (P=0.0001) and serum creatinine (P=0.001)than in those in the lowest ones. Moreover, tHcy levels were significantly lower in subjects in the highest than in the lowest folate quartile-groups (P=0.02). tHcy levels significantly correlated with age (r=0.174; P=0.001), systolic BP (r=0,162; P=0.002), serum creatinine (r=0.267, P=0.000) levels, creatinine clearance (r=-0.119; P=0.025) and marginally with vitamin B12 levels (r=-0.102, P=0.05). Conversely, tHcy levels did not significantly differ according to BMI, alcohol intake, current smoking, diabetes duration, HbA1c and glucose concentrations, and according to treatment of diabetes. The MTHFR C677T genotype distribution, consistent with the Hardy-Weinberg equilibrium, was similar in diabetic and control subjects (P=0.698), with a TT homozygous frequency of 22% in type 2 diabetic subjects and 19 % in controls. Overall, tHcy plasma levels did not significantly differ according to MTHFR genotypes, even in the lowest folate quartile group. After multivariable adjustment, tHcy levels and the prevalence of TT genotype were not significantly different in diabetic subjects with any type of documented long-term complication than in those without.
Conclusion: Age, sex, vitamin status, systolic blood pressure and renal function are the main determinants of tHcy plasma levels in type 2 diabetic subjects, as in non diabetic individuals. Conversely, after controlling for major confounders, diabetes-related variables and the presence of diabetic complications do not affect tHcy plasma concentrations.

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Resistin – determination in persons with diabetes mellitus of Type 2 or in individuals with acute inflammatory disease.

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Background and Aims: Resistin is a recently discovered signal molecule, which could help elucidation of pathophysiology of the origin of insulin resistance and its correlation with obesity. As little information was available about resistin determination in venous blood at the time of our study, we focused on the question whether any correlation exists between persons with type 2 diabetes mellitus, with systemic inflammation, healthy persons and resistin concentration and laboratory markers of inflammation, leptine, BMI. Differences of resistin values in these types of probands were studied as well.
Materials and Methods: Persons under study were divided into 3 groups: group A - with clinical signs of inflammatory disease of respiratory tract, leukocytosis > 10000/ul and CRP concentration > 50 mg/l (35 persons); group B – with satisfactorily compensated type 2 DM treated by PAD, without clinical signs of inflammation (12 persons); group C - without clinical signs of inflammation (77 persons). For all probands we determined BMI index and examined resistin, leptin, interleukin 6, TNF-alpha, Na, K, Cl, insulin, cholesterol, HDL, LDL, triacylglycerols, creatinine, uric acid, ALT, AST, GMT, P, Mg and albumin.
Results: Persons with clinical signs of severe inflammation had higher concentrations of Il6, CRP, resistin and a markedly lower BMI, decreased values of glucose, sodium, triacylglycerols, cholesterol, LDL and HDL compared to diabetics of type 2 ($p<0.05$). Persons with clinical signs of severe inflammation showed significantly higher concentrations of TNF-alpha, Il6, CRP, resistin, glucose, leptin and considerably lower values of albumin, sodium and HDL than healthy individuals ($p<0.05$). Persons with type 2 DM had markedly higher values of BMI, CRP, glucose, triacylglycerols, LDL, GMT and leptin, compared to healthy probands ($p<0.05$). None of the three groups differed markedly in age or sex. The group of healthy probands showed a significant correlation between leptin and resistin (correlation coefficient 0.82); this correlation was not found in patients with inflammation and type 2 DM. The group of probands with inflammations was found to have a significant positive correlation between resistin and inflammatory markers (correlation coefficient 0.3-0.5) and negative correlation between resistin and cholesterol. We also found positive correlations between leptin and BMI as well as negative correlations between leptin and CRP. No significant correlations between resistin and other studied parameters were found in DM of type 2.
Conclusion: Patients with severe inflammatory disease displayed correlation between resistin concentration and laboratory markers of inflammation. No correlation was found between leptin and resistin, which occurs in healthy population. Resistin concentration in the serum of these patients is significantly higher compared to healthy subjects and compensated type 2 diabetics with signs of insulin resistance. This may be due to a direct effect of inflammatory cytokines on resistin production. In patients with type 2 DM no significant correlations were found among resistin, insulin sensitivity markers, BMI or leptin.

PS 96

Platelets, Coagulopathy and Endothelium in Diabetes

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Endothelin mediated remodeling in the aorta of diabetic rats.

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Background and Aims: Sustained hyperglycemia in diabetes may lead to upregulation of potent vasoactive factors, such as endothelins (ETs). In addition to vasoactivity, ET stimulates increased extracellular matrix (ECM) protein production and smooth muscle cell proliferation. Remodeling of the ECM may be associated with increased production of fibronectin (FN), a predominant ECM protein, and specific splice variant of fibronectin i.e., oncofetal fibronectin (fFN). Proliferating smooth muscle cells also express synthetic non-muscle type myosin heavy chain (SMemb). Both fFN and SMemb are considered as the indicator of vascular remodeling. We evaluated the role of ET_A, the predominant receptor on smooth muscle cells, in diabetes associated vascular hypertrophy in the rat aorta.
Materials and Methods: Streptozotocin-induced diabetic Sprague-Dawley rats were treated with a highly selective ET_A receptor antagonist, TBC3214 for 26 weeks. Age- and sex-matched rats were used as controls. Aortas were obtained and mRNA levels of genes which are associated with vascular hypertrophy, ET-1, ET-3, transforming growth factor beta (TGF-β), angiotensinogen, and SMemb were quantified using real time RT-PCR. As an assessment of increased ECM protein deposition, we also quantified mRNA levels of FN, fFN, and plasminogen activator inhibitor 1 (PAI-1), an inhibitor of ECM protein degradation. In addition, medial thickness of aortas was measured by trichrome stain and histomorphometry.
Results: mRNA levels of ET-1, ET-3, TGF-β, angiotensinogen, and SMemb were significantly (ANOVA/post hoc; $p<0.05$) increased in the aortas of diabetic rats ranging from 2.8 to 8 fold compared with control. In addition, FN, fFN, and PAI-1 expression were also significantly upregulated. In parallel to these results, medial thickness was increased in aortas of diabetic rats (1.5 fold to control). Treatment with TBC3214 attenuated the increase in mRNA levels approaching those in control rats of all genes except that of ET-3. Furthermore, TBC3214 treatment reduced medial thickness to levels comparable to controls.
Conclusion: These results indicate that diabetes-induced ET alteration is associated with vascular remodeling and vascular wall thickening via ET_A receptor mediated signaling. These data further demonstrate that these changes in macrovasculature in diabetes may be mediated via ET dependent alteration of TGF-β and angiotensinogen.
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Second messenger pathways and transcription factors involved in PDGF-β receptor upregulation in raised glucose conditions.

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Background and Aims: Atheroma formation has a premature onset in diabetic subjects and involves vascular smooth muscle cell (VSMC) migration into the lesion. In chemotaxis assays VSMC migration to serum is potentiated by elevated glucose conditions and can be blocked by PDGF-β receptor antibodies. The aim of this study was to assess the pathways and transcription factors involved in high glucose-induced PDGF-β receptor upregulation in human aortic VSMC.
Materials and Methods: Cells were cultured in normal (NG, 5 mM) or high (HG, 25 mM) glucose with or without second messenger inhibitors (24 h). Protein levels of receptors and second messengers were assayed by immunoprecipitation (IP): PDGF-β receptor, PI3K and MAPK cascade proteins (Akt and ERK respectively), and conventional PKC isoforms (α, βI, βII, and γ). PDGF-β receptor mRNA level (RT-PCR) and transcription factor binding (EMSA) were also assessed.
Results: HG exposure for 24 h led to a significant increase in PDGF-β receptor protein (+86 ± 26%, $p<0.05$: n=6) and mRNA (+45 ± 7%, $p<0.0001$: n=14). PI3K inhibitor (LY294002) and p70S6K inhibitor (rapamycin) significantly decreased the receptor (protein and mRNA) level in HG ($p<0.05$: n=7; in each case); MAPK inhibitors (PD98059 and mevastatin) and the PKC-βII inhibitor (LY379196) also significantly decreased the receptor level in HG ($p< 0.05$, in all cases). Activation of Akt, ERK and PKC-βII by HG were noted at time periods as short as 2 h ($p<0.05$: n=4; in all cases). PI3K or p70S6K inhibition significantly decreased the activation (phosphorylation) of both ERK and PKC-βII in HG ($p<0.05$: n=4-6; in each case); PI3K inhibition also significantly decreased the activation (phosphorylation) of Akt in HG ($p<0.05$: n=4). MAPK inhibition significantly decreased the level of phosphorylatd ERK in HG ($p<0.05$: n=4; for both inhibitors) but did not affect Akt or PKC. PKC inhibition either with general PKC (calphostin C) or specific PKC-βII (LY379196) inhibitors did not affect phosphorylation of Akt or ERK. The aldose reductase inhibitor, sorbinil, significantly reduced the HG-induced increase in PDGF-β receptor level and activation of Akt, ERK, and PKC-βII ($p<0.05$: n=4-6; in each case). Nuclear binding of the transcription factors STAT-1 and AP-1 significantly increased in HG ($p<0.05$: n=4-6; in each case), and returned to basal levels after PI3K, p70S6K or MAPK inhibition. PKC inhibitors had no effect on these transcription factors in HG.
Conclusion: ERK (activated through PI3K, p70S6K and MAPK) increases AP-1 and STAT-1 nuclear binding and subsequent PDGF-β receptor upregulation in high glucose conditions. PKC-βII (activated through PI3K and p70S6K) also controls PDGF-β receptor upregulation in high glucose conditions but by a different transcriptional mechanism. All of the above effects noted with HG were dependent on increased flux through the sorbitol pathway.

1122

Cationic propyl gallate-induced platelet aggregation, its determination and use in risc patients with acute coronary syndrome (two-year follow-up).

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Background and Aims: Recently, resistance to acetylsalicylic acid (ASA) administration has been widely discussed. However, the assessment of ASA non-respondents is rather difficult and routine examination methods may be encumbered with high pre-analytical, analytical and post-analytical errors. Therefore it is suggested to use aggregometry after induction by cationic propyl gallate (CPG); it is a robust method with high sensitivity and specificity for the assessment of ASA resistance and is not supposed to involve any problems associated with other commonly used methods. As we dispose of this system, we verified and tested it and tried to answer the following questions:
a) Do patients with acute coronary syndrome (ACS) have more frequently ASA resistance than healthy volunteers? (evaluated by aggregometry after CPG induction); b) Are the aggregation values in persons with various disorders of metabolic homeostasis and risk factors of atherosclerotic complications different? c) Is it possible to assess ASA resistance using a single examination of aggregation after CPG induction or is a repeated examination required? d) Do the persons with ASA resistance and ACS in case history have a higher occurrence of recurrent complications during a two-year follow-up? e) Is it possible to use a single examination of platelet aggregation after CPG induction for prediction of recurrent cardiovascular complications in ACS patients during the follow-up?
Materials and Methods: We examined 103 patients of mean age of 69 years. They were ACS persons without ST segment elevation and subjected to conservative therapy; all probands were administered 100 mg of ASA daily, in addition to other medicines. Probands were examined at ACS origin, after 3, 12 and 24 months. The examination consisted of case taking, clinical examination, BMI determination, laboratory assessment of cholesterol, HDL, LDL, triacylglycerols, glucose, and of examination of platelet aggregation under standard conditions
Results: a) Patients with acute coronary syndrome display more frequently ASA resistance than healthy volunteers (45% vs 6%, $p<0.001$). b) Patients with type 2 DM, smokers, individuals with decreased HDL cholesterol or higher triacylglycerol concentration have more frequently ASA resistance ($p<0.05$). c) To assess the ASA resistance, a single examination of aggregation after CPG induction can be used.d) During the next two years after case taking, persons with acute coronary syndrome had a higher percentage of recurrent cardiovascular complications ($p<0.001$).
Conclusion: A single determination of platelet aggregation after CPG induction shows a highly positive predictive value for assessment of risk of recurrent cardiovascular complications during the next two years in patients with acute coronary syndrome (positive predictive value slope > 48%/min 100%, sensitivity 53.2%, specificity 100%.).

1123

Expression of cyclooxygenase in rat aorta, platelets and mononuclear cells is increased in an experimental model of diabetes.

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Background and Aims: Diabetes represents a major risk factor in the development of cardiovascular disease. Several studies have shown that diabetes is associated with increased vasoconstrictor activity and decreased vasodilator capacity of arteries and resistance vessels. Such changes are likely to compromise tissue perfusion and contribute to damage or dysfunction. There is also evidence that platelet activity is enhanced resulting in hypercoagulability that may exacerbate vascular damage. Prostanoids play a crucial role in the regulation of vascular tone and platelet activity and also function as mediators of the inflammatory response of mononuclear (MN) cells. Our laboratory has shown previously that there is an age-related increase in the expression of cyclooxygenase (COX) 1 and 2 in rat aorta, platelets and MN cells. The aim of the present study was to determine whether the expression of COX-1 and COX-2 in aorta, platelets and MN cells is altered in an experimental model of diabetes.
Materials and Methods: Male Sprague-Dawley rats (180 - 200 g) were treated with a single injection of streptozotocin (STZ, 60 mg/kg in citrate buffer, i.p.) to induce diabetes. Control animals were given equal volumes of vehicle. Studies were performed at 2 and 8 weeks following induction of diabetes that was confirmed by glycosuria and increased blood glucose levels. Animals were anaesthetized with sodium thiopentone (100 mg/kg). Segments of thoracic aorta were isolated, cleared of surrounding tissue and processed for immunohistochemistry using specific antibodies to assess the levels of COX-1 and COX-2 in these vessels. Platelets and MN cells were isolated from blood samples. Western blot analysis was used to detect changes in expression of COX-1 and COX-2 in platelets and MN cells respectively. Changes in the level of protein expression between diabetics and controls were quantified using densitometry.
Results: Immunohistochemistry revealed that both COX-1 and COX-2 staining was significantly greater in the smooth muscle layer of aortas isolated from 8-weeks STZ-treated rats compared with controls ($p < 0.05$, $n = 5$). No changes between diabetics and controls were observed at 2 weeks of STZ treatment. Western blot analysis on isolated platelets and MN cells also demonstrated an elevated COX-1 and COX-2 protein expression, respectively, from 8 weeks STZ-treated rats ($p < 0.05$, $n = 5$) but not after 2 weeks.
Conclusion: These findings suggest that the increased expression of COX in the aorta, platelets and MN cells is one of the progressive changes in response to the diabetic condition. Such an increase may result in alterations in prostanoid production that may, in turn represent a contributing factor in the development of diabetic vascular complications.

1124

Abciximab and endogenous thrombin potential assessment in diabetic patients.

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Background and Aims: Atherosclerosis and arterial thrombosis are increased in diabetic patients with a pathogenesis that includes platelet hyper-reactivity. Abciximab produces the same degree of platelet inhibition in diabetic patients and non-diabetics volunteers, but in diabetics appears to be associated with a significant reduction in mortality at 30 days. We investigated the effects of abciximab on thrombin generation in platelet rich plasma from type1-diabetic and non-diabetic subjects as a possible explanation for the mortality lowering effect seen in diabetic patients with acute coronary syndromes.
Materials and Methods: In vitro thrombin generation assays were carried out in platelet rich plasma of type1-diabetic patients (n=10) and non-diabetic volunteers (n=15) in the presence and absence of abciximab, with and without sodium arachidonate as a platelet activator. Thrombin generation, measured as the endogenous thrombin potential (ETP), was assayed using an intrinsic coagulation procedure similar to the Hemker method without employing tissue factor.
Results: Abciximab inhibited thrombin generation in non-activated platelets, with reduction in peak thrombin generation only in diabetic subjects (median reduction: -13.4% at 4 µg/ml); time to peak of thrombin generation was also significantly prolonged (19.75% and 12.85%, for abciximab concentrations of 3 and 4 µg/ml respectively) only in diabetics. The area-under-the-curve (AUC) for thrombin concentration / time [0 to 28.4 minutes] (endogenous thrombin potential) was reduced only in diabetics (median reduction: -18.6% for 4 µg/ml).
Conclusion: There is a selectively increased thrombin inhibitory effect of abciximab on platelets derived from diabetic patients. We hypothesize that reductions in thrombin potential mirror a higher proportion of GPIIb-IIIa molecules exposed, increasing the efficacy of abciximab and potentially explaining the observed reduction in mortality in diabetic patients with acute coronary syndromes given abciximab.

1125

The role of platelet-derived growth factor in diabetes-associated atherosclerosis.

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Background and Aims: The molecular mechanisms by which diabetes promotes atherosclerosis are not fully understood. Platelet-derived growth factor (PDGF) has been shown to play a major role in the pathology of vascular diseases, but whether it plays a role in atherosclerosis associated with diabetes remains unknown. The aims of this study were to assess whether PDGF-dependent pathways are involved in the development of diabetes-induced atherosclerosis and to determine the effects of PDGF receptor antagonism on this disorder.
Materials and Methods: Diabetes was induced by injection of streptozotocin in six-week old apolipoprotein E-knockout (apo E-KO) mice. Diabetic animals received treatment with a tyrosine kinase inhibitor which inhibits PDGF action, imatinib (STI-571, 10 mg/kg/day) or no treatment for 20 weeks. Non-diabetic apo E-KO mice served as controls.
Results: Induction of diabetes was associated with a five-fold increase in the aortic plaque area (diabetic apo E-KO, 22.0±1.8% vs. control apo E-KO, 4.7±0.4%; $P<0.05$) in association with an increase in PDGF-B (5fold) and PDGF-beta receptor gene and protein (6fold) expression as well as other prosclerotic cytokines, such as connective tissue growth factor (2.4 fold) and the chemokine, monocyte chemoattractant protein-1 (95 fold). Imatinib treatment reduced the development of atherosclerotic lesions (13.4±0.7%; $P<0.05$ vs. diabetic apo E-KO) in association with decreased expression of growth factors and chemokines.
Conclusion: Antagonism of the PDGF pathway using imatinib appears to be a novel therapeutic option to retard the development of atherosclerosis, specifically in the context of diabetes.

PS 97

Atherosclerosis and Smooth Muscle Cells in Diabetes Mellitus

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High glucose potentiates nitric oxide synthase II induction by interleukin-1β in cultured human aortic smooth muscle.

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Background and Aims: Diabetic vasculopathy is actually considered as a chronic inflammatory disease of the vascular wall. Circulating levels of pro-inflammatory cytokines, including interleukin-1β (IL-1β), are elevated in diabetic patients. Previous works have shown that pro-inflammatory cytokines increase the expression of nitric oxide synthase II (NOS II) in vascular smooth muscle cells. In the present work, we aimed to investigate whether high D-glucose levels may modulate the inflammatory mechanisms induced by IL-1β, particularly those related to NOS II, in human vascular smooth muscle.
Materials and Methods: Human aortic smooth muscle cells (HASMC) were obtained by enzymatic dissociation from five organ donors. NOS II levels were measured by Western blotting and visualized by indirect immunofluorescence. Nitric oxide (NO) production was assessed by the Griess method.
Results: Treatment of HASMC with IL-1β (10 ng/ml) for 18 h resulted in a marked enhancement of NOS II levels (around 18-fold increase over basal; $p<0.05$). Such an effect was potentiated by around 75 % when IL-1β was co-incubated with 22 mM D-glucose ($p<0.05$ vs IL-1β alone). D-glucose alone did not modify basal NOS II levels. This increase in NOS II levels induced by IL-1β was accompanied by a parallell increase in NO production, which was also further enhanced by D-glucose. The enhancement of NO production was mainly due to NOS II activation,as it was dramatically reduced by the NOS II specific inhibitor 1400W (10 μM). L-glucose, used as osmotic control, did not mimick the effects of D-glucose. The potentiation of IL-1β effects by high D-glucose was confirmed by indirect immunofluorescence, using a monoclonal antibody raised against NOS II.
Conclusion: High D-glucose potentiates the induction of NOS II elicited by IL-1β. These results suggest that hyperglycemia can play a direct role in the development of diabetic vascular complications by exacerbating the inflammatory pathways mediated by IL-1β.

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Effect of high glucose and aldose reductase inhibitor on HB-EGF mRNA expression in vascular smooth muscle cells.

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Backgrounds and Aims: The increased polyol pathway activity has been implicated in the pathogenesis of diabetic microangiopathy, but the relation with diabetic macroangiopathy remains unclear. The proliferation of smooth muscle cells (SMCs) is considered as one of initiating factors of the diabetic macroangiopathy. HB-EGF is a mitogen for SMCs proliferation that can be induced by high glucose or 3-DG and MG. Our study was conducted to investigate whether the activation of polyol pathway induced by high glucose could produce the expression of HB-EGF mRNA in mediation of 3-DG.
Material and Methods: Rat vascular SMCs were grown in medium containing 5.5mM or 40mM glucose with or without an aldose reductase inhibitor, SNK-860 (0.1μM or 1μM) or aminoguanidine (100μM) for 2 hours after confluence. The proliferation of SMCs was measured as the incorporation of [^{3}H]-thymidine. The intracellular 3-deoxyglucosone concentration was determined by high-performance liquid chromatography. The expression of HB-EGF mRNA was investigated by Northern blotting assay.
Results: SMCs cultured with 40mM glucose demonstrated the accelerated thymidine incorporation compared with that of SMCs in 5.5mM glucose. The intracellular concentration of 3-DG was significantly elevated in SMCs cultured with 40mM glucose above the levels seen in the 5.5mM glucose group. A stronger HB-EGF mRNA expression was found in the high glucose group as compared with the normal glucose group. All of these abnormities were prevented by SNK-860 and aminoguanidine.
Conclusion: The administration of SNK-860 and aminoguanidine lowers the intracellular 3-DG, which may finally decrease the acceleration of HB-EGF mRNA induced by high glucose. The results suggest that the polyol pathway may play a substantial role in the SMCs proliferation in mediate of reactive intermediate metabolites.

1128

The effect of high glucose and cyclic stress on pathways dependent on extracellular-regulated kinase (ERK) activation in vascular smooth muscle cells.

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Background and Aims: Hypertension and hyperglycaemia are important aetiological factors in the premature onset of atherosclerosis in diabetes. PDGF-β receptor and β3 integrin levels are both increased by high glucose (25 mM; HG) or by flexion in vascular smooth muscle cells (VSMC) grown in culture. The second messenger pathways leading to increased PDGF-β receptor and β3 integrin level are known to be ERK1/2 dependent. The aim of this study was to determine how extracellular structural proteins, flexion, and glucose in combination may alter these pathways in VSMC.
Materials and Methods: Primary, thoracic aorta-derived porcine VSMC, were plated on surfaces coated with surrogate basement membrane (Matrigel) or on individual proteins of the extracellular matrix. The cells were exposed to normal glucose (5 mM; NG) or HG and various degrees of cyclic stretch within the physiological and pathophysiological range, using the Flexercell apparatus, or left unflexed for 24 h. Immunoprecipitation was carried out for the PDGF-β receptor, β1 and β3 integrins and the second messenger proteins Akt (in the PI3K cascade) and ERK1/2 (in the MAPK cascade). In all comparisons NG, unflexed was set at 100% (Wilcoxon signed-rank test; mean ±SE).
Results: The PDGF-β receptor, β1 and β3 integrins were significantly increased in HG conditions (+131%, +89%, and +48% respectively, all n=4-8, $p<0.05$). In the presence of HG MAPK (mevastatin, PD98059) and PI3K (LY294002) inhibitors reduced the PDGF-β receptor and β1 integrin levels significantly (n=4-8, $p<0.05$ in all cases); the β3 integrin was decreased in the presence of the MAPK inhibitors ($p<0.05$), but was not affected by the PI3K inhibitor (n=4-8). When grown on Matrigel in HG, PDGF-β receptors were increased by 147% compared with NG (247±73%, $p<0.05$, n=4). Flexion increased receptor levels in both NG and in an additive manner in HG (NG: 327±135%, and HG: 504±179%, both $p<0.05$, n=4); of the individual protein-coated surfaces tested, cells grown on collagen showed the most response to flexion in HG (461±128%, $p<0.05$, n=4). At high levels of cyclic stretch the effects of HG and flexion on PDGF-β receptor upregulation were no longer additive; this may indicate saturation of the mechanisms involved. The β1 integrin levels were increased in cells grown on Matrigel in HG, flexion had no additive effect (unflexed: 200±11%, and flexed: 189±55%, both $p<0.05$, n=4); β3 integrin levels were also increased in HG. Activated (phosphorylated) levels of Akt were increased in HG (without flexion: 122±17%, and with flexion: 130±29%, both $p<0.05$, n=4). Activated (phosphorylated) levels of ERK1/2, showed a significant difference among all conditions (HG: 198±38%, flexion in NG: 340±122%, and flexion in HG: 497±200%, all $p<0.05$, n=4).
Conclusion: Flexion and HG were additive for the upregulation of the PDGF-β receptor through the PI3K/MAPK pathway at low but not high levels of flexion, on a complete basement membrane coating. β1and β3 integrins, were upregulated by HG; the PI3K pathway was involved in β1 but not β3 upregulation.

1129

Serum from patients with Type 2 diabetes mellitus has proapoptotic effects on human arterial smooth muscle cells.

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Background and Aims: Previous work has shown that serum from type 2 diabetic patients with neuropathy promotes apoptosis of cultured neuronal cells, compared to serum from diabetic patients without neuropathy and from healthy subjects. We tested the hypothesis that serum from type 2 diabetic patients similarly induces apoptosis of human aortic vascular smooth muscle cells (HAVSMCs). We also examined the relation between apoptosis and severity of coronary artery disease (CAD).

Material and Methods: We studied serum from 30 asymptomatic type 2 diabetic patients who underwent coronary angiography after a positive screening for silent myocardial ischemia. Serum from 15 healthy adult volunteers were used as controls. HAVSMCs were incubated in the presence of 10% serum from individuals of the diabetic and control groups. Apoptosis was detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL), by annexin-V labelling and by caspase 3 active fragment immunoblotting.
Results: The percentage of apoptotic nuclei assessed by TUNEL was higher in presence of serum from diabetic patients (increasing from 11±5% to 18±9% at 48 hours, $p<0.05$ versus healthy subjects). Annexin-V labelling and active caspase 3 immunodetection confirmed these results consistently. However, the proapoptotic effect was observed only in patients with CAD (n=20).
Conclusions: Serum from type 2 diabetic patients contains a factor that induces apoptosis in arterial smooth muscle cells. This factor is associated with CAD. We speculate that this proapoptotic effect could be implicated in the fragilization and rupture of atherosclerotic plaques.

1130

Glucose increases cytosolic free Ca^{2+} concentration and epalrestat can reverse it in cultured rabbit smooth muscle cells.
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Background and Aims: Hypertension is frequently associated with diabetes mellitus. Recently, several investigators reported that the abnormalities of polyol and myo-inositol metabolism due to hyperglycemia reduced Na^+/K^+-ATPase activity of cell membrane through phosphoinositide metabolism or proteinkinase C, and that reduced Na^+/K^+-ATPase activity also disturbed calcium metabolism. Especially, abnormality of calcium metabolism may induce hypertension. To assess whether hyperglycemia can influence calcium metabolism of vascular smooth muscle cells (SMC), we cultured rabbit aortic SMC under high glucose condition and measured cytosolic free calcium concentrations ($[Ca^{2+}]$ i). Furthermore, we examined the effect of epalrestat, an aldose reductase inhibitor, on calcium metabolism.
Materials and Methods: *1. Cell Culture;* SMC from male New Zealand rabbits were cultured by the procedure described by Ross. The cells on glass slides after reaching confluence were incubated for 72 hours in the media described below, respectively. A) glucose 5.5 mM DMEM, B) glucose 5.5 mM DMEM + epalrestat 0.1 mM, C) glucose 30 mM DMEM, D) glucose 30 mM DMEM + epalrestat 0.1 mM. *2. Ca^{2+} measurement;* SMC were loaded with 5 μmol/L fura-2 for 60 min. The excitation wavelengths were 340 and 380 nm at 200-ms intervals, and the emission was detected at 510 nm by a photomultiplier. Then fluorescence ratio was calculated and converted to $[Ca^{2+}]$i.
Results: *1. Effect of high glucose concentration of media on $[Ca^{2+}]$i;* When SMC were incubated with (C), a significant increase of $[Ca^{2+}]$i was observed ($p<0.001$, ϖσ A). No significant change was observed the SMC incubated in (A). *2. Effect of epalrestat on $[Ca^{2+}]$i;* $[Ca^{2+}]$i of SMC cultured in (B) had no change compared with (A). Though (C) increased $[Ca^{2+}]$i by 90 %, adding 0.1 mmol/L epalrestat (D) could reverse increased $[Ca^{2+}]$i due to (C).
Conclusion: Our results show that high glucose concentration in medium increased $[Ca^{2+}]$i of cultured SMC, and that addition of epalrestat corrected increased $[Ca^{2+}]$i. Because hyperglycemia decreased membrane Na^+/K^+-ATPase activity of SMC through abnormality of polyol and myo-inositol metabolism, the change of cation transport system may increase $[Ca^{2+}]$i. The constriction level of SMC rises in proportion to a rise in $[Ca^{2+}]$i . Therefore, hypertension may be more common in diabetic patients than in non-diabetic populations. It is suggested that aldose reductase inhibitor could prevent these abnormalities, and might treat hypertension of diabetic patients.

Effect of glucose and epalrestat on [Ca2+]i

medium	$[Ca^{2+}]$i (nM)
A	67.9 ± 6.3
B	69.0 ± 13.2
C	129.2 ± 10.4
D	55.4 ± 15.5

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Vascular smooth muscle cell chemotaxis is potentiated by glucose via Phosphoinositide 3-kinase-p110β, Akt, ERK1/2 and protein kinase CβII dependent pathways.
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Background and Aims: Chemotaxis of vascular smooth muscle cells (VSMCs) plays a major role in atheroma formation. VSMCs exposed to 5mM glucose do not show chemotaxis to FCS without prior serum starvation, while those exposed to 25mM glucose show chemotaxis. PI3K consists of a p85 adapter subunit and a p110 catalytic subunit. There are 3 isoforms of p110, α, β and δ. Akt is a known downstream target of PI3K. High glucose concentration is known to increase the activity of PKCβ. The aim of this study was to investigate the role of the p110 isoforms, Akt and PKCβ in glucose-potentiated VSMC chemotaxis.
Materials and Methods: P110α, β, δ, Akt, ERK1/2, PKCβI and βII neutralising antibodies were microinjected into VSMC, which were then placed in the Dunn chemotaxis chamber in 25mM glucose (FCS was used as the chemoattractant). As controls, VSMCs were injected with non-specific rabbit IgG. The role of PKCβII was further investigated using the PKCβII specific inhibitor LY379196, while the role of the PI3K isoforms were further investigated using the p110δ specific inhibitor D000. In addition the general PI3K inhibitor LY294002 and the general MAPK inhibitor PD-98059 were used.
Results: Western blotting revealed that all three isoforms of p110 are expressed in human VSMC, and that exposure to 25mM glucose increased Akt and ERK1/2 phosphorylation. The P13K inhibitor LY294002 (inhibits all p110 isoforms) and the MAPK inhibitor PD-98059 blocked VSMC chemotaxis to FCS. Microinjection with non-specific rabbit IgG or with antibodies against p110α, p110δ and PKCβI did not affect chemotaxis. In contrast, microinjection of the anti-p110β, anti-Akt, anti-ERK1/2 and anti-PKCβII antibodies inhibited chemotaxis. No inhibition was observed when the antibodies were incubated with their cognate peptide prior to microinjection. All control experiments showed chemotaxis.. Treatment with LY379196 (200nM) inhibited chemotaxis but did not affect the velocity of cytokinetic movement, while D000 did not inhibit chemotaxis.
Conclusion: In conclusion elevated glucose sensitises VSMCs to serum factors, inducing chemotaxis via pathways involving PI3K-p110β, Akt, ERK1/2 and PKCβII.

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Adenovirus-mediated expression of dominant-negative Pim-1 inhibits smooth muscle cell proliferation.
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Background and Aims: Hyperglycemia is an important causative factor for macrovascular complications in diabetes. Whereas proliferation of vascular smooth muscle cells (VSMC) is an essential event in the development of atherosclerosis, hyperglycemia-induced oxidative stress has been shown to stimulate migration and proliferation of VSMC. By use of subtractive RT-PCR approach, we recently identified p100, a well-known coactivator of c-Myb, as an oxidative stress-responsive gene expressed in VSMC. p100 is a direct substrate of the proto-oncogene Pim1, which serves as a serine/threonine protein kinase, and thereby mediates Pim1-dependent activation of c-Myb. Indeed, Pim1 is an essential factor for hematopoiesis in vivo probably by activating Pim1 - p100 - c-Myb and/or Pim1 - c-Myc axis. In this study, we examined a possible role of Pim1 in the oxidative-stress dependent progression of atherosclerosis.
Materials and Methods: Pim-1 protein expression was examined by Western blotting (for cultured VSMC) and immunocytochemistry (for cultured VSMC and neointima of the balloon-injured rat carotid arteries). To examine effects of the blockade of Pim-1 function, we have generated an adenovirus expressing dominant-negative form of Pim-1 (Ad/DN-Pim-1). VSMC were isolated from the thoracic aortas of male Sprague-Dawley rat (passage<5). After culturing in DMEM with 0.1% FCS for 48 hours to induce quiescence, the cells were infected with either Ad/DN-Pim-1 or the control Ad/lacZ (MOI=100). Then, the cells were stimulated by H_2O_2 (0-100μM) for 24 hours. DNA synthesis rates were assessed by determination of $[H^3]$-thymidine incorporation using liquid scintillation counting and cell cycle progression was assessed using FACS.
Results: In vivo, at 14 days after balloon injury, intima thickening was clearly visible and Pim-1 expression was markedly induced in the

neointima of injured arteries. On the other hand, in vitro treatment of VSMC with serum (10%, 24 hours) or with H_2O_2 (10μM, 24 hours) also markedly induced Pim-1 expression in those cells. Whereas the H_2O_2 or serum stimulation increased DNA synthesis (175% and 321% vs. unstimulated control (100%), respectively), this was totally blocked by the pre-infection of Ad/DN-Pim-1 (94%, 97% vs. control, respectively). Similarly, H_2O_2 also enhanced G(1)-S cell cycle progression in VSMC, which was again fully blocked by pre-infection of Ad/DN-Pim-1.
Conclusion: Expression of the proto-oncogene Pim-1 expression is induced in neointima of the balloon-injured rat carotid arteries. Because specific inhibition of Pim-1 function suppresses VSMC proliferation, Pim-1 is likely to play a pivotal role in the development of atherosclerosis. Also, the dominant negative Pim-1 may have potential clinical benefit.

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Experimental Atherosclerosis

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Oxidative stress in diabetic patients with and without peripheral vascular disease.
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Background and Aims: The aim of this study was to compare antioxidative status in diabetic and non-diabetic patients, as well as to compare antioxidative status in diabetic patients with and without peripheral vascular disease.
Materials and Methods: Our study included 211 participants, from which 109 were diabetics patients. Diabetic patients were subdivided into 2 groups: with peripheral vascular disease (DM+PVD+) and without peripheral vascular disease (DM+PVD-). Diagnosis of peripheral vascular disease has been established by Doppler sonographic analysis. Antioxidative status has been presented through establishing key antioxidative enzimes: superoxide dismutase (SOD), catalase, glutathion peroxidase (GLPX), total antioxidative status (TAS) by Randox tests RANSLE and RANSOD. The participants have been examined and the key parameters have been established: weight, hight, BMI, age, type of diabetes, data about the lenght of diabetes, smoking, art. hypertension, as well as Doppler sonography of peripheral arteries, ECG, antioxydative status, lipid prophile, fibrinogen, glucosis, HbA1C, biuret. The participants have also been asked about history of CAD, stroke and diabetic retinopathy. The statistical analysis was performed using Mann-Whitney test.
Results: Diabetic patients had mean rang of TAS 83,06, whereas non-diabetic patients had mean rang of TAS 130,51 (p=0,000). Diabetic patients had mean rang of SOD 122,56, whereas non-diabetic patients had mean rang of SOD 88,31 (p=0,000). Diabetic patients had mean rang of catalase 95,22, whereas non-diabetic patients had mean rang of catalase 117,52 (p=0,008). Diabetic patients had mean rang of GLPX 84,29, whereas non-diabetic patients had mean rang of GLPX 129,20 (p=0,000). DM+PVD+ group had mean range of TAS 54,01, whereas DM+PVD- group had mean range 55,94 (p=0,750). DM+PVD+ group had mean range of SOD 56,82, whereas DM+PVD- group had mean range 53,28 (p=0,555). DM+PVD+ group had mean range of catalase 62,59, whereas DM+PVD- group had mean range 47,81 (p=0,015). DM+PVD+ group had mean range of GLPX 53, 68, whereas DM+PVD- group had mean range 56,25 (p=0,676).
Conclusions: Our study has shown the statistically significant lower activity of antioxidative enzimes in diabetic patients in comparison to non-diabetic patients. We demonstrated the presence of oxidative stress through statistically significant antioxidative status disbalance irrespective of the presence of peripheral vascular disease, which underlines his major role in pathogenesis of vascular complications in DM, prior to clinical manifestation.

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Polymorphisms in the 5'-upstream region of the PKC beta gene and vascular complications in Japanese patients with Type 2 diabetes.
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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. In this study, we investigated polymorphisms in the 5'-upstream region of the PKC beta gene as well as their associations with diabetic vascular complications in Japanese populations.
Materials and Methods: One hundred healthy subjects with normal fasting plasma glucose levels (38 men and 62 women, mean age ± SD: 52 ± 15 years) and 204 patients with type 2 diabetes (104 men and 100 women, 59 ± 11 years) were recruited. All subjects were Japanese and resided in the same area (Kochi Prefecture, Japan), and gave informed consent to participate prior to the study. We detected the nucleotide sequence in the 5'-upstream region of the PKC beta gene using a walking upstream method. Variations in the upstream region (-1066/ +256) were examined in 60

diabetic patients by means of a cycle sequencing method. For screening of polymorphisms in remaining patients and all healthy controls, a PCR and RFLP method or a cycle sequencing method was performed.
Results: Five single nucleotide polymorphisms; C(-238)G, C(-287)T, A(-348)G, C(-546)G, and C(-853)T, were identified in the upstream region. The C(-287)T and A(-348)G polymorphisms were in perfect linkage disequilibrium. There were no significant differences in allele frequencies of the 5 polymorphisms among patients with type 2 diabetes and healthy subjects. However, diabetic patients with -238GG or -287CC/ -348GG genotype showed significantly higher frequencies of macrovascular diseases as compared to patients with other genotypes. In a stepwise regression analysis for the presence of macrovascular diseases using known risk factors as independent variables, both C(-238)G and C(-287)T-A(-348)G polymorphisms were significant contributors, as were age, diabetes duration, and the presence of proteinuria.
Conclusion: We located 5 single nucleotide polymorphisms in the 5'-upstream region of the PKC beta gene, and found that the common polymorphisms C(-238)G and C(-287)T-A(-348)G were associated with the presence of macrovascular diseases in type 2 diabetic patients. These PKC beta genetic polymorphisms may have an effect on the susceptibility of diabetic vascular complications through an alteration of tissue PKC beta density or function.

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Alteration of K_{ATP} channel function in internal mammary artery from patients with Type 2 diabetes.
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Background and Aims: It is well known that diabetic patients suffer frequently from cardiovascular complications. Internal mammary arteries (IMA) are used as coronary bypass graft material. ATP-sensitive $K^+(K_{ATP})$ channels have important role in the control of vascular tone and therefore of blood pressure. Opening of vascular K_{ATP} channels exerts vasodilator response under ischaemic conditions. Our aim was therefore to compare K_{ATP} channel function in diabetic IMA with that of nondiabetic IMA, in vitro.
Materials and Methods: IMAs were supplied from patients undergoing coronary bypass surgery. Endothelium-denuded rings were mounted in organ baths for isometric tension recordings. Cumulative concentration-response curves for cromakalim (CRO), a K_{ATP} channel opener, were obtained in the presence or absence of 10 μM glibenclamide (GLI), a K_{ATP} channel blocker, in IMA rings pre-contracted with phenylephrine (PE). Concentration-response curves for PE or sodium nitroprusside (SNP) were also evaluated to investigate if there was any change in the reactivity to these agonists. Agonist pEC_{50} values (apparent agonist affinity constant: -log EC_{50}) were calculated by lineer regression analysis of the curves and taken as a measure of the sensitivity of IMAs to the agonists.
Results: Concentration -response curves for CRO were significantly shifted to the right in diabetic IMA. % of maximum relaxation (Emax) was decreased by 36 ± 4 % ($p<.05$, n:5 for each). In addition, GLI was found to be less effective in diabetic IMA in inhibiting of CRO-induced relaxations. Emax of CRO (at 3μM) was inhibited by 48 ± 2 % in nondiabetic and 28 ± 4 % in diabetic IMA after 30 min GLI incubations ($p<.05$, n:5 for each). On the other hand, PE-induced contractions increased significantly in diabetic IMA. SNP responses were similar in both group of arteries.
Conclusion: The results indicate that diabetes causes an impaired dilatation of human IMA through K_{ATP} channels. Regarding their vasodilator role against hypoxic and / or ischaemic insults, a diminished response through K_{ATP} channels together with an increased contractile response may contribute to diabetes-induced vascular events such as vasospasm and even hypertension. Additionally, since IMAs are used as conduit vessels in coronary bypass graft surgery, our findings therefore suggest that the defective dilatation and vasoconstriction may also affect the performance of IMA grafts adversely in type 2 diabetes. *Supported by grant of Scientific and Technical Research Council of Turkey (no:SBAG-AYD403).*

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Effect of hyperglycaemia on the vasodilatory function in the canine coronary circulation.
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Background and Aims: Effect of hyperglycaemia on coronary reactivity was investigated in an *in vivo* canine model.
Materials and Methods: Four different experimental groups of pentobarbital anesthetized (133 μmol/kg Nembutal iv., Sanofi) mongrel dogs of either sex (n=25) were set up. Diabetes (n=12) was induced by a single iv. infusion of alloxan monohydrate (560 μmol/kg, Sigma), 3 months prior to the acute experiments. Six diabetic dogs remained untreated (D), further 6 diabetic dogs (ITD) were treated with insulin (0.5-1.0 IU/kg/day, Insulatard s.c.), started a week after alloxan administration. Acute topical hyperglycaemia (25 mmol/l glucose, HG, n=7) was induced in metabolically healthy dogs by infusing glucose into the left anterior descending coronary artery (LAD). Finally, normoglycaemic control dogs (NG, n=6) were investigated. Mean arterial blood pressure and heart rate were monitored (Statham p23Db), LAD coronary blood flow (CBF) was measured by an electromagnetic flow probe (Gould-Statham SP2202). Drugs were infused into the LAD by a syringe pump (Terumo STC 526) via a catheter introduced through a diagonal branch. Endothelium-dependent vasodilation was tested by infusing acetylcholine (ACh, 2.25-36 pmol/kg/min) into the LAD. Sodium nitrate was infused (0.5-3.75 pmol/kg/min) to test non-endothelium-dependent vascular relaxation.
Results: The rise in CBF induced by ACh proved to be smaller ($p<0.05$) in both the D and HG groups as compared to NG control dogs (D:123±11%, HG:151±24% vs. NG:205±15%). In case of ITD dogs kept normoglycaemic with insulin treatment (fasting blood glucose: 4.27±0.67 mmol/l), the ACh-induced vasodilation (173±27%) was similar to those of the metabolically healthy controls. Vasodilation to sodium nitrate did not differ among the groups.
Conclusion: It could be concluded that hyperglycaemia may have a pathogenic role in the development of the alterations in coronary reactivity in diabetes. Adequate insulin treatment could prevent the impairment in endothelium-dependent vasodilation seen in the untreated diabetic state.

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Omapatrilat, an angiotensin-converting enzyme and neutral endopeptidaze inhibitor attenuates atherosclerosis in diabetic and in non-diabetic LDL receptor-deficient mice.
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Background & Aims: Omapatrilat is a vasopeptidase inhibitor that inhibits both angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP). ACE inhibitors have been shown to inhibit atherosclerosis in apo E-deficient mice and in several other animal models but failed in LDL receptor-deficient mice despite effective inhibition of the renin-angiotensin-aldosterone system.
The aim of the present study was to examine the effect of omapatrilat on atherogenesis in diabetic and non-diabetic LDL receptor-deficient mice.
Materials & Methods: LDL receptor-deficient male mice were randomly divided into four groups (n=11 each). Diabetes was induced in two groups by low-dose STZ, the other two groups served as non-diabetic controls. Omapatrilat (70mg/kg/day) was administered to one of the diabetic and to one of the non-diabetic groups. The diabetic and the non diabetic mice were sacrificed after 3 and 5 weeks respectively. The aortae were examined and the atherosclerotic plaque area was measured.
Results: The atherosclerotic plaque area was significantly smaller in the omapatrilat treated mice both diabetic and non diabetic, as compared to non treated controls. The mean plaque area of omapatrilat treated non-diabetic mice was 9357±7293μm^2 vs 71977±34610μm^2 in the non-treated mice ($p=0.002$). In the diabetic animals the plaque area was 8887±5386μm^2 vs 23220±10400μm^2 respectively for treated and not treated mice ($p=0.001$). Plasma lipids were increased by omapatrilat. Mean plasma cholesterol in treated mice, diabetic and non diabetic combined, was 39.31±6.00 mmol/L vs 33.12±7.64 mmol/L in the non treated animals ($p=0.008$). The corresponding combined mean values of triglycerides were 4.83±1.93 vs 3.00±1.26 mmol/L ($p=0.02$). Omapatrilat treatment did not affect weight or plasma glucose levels.
Conclusion: Treatment with omapatrilat inhibits atherogenesis in diabetic as well as non-diabetic LDL receptor-deficient mice despite an increase in plasma lipids, suggesting a direct effect on the arterial wall.

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Superoxide production in vasculature of Type 2 diabetic Goto-Kakizaki rats: roles for insulin and PI3-kinase.

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Background and Aims: Reactive oxygen species (ROS) are involved in diabetes-associated vascular complications. The aims of this study were to identify sources of superoxide ($O_2^{-\cdot}$) production by vascular tissue derived from normal and diabetic Goto-Kakizaki (GK) rats. The GK rat is a non-obese animal model of type II diabetes.
Methods and Meterials: Hyperglycaemia was confirmed in 10 and 12-week old male diabetic rats by blood glucose measurements. Insulin levels were measured by ELISA. Mesenteric artery rings, 2mm 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. $O_2^{-\cdot}$ levels in mesenteric artery branches were measured by lucigenin (5μM) chemiluminescence and expressed as arbitrary units/mg wet wt tissue. Data were analysed using the statistical package PRISM: a p-value <0.05 was taken to indicate significance.
Results: At 10 and 12 weeks diabetic blood glucose levels (mM) were significantly higher than their age matched Wistar controls (6.1±0.3 vs. 10.3±0.5, 6.7±0.2 vs. 10.7±0.5 respectively, both p<0.001). Insulin levels (μg/L) were higher in control animals at 10, but not 12 weeks (1.2±0.2 vs. 0.6±0.1, p<0.05, 0.1±0.02 vs. 0.1±0.03). Diabetic rats had impaired EDR compared with age-matched controls (R_{max}, maximal relaxation 59.0±4.6%, vs. 76.1±5.9%, respectively p<0.05, n=7). NADPH (100 μM) did not increase $O_2^{-\cdot}$ production in control or diabetic vessels, in contrast to the flavoenzyme inhibitor, diphenyleneiodonium chloride (100 μM), which alone significantly raised $O_2^{-\cdot}$ in both cases (p<0.05). In control and diabetic tissue, 5mU/ml insulin significantly increased $O_2^{-\cdot}$ production (control 912.6±139.3 vs. 44.8±29.2, p<0.001; diabetic 827.5±164.4 vs. 42.8±18.7, p<0.01, n=4). When control tissue was incubated with the PI3-kinase inhibitor wortmannin (50nM), the insulin effect was greatly reduced (from 912.6±139.3 to 180.2±69.7, p<0.001, n=4), whereas in diabetic tissue there was no significant reduction.
Conclusions: The endothelial dysfunction exhibited by diabetic GK rats does not appear to be due to increased tissue $O_2^{-\cdot}$ production via the NADPH/NADH oxidase pathway. Insulin however, increases $O_2^{-\cdot}$ production in control tissues and this effect is inhibited by the PI3-kinase inhibitor wortmannin, indicating that insulin produces $O_2^{-\cdot}$ via the PI3-kinase pathway in mesenteric arteries. The lack of a wortmannin effect in diabetic tissue, may indicate an alternative pathway for insulin-induced $O_2^{-\cdot}$ production in diabetic vasculature.
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Enhanced cardiomyocyte apoptosis after myocardial infarction in diabetic rats is associated with adverse left ventricular remodeling.

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Background and Aims: Cardiovascular diseases are leading cause of morbidity and mortality in diabetic patients and diabetes is known to reduce survival after myocardial infarction (MI). In this work, we wanted to study whether diabetes is associated with enhanced cardiomyocyte apoptosis after MI and thus interferes with the post-MI remodeling process in insulin-deficient hyperglycemic rats.
Materials and Methods: Diabetes was induced by intravenous streptozotocin (45mg/kg, diabetic groups) injection and diabetic rats were treated daily with 4 units of long-acting insulin. 4 weeks after streptozotocin or citrate buffer (control groups) injection, MI was produced by ligation of left descending coronary artery. Only animals with glucose >20mmol/l were included in the diabetic groups. Echocardiography was performed pre-operatively and 30 days after operation to all animals. The rats were sacrificed 1, 4 or 12 weeks after the operation. Cardiomyocyte apoptosis was quantified by TUNEL and caspase3 -methods both in the border zone of MI and in the remote area of the left ventricle (LV). Fibrosis from the non-infarcted area of LV was measured by collagen volume fraction (CVF) method and connective tissue growth factor (CTGF) was determined immunohistochemically 12 weeks after MI.
Results: The mean glucose in diabetic rats was 28.1 mmol/l vs. 5.6 mmol/l in non-diabetic rats 12 weeks after MI. One week after MI the number of apoptotic cells was equally high in diabetic and non-diabetic rats. 4 weeks after MI, the number of apoptotic cells in non-diabetic rats had started to decrease, whereas in diabetic rats apoptosis remained high. At 12 weeks after MI the number of apoptotic cardiomyocytes was 2.4-fold higher in the diabetic compared with non-diabetic rats measured by TUNEL and caspase3-methods both in the border zone of MI (p<0.001) and in the remote area (p<0.01). Echocardiographically measured LV end diastolic diameter and LV end diastolic volume were significantly larger in diabetic MI rats compared with non-diabetic MI rats (9.97±0.21 vs. 9.59±0.17 mm; p<0.05 and 933±64 vs. 796±41μl; p<0.05). CVF was increased in diabetic as compared to non-diabetic MI rats (5.8±1.2 vs. 3.7±0.7%, p<0.05). Likewise, the CTGF score (1-4) was higher in diabetic vs. non-diabetic MI rats (2.7±0.5 vs. 0.8±0.4; p<0.01). There were no differences in the size of MI between diabetic and non-diabetic rats.
Conclusions: The novel finding in the present study is that experimental diabetes leads to prolonged cardiomyocyte apoptosis after MI. The enhanced apoptosis in diabetic rats with MI takes place in parallel with LV enlargement and increased fibrosis, which can be explained, at least in part, by increased myocardial CTGF expression. Thus, apoptotic myocyte loss may be an important mechanism contributing to progressive dilatation of the heart and poor prognosis after MI in diabetes.

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Advanced glycation end products (AGEs) and longevity in C. elegans.

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Background and Aims: Advanced Glycation End Products (AGEs) accumulate during aging and in an accelerated way in diabetes mellitus. One defined AGE-protein is carboxymethyllysine (CML). Caloric restriction and insulin resistance (IR) contributes to longevity in several species. The increase in life span might be due to reduced AGE-accumulation and reduced formation of reactive oxygen species (ROS). Aim of this project is to investigate AGE-formation in the nematode C. elegans during aging and to evaluate the effects of IR, caloric restriction and inhibitors on AGE-formation and life time in C. elegans.
Materials and Methods: CML-accumulation was determined by ELISA in extracts of wild type C. elegans (N2) in „young“ C. elegans and in „old“ C. elegans. CML was determined in C. elegans put on „caloric restriction“, insulin-resistant C. elegans (daf-2-mutants) and in C. elegans treated with the AGE-inhibitor tenilsetam. Furthermore, the effects of caloric restriction, IR and AGE-inhibition on life span were determined.
Results: We could demonstrate a significant increase in AGE (CML)-formation during aging in C. elegans. AGE-accumulation was significantly lower in insulin resistant mutants, in C. elegans put on caloric restriction and in C. elegans treated with an AGE-inhibitor than in wild type C. elegans. The decreased AGE-formation was associated with an increased life-span in C. elegans „on diet“ and in insulin resistant C. elegans.
Conclusion: Our data demonstrate that AGEs accumulate during aging in C. elegans. Reduction of AGE-formation by caloric restriction or IR is associated with an increased lifespan. If reduced AGE-formation is causal for prolongation of life span or if it is an epi-phenomenon requires further investigation.

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Diabetic retinopathy and diabetic macular edema progression rates in recent placebo-controlled clinical trials.

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Background and Aims: The PKC-DRS was a 252 patient, multi-center, multi-national, double-masked, placebo-controlled, parallel, four-arm study to determine whether 36-48 months of treatment with an oral PKC β inhibitor, ruboxistaurin (RBX, LY333531) mesylate, would delay the progression of diabetic retinopathy in patients with moderately severe to very severe nonproliferative diabetic retinopathy (ETDRS retinopathy severity grades 47B-53E). The PKC-DMES was a 686 patient multi-center, multi-national, double-masked, placebo-controlled, parallel, four-arm study to determine whether 30-52 months of treatment with RBX would delay the progression of diabetic macular edema (DME) in patients with DME not involving or imminently threatening the center of the macula, and mild to moderately severe nonproliferative DR (ETDRS levels 35-47A).
Materials and Methods: In both studies, reading center masked grading of ETDRS 7 standard field stereo photographs was used to determine progression of DR and DME.
Results: At 36 months, the Kaplan-Meier event rate estimate of progression by ≥ 3 steps on the ETDRS scale was 55% in PKC-DRS placebo-treated patients, compared with a 36 month estimate of 64% from ETDRS data on eyes assigned to deferral of photocoagulation in the ETDRS study. In the PKC-DMES, rates of DME progression in placebo-treated patients were 29% at 1 year and 55% at 3 years, compared with a 1-year estimate of 33% from data from the ETDRS trial. Progression rates of DR and DME for various subgroups of the studies will be presented. Baseline demographic factors that influence the rates of DR or DME progression include (but may not be limited to) ETDRS retinopathy severity level, DME severity level, HbA_{1c}, blood pressure, gender, BMI, diabetes type, and diabetes duration.

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Younger age, insulin deficiency and more severe hyperglycemia predict development of microangiopathy in Indian Type 2 diabetic subjects.

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Aims: To study incidence and predictors of diabetic retinopathy and nephropathy over 10 years in Indian type 2 diabetic subjects.
Material & Methods: We followed 189 type 2 diabetic subjects for 10 years from diagnosis. We recorded clinical and biochemical-endocrine parameters at 0, 1, 5 & 10 years. Pancreatic β-cell function (B) and insulin sensitivity (R) were assessed by Homeostasis Model Assessment (HOMA). Retinopathy was diagnosed by direct ophthalmoscopy through dilated pupils; nephropathy by urinary albumin excretion rate (UAER) on overnight 8h urine collection. All patients were advised on diet and physical activity and treated with antidiabetic drugs as appropriate.
Results: Twenty two subjects died and 23 were lost to follow-up. At 10 years, 26/144 (18%) subjects showed retinopathy, 21 (14%) nephropathy (UAER > 20 µg/min, 8h overnight urine collection), 6 (4%) showed both. These subjects were younger (41 v 46 y; p=0.001), thinner (triceps skinfold 13.9 v 16.5 mm, p=0.047) at diagnosis, and more hyperglycemic and insulin deficient at diagnosis and 10 years later (fasting plasma glucose 183 v 159 mg/dl; p=0.014 at diagnosis, HbA1 9.5 v 8.2% at 10 years, p<0.001; HOMA-B 6.3 vs 15.0, p=0.007 at diagnosis, 2.8 vs 6.1, p=0.002 at 10 years) compared to those without microangiopathy. BMI, blood pressure, plasma lipid levels and insulin sensitivity did not differ between two groups. Multivariate analysis showed younger age (p=0.008) and lower HOMA-B (p=0.043) to be predictive of microangiopathy at 10 years. Cardiovascular disease was predicted in these subjects by older age, central obesity and higher systolic blood pressure, plasma glucose and total cholesterol concentrations.
Conclusions: Younger, more insulin deficient and poorly controlled type 2 diabetic patients were at higher risk of microangiopathy. Earlier diagnosis and intensive treatment is warranted.

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Metabolic control, young age at onset and signs of nephropathy and retinopathy in patients with childhood onset Type 1 diabetes. A population-based study in northern Sweden.

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Background and Aims: To study the impact of metabolic control (HbA1c) also early in disease and effect of age at onset on the occurrence of incipient diabetic nephropathy (IN) and background retinopathy (BRP) in young patients with type 1 diabetes treated according to a nationwide diabetes care programme.
Materials and Methods: All children diagnosed 0-14 years in a geographically defined area in nothern Sweden between 1981-1992, were identified using The Swedish Childhood Diabetes Registry. After 1981 a nation wide childhood diabetes care program was introduced and recommended intensified insulin treatment and HbA1c, urinary microalbumin analyses and fundus photography to be followed routinely. Data on these 94 patients were retrieved by retrospective study of medical records and laboratory reports.
Results: During the follow-up period; mean 11.8±3.5 (range 5-19) years, 17 patients (18%) developed IN and 45 patients (48%) BRP. A Cox proportional hazard regression, modelling duration to event of IN or BRP, showed that metabolic control (mean HbA1c) during the follow up was significantly associated with both IN and BRP when adjusted for gender, age at onset and tobacco use as potential confounders. During the first five years of diabetes mean HbA1c was a near-significant determinant for development of IN (HR 1.41, p=0.083) and a significant determinant of BRP (HR 1.32, p=0.036). The age at onset of diabetes significantly influenced the risk of developing BRP (HR 1.11, p=0.021) and in a Kaplan-Meier analysis onset of diabetes before the age of five, compared to the age groups 5-11 and after 11 years, seemed to prolong time to event (p=0.015) for BRP but no clear tendency was seen for IN perhaps due to lower statistical power.
Conclusion: More than 50% of patients with childhood onset type 1 diabetes develop early signs of diabetic complications after ~ 12 years of diabetes, despite modern insulin treatment. metabolic control, also during the first five years of diabetes, seems to be of importance whereas a young age at onset seems to delay development of microvascular complications.

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Complications status in subjects with diabetes cared for at the primary and secondary/tertiary level - a snapshot of the Diabcare-Asia 1998 and 2001 data.

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Background & Aims: To establish a baseline status of diabetes control, diabetes management and complications in diabetes patients managed by specialists or endocrinologists and general physicians.
Materials & Methods: A cross-sectional survey was carried out by Novo Nordisk Asia Pacific and participating regions through their national diabetes associations in 1998 (12 regions) and 2001 (9 regions). Valid data were obtained from ~20,000 patients seen by specialists (SP) in the 1998 study and from ~8500 patients seen by general practitioners (GP) in the 2001 study. Information collected included basic patient data (date of birth, gender), type of diagnosed diabetes & risk factors, recent 12 months clinical measurements (HbA_{1c}, fasting blood glucose, lipids, blood pressure, etc), types of complications present, diabetes education received, type of treatment received and self-monitoring of glucose levels. Non-parametric and multiple regression analyses (linear and logistic, with adjustments for age, sex and diabetes duration) were carried out. We present the results of the complications status in patients managed by SP and GP.
Results: The findings from both surveys suggested that the profile of diabetes patients managed by GP was slightly different from the profile of diabetes patients managed by SP. In both cohorts, at least 90% of the patients were diagnosed as type 2 diabetes. Patients managed by GP were slightly older (57.5 ± 0.08 [SE] yrs vs 60.1 ± 0.12, p<0.05) and had diabetes for slightly shorter duration (9.2 ± 0.05 [SE] yrs vs 8.0 ± 0.06, p<0.05). Mean BMI in both cohorts was similar (24.4 ± 0.03 [SE] kg/m^2 vs 24.4 ± 0.04 kg/m^2, p>0.05). The glycaemic control in both cohorts were sub-optimal, as shown by the indices of control, HbA_{1c} (8.5 ± 0.01 [SE]% vs 8.1 ± 0.02%, p<0.05) and FBG (8.9 ± 0.03 [SE] mmol/l vs 8.9 ± 0.04 mmol/l, p>0.05). In general, complications were more frequently reported

in the SP cohort. Neuropathy (36% vs 30%, odds ratio [OR] of SP/GP=0.75; 95% CI 0.71–0.79), background retinopathy (21% vs 12%, OR=0.53; 95% CI 0.49–0.58), cataract (27% vs 25%, OR=0.77; 95% CI 0.73–0.83) and microalbuminuria (presence of 20–300 mg/l albumin in the urine, 45% vs 38%, OR 0.73; 95% CI 0.61–0.82) were the most commonly reported complications in both cohorts of patients. The frequency of the other types of complications was low (<7%).
Conclusions: Although the studies were not conducted at the same time, it showed that in the GP cohort, fewer complications were reported.

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Optical coherence tomography is a new and reliable method for the assessment of diabetic maculopathy.
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Background and Aims: Patients with diabetes develop macular changes antecedently or parallell with diabetic retinopathy. Our aims were to screen, assess and follow-up retinal thickening due to diabetic maculopathy with the use of a new method, Optical Coherence Tomography (OCT).
Patients and Methods: 140 eyes of 74 patients with diabetes were examined between March 1. and August 31. 2002, independent of diabetic maculopathy seen by fundus examination. We obtained six radial scans of the macula. Type of diabetes, best corrected visual acuity, and fundus photograph were recorded of each patient. We examined foveolar thickness and total macular volume with the help of the OCT software.
Results: Intact macula was found in 30 eyes of type 1 diabetes patients (76%), and 53 eyes of type 2 diabetes patients (53%). In type 1 diabetes pathological findings were: diffuse macular edema (4 eyes=10%), cystoid macular edema (5 eyes=12%), serous macular detachment (2 eyes=5%), and epiretinal membranes (3 eyes=8%). In type 2 diabetes diffuse macular edema (23 eyes=23%), cystoid macular edema (16 eyes=16%), serous macular detachment (1 eye=1%) and epiretinal membrane (9 eyes=9%) was found. Both in type 1 and type 2 diabetes foveal thickness and macular volume correlated with best corrected visual acuity (R^2=0,57 and R^2=0,55, respectively for type 1 diabetes and R^2=0,34 and R^2=0,43, respectively for type 2 diabetes).
Conclusion: With the help of OCT both foveal and extrafoveal macular thickening can be measured objectively. Macular thickening correlates with visual acquity. OCT is a quick and precise, reliable method for quantitative and qualitative assessment and also follow-up of diabetic maculopathy.

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Impact of diabetes in patients undergoing eye procedures.
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Background and Aims: Diabetic eye complications such as cataract and diabetic retinopathy are a leading cause of blindness and visual impairment among adults in industrialised countries. To determine the current impact of diabetes in patients undergoing eye procedures, we analyzed the data of the 45 837 consecutive in-patients recorded in a national eye Center from January 1997 to December 2000.
Materials and Methods: The demographic characteristics of the study population were registered in a database. The 4 years-evolution of main causes of hospitalization and their respective length of stay according to the type and treatment of diabetes were analyzed.
Results: There were 4462 in-patient stays with diabetes corresponding to 9.7% of all the hospitalizations. Very few patients (5%) had type 1 diabetes; 90% had type 2 and 17% of which were taking insulin at the time of the study. Diabetic patients (65.3 ± 13.1 yr) were hospitalized 1.43 time during the 4 years of the study (vs 1.30 time in non-diabetics; p<0.001). Cataract surgery, retinal diseases and ocular hypertension were respectively the first (46%), the second (19%) and the third (7%) cause of hospitalization in the whole study population. Patients with diabetes underwent ophthalmologic procedures as cataract surgery and vitrectomy with increasing frequency compared to those without diabetes (p<0.001). Among diabetic patients with retinal disease, 92% of them with type 1, 80% with type 2 taking insulin and 40% with hypoglycemic agents were hospitalized for the treatment of a severe diabetic retinopathy. The rate of hospitalization for ocular hypertension did not differ between diabetic and non-diabetic population. The 4 years-evolution of the annual incidence of diabetic patients hospitalized in our eye Center showed a consistent raise: 8.8% in 1997 to 10.5% in 2000 and concerned especially type 2 with diabetic retinopathy. Furthermore, a 2-year increase of the mean age was observed in patients with type 2 diabetes during the study period. Diabetic patients, in particular those with insulin therapy, retinal diseases and/or one-eyed, stayed one day longer than non-diabetic patients (p<0.001).
Conclusion: In the next future, an increasing number of elderly patients with type 2 diabetes will undergo eye procedures in ophthalmological departments and require specific management related to associated disorders. This demographic evolution will probably worsen as a result of the epidemic explosion of diabetes.

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Progression of posterior vitreous detachment aids prognosis of diabetic retinopathy.
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Background and Aims: Despite lengthy diabetes mellitus with poor blood glucose control, some patients have minimally progressing diabetic retinopathy (DR), while others have rapidly progressing DR even with excellent blood glucose control. During our study in which we classified posterior vitreous detachment (PVD), we observed that proliferative DR is rare in patients with complete PVD, which prompted us to study the relation between PVD and progression of DR.
Materials and Methods: The medical records of patients with diabetes in our hospital were reviewed for the relation between progression of DR and status of PVD and HbA1c for 3 years. PVD was classified into five types according to the previous study (Kakehashi, Brit J Ophthalmol 1997): no PVD, complete PVD with collapse, complete PVD without collapse, partial PVD with a thickened posterior vitreous cortex, and partial PVD without a thickened posterior vitreous cortex. DR was classified into four types: no DR, simple DR, preproliferative DR, and proliferative DR. When the degree of DR increased from the lower to higher categories or when laser treatment or vitreous surgery was applied, the DR was defined as progressed.
Results: Progression of DR over 3 years was observed in 128 of 292 (43.8%) eyes with no PVD, 0 of 14 (0%) eyes with complete PVD with collapse, 2 of 8 (25%) eyes with complete PVD without collapse, 15 of 15 (100%) eyes with partial PVD with a thickened posterior vitreous cortex, and 19 of 74 (25.7%) eyes with partial PVD without a thickened posterior vitreous cortex. Progression of DR was significantly higher in eyes with partial PVD with a thickened posterior vitreous cortex than in eyes with complete PVD with collapse (p<0.0001). The HbA1c level was not significantly different between these two groups (7.4±0.9% for partial PVD with a thickened posterior vitreous cortex vs. 7.5±0.9% with complete PVD with collapse; p=0.7), although it was higher in patients with progression of DR when simply compared among all patients with (7.8±1.8%) or without (7.5±1.5%) progression of DR (p=0.04).
Conclusion: Complete PVD is a strong negative risk factor for DR. It is important to evaluate PVD status in patients with diabetes.

1148

Sensitivity and specificity of insufflation tonometry in detecting raised intraocular pressure within a diabetic retinopathy screening programme.
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Background and Aims: Previous studies suggest that the prevalence of glaucoma is about 4% among diabetic people. Hence, the need to screen for raised intraocular pressure (IOP). The aim of this study was to compare the results of insufflation versus applanation tonometry as a screening tool to detect raised IOP in a diabetic population.
Materials and Methods: 3817 consecutive patients underwent screening for diabetic eye disease between 1/9/01 and 31/10/02. Of these, 3752 (98.3%) were also subjected to insufflation tonometry. Patients with IOP ≥ 23 mmHg at insufflation in either eye were referred for further assessment, including applanation tonometry and slit-lamp biomicroscopy.
Results: The mean ± SD insufflation IOP in 7485 eyes was 16.75 ± 3.96 mmHg (95% CI 16.66-16.84). IOP was ≥ 23 mmHg in 310 eyes (4.1%) of 195 patients (5.1%). History of glaucoma was already known in 42 of them, who were referred back to their ophthalmologists. The other patients were

recalled for applanation tonometry. In the 226 eyes of the 137 (90%) patients who returned, the mean IOP was 21.23 ± 4.38 mmHg (95% CI 20.66-21.80). An IOP ≥ 23 mmHg was confirmed in 96 (42.4%) eyes of 55 patients.Topical treatment was prescribed for 50 patients (91 eyes). As a result, IOP decreased to ≤ 22 in 65 (71.4 %) eyes, and remained ≥ 23 mmHg in the reminder.To estimate sensitivity, applanation tonometry was repeated in the 72 fellow eyes which had an IOP ≤ 22 mmHg at insufflation. Normal IOP was confirmed in 66 (92%) eyes. Overall, insufflation tonometry had 94% sensitivity, 34% specificity, 42% positive predictive value and 92% negative predictive value.
Conclusion: Insufflation tonometry is a low specificity screening test but permits to identify and treat diabetic patients with previously unknown raised IOP.

PS 100
Clinical Observations on Retinopathy

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The THOR Effect: thyroxine-treated hypothyroidism offsets diabetic retinopathy.
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Background and Aims: Despite evidence that strict control of blood glucose and blood pressure delays development of diabetic retinopathy, aetiological factors in the pathogenesis and progression of diabetic retinopathy (DR) have not been completely elucidated. Role of thyroid status in macrovascular disease both in diabetic and non diabetic subjects have previously been demonstrated, however its effect on diabetic microvascular complications has not been explored. Based on our anecdotal observation and pilot study, we compared the prevalence and time to development of DR in patients with diabetes with and without treated hypothyroidism.
Materials and Methods: Data on patients with coexisting type 2 diabetes and thyroxine treated hypothyroidism (THD) (n=147) and duration of diabetes matched euthyroid controls (n=383) were identified from our diabetes clinics. Prevalence of DR was analysed by chi square: time to DR by survival analysis (Kaplan-Meier and Cox Regression).
Results: Prevalence of DR was 27.9% in HD and 55.1% in the control group ($p<0.0001$). THD was 30% less likely to have DR compared to controls (OR=0.32; 95% CI=0.21-0.48; $p<0.001$). There was significant difference in time to DR between THD and control group (18.0 yrs vs.14.7 yrs: Log-Rank $p<0.0001$). The risk of developing DR in THD was two-fifths to those of controls (hazard ratio =0.418: $p<0.001$) in a time-dependent variable analysis. There was no difference in the mean HbA1c (8.1 ± 1.2 vs. 8.0 ± 2.2; p= 0.63) or duration of diabetes (12.9±7.8 vs. 13.0±7.3) between the THD group and the controls.The difference was apparent despite the THD group being older (66.2 ± 10.2 vs.62.6 ± 11.2) and higher systolic blood pressure (147.6±18.1 vs.144.2 ±15.9 (in mmHg)) as compared to the controls
Conclusion: Significant sparing effect of concomitant thyroxine treated hypothyroidism and diabetes was noted on the development of DR in a temporally-explicit analysis. Exact mechanism for this is unclear but may be due to action of exogenous thyroxine on the hypothalamo-pituitary axis. Further studies are needed to elucidate a possible role of thyroid status in microvascular complications in patients with diabetes.

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To what extent does initiation of insulin therapy worsen retinopathy in Type 2 diabetes?
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Background & Aims: Universal worsening of retinopathy after commencing insulin therapy in type 2 diabetes has been suggested by previous work. We studied 211 such patients from the time of initiation of insulin for 3 years (age 62.5±0.7 years, duration of diabetes 8.1±0.3 years).
Materials & Methods: Retinal photographs were graded using the EURO DIAB system at baseline, 1, 2 and 3 years.
Results: HbA1c (%) was 9.9±1.3 at baseline and 8.3±1.1, 8.4±1.3, 8.4±1.4 on successive years. At baseline, 64.5% (136/211) had no retinopathy, 26% early nonproliferative (NPDR), 5.7 % moderate to severe NPDR and 3.8% proliferative retinopathy. Mean EURO DIAB scores (range 0-5) were 0.54 at baseline but progressively increased to 0.64, 0.75 and 1.1 over successive years. Over 3 years, 48.9% showed no progression but 9.5% developed severe NPDR or proliferative retinopathy. Only 3/136 without retinopathy at baseline progressed to severe NPDR or proliferative retinopathy, whereas 8/55 patients with early NPDR at baseline (chi square 10.9, $p<0.001$) and 16/67 with any degree of NPDR (chi square 18.9, $p<0.0001$) progressed.

Progression was not related to HbA1C at baseline nor to change in HbA1C.
Conclusions: We conclude that on initiation of insulin treatment in type 2 diabetes, clinically significant worsening of retinopathy occurs in 14.5% with early NPDR and 66.6% with moderate NPDR but is uncommon in those with no retinopathy at baseline. This is the largest study of this topic and provides definitive information upon which to base clinical decisions.

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Effects of pancreas transplantation on advanced diabetic retinopathy.
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Background and Aims: Pancreas transplantation (PTx) normalizes blood glucose levels and improves quality of life in Type 1 diabetic patients. The effects of PTx on diabetic late microvascular complications are still unclear.
Materials and Methods: We studied the evolution of diabetic retinopathy (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).
Results: 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). Post-transplant DR improvement (defined as a regression to a lower retinopathy grade in the NPDR group, and as a reduction of retinal lesions in the PDR group) was observed in 6 patients, 25%, in the NPDR group, and in 21 patients, 80% ($p<0.05$ vs pre-transplant), in the PDR group. Progression of DR occurred in 1 patient in the NPDR group, and no measurable change was registered in all the other pancreas recipients. Visual acuity increased slightly, but significantly ($p<0.05$) from 0.82±0.55 (pre-transplant) to 0.87±0.44 (post-transplant), with 7 eyes (4 in the NPDR group and 3 in the PDR group) improving of 2 lines or more.
Conclusion: In conclusion, despite a relatively short follow-up period, successful PTx in our cohort of patients improved or stabilized advanced diabetic retinopathy.

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Effect of pregnancy on diabetic retinopathy progression: changes in vasoactive hormones.
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Background and Aims: To evaluate the role of systemic vasoactive hormones in relation to changes in diabetic retinopathy during pregnancy and postpartum.
Materials and Methods*:* A prospective follow-up study of 69 pregnant women with insulin-dependent diabetes and 11 non-diabetic pregnant women. 36 women were treated with insulin lispro and 33 were treated with regular insulin. Diabetic retinopathy was graded from color fundus photographs acording to the Diabetes Control and Complications Trial (DCCT). Plasma natriuretic peptides (ANP, BNP, CNP), markers of renin-angiotensin system (plasma renin activity, PRA), aldosterone, angiotensin II) and adreonomedullin (AM) and HbA1c were measured during the first, and third trimester and 3 months postpartum. Retinal blood flow was measured with confocal scanning laser Doppler system.
Results: 41 (59.4%) of diabetic women had mild retinopathy (DCCT<4) and 28 (40.6%) had more severe retinopathy (DCCT>3) at first trimester. Although compared to regular insulin, insulin lispro improved glycemic control, this was not translated in progression of diabetic retinopathy nor changes in plasma levels of vasoactive hormones. Concentrations of PRA ($p<0.0001$), and ANP ($p=0.04$) were significantly lower in diabetic women than in healthy women throughout pregnancy and postpartum. 7 (10.1%) of diabetic women developed proliferative changes during study period. The change in retinopathy level (DCCT) correlated with PRA in diabetic women. There were no significant differences in angiotensin-II, aldosterone, AM, BNP, or CNP concentrations between the two groups. During the third trimester, there was a positive correlation between ANP and the retinal blood flow in diabetic women ($r=0.381$, $p=0.035$).
Conclusions: We conclude that diabetic pregnancy is associated with lower levels of PRA and ANP compared to normal pregnancy. Worsening of retinal condition in pregnant diabetic women could to be related to changes in PRA and ANP.

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Retinopathy is more prevalent in overweight than in normal weight Type 1 diabetic patients.
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Background and Aims: Retinopathy may not only be related to glycemic control and diabetes duration, but also to blood pressure and BMI, as was shown by the UKPDS and the HOORN study. However, information on the possible pathogenic role of BMI on retinopathy in type 1 diabetes is scarce.
Patients and Methods: We studied 500 type 1 diabetic patients, but 25 subjects with major cardiovascular disease, amputation, or serum-creatinin >1.5 mg/dl were excluded (M/F: 265/210; mean age: 41±12 y; duration: 19±11 y; HbA1c: 7.8±1.1 %). Patients were subdivided according to BMI: 132 normal weight men (BMI<25), 108 normal weight women, 133 overweight men and 102 overweight women. Retinopathy was examined by fundoscopy (Airlie House classification), neuropathy by electromyography, blood pressure was taken after 5 minutes rest and a mean of 4 measurements was used.
Results: Hypertension (>130/80 mmHg) was present in 41%, retinopathy in 53%, and neuropathy in 41% of patients. Overweight subjects had more retinopathy (63% vs 45%, $p<0.0001$, OR=2.1) and neuropathy (47% vs 36%, $p=0.02$, OR=1.6) than normal weight patients. Patients with retinopathy were older (46±11 vs 36±11 y, $p<0.0001$), had a longer diabetes duration (25±10 vs 13±9 y, $p<0.0001$), a higher HbA1c (8.0±1.0 vs 7.7±1.2%, $p=0.004$) and a higher BMI (26.0±4.2 vs 24.8±4.4, $p=0.005$) than those without retinopathy. Logistic regression analysis showed that diabetes duration ($\beta=0.14$, $p<0.0001$), blood pressure ($\beta=0.21$, $p=0.013$), HbA1c ($\beta=0.25$, $p=0.019$), and gender ($\beta=0.47$, $p=0.043$), but not BMI or age were independent risk factors for retinopathy.
Conclusions: Retinopathy is more prevalent in overweight (BMI≥25) type 1 diabetic subjects. However, logistic regression analysis showed that diabetes duration, blood pressure, HbA1c and gender were the main determinants of retinopathy. BMI was no independent risk factor for this complication.

1154

Retinopathy is associated with impaired myocardial perfusion in young adults with long lasting Type 1 diabetes.
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Background and Aims: Long standing diabetes mellitus and microvascular complications remarkably increase cardiovascular incidents in younger population. The aim of this study was the assessment of blood supply to heart muscle with non-invasive diagnostic methods and plasma concentration of VEGF (vascular endothelial growth factor) in asymptomatic patients with long lasting type 1 diabetes, with (Ret+) or without retinopathy (Ret-).
Materials and Methods: 41 type 1 diabetic patients (23 female, 18 male), aged 30±7.6, duration of disease 15.2±5.5 years, HbA1c 8.25±1.82 % were recruited to this study. 27 patients Ret+ (15 females, 12 male) and 14 Ret- (8 females, 6 males). We performed the following investigations: 24-hour ECG tape, exercise treadmill test, echocardiography with dobutamine and atropine challenge, single photon emission computer tomography (SPECT) at rest and after dipirydamol induction of ischaemia. Serum VEGF concentration was measured using ELISA method.
Results: There were not any significant differences in results of 24-hour ECG tape study. All exercise and echocardiography stress tests with dobutamine and atropine were negative. There were significant differences in SPECT between Ret+ and Ret- group. The results are presented in the table:

	Ret+	Ret-	p
Total perfussion SPECT %	1.04 ± 0.14 48	-1.64 ± 0.22 7	p<0.05 p<0.05
VEGF pg/ml	89.99 ± 7.20	78.59 ± 27.3	ns

The presence of retinopathy increased 18-fold the probability of the changes in SPECT (OR: 18.2, 95%CI: 1.67-198.87; $p=0.0173$).
Conclusion: The results show that asymptomatic patients with long standing type 1 diabetes and retinopathy have a risk of the early disturbances in heart muscle perfusion.

1155

Longterm Mg supplementation influences favourably the natural evolution of neuropathy and retinopathy in Mg depleted Type 1 diabetic patients.

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Background and Aims: 20-25% of type 1 diabetic patients (T1dm) have chronic Mg depletion and low circulating Mg levels have been linked to more severe retinopathy (DR) and neuropathy(DN). Shortterm supplementation studies have suggested a decrease of DN signs and a stabilisation of DR.The aim of this study is to determine if longterm Mg supplements can normalize the Mg status and influence the evolution of DR and DN in a large cohort of depleted T1dm.
Materials and Methods: 110 T1dm (60M,50W) with chronic Mg depletion(erythrocyte Mg<2.3mMol/l)were randomized to receive 300 mg Mg++ daily or no supplement for a period of 5 years.Follow-up was organized by the same physician:stabilisation of the metabolic control(HbA1c every 3 months+Mg status) and kidney function,eye-fundi,EMG and neurologic control every year.DN and DR were staged following a fixed system.Drop-out was decided for the following reasons:recurrent DKA,diseases or drugs interfering with Mg status,side-effects of Mg supplement.
Results: 97 patients(53M,44W) finished the study: 49(27M,22W)were supplemented(=groupA) and 48(26M,22W)served as controls(=groupB). ANOVA did not show significant differences in regard of age, duration of diabetes,HbA1c,Mg status or presence of DR and DN between the 2 groups at the start.HbA1c after 5 years (A:7.8%,sd0.8;B:7.75%,sd0.75)was not significantlly different from the start.Erythrocyte Mg rose significantly ($p<0.0001$) in group A to normal levels(from 2.02 mM/l,sd0.14 to 2.37 sd0.16 after 5y) but remained low in groupB.Staging of DN after 5y shows a decrease in 39%,a statuquo in 49% and a worsening in 12% in groupA and 8,31 and 61% in groupB.(Fisher Exact test:$p<0.0001$)Staging of DR after 5y shows resp.6,80 and14% in groupA and 0,63 and 37% in groupB (Fisher Exact test:$p<0.05$)
Conclusion: Under stable metabolic control,longterm Mg supplementation is able to normalize incipient DN and to stabilize DR as compared to non-supplemented Mg depleted T1dm controls.

PS 101
Retinopathy: Associated Factors

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No association between MTHFR gene polymorphism and diabetic retinopathy in Japanese Type 2 diabetes mellitus.

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Background and Aims: The development of diabetic retinopathy varies among individuals due to not only metabolic control but also genetic factors. Methlenetetrahydrofolate reductase (MTHFR) is an enzyme involved in remethylation of homocysteine to methionine. Impaired activity of MTHFR can result in hyperhomocysteinemia, which may lead to macroangiopathy. A point mutation (C677T) in the MTHFR gene has been reported to be correlated with macroangiopahty in diabetes. However, such an association is poorly understood in microangiopathy. We investigated the relationship between the MTHFR gene polymorphism and diabetic retinopathy in Japanese type 2 diabetic patients.
Materials and Methods: We studied 336 subjects with type 2 diabetes, whose mean age was 60.0 years, mean diabetic duration of 11.7 years, and mean HbA1c level of 7.3 %. To avoid the confounding effect of nephropathy, subjects with advanced nephropathy were excluded. Diabetic retinopathy (DR) was defined as non-diabetic retinopathy (NDR), non-proliferative retinopathy (NPDR), and proliferative retinopathy (PDR). Real time polymerase chain reactions were used to detect (C677T) polymorphism of the MTHFR gene. Statistical difference in genotype distribution and allele frequencies among the groups was assessed by χ^2-test.
Results: The allelic frequency of the C677T mutation was 0.39, and the genotypes were Hardy-Weinberg equilibrium (677C/677C, 36.3%, n=133; 677C/677T, 49.7%, n=182; 677T/677T, 14.0%, n=51). Of our 366 diabetic patients, 14.2% (n=52) had NPDR, 12.6% (n=46) had PDR, and the remaining 73.2% (n=268) had NDR. There was no association between the genotypes and clinical parameters such as age, duration of diabetes, HbA1c, serum lipids, and serum creatinine. The frequency of MTHFR (C677T) polymorphism in the patients with DR did not significantly differ from that in patients without DR (patients with DR: 677C/677C, 33.7%; 677C/677T, 51.0%; 677T/677T, 15.3% versus patients without DR: 677C/677C, 37.3%; 677C/677T, 49.3%; 677T/677T, 13.4%; χ^2 test, p=0.78. After adjustment for duration of diabetes, HbA1c level and blood pressure, multiple regression analysis also showed no significant correlation between MTHFR gene polymorphism and diabetic retinopathy (p=0.98).
Conclusion: These data indicate that MTHFR gene polymorphism is not associated with diabetic retinopathy in Japanese type 2 diabetic patients.

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Angiotensin converting enzyme (ACE) insertion / deletion (I/D) and Gly82Ser AGE-receptor polymorphism in subjects with Type 1 diabetes and early diabetic retinopathy.

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Background and Aims: Epidemiological and pathophysiological studies have suggested that specific genetic factors may be involved in the development of diabetic proliferative retinopathy. We aimed to evaluate the relationship between the ACE insertion/deletion polymorphism and the frequency of Gly82Ser polymorphism in exon 3 of the receptor for AGE (RAGE) gene and early proliferative diabetic retinopathy in patients with type 1 diabetes.
Materials and Methods: Therefore, we compared 50 patients with longstanding (>20 years) type 1 diabetes who had only mild or no background (No/mildDR) retinopathy with 34 patients who developed proliferative diabetic retinopathy (PDR) within 15 years of diagnosis and underwent laser coagulation. The polymorphic region in intron 16 of the ACE gene (17q23) was analyzed using the polymerase chain reaction

(PCR). Genotype frequencies of Gly82Ser polymorphism were studied by PCR amplification and restriction fragment length polymorphism analysis using AluI enzyme.
Results: Mean age was 52±12 yrs in No/mildDR, and 43±12 yrs in PDR, while diabetes duration was 32±10 vs. 23±6 yrs; 41 subjects were males. There were no differences in glycaemic control (HbA1c 8.2±1.0 vs. 8.3±1.1%) or visual accuracy (VODS 0.90 ± 0.15 vs. 0.82 ± 0.27). Both groups had similar prevalence of hypertension and macrovascular disease, although nephropathy was more prevalent in the PDR group (32% vs 11%, p=0.021). The ACE genotype distribution in patients with PDR (DD 35.3%, ID 47.1%, II 17.6%) was not different from that of patients with minimal or no retinopathy (DD 23.5%, ID 58.8%, II 17.6%). The frequency of I allele was 0.412 vs. 0.471 and D allele was 0.588 vs. 0.529 among subjects with and without retinopathy, respectively. The frequency of the Ser82 allele was 15% in the PDR group compared to 9% in the No/mildDR group (P=.34).
Conclusion: These results do not support the hypothesis that there is an association between ACE gene I/D polymorphism and Gly82Ser polymorphism in the RAGE gene and the early development of diabetic retinopathy in type 1 diabetic patients.

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Increased plasma concentrations of Osteoprotegerin in Type 2 diabetic patients with microvascular complications.
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Background and Aims: Osteoprotegerin (OPG) is a newly identified inhibitor of bone resorption. Recent studies indicate that OPG also acts as an important regulatory molecule in the vasculature. Plasma levels of OPG seem to be elevated in subjects with diabetes as well as in non-diabetic subjects with cardiovascular disease. The aim of the present study was to examine the association between plasma OPG levels and microvascular complications and glycaemic control in patients with type 2 diabetes.
Materials and Methods: Four groups of each 20 subjects, individually matched for age and gender, were included in the study: (i) subjects with normal glucose tolerance (NGT); (ii) subjects with impaired glucose tolerance (IGT); (iii) type 2 diabetic patients without retinopathy; (iv) type 2 diabetic patients with diabetic maculopathy (DMa). Plasma concentration of OPG was measured in duplicate by a sandwich ELISA method (R&D Systems, Minneapolis, MN, USA). Furthermore, fundus photography, flourescein angiography, and measurements of urinary albumin excretion rate (RIA) were performed.
Results: Plasma OPG was significantly higher in diabetic (iii+iv) than in NGT (i) subjects (3.04 ± 0.15 vs. 2.54 ± 0.16 ng/l, $P < 0.05$). Plasma OPG was significantly higher in the DMa (iv) group than in the NGT (i) group (3.25 ± 0.23 vs. 2.54 ± 0.16 ng/l, $P = 0.01$). Moreover, plasma OPG was significantly higher (3.61 ± 0.36 ng/l) in the group of diabetic subjects with both microalbuminuria and DMa ($n = 7$) than in the NGT (i) (2.54 ± 0.16 ng/l, $P < 0.01$), IGT (ii) (2.82 ± 0.21 ng/l, $P < 0.05$), and no retinopathy (iii) groups (2.83 ± 0.20 ng/l, $P < 0.05$).
Conclusion: We found increased levels of OPG in plasma from type 2 diabetic patients with microvascular complications. This finding indicates that OPG may be involved in the development of vascular dysfunction in diabetes.

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Association of leptin with nephropathy and retinopathy in Type 2 diabetic subjects.
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Background and Aims: Leptin is a hormone that may be associated with nephropathy and diabetic retinopathy. Some recent data has shown positive association of serum leptin level with diabetic nephropathy and diabetic retinopathy. In the present study, we have explored the relationship of serum leptin with microvascular complications of diabetes mellitus (DM).
Materials and Methods: Two hundred and forty two subjects were recruited. Fifty were healthy controls, of whom 26 were female. One hundred and ninety two type 2 diabetic subjects were divided into 4 groups. All 4 groups were matched for age, sex and BMI. The first group (n=54) consisted of subjects with DM without any evidence of nephropathy or diabetic retinopathy (DR). The second group (n=54) consisted of patients with DM with DR without nephropathy. The third group (n=44) was DM with nephropathy without DR and the last group (n=40) was DM with both nephropathy and DR. Patients with ischemic heart disease were excluded from the study.
Results: Mean (± SE) serum leptin concentration of healthy control population was 12495.29 (± 1687.46) pg/ml. Mean (± SE) of serum leptin in male and female were 10944.53 (± 1340.35) pg/ml and 30681.04 (± 2510.97) pg/ml respectively. Mean serum leptin concentration was 15601 (± 1659.81) pg/m for subjects with a BMI of < 25 kg/m^2 and 27437.67 (± 2773.67) pg/ml for those with a BMI of ≥ 25. Distribution of serum leptin concentration was skewed; therefore, log transformation of serum leptin has been done. Females had significantly higher level of serum leptin than male (P = <0.001). Log serum leptin correlated positively with BMI (P = <0.001) and was significantly higher (P = <0.001) in overweight and obese individuals, who had a BMI ≥ 25 [4.25 (± 0.41)] than individuals with BMI < 25 [3.96 (± 0.44)]. There was no correlation with waist hip ratio (P = 0.100). Diabetic patients with nephropathy had significantly higher value than patients with DM without nephropathy or DR [4.31 (± 0.48) vs. 4.02 (± 0.39), P = 0.006] and in patients with DM with DR [4.31 (± 0.48) vs. 4.03 (± 0.42), P = 0.007]. Log serum leptin of patients with DM with both nephropathy and DR, was also significantly higher than patients with DM without any of these two complications [4.24 (± 0.56) vs. 4.02 (± 0.39), P = 0.031] and patients with DM with DR [4.24 (± 0.56) vs. 4.03 (± 0.42), P = 0.038].
Conclusion: Serum leptin level does not differ in diabetic and non-diabetic population. In type 2 diabetes mellitus, serum leptin concentration is associated with nephropathy. No association was found between serum leptin level and diabetic retinopathy.

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T cells in the vitreous fluid of diabetic patients: comparison with peripheral bood and relationship with retinopathy activity and clinical outcome.
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Background and Aims: 1) To compare CD4/CD8 and CD28 expression in T cells infiltrating the vitreous fluid in diabetic patients with proliferative diabetic retinopathy with those obtained in samples of peripheral blood from the same patients. 2) To evaluate the relationship between the infiltrating T cells and both the activity of PDR and the clinical outcome.
Materials and Methods: Both vitreous and peripheral blood samples were simultaneously obtained from 22 consecutive type 2 diabetic patients and analysed by flow cytometry (cellQuest Program, Becton Dickinson). Six non-diabetic patients requiring vitrectomy were also evaluated. For the purpose of the study diabetic patients with vitreous hemorrhage were analysed separately. The identification of vitreous hemorrhage was made when hemoglobin was detected by spectrophotometry whitin the vitreous fluid (lower limit of detection 0.03 mg/ml). Patients who had undergone previous vitreoretinal surgery or had received photocoagulation in the previous 3 months were all excluded. Retinopathy was graded intraoperatively by the same ophthalmologist, taking into consideration the presence of active neovascularization whenever perfused preretinal capillaries were found and quiescent retinopathy whenever non perfused vessels or fibrosis were present. After vitrectomy an ophthalmologic evaluation was systematically performed during follow-up (8±2 months). Statistics: For comparisons between peripheral blood and vitreous fluid in the same patient the Wilcoxon test was used. Data are presented as median and range.
Results: T lymphocytes were detected in all diabetic patients with vitreous hemorrhage, in the 54% of diabetic patients without hemovitreous, but in none of the non-diabetic patients. The percentage of T cells (CD3+), TCD4+ (CD3+ CD4+) and TCD8+ (CD3+ CD8+) subsets, as well as the expression of CD28 were similar in the vitreous fluid and in the peripheral blood in those patients with vitreous hemorrhage. However, in patients without vitreous hemorrhage the percentage of CD4+ CD28- T cells in the vitreous fluid was significantly higher than in the peripheral blood [33.34 (20.75-100.00) vs. 8.45 (2.43-56.59; p=0.02), and it was observed in every patient. In addition, all these patients presented quiescent retinopathy and their outcome was better than either those patients with hemovitreous or patients in whom intravitreous T cells were undetectable.
Conclusion: T cells infiltrating the vitreous of diabetic patients without vitreous hemorrhage not only show a different pattern than in the peripheral blood but also seem to improve the prognosis of PDR.

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Effect of diabetic nephropathy and blood pressure on the prevalence of circulating antipericyte autoantibodies at various grades of retinopathy.

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Background and Aims: As diabetic retinopathy is related to microalbuminuria, elevated blood pressure and HbA1c level, the effect of albumin:creatinine clearance ratio in relation to the degree of diabetic retinopathy, blood pressure and HbA1c levels on the prevalence of anti-pericyte autoantibodies in diabetic patients was determined.
Materials and Methods: Three-hundred and seventy-five subjects, 20% with type 1 and 80% with type 2 diabetes participated in this study. Serum anti-pericyte autoantibodies were detected by immunofluorescence on tissue cultured bovine retinal pericytes. Pericyte Autoantibody prevalence was stratified by HbA1c levels, systolic and diastolic blood pressure and albumin:creatinine clearance ratio at various ETDRS grades.
Results: A difference in anti-pericyte autoantibody prevalence stratified by albumin:creatinine (A:C) clearance ratio was not quite statistically significant in male patients ($p = 0.054$), but was statistically very significant in female patients ($p = 0.0009$). The effect of elevated A:C clearance ratio on antibody prevalence at particular ETDRS grades was determined and found to be associated with a decrease in antibody prevalence independently of retinopathy grade ($p=0.019$). There was a positive correlation with increased systolic blood pressure ($p=0.0082$) and HbA1c levels were inversely associated with autoantibody prevalence.
Conclusion: The increased prevalence of antipericyte autoantibodies in patients with hypertension suggests that these autoantibodies are related to tissue damage and repair and the decline in prevalence with severe retinopathy might serve as a marker of advanced microangiopathy. The finding that increased A:C clearance ratio is associated with a lower autoantibody prevalence independently of retinopathy may be a useful marker for molecular studies of interactions of diabetic nephropathy with retinopathy.

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Hyperglycemia facilitates angiotensin II activation of chemotaxic response of bovine endothelial cells.

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Background and Aims: Clinically, Angiotensin II (Ang II) receptor blocker have been shown to slow down the development and progression of diabetic vascular complications, beyond their hypotensive effects. The finding implies existence of a molecular link between hyperglycemia and Ang II signaling, which is causally related to the development of the diabetic complications.
Materials and Methods: We studied the directional migratory effect and activation of intracellular signaling of hyperglycemia and Ang II on cultured bovine coronary artery endothelial cells (BCAEC) by two-chamber method and immunoprecipitation, respectively.
Results: Interestingly, the cell moved in the reverse direction against Ang II (100 nmol/l). Namely, Ang II induced negative chemotaxic response of BCAEC. On the other hand, Exposure to high glucose (30 mmol/l) activated the omnidirectional migration (positive chemokinesis) of BCAEC. Moreover, high glucose enhanced Ang II-induced negative cell migration. Next, we found that each of high glucose and Ang II activated mitogen-activated protein kinase (MAPK) is one of important molecules of intracellular signal transduction. Hyperglycemia promotes Ang II-induced MAPK activation is exaggerated. PD98059, a MAPK kinase inhibitor inhibited Ang II-induced migration, RNH-6270 (an Ang II type 1 receptor (AT1) blocker) completely suppressed Ang II-induced migration. AT1/MAPK pathway should be responsible for the effect of Ang II on the cell migration. It has been reported that Ang II activates a tyrosine phosphatase, SHP-2, and PYK2 is constitutively associated with a non-receptor tyrosine kinase PYK2. PYK2 functions as a regulator of ion channels, cellular adhesion, and mitogenic reactions. Treatment of the cell with high concentration of glucose caused dissociation of PYK2 and SHP-2. An imposed dissociation of PYK2 and SHP-2 by overexpression of the phosphatase inactive form of SHP-2 in the BCAEC significantly enhanced Ang II-induced MAPK activation. Hyperglycemia may enhance the proliferative action of Ang II primarily by modifying interaction between PYK2 and SHP-2.
Conclusions: The endothelial dysfunction in diabetes may result from hyperglycemic facilitation of Ang II-activated chemotaxis of the endothelial cell.

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Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of cardiovascular complications in Type 1 diabetes mellitus.

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Background and Aims: Diabetes mellitus (DM) is a major risk factor for cardiovascular disease and is frequently associated with impaired collateral formation and neovascularization. Recently, it has become clear that neovascularization does not exclusively rely on angiogenesis, but also involves the recruitment of endothelial progenitor cells (EPC). Hence, circulating EPC may be pivotal for normal vascular function by contributing to vascular maintenance and repair. We assessed the hypothesis that, in addition to endothelial dysfunction, a reduced vascular regenerative potential due to 'EPC dysfunction' may contribute to the development of cardiovascular complications in type 1 DM.
Materials and Methods: To explore the concept of EPC dysfunction we have analysed gene expression profiles and function of circulating EPC of type 1 DM patients (n=9, age 28.1± 10.4 yrs) and age-matched healthy volunteers (n=10, age 28.6± 7.6 yrs) using Affymetrix high-density oligonucleotide microarrays. Mononuclear cells (MNC) were isolated from peripheral blood and EPC were obtained after four days differentiation culture.

Results: We observed 40% reduction in the number of EPC in type 1 DM patients compared to healthy volunteers (2.2 x 10^6± 1.16 x 10^6 (n=26) vs. 3.6 x 10^6 ± 1.35 x10^6 EPC (n=28) per 50 x 10^6 MNC, respectively; $p < 0.001$). This reduction was inversely correlated with patient levels of hemoglobin A1C ($P< 0.05$). In two independent experiments, total RNA of EPC from 4-5 age-matched subjects per group was pooled and gene expression profiles were analysed and compared. Despite culturing EPC in differentiation medium with normal glucose concentration (5.5 mmol/l) for 4 days, we repeatedly observed changes in expression of genes that have previously been functionally associated with insulin, glucose and fatty acid metabolism. These data suggest that circulating EPC can act as biosensors for the adverse metabolic state in type 1 DM. Moreover, we observed altered expression in clusters of genes that imply increased apoptosis and a reduced capacity of diabetic EPC to proliferate, traffic and home. Finally, conditioned medium of the diabetic EPC were significantly reduced in their capacity to support tube formation in an in vitro angiogenesis assay.
Conclusion: Metabolic stress factors associated with type 1 DM lead to a reduced number and impaired function of circulating EPC and thereby contribute to vascular ischemic complications.

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Expression of costimulatory molecules CD28/CTLA-4 and ICAM-1/LFA-1 on peripheral blood mononuclear cells in the course of Type 1 diabetes in children and adolescents.
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Background and Aims: Insulin - dependent Type 1 diabetes is mediated by autoreactive - T lymphocytes recognizing pancreatic islet cell antigens. The process of T cells proliferation is initiated after T-cell receptor (TCR) biding to antigen (I signal) and stimulation of costimulatory molecules CD28/CTLA-4 (II signal). ICAM-1/LFA-1 not only participate in the lymphocytes T proliferation but also mediate leucocyte migration to the site of inflammation. An increasing number of studies have documented the central role of T cell costimulation in autoimmunity. In the study: (a) we examined the expression of CD28, CTLA-4, LFA-1 on lymphocytes T and the expression of ICAM-1 on monocytes in the course of Type 1 diabetes in children and adolescents; (b) we assessed a relationship between the percentage of CD28, CTLA-4 and ICAM-1, LFA-1 on mononuclear cells and the evolution of vascular complications (microalbuminuria, arterial hypertension, diabetic retinopathy); (c) we tried to elucidate weather the expression of CD28/CTLA-4 and LFA-1/ICAM-1 could be a potential early marker of autoimmunological process and the development of diabetic vascular complications?
Material and Methods: The study was carried out in three groups of subjects 60 children (aged 9 - 20) with diagnosed Type 1 diabetes: (a) (20 n) lasting less than 5 years, (b) (20 n) lasting over 5 years, (c) (20 n) with Type 1 diabetes and vascular complications (microalbuminuria, arterial hypertension, diabetic retinopathy). 20 healthy volunteers (control group), (aged 6-17). The expression of adhesion molecules has been evaluated by using three-color flow cytometry (Coulter EPICS XL). HbA_{1C} concentration has been analyzed by liquid chromatography technique HPLC - Variant (Bio-Rad).
Results: In the study, the expression of CTLA-4 receptor was enhanced in children with diabetes lasting less than 5 years ($p < 0,005$) and over 5 years ($p < 0,01$) versus healthy patients. In the group of children with vascular complications we did not observe a significant increase in the expression of CTLA-4 receptor. In contrast, the expression of costimulatory molecule CD28 and ICAM-1, LFA-1 in children with Type 1 diabetes lasting < 5 years was decreased versus control group ($p < 0,01$). In children with diabetic vascular complications we also found decreased expression of CD28 molecule ($p < 0,01$) and the lowest expression of LFA-1($p < 0,0001$). In this group, a positive correlation between the percentage of LFA-1 and arterial hypertension was detected ($r = 0,58$, $p < 0,05$).
Conclusion: The abnormal expression of adhesion molecules on mononuclear cells of peripheral blood found in the first stage of disease (diabetes lasting less than 5 years), was maintained in the course of Type 1 diabetes and suggested that immunological disorders lasted throughout the disease. The evaluation of the percentage of adhesion molecules on mononuclear cells could be a relatively sensitive marker of risk or / and development of Type 1 diabetes in children and adolescents.

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Expression of L-selectin on peripheral blood lymphocytes T in the course of Type 1 diabetes in children and adolescents.
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Backgrounds and Aims: Type 1 diabetes mellitus is the autoimmune–mediated organ specific destruction of pancreatic beta islets in which autoreactive T cells play a critical role. The extravasation and the migration of autoreactive T cells is of paramount importance in the etiology of organ-specific autoimmune disorders. L-selectin initiates lymphocyte migration to the sites of inflammation and is a homing receptor. The aim of this study was (a) to evaluate the expression of L-selectin on T cells in the course of Type 1 diabetes (lasting less than 5 years and over 5 years); (b) to assess if there is any relationship between the percentage of L-selectin receptor and the evolution of vascular complications (microalbuminuria, arterial hypertension, diabetic retinopathy); (c) to find out weather the expression of L-selectin on T cells could be a potential early marker of autoimmunological process and the development of diabetic vascular complications?
Materials and Methods: The study was carried out in three groups of subjects - 60 children (aged 9-20), with diagnosed Type 1 diabetes: (a) (20 n) lasting less than 5 years, (b) (20 n) lasting over 5 years, (c) (20 n) with Type 1 diabetes and vascular complications (microalbuminuria, arterial hypertension, diabetic retinopathy). 20 healthy volunteers (control group),(aged 6-17). The expression of adhesion molecules has been evaluated by using three-color flowcytometry (Coulter EPICS XL). HbA_{1C} concentration has been analyzed by HPLC - Variant (Bio-Rad).
Results: L-selectin expression on lymphocytes T in children with Type 1 diabetes lasting less than 5 years showed increased values versus healthy controls ($p < 0,05$). The percentage of T lymphocytes expressing L-selectin was still enhanced in children with Type 1 diabetes lasting over 5 years. In the group with vascular complications (microalbuminuria, arterial hypertension, diabetic retinopathy) we detected the highest percentage of L-selectin on lymphocytes T ($p < 0,001$ versus healthy controls). In addition, we found a positive correlation between L-selectin and arterial hypertension ($r = 0,76$, $p < 0,05$) in this group.
Conclusions: An increased percentage of T lymphocytes expressing L-selectin was detected in children with Type 1 diabetes lasting less than 5 years and was maintained enhanced in the course of Type 1 diabetes which could suggest that the elevated L-selectin expression could have a genetic background. Futhermore, the highest values of L-selectin in children with vascular complications and positive correlation with arterial hypertention may indicate the metabolic and immunological disorders and could be implemented as an early marker of diabetic complications in the course of Type 1 diabetes.

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Diabetic low-density lipoprotein triggers apoptosis in vascular endothelial cells.
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Background and Aims: Hyperglycemia, assumed to trigger the development of diabetic vascular complications, also increases glycation of plasma (lipo-)proteins. Thus, this study compared the effects of low-density lipoprotein (LDL) glycated either *in vitro* (LDL_{iv}) or *in vivo* in diabetic patients (LDL_D) on apoptosis, proliferation and associated protein expression in human umbilical vein endothelial cells (HUVECs) in the absence/presence of lipoprotein lipase (LPL).
Materials and Methods: Apoptosis, proliferation and protein expression were determined by DNA fragmentation assays, ^{3}H-thymidine incorporation and Western blot analyses, respectively.
Results: At 100mg/l, both LDL species considerably increase endothelial apoptosis (LDL_{iv}: +63%; LDL_D: +40%; $p<0.05$) compared to intraindividual non-glycated LDL subfractions. Considering its lower degree of glycation (LDL_D: 5-10%; LDL_{iv}: 42%), LDL_D's relative proapoptotic activity is 2.7-fold greater than that of LDL_{iv}. Glycated LDL-induced apoptosis is associated with increased expression of apoptosis promotors (LDL_{iv}: bak: +88%, CPP-32: +49%; LDL_D: bak: +18%, CPP-32:

+11%; p<0.05) and is attenuated by caspase inhibitors. Glycated LDL's antiproliferative activity (LDL_{iv}: -34%; LDL_D: -9%; p<0.01) relates to reduction (p<0.05) of cyclin D3 (LDL_{iv}: -27%; LDL_D: -24%), of hypo- (LDL_{iv}: -22%; LDL_D: -19%) and hyperphosphorylated (LDL_{iv}: -53%; LDL_D: -22) Retinoblastoma protein and is paralleled by reduced expression of endothelial NO-synthase (LDL_{iv}: -30%; LDL_D: -23%). In response to LPL (100U/l), LDL_D more markedly triggers endothelial apoptosis (27.1-fold) compared to LDL_{iv}, suggesting that LDL_D owns a higher potential for endothelial cell damage than LDL_{iv}.
Conclusion: The observed behaviour of LDL_D versus LDL_{iv} could be of clinical importance and relate to differences in structure and cellular uptake of LDL_D compared to LDL_{iv}. Diabetic LDL's proapoptotic activity could trigger development and progression of endothelial dysfunction preceding manifest vascular disease.

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Upregulation of oncofetal fibronectin in target organs of diabetic complications.
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Background and Aims: Increased extracellular matrix (ECM) protein synthesis is a key feature of diabetic complications. Fibronectin (FN), a predominant ECM constituent, has been shown to undergo alternative splicing to produce embryonic isoforms in various pathological conditions. These isoforms are referred to as oncofetal variants. The major oncofetal isoform, characterized by the inclusion of extra domain B (EDB), is the focus of the present study. We have investigated the expression of EDB^+ FN in three major target organs of diabetic complications, retina, kidney, and heart. In addition, we have studied the role of diabetes-induced endothelin-1 (ET-1) in regulating preferential expression of oncofetal FN.
Materials and Methods: Male Sprague-Dawley rats were made diabetic by single intravenous injection of streptozotocin. Diabetic rats were randomly divided into two groups, diabetic and diabetic on dual ET-receptor antagonist, bosentan. Age- and sex-matched rats were used as controls. Following three month follow-up, rats were euthanized and tissues were harvested. RNA was extracted from retinal, kidney, and heart tissues and subjected to real time quantitative RT-PCR.
Results: Here we show that expression of EDB^+ FN, relative to total, is upregulated in all three major target organs of diabetic complications. Our retinal, kidney, and heart mRNA data indicates that diabetes leads to upregulation of EDB^+ FN (3-16 fold increase; $p<0.05$) and ET-1 (4-8 fold increase, $p<0.05$) mRNA levels. Treatment of diabetic rats with ET-receptor antagonist significantly reduced mRNA levels of both EDB^+ FN and ET-1 (p<0.05).
Conclusion: Our data showed that diabetes-induced upregulation of oncofetal FN, potentially mediated by ET-1, is a novel mechanism which might be involved in the pathogenesis of diabetic complications.

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Lipoapoptosis of human coronary artery endothelial and smooth muscle cells: effects of saturated and unsaturated free fatty acids.
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Background and Aims: Insulin resistance and type 2 diabetes are often associated with cardiovascular disease. Recently, it was shown that apoptosis is involved in the progression of atherosclerotic lesions and in complications of advanced atheroma. Since elevated free fatty acids (FFA) induce peripheral insulin resistance on the one hand and apoptosis in certain cell types such as beta-cells on the other hand, we investigated whether human coronary artery endothelial (CAEC) and smooth muscle cells (CASMC) undergo FFA-induced apoptosis as well.
Materials and Methods: CAEC and CASMC were purchased from Clonetics/BioWhittaker and cultured according to the manufacturer's instructions. Cells were incubated with saturated (palmitate, stearate) and unsaturated (palmitoleate, oleate, linoleate) FFA (1 mM, 24 h) and apoptosis was assessed by flow cytometry (quantification of sub-G1 DNA content). Necrosis was discriminated from apoptosis by flow cytometry after double staining with propidium iodide and annexin-V.
Results: In CASMC, stearate treatment increased apoptosis 9-fold compared to control cells (n = 3, p < 0.01, t-test). Furthermore, stearate-dependent lipoapoptosis was superimposed by necrosis. Incubation with palmitate or the polyunsaturated FFA linoleate triggered apoptosis significantly but to a much lower extent than stearate (2-fold increase over basal, n = 3, p < 0.05, t-test). The monounsaturated FFA palmitoleate and oleate had no pro-apoptotic effect on CASMC. In CAEC, only stearate exerted significant pro-apopotic effects (4-fold increase over basal, n = 3, p < 0.05, t-test). Stearate-induced necrosis was not detectable. Treatment with palmitate or unsaturated FFA did not induce apoptosis in CAEC. Even more, unsaturated FFA completely prevented stearate-induced apoptosis.
Conclusion: Certain FFA are able to induce apoptosis in CAEC and CASMC. The pro-apoptotic properties depend on FFA's chain length and degree of saturation as well as the target cell type. Stearate exerts lipoapoptotic effects in both cell types tested with CASMC being more sensitive than CAEC.

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The relation between increment of postprandial glycaemia and polymorphonuclear neutrophils function in Type 2 diabetes.
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Background and Aims: Postprandial hyperglycemia, inflammatory process and polymorphonuclear neutrophils (PMN) play an important role in the development of late diabetic complications. The aim of this study was to evaluate the relation between increment of postprandial glycaemia induced by low fat meal (30 g carbohydrates, 6 g fat, 8 g proteins) and PMN's functions in type 2 diabetes.
Materials and Methods: The study was performed in 25 obese type 2 diabetic patients (18 women and 7 men, aged 61.7±10.5 years, diagnosed of diabetes 16.0±7.5 years, BMI 34.8±6.4 kg/m^2, HbA1c 8.4±0.93 %, FPG 8.7±2.8 mmol/l, 2hPPG 12.6±4.4 mmol/l, total cholesterol 6.2±1.0 mmol/l, LDL 3.7±1.0 mmol/l, triglyceride 3.1±1.9 mmol/l, HOMA-IR 12.95). PMN were isolated from the blood by single-step gradient centrifugation and resuspended in Hanks Balanced Salt Solution to 5 x 10^6 cells/ml. We assessed the following functions of unstimulated and zymosan stimulated PMN: adhesion to plastic surfaces acc. to Bath et al., chemotactic activity acc. to Boyden technique, superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) production by PMN acc. to Pick et al., and metabolites of nitric oxide production by PMN (measured spectrophotometrically acc. to Fosterman at al.). Moreover, we assessed enzyme release by PMN (lisozyme, glucuronidase, mieloperoxydase).
Results: We observed significant correlation between increment of glycaemia and chemtotaxis (r=0.41, p<0.05), H_2O_2 production by unstimulated PMN (r=0.43, p<0.05), O_2^- production by unstimulated PMN (r=0.52, p<0.05), lisozyme (r=0.46, p<0.05).
Conclusion: The obtained results suggest that increment of postprandial glycaemia stimulates PMNs in type 2 diabetes. It might be confirmation that toxic effect of postprandial hyperglycaemia is also connected with stimulation of PMN.

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Oxidative Stress in Vascular Disease

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Oxygen radical scavenger, apocynin, retards progression of vascular lesions in Type II diabetic rats complicated with NO dysfunction.

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Background and Aims: Recent mega-trials have revealed that strict control of complicated coronary risk factors such as hyperlipidemia and hypertension is important to the prevention of diabetic vascular lesions. We speculated that one of the pathogenic responsible factors for the risk burden of these complicated coronary risk factors is an unbalance of the NO-oxygen radical system in vessels. Objective: We investigated the relevance of NO and O2- in the development of vascular lesions in type II diabetic rats with or without dysfunction through the effect of NADPH oxidase inhibition. We used NO synthase inhibitor, L-NAME, to make the model of endothelial dysfunction in diabetes complicated with other coronary risk factors such as hypertension or hyperlipidemia, and apocynin was investigated as the candidate treatment tool for diabetic vascular lesions.
Materials and Methods: Male OLETF (type II diabetic rats) and littermate LETO (28 weeks old) were divided into six groups. LETO was fed regular chow w/wo apocynin (Gp C, Gp C-apo). OLETF was fed regular chow w/wo apocynin (Gp DM, Gp DM-apo). OLETF was fed regular chow plus LNAME (NO synthase inhibitor) w/wo apocynin (Gp DMLN, Gp DMLN-apo). Five days after [maintenance under the above conditions, the peritoneal macrophages of the rats were stimulated with thioglycolate. Two days after the injection, the rats were evaluated. Plasma glucose and lipid levels were not changed throughout the experimental period. Intimal thickening was observed in aortae from Gp DM and Gp DMLN, however, there was little thickening in aortae from Gp DMLN-apo. Tone-related basal NO release and plasma NOx (sum of NO_2^- and NO_3^-) were higher in Gp C, C-apo, DM-apo, DMLN-apo than in Gp DM and Gp DMLN. Acetylcholine-induced NO-dependent relaxation was improved in Gp DMLN-apo compared with that in Gp DMLN(Max relaxation GpC: 72.5±3.8, Gp C-apo74.6 ±4.2 , GpDM 51.0±4.5*, GpDM-apo 63.2± 3.9, Gp DMLN 35.8 ±4.6* , Gp DMLN-apo 62.1± 5.1% (*P<0.05 vs GpC). The amount of superoxide anion released from peritoneal macrophages was increased in the DM groups and especially in Gp DMLN. However, it was decreased in Gp DM-apo and Gp DMLN-apo. 8-epi PGF2α was decreased and TNF α was increased in Gp DM, especially Gp DMLN, however they were decreased and restored to normal levels in Gp DM-apo and Gp DMLN-apo.
Conclusion: Apocynin retards the progression of diabetic angiopathy in OLETF rats without changing plasma glucose and lipid levels. NO and O2- may play a role in important underlining mechanisms by decreasing TNF α levels.

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Alpha lipoic acid reduces expression of hypoxia inducible factor Type 1 alpha in the sciatic nerve of streptozotocin induced diabetic rats.

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Background and Aims: Peripheral nerve damage leading to diabetic neuropathy is a pathological consequence of diabetes. The known association between peripheral nerve damage and oxidative stress mediated by high glucose concentrations has previously been demonstrated. Hypoxia inducible factor type 1 alpha is a transcription factor that is known to regulate angiogenic growth factors e.g. vascular endothelial growth factor and key glycolytic enzymes in conditions of low oxygen. We wished to determine if hypoxia inducible factor type 1 alpha was present in the sciatic nerve of diabetic rats and determine if the use of an anti-oxidant altered the expression of this transcription factor.
Materials and Methods: In this study, the sciatic nerve was removed from control rats, streptozotocin-diabetic rats and streptozotocin-diabetic rats that had been fed the anti-oxidant, alpha lipoic acid (300mg/kg). Samples were taken 3 months following induction of diabetes. Immunohistochemistry was carried out on cryopreserved sections of sciatic nerve using a specific monoclonal antibody for hypoxia inducible factor type 1 alpha and a polyclonal von Willebrand factor antibody. Each assay was carried out in triplicate with 6 animals per test group. The protein-antibody complex was visualised with an alkaline phosphatase detection system and analysed with microscopy.
Results: There was a noticeable increase in the level of hypoxia inducible factor type 1 alpha in the vasculature of the sciatic nerve of the diabetic rat. Control animals had either no staining or a low intensity of staining while 5/6 of the diabetic rats showed intense staining which co-localised with von Willebrand factor specific staining. Sciatic nerve samples from diabetic rats treated with alpha lipoic acid showed a marked decrease in the level of hypoxia inducible factor type 1 alpha in all of the animals examined (6/6) which were comparable to the control group.
Conclusion: The expression of hypoxia inducible factor type 1 alpha correlated with the increasing oxidative stress of the sciatic nerve in the diabetic rat and was reduced when an anti-oxidant was used. These data demonstrate that this transcription factor plays a key role in the response of the sciatic nerve to oxidative stress. Further studies to elucidate the precise mechanism of hypoxia inducible factor type 1 alpha expression will facilitate our understanding of the mechanism of diabetic neuropathy and associated vascular disease.

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Metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in microvascular complications of Type 2 diabetic patients: their relation to oxidative stress.

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Background and Aims: Matrix metalloproteinases (MMPs) are a family of secreted zinc proteases capable of degrading collagen and other matrix components. MMP-9 is one of the family that produced by inflammatory and stimulated connective tissue cells as that the naturally occurring inhibitor, the tissue inhibitor of metalloproteinase-1 (TIMP-1). Diabetes mellitus (DM) is known to be a primary risk factor for vascular complications. The increased risk of microvascular complications in type-2 DM may be attributed to many interrelated metabolic and vascular mechanisms. Over expression and the imbalance between MMP-9 and TIMP-1, in addition to their relationship to other immunological mediators and oxidative stress are expected to play a major role in endothelial damage. Our aim was to study the role of MMP-9 and TIMP-1 in microvascular complications of type-2 DM and their relation to oxidative stress: advanced oxidation protein products (AOPP) as a reliable marker of oxidant mediated protein damage and thiobarbituric acid reactive substances (TBARS) as a measure of lipid peroxidation.
Materials and Methods: The study included 39 type-2 diabetic patients categorized in 3 equal groups according to UAE into normo, micro and macroalbuminuric. In addition, 13 individuals of matched age and sex were selected to serve as control group. Serum MMP-9 and TIMP-1 were assayed by ELISA technique (Quantikine R&D;). Plasma AOPP was determined by an adopted semiautomated method (Witko-Sarsat et al;1996) expressed in µmol/L of chloramine-T equivalents (Sigma). The assay of plasma TBARS depends upon the reaction of TBA with malondialdehyde (MDA) and MDA like substances formed by lipid peroxidation process.
Results: Results revealed that serum MMP-9 concentrations were increased in the three studied diabetic groups than controls with higher values among the macro- then the micro- and lastly the normoalbuminuric group. The difference between the groups was statistically significant (X2(3)=18.283, p=<0.001). TIMP-1 showed statistical significant difference between groups ((X2 (3)=8.064, p=0.045). Diabetic microvascular complications were associated with more oxidative stress. The difference between the groups was statistically significant (AOPP: F=22.766, p=< 0.001 and TBARS: X2 (3)=21.936, p=< 0.001).There was a significant positive correlation between MMP-9 and both AOPP and TBARS only in diabetic group with macroalbuminuria (r=0.919, p=< 0.001 and r=0.569, p=0.042 respectively).
Conclusion: These data suggest that MMP-9 plays an important role in diabetic nephropathy and we can make use of the relationship to oxidative stress to provide a rationale of therapeutic strategies in order to modulate the progression of endothelial damage in type-2 diabetes.

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Serum extracellular superoxide dismutase in patients with Type 2 diabetes: relationship to the development of micro- and macrovascular complications.

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Background and Aims: Extracellular-superoxide dismutase (EC-SOD) is a secretory glycoprotein with an affinity for heparan-like substances, and is the principal enzymatic scavenger of superoxide in the extracellular space. The aim of this study was to determine the distribution of serum extracellular superoxide dismutase (EC-SOD) concentrations in patients with type 2 diabetes and to assess whether increased EC-SOD concentration is associated with the development of diabetic vascular complications.
Materials and Methods: Serum EC-SOD concentrations were determined in 222 patients with type 2 diabetes and in 75 healthy control subjects by an enzyme-linked immunosorbent assay (ELISA). The Arg213Gly genotype was analyzed by the polymerase chin reaction (PCR)-restriction fragment length polymorphism (RFLP) method described by Marklund et al.
Results: The serum EC-SOD concentrations showed a distinct bimodal distribution in both patients with diabetes and in control subjects. All subjects with the high-level phenotype carried the Arg213Gly mutation. The frequency of this variant was similar in the diabetes and control groups. Within the group of subjects with the common EC-SOD phenotype, the mean serum EC-SOD concentration was significantly higher in patients with type 2 diabetes (99.3 ± SEM 1.3 ng/ml) compared to the controls (68.4 ± 2.3 ng/ml, $p<0.01$). Stepwise multiple regression analysis of the data of diabetic common phenotype group showed a significant relationship between serum EC-SOD concentration and duration of diabetes ($F=5.31$), carotid artery intimal-media thickness ($F=8.24$), and severity of nephropathy ($F=16.05$) and retinopathy ($F=4.43$).
Conclusion: We observed a strong relationship between the serum concentration of EC-SOD and the severity of both microvascular and macrovascular diabetic complications. These findings suggest that serum EC-SOD concentration levels may be a marker of vascular injury, possibly, reflecting hyperglycemia induced oxidative injury to the vascular endothelium and decreased binding of EC-SOD to the vascular wall.

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Xanthine oxidase increases AP-1 levels in human cultured aortic smooth muscle: prevention by antioxidants.

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Background and Aims: Functional and remodelling alterations of the vessel wall represent one of the main complications of long-term diabetes mellitus Reactive oxygen species (ROS) seem to play a key role in such vascular alterations. Recently (Desco et al., Diabetes 51:1118, 2002), it has been shown that in diabetes the ROS-generating enzyme xanthine oxidase (XO) released by the liver can bind endothelial cells. Our aim was to analyse whether endothelium-bound XO can alter the behaviour of the underlying smooth muscle. In particular we studied growth-related mechanisms by determining the levels of the transcription factor AP-1.
Materials and Methods: Cultured human aortic smooth muscle cells (HASMC) were obtained by enzymatic dissociation from five organ donors. AP-1 levels were determined by Western blotting. XO-induced release of superoxide anions was determined in a cell free system by the cytochrome c reduction method, whereas intracellular superoxide anions were detected with the fluorescent probe hydroethidine.
Results: HASMC were stimulated with xanthine/xanthine oxidase (X/XO, 100 μM and 50 μU/ml, respectively) or 10% fetal calf serum (FCS) as positive control and AP-1 levels were measured after 1, 2 and 4 hours of treatment. X/XO maximally increased AP-1 levels at 2 hours (151.47±35.43% vs time 0), followed by a rapid decay. In the presence of FCS, AP-1 levels peaked at 1 hour (136.23±43.12% vs time 0) and remained constant over time. The release of ROS by X/XO was demonstrated by incubation of xanthine (100 μM) with growing concentrations of XO (50 - 500 μU/ml) in a cell free system, which resulted in a linear concentration-dependent increase of superoxide anions generation ($r= 0.98$). This effect was abolished by both superoxide dismutase (SOD, 200 U/ml) and allopurinol (100 μM). Furthermore, to confirm that upon stimulation with XO (50, 250, and 500 μU/ml), superoxide anions were found inside HAMSC, a concentration-dependent increase in intracellular fluorescence was observed from 1 hour after stimulation.Finally, to assess the role of ROS in XO-induced AP-1 increased levels, the experiments were performed in the presence of SOD (200 U/ml) and TEMPOL (1 μM), which can act as extracellular and intracellular scavengers of superoxide anions, respectively. Both agents prevented the stimulatory effect of XO on AP-1 levels after 2 hour of treatment.
Conclusion: In conclusion, XO enhances AP-1 levels in human cultured vascular smooth muscle, by a mechanism that directly involves the production of superoxide anions. We propose that increased oxidative stress due to enhanced vascular XO activity in diabetes can alter vascular smooth muscle growth. XO could therefore have a role in the development of diabetic vasculopathy, by acting through vascular remodelling processes.

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Cytochrome P4502E1 participate to the oxidative stress in the kidney of the diabetic rat. Antioxidant effects of taurine and N-acetylcysteine.

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Background and Aims: An increase of Reactive Oxigen Species (ROS) and a reduction of antioxidants have been described in diabetes, and participate to the evolution of diabetic nephropathy. Many pathways have been identified as superoxide anion producers, but their relevance to the oxidative stress in diabetes is still under debate. An increase of the Cytochrome P4502E1 (CYP2E1) has been described in diabetes, and can contribute to the generation of the superoxide anion. Taurine (T) is known as a scavenger of carboxyl radicals, N-acetylcysteine (NAC), a potent ROS scavenger, is also a precursor of both T and reduced glutathion (GSH). Aims: - to assess the possible relation between CYP2E1 and oxidative stress in the kidney of the diabetic rat; - to evaluate the effects of a T+NAC association on the CYP2E1 expression and on the oxidative stress.
Materials and Methods: Three groups of rats have been studied. Diabetic (D), diabetic treated with T+NAC (DTN), and control rats (C). The duration of diabetes was 4-8 months. The following parameters have been analyzed. CYP2E1 (by Western blot), Malonyldialdheyde (MDA, by HPLC); protein content of carbonyl groups (C-PROT), Superoxide dismutase (SOD) and Glutathione reductase activities (G-RED) by spectrophotometry. Fasting blood glucose (FBG) and HbA1 were also determined.
Results: The CYP2E1 significantly increases in the kidney of diabetic rats and is reverted to normal by the treatment with T+NAC (C:1977±1699, D:3778±2071, DTN:2460±1980 OD/mg of prot; $F=3.4$, $p=0.04$). The C-PROT also significantly increases in the kidney of diabetic rats and is reduced by the treatment with T+NAC (C:0.75±0.45, D 1.55±0.75, DTN:1.09±0.39 nmol/mg of prot; $F=9.5$, $p=0.0003$). MDA is not modified by diabetes nor by T+NAC treatment (C:31±22, D:31±16, DTN:30±17 pmol/mg of prot; $F=0.01$, n.s.). SOD activity is dramatically reduced in diabetes, and the T+NAC treatment does not restore it (C:261±156, D:48±22, DTN:43±21 nmol/mg of prot; $F=27.5$, $p:0.0001$). The G-RED activity increases in the kidney of diabetic rats, and even more in the T+NAC treated animals (C:530±189, D:723±279, DTN:1095±505 U/mg of prot; $F=12$, $p=0.0001$). The expression of CYP2E1 highly correlates with the C-PROT content in all the subjects ($r:0.54$, $p<0.001$), and also in the individual sub-groups (C $r:0.56$, $p<0.05$, D $r:0.49$, $p<0.05$, DTN $r:0.46$, $p<0.05$). HbA1 correlates with C-PROT ($r:0.32$, $p<0.05$), SOD ($r:-0.57$, $p<0.001$) and G-RED ($r:0.34$, $p<0.01$). FBG correlates with CYP2E1 ($r:0.28$, $p<0.05$), C-PROT ($r:0.45$, $p<0.001$) SOD ($r:-0.73$, $p<0.001$) and G-RED ($r:0.34$, $p<0.01$).
Conclusion: The expression of CYP2E1 is highly related to the oxidative stress through its relation with the oxidation of proteins (C-PROT). The treatment with T+NAC reduces both the CYP2E1 expression and the carbonyl stress probably increasing the efficiency of the glutathione pathway.

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Ferric iron and its complexes induce para-, meta-, orto-tyrosine formation from phenylalanine in the presence of hydrogen peroxide. Role of the superoxide free radical in the reaction.

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Background and Aims: Oxidative stress plays an important role in the pathogenesis of diabetic complications due to damaging effect of free radicals such as hydroxil free radical (OH). ˙OH can be produced in the Fenton reaction in the presence of ferrous iron (Fe^{2+}) and hydrogen peroxide (H_2O_2). However, in the living cell the redox-active iron is mainly present in ferric form. ˙OH can generate para-, meta-, orto-tyrosine (p-, m-, o- Tyr) from the essencial amino acid phenylalanine (Phe). In our experiments we planned to investigate in an in vitro model formation of Tyr due to ferric iron (Fe^{3+})-H_2O_2 reaction from Phe, the effect of different iron chelators and superoxide dismutase (SOD).
Materials and Methods: Amino acids were detected upon their autofluorescence (Tyr: Ex. 275nm, Em. 305nm and Phe: Ex. 258nm, Em. 288nm).With a fluorescent spectophotometric method we measured the total Tyr production due to Fe^{3+}, Fe^{3+}-EDTA, Fe^{3+}-citrate complexes and H_2O_2. Using fluorescent HPLC we measured the production of the three Tyr isomers and consumption of phenylalanine. We also examined the effect of SOD.
Results: Fluorescent photometric data: In our experiments 100, 50, 25 μM Fe^{3+} caused a concentration-dependent increase in relative fluorescence (736±58 %, 430±43 %, 219±10 %, vs. baseline) in the total Tyr production from Phe in the presence of H_2O_2, however H_2O_2 alone had only a slight effect (147±1 %). Fe^{3+}-citrate + H_2O_2 increased Tyr production (2473±103 %, $p<0.001$ vs. Fe^{3+}+ H_2O_2). Addition of different concentrations of SOD (1.62, 6.25, 25, 100 U/ml) decreased the Tyr production (63±6.5, n.s.; 50±6.7, n.s.; 33±1.8, $p<0.01$; 27±2.3 %, $p<0.01$ vs. control), while 50 minute heating of the SOD (100 U/ml) prevented its effect (103±6.1 %, p=1.000 vs. control). Fluorescent HPLC data: We found that in comparison to Fe^{3+} (p-Tyr, 5.06±0.18; m-Tyr, 5.69±0.12; o-Tyr, 7.41±0.13 μM), Fe^{3+}-citrate increased (12±0.46, 13.41±0.54, 17.73±0.72 μM, $p<0.005$ for all vs. Fe^{3+}), Fe^{3+}-ATP decreased (0.18±0.04, 0.20±0.05, 0.28±0.05 μM, $p<0.001$ for all vs. Fe^{3+}), Fe^{3+}-EDTA decreased (0.99±0.09, 1.06±0.08, 1.42±0.09 μM, $p<0.001$ for all vs. Fe^{3+}) the p-, m- and o-Tyr production in the presence of H_2O_2. Phe consumption due to ˙OH was increased in Fe^{3+}-citrate medium compared to the Fe^{3+} (16.67±1.76 vs. 2.33±1.20 μM, $p<0.01$).
Conclusion: Summarizing, we found that ferric iron and H_2O_2 were able to produce p-, m-, o- Tyr from Phe. Iron chelators ATP and EDTA decreased, while citrate increased Tyr formation from Phe. Addition of SOD decreased Tyr formation, while its heating prevented its effect. Thus, our data support that superoxide free radical plays an important role in the reaction. Pathophysiologic role of this ˙OH producing pathway needs to be further investigated.

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Effects of hyperglycaemia on F_2-isoprostanes in Type 2 diabetes mellitus.

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Background and Aims: There is still no consensus regarding the link between hyperglycaemia and diabetic complications. Recently, oxidative stress (OS) has been a subject of a great interest, however, the results of different studies evaluating this pathogenic pathway have shown inconclusive results probably related to the lack of specificity and sensitivity of the current methods available. Nowadays, isoprostane measurement appears the best marker of OS available in vivo, thus the aim of this study was designed to evaluate the effect of hyperglycaemia on 8-epi-$PGF_2\alpha$ (as an integrated index of F_2-isoprostane production) in Type 2 diabetes mellitus
Materials and Methods: We studied 12 type 2 diabetic patients (4 males and 8 females) with a mean age of 58.7 ± 8.2 years, evolution of disease of 11.7 ± 5.2, body mass index 27.0 ± 5.2 admitted to the Clinic Hospital for starting insulin therapy due to secondary failure to hypoglycaemic agents. Twelve age-matched non diabetic subjects (5 males and 7 females) with a mean age of 59.0 ± 6.1 were also studied as the control group. Current smokers and patients with evidence of micro-macrovascular disease were excluded from the study. Before starting insulin therapy all diabetic patients and control subjects performed a 24-h urinary collection to determine 8-epi-$PGF_2\alpha$, creatinine and albumin excretion. Fasting blood sample was also obtained to measure glucose, HbA_{1c} and lipid profile. In the diabetic patients all the above mentioned parameters were also evaluated 12 weeks after starting insulin therapy, when glycemic control improved
Results: F_2-isoprostanes were measured using mass spectometry. 8-epi-$PGF_2\alpha$ excretion in diabetic patients showed higher levels than control subjects (67.7 ± 29.8 vs. 37.3 ± 11.8 $p < 0.05$). Insulin therapy induced a significant reduction in the mean HbA_{1c} level at 12 weeks after insulinization (10.3 ± 1.4 vs. 7.3 ± 0.9 %, $p < 0.001$), and this improved glycemic control was associated with a 24% of reduction in the 8-epi-$PGF_2\alpha$ excretion levels at 12 weeks (89.1 ± 39.3 vs. 67.7 ± 29.8 p =0.07).
Conclusion: OS is higher in Type 2 diabetic patients without evidence of diabetic complications than matched control subjects. Glycemic control reduced the OS, but normalisation was not achieved, indicating continued oxidant injury despite optimal control of the diabetes.

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Evidence of oxidative stress in diabetic mice: differences between two diabetic models and favorable anti-oxidative effect of rice bran extract.

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Background and Aims: Recently, oxidative stress is considered to be a key factor in the development and complications of diabetes. On the other hand, rice bran is known to contain large number of active components that have contributed to the maintenance of health for Japanese people. And there is possibility that rice bran extract has favorable anti-oxidative effect. The aim of present study was to investigate oxidative damage in diabetic mice and anti-oxidative effect of rice bran extract. Ferulic Acid and Ricetrienol (crude lipophilic extract containing alpha-tocopherol, tocotrienol et al) were used as a represent of rice bran extract.
Materials and Methods: As a model of type 1 diabetes, streptozotocin-induced diabetic mice (STZ mice) and as a model of type 2 diabetes, KKAy mice were used. STZ group was divided into three sub- groups (STZ mice with normal diet, with diet including 0.3% Ferulic Acid and non-diabetic mice with normal diet). In the same way, KKAy group was divided into three sub-groups too (KKAy mice with normal diet, with diet including 0.1% Ricetrienol and non-diabetic C52BL mice with normal diet). After six weeks of diabetes, body weight, plasma glucose, HbA1c were measured. To investigate oxidative damage, plasma malonydialdehyde (MDA), 8-isoprostane and 8-OHdG in the urine were measured. Furthermore, the mRNA expressions of antioxidant isoenzymes [superoxide dismutase (SOD) and glutathione peroxidase (GPx)] in the kidney were quantified using real-time PCR method.
Results: In STZ group, STZ mice demonstrated severe high plasma glucose, low body weight and low lipidemia. 8-isoprostane and 8-OHdG were significantly increased compared with controls and this was suppressed by 0.3%Ferulic Acid. The mRNA expression of SOD and GPx remained unchanged in all three sub-groups. In KKAy group, KKAy mice demonstrated moderate high plasma glucose, high body weight and hyperlipidemia. MDA, 8-isoprostane and 8-OHdG were significantly increased compared with controls and this was suppressed by 0.1%Ricetrienol. The mRNA expression of SOD and GPx were increased compared with controls too. And 0.1%Ricetrienol decreased SOD expression and increased GPx expression. Average of HbA1c in STZ group was higher than that in KKAy group.
Conclusion: Oxidative stress in diabetic mice was confirmed. And rice bran extract (Ferulic Acid and Ricetrienol) protected oxidative damage. There was clear difference in the response of antioxidant enzymes to oxidative stress between two diabetic models. This difference supposed to come from the difference of diabetic severity.

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AGE accumulation is poorly reflected by long term metabolic control in Type 2 diabetic patients.

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Background and Aims: In a DCCT-substudy, collagen AGE (advanced glycation endproducts) accumulation in skin biopsies explained an unexpectedly high percentage of variance in the incidence of diabetic complications, also after adjustment for HbA1c levels. They further demonstrated that accumulation of different AGE was poorly predicted by HbA1c levels over several different time periods. In our study, we related skin AGE levels, measured with a validated non-invasive autofluorescence method, to HbA1c values of a period of four years prior to the AGE measurement in a population of type 2 diabetic patients to study whether the above conclusions are similar in type 2 diabetes mellitus.
Materials and Methods: Skin AGE-levels were assessed non-invasively by quantification of skin-autofluorescence. Autofluorescence (AFr) of the skin was performed by illumination of the lower arm with a fluorescent tube (peak intensity ~365 nm) and was calculated by correcting the mean of intensities of the emission light (420-600 nm) for the amount of reflected excitation light in the range 300-420 nm. Type 2 diabetic patients of a follow-up study in a shared-care setting, that participated in all yearly screenings during the last four years and who had a skin autofluorescent measurement in the fourth year, were included in this analysis.
Results: 451 patients (male 202, mean age 68.3 years, median known diabetes duration 7.6 years), had mean HbA1c (5th, 95th percentile) in the four years of, respectively 7.3 (5.8, 9.4), 7.4 (5.8, 9.6), 7.3 (5.7, 9.5) and 7.2(5.6, 9.6)%. Table 1 shows the amount of variance in AFr which could be explained by different HbA1c on different dates and by the variation in HbA1c levels.

Table 1.

AFr versus *(adjusted for age)*	R^2 (%)	P
Mean HbA1c up to AFR-measurement (=over 4 years)	3.4	<0.001
Mean HbA1c over the past year	4.2	<0.001
HbA1c nearest to AFR-measurement (T=0)	3.7	<0.001
HbA1c (T= -4years)	1.4	<0.006
Variance of HbA1c (over 4 years)	3.2	<0.001
Variance of HbA1c (over 4 years) *(adjusted for age and mean HbA1c)*	1.4	<0.008

Conclusion: In this population of Type 2 diabetic patients, the contribution of cumulative and fluctuating HbA1c to AGE accumulation expressed as AFr is rather low. The poor relation between HbA1c and AGE in this group is in agreement with the results of the DCCT in type 1 diabetic patients. AGE accumulation might reflect other mechanisms such as oxidative stress. Follow-up studies will show whether this new marker will be more useful in predicting diabetic complications than HbA1c in type 2 diabetes mellitus.

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Association between serum concentrations of advanced glycation end products and C-reactive protein in Type 2 diabetes mellitus.

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Background and Aims: Recent studies have consistently shown that markers of inflammation are elevated in patients with type 2 diabetes but the causal mechanisms are not well understood. Since in vitro studies have demonstrated that advanced glycation end products (AGEs) can trigger inflammatory responses, we have determined whether serum concentration of advanced glycation end products is an important determinant of inflammatory marker like C-reactive protein (CRP) level in patients with type 2 diabetes.
Materials and Methods: 204 patients with type 2 diabetes mellitus and 82 healthy non-diabetic controls of similar body mass index (BMI) were recruited. Serum AGEs were assayed by competitive ELISA using a polyclonal rabbit antisera raised against AGE-RNase. Plasma high sensitivity CRP was measured by an immunoturbidimetric assay and interlukin-6 (IL-6) by ELISA.
Results: Serum AGEs were increased in diabetic patients compared to controls (4.24 ± 0.88 unit/ml vs 3.20 ± 0.78 respectively, mean ± SD, $p<0.01$). Both plasma CRP [1.55 (0.81 - 2.95) mg/l vs 0.85 (0.43 - 1.67) respectively, median (interquartile range), $p<0.01$] and IL-6 [0.80 (0.68 - 0.97) pg/ml vs 0.61 (0.44 - 0.81) respectively, $p<0.01$] were also elevated. In the diabetic patients, log(CRP) correlated with AGEs ($r = 0.22$, $p = 0.002$) and with log (IL-6) ($r = 0.29$, $p<0.001$). Forward stepwise linear regression analysis using age, gender, BMI, smoking, AGEs and log(IL-6) showed that only BMI, log(IL-6) and AGEs were significant independent determinants of log(CRP).
Conclusion: Serum concentration of AGEs is increased in patients with diabetes and is an independent determinant of plasma CRP levels. Subclinical inflammation in patients with diabetes may therefore be partly due to activation of the inflammatory response by AGEs.
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Muscle protein is protected from increased chemical modification in diabetic rats.

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Background and Aims: Although muscle is a major site of glucose disposal, little information is available on whether muscle protein is damaged by increased chemical modification in diabetes. Our aim was to measure the effect of long-term diabetes on both glycation and advanced glycation and lipoxidation end-products (AGE/ALEs) on muscle protein.
Materials and Methods: Female Sprague-Dawley rats were made diabetic with streptozotocin (DB, n = 8) at 6 weeks of age; blood glucose was maintained at ~24 mM by insulin injections, compared to 5.5 mM in age-matched, non-diabetic animals (ND, n = 8). Animals were sacrificed after 7 months, and gastrocnemius muscles were isolated. Isotope dilution mass spectrometry was used to quantify glycation (fructoselysine, FL), the AGE/ALEs N^{ε}-(carboxymethyl)- and (carboxyethyl)-lysines (CML, CEL), the ALE, malondialdehyde-lysine (MDA-Lys), as well as a novel cysteine derivative, S-(carboxymethyl)cysteine (CMC), in total muscle protein and in myofibrils, and in insoluble skin collagen.
Results: For ND rats, FL in both total muscle and myofibrils was ~ 0.2 mmol FL/mol Lys and increased to ~0.6 mmol FL/mol Lys in DB rats. This 3-fold increase was less than the 4.4 fold increase in average plasma glucose in DB animals, and even lower than the 5-fold increase in glycation of long-lived skin collagen (4.4 vs. 23.2 mmol FL/mol Lys, ND vs. DB). CML and CMC were each ~0.02 mmol/mol Lys in myofibrils, about twice the levels in total muscle protein. Both adducts increased in these fractions by 40-100%, $p \leq 0.025$ in DB compared to ND muscle. In contrast, levels of CML and CMC were significantly higher in ND skin collagen (~0.06 and 0.04 mol/mol Lys) and increased ~4 fold and 2 fold in DB rats (0.25 and 0.09 mmol/mol Lys), respectively. CEL and MDA-Lys were lower in myofibrils (0.01 mmol CEL, 0.007 mmol MDA-Lys /mol Lys, respectively) compared to total muscle protein (0.03 mmol CEL, 0.014 MDA-Lys/mol Lys), and were essentially unchanged in diabetes. CEL was increased 5-fold, to 0.128 mmol/mol Lys in DB skin collagen; MDA-Lys was not detectable in ND collagen but averaged 0.01 mmol/mol Lys in DB collagen.
Conclusion: Muscle protein is modified by glycation, glycoxidation and lipoxidation reactions. The distribution of adducts in diabetic muscle was variable, showing similar FL, higher CML and CMC, and lower CEL and MDA-Lys in total muscle protein vs. myofibrils. These results indicate that: 1) glycation and AGE/ALE formation proceed inside myocytes; 2) glycation and AGE/ALE formation were lower in ND muscle protein, compared to skin collagen; and 3) compared to skin collagen, muscle protein is protected from increased chemical modification by glycation and AGE/ALE formation in diabetes. Our data suggest that muscle protein is protected from chemical modification by glycation and AGE/ALE

formation under normal conditions, and that impaired glucose uptake limits protein damage in diabetic muscle. Other tissues that are insulin-dependent for glucose uptake may also be spared from damage by glycation and AGE/ALE formation in diabetes.

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High dose thiamine therapy suppresses the accumulation of advanced glycation endproducts and oxidative biomarkers in the glomeruli of streptozotocin-induced diabetic rats on insulin maintenance therapy.

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Background and Aims: Accumulation of advanced glycation endproducts (AGEs) and oxidative stress has been implicated in the development of diabetic nephropathy. Accumulation of triosephosphates in glomerular endothelial cells and pericytes may be linked to increased AGE formation and oxidative stress. In this case, High dose thiamine therapy may counter these processes and suppress AGE accumulation and oxidative damage. The aim of this study was to determine the effect of high dose thiamine on the accumulation of AGEs and oxidative biomarkers (methionine sulfoxide MetSO and dityrosine Dityr) in streptozotocin (STZ) induced diabetic rats on insulin maintenance therapy.
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 stabilized by injection s.c. of 2 U of Ultralente insulin every 2 days. Thiamine was given orally, mixed with the chow over 24 weeks. Study groups were: controls (C), controls + 70 mg/kg/day thiamine (C+T), diabetic (D), diabetic + 7 mg/kg/day thiamine (D+T7) and diabetic + 70 mg/kg/day thiamine (D+T70); n = 8 in each study group. Protein glycation and oxidation biomarkers were assayed in enzymatic digests of cytosolic glomerular protein by LC-MS/MS with stable isotope-substituted standard calibration. Biomarkers assayed were: fructosyl-lysine (FL), hydroimidazolones derived from methylglyoxal, glyoxal and 3-deoxyglucosone, MG-H1, G-H1, 3DG-H1, N_{ε}-carboxymethyl-lysine (CML), N_{ε}-carboxymethyl-lysine (CEL), MetSO and Dityr. Statistical analysis: P and P', is the significance with respect to normal controls and diabetic controls, respectively (*t*-test).
Results: Plasma glucose concentration was increased 3-fold and HbA_1 increased one-fold in STZ diabetic rats, respectively (P<0.001); neither were decreased significantly by thiamine. In normal controls, biomarker concentrations were (pmol/mg protein, mean ± SEM): FL 157 ± 21, MG-H1 55 ± 14, CEL 15 ± 4, G-H1 22 ± 6, CML 135 ± 25, 3DG-H1 16 ± 4, MetSO 1486 ± 343 and Dityr 27 ± 6. All these glycation and oxidation biomarkers were increased in glomerular protein of diabetic rats: FL 168% (P<0.001), MG-H1 558% (P<0.001), CEL 316% (P<0.001), G-H1 128% (P<0.05), CML 37% (P<0.05), 3DG-H1 333% (P<0.001), MetSO 147% (P<0.01) and Dityr 98% (P<0.05). Thiamine therapy decreased the AGE biomarkers significantly in the diabetic rats but not in the controls – excepting G-H1 was not decreased significantly; the increased glomerular levels of MG-H1, CEL and 3DG were decreased to control levels. FL was not decreased significantly by thiamine therapy. MetSO and Dityr were also decreased to control levels by thiamine. High dose thiamine therapy prevented the development of incipient nephropathy, as judged by microalbuminuria, under these conditions.
Conclusion: AGEs and oxidative biomarkers accumulated markedly in glomerular protein of STZ diabetic rats. AGE accumulation – particularly derived from dicarbonyl compounds – and oxidative damage may contribute to the development of diabetic nephropathy and high dose thiamine prevents this.

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N-(Carboxymethyl)valine adduct in hemoglobin (CMV-Hb): a new marker reflecting accumulation of oxidative stress.

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Background and Aims: Hyperglycemia may induce reactive oxygen species (ROS) production, which may be closely associated with diabetic complications. However, current markers of ROS such as urinary 8-OH-2'-deoxyguanosine or 8-iso-PGF2α showing short-term oxidative stress. To evaluate whether N-(Carboxymethyl)valine adduct in hemoglobin (CMV-Hb), a kind of advanced glycation end products (AGE), may be a useful marker reflecting accumulation of ROS for longer period, we evaluated characteristics of CMV-Hb in vitro and in vivo.
Materials and Methods: Purified hemoglobin was incubated at 37 °C for 2-4 wk with glucose (0-500 mg/dl) and/or hydrogen peroxide (H_2O_2, 0-1000 μM), and in vitro formation of CMV-Hb were evaluated. For in vivo study, 1,163 type 2 diabetic patients and 486 healthy non-diabetic subjects were measured HbA1c and CMV-Hb extracted from peripheral red blood cells by HPLC and a latex-immunoassay using specific monoclonal antibody for CMV-Hb which we made, respectively, and cross-sectional analysis was done. Furthermore, 15 diabetic patients showing remarkably higher CMV-Hb level were extracted from the total diabetic group, and evaluated the effect of 6 months treatment of anti-oxidant agents on CMV-Hb level.
Results: In vitro CMV-Hb increased in glucose dependent manner for 4 wk (15.7-44.8 pmolCMV/mgHb) and in H_2O_2 dependent manner for 2 wk (15.8-21.3). Co-incubation of glucose and H_2O_2 for 2 wk additively formed CMV-Hb (30.0-45.5). CMV-Hb levels from peripheral blood cells in the diabetic patients were significantly higher than those in the non-diabetic control subjects (22.4±7.9 vs. 14.6±3.1 pmolCMV/mgHb, P<0.001). CMV-Hb level was associated with serum creatinine but not with HbA1c from multiple regression analysis. Levels of CMV-Hb of 15 diabetic patients significantly decreased by 6 months anti-oxidant agents (from 34.1±3.3 to 30.3±3.8, P<0.01), while HbA1c did not change during the period.
Conclusion: These results demonstrate that CMV-Hb may be formed by hyperglycemia and/or reactive oxygen species and this may be a new clinical marker reflecting accumulation of oxidative stress in diabetic subjects.

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AGE mediated atherosclerosis does not involve direct stimulation of vascular smooth muscle cells.

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Background and Aims: The prevalence of diabetes mellitus is increasing worldwide. In Australia, 7.6% of adults are affected. Individuals with diabetes are at least 2-4 times more likely to suffer cardiovascular disease for reasons that are largely unknown. Advanced Glycation End Products (AGEs) are a heterogeneous group of advanced products of the Maillard reaction. AGEs accumulate *in vivo*, as a consequence of aging and at an accelerated rate in the setting of diabetes. The potential role of a direct action of AGEs on vascular smooth muscle cells in initiating the atherosclerotic cascade was investigated.
Materials and Methods: Several AGEs were constructed to mimic those formed *in vivo*. BSA-AGE was formed by a 60 day incubation of BSA with glucose. N^{e}-(carboxymethyl)lysines (CML) with two degrees of glycation were prepared by 24 hour incubation of BSA with sodium cyanoborohydride and glyoxylic acid. Constructed AGEs were characterised fully to determine protein content, level of free amino groups as a measure of glycation, fluorescence and size (SDS-PAGE). Human vascular smooth muscle cells were characterised by immunohistochemical analyses for smooth muscle α-actin and receptor for AGE (RAGE). RAGE was also detected by Western blotting. The effects of AGEs on glucose consumption, *de novo* protein synthesis and proteoglycan biosynthesis in human vascular smooth muscle cells were investigated in a normoglycemic environment (5mM glucose) and under high glucose conditions (25mM) as found in the diabetic state.
Results: Glucose consumption was not significantly altered in the presence of BSA-AGE (1-100μg/mL) or CML (30-100μg/mL) in low or high glucose but was increased by TGF-β as a positive control. Protein synthesis was increased in low glucose conditions in the presence of least and most glycated CML (100μg/mL) and BSA-AGE (100μg/mL), 25%(p<0.01), 28%(p<0.01) and 19%(p<0.01) respectively, relative to basal control. However, BSA control proteins also resulted in significant stimulation (20% and 23%, p<0.01), rendering stimulation by AGEs not due to protein glycation and therefore not significant (p>0.05). In both low and high glucose conditions, proteoglycan synthesis was stimulated 73% and 68% by least and most glycated CML (100μg/mL) respectively. Again BSA control protein showed significant stimulation (56%), demonstrating no significant stimulatory effect due to glycation.
Conclusion: Many cell types are involved in atherogenesis and the direct action of AGEs on vascular smooth muscle cells may not be a critical mechanism for AGE-mediated atherogenesis. The actions of AGEs on other critical cells may be more important.

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Skin-collagen pentosidine and fluorescence at 370/440nm as markers of diabetes duration and complications in a French population.

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Background and Aims: Collagen advanced glycoxidation end products (AGEs), modifying physico-chemical properties of the protein, are expected to be associated with microvascular complications in diabetes. Therefore we tested pentosidine (P) and fluorescence intensity at 370/440nm (F) in skin collagen as markers of complications in a cohort of french diabetics (D) and age-and sex-matched normal controls (NC).
Materials and Methods: Subjects: 30 D, 15 type-1 D [D1; mean age=37.6y, range (26-57); mean diabetes duration: 14.5y (2.5-31); mean HbA1c 9%,(6-15.3)], 15 type-2 D [D2; mean age=51.9y (35-62); diabetes duration: 8.2y (0.1-23); HbA1c 8.1% (6.2-12.5)] and NC were studied after written consent with approval by the hospital ethics comitee CCPPRB. Renal insufficiency (creatinine clearance < 60 ml/min) was an exclusion criterium. Methods: Skin punch-biopsy (4mm diameter) was carried out from the buttock, the specimen soaked in saline for 1h, then frozen in liquid nitrogen. Collagen was extracted, F, P and hydroxyproline (H) measured according to Monnier et al. (Methods in aging research, 1999, CRC Press).
Results: In NC, P/H was correlated with age (p=0.05), but F/H was not. In D neither P/H nor F/H were correlated with age. Paired student test showed extremely significant differences between D and NC for P/H (p=0.0014) and F/H (p=0.0001). P/H was correlated with F/H more strongly in D (p<0.0001) than in NC (p=0.066). Neither P/H nor F/H were correlated with HbA1c percentage measured at the time of biopsy. In D, P/H was more tightly correlated with diabetes duration (p=0.017) than was F/H (p=0.040). However F/H was significantly correlated with retinopathy score in D (p=0.023) whereas P/H was not. F/H was also correlated with microalbuminuria, but only in D1, at the limit of significance (p=0.062), whereas P/H was not.
Discussion and Conclusion: The skin-collagen parameter F/H was correlated with retinopathy score in D much better than HbA1c was (p=0.086 vs 0.023). Besides it was correlated with microalbuminuria in D1 slightly better than HbA1c was (p=0.073 vs 0.062). Our data are in agreement with the report of Sell et al concerning a D1 american population which appeared during the course of this work. Skin-collagen pentosidine and fluorescence appear to be useful as markers of diabetes duration and also renal and mainly retinal microangiopathy.

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Structure-activity relationships of Pyridorin™ and related novel compounds in relation to their mechanism of AGE inhibition.

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Background and Aims: Pyridorin™ (pyridoxamine dihydrochloride, PM), is a powerful inhibitor of advanced glycation end-product (AGE) formation, particularly those that arise from the breakdown of glycated proteins via the Amadori pathway. Currently, PM is progressing through phase 2 clinical trials of diabetic nephropathy, having previously demonstrated marked efficacy in several animal models of type 1 and type 2 diabetic nephropathy. PM has recently also shown efficacy in preclinical models of diabetic retinopathy and neuropathy. We present here in vitro studies designed to elucidate the structure-activity relationships (SAR) of PM's inhibition of AGE formation.
Materials and Methods: Two methods were used to measure the inhibition of AGE formation using anti-AGE ELISA detection. Four sites of modification on the PM molecule (the hetero groups) were investigated: 4'-aminomethyl, 3-phenol, pyridine-N, and 5'-hydroxymethyl. We synthesized derivatives to explore the importance of each functional group using the strategies: 1) "loss of function," (analogs missing each functional group); 2) "single function," (analogs containing only one functional group at a time); 3) sensitivity to chemical modifications, such as alkylation; and 4) modulation of the pK of ionizing groups by changing substituents.
Results: We found that: 1) single-function analogs are inactive; 2) bifunctional analogs combining the 4'-aminomethyl nitrogen (substituted or unsubstituted) and the 3-phenol are the most active; 3) the pyridine-N is not critical but greatly facilitates the activity by modulating the acidity of the phenolic group; 4) the 5'-hydroxymethyl is not essential.
Conclusion: These findings provide critical insights into the mechanism of inhibition of post-Amadori glycoxidation by PM and related compounds. The bifunctional requirement implicates binding of redox metal ions, but not simple chelation, in the activity. These results thus establish a basis for the de novo design and lead optimization of the second-generation of AGE inhibitors that may display greater potency and efficacy for the treatment of diabetic complications.

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Pyridoxamine inhibits chemical modification of proteins by lipids in obese and diabetic rats: mechanism of action of pyridoxamine.

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Background and Aims: Increased chemical modification of proteins by advanced glycation and lipoxidation end-products (AGEs/ALEs) is implicated in the development of diabetic complications. In previous work, we have shown that pyridoxamine (PM), an inhibitor of AGE/ALE formation, inhibits the formation of AGE/ALEs in skin collagen and development of nephropathy in diabetic and obese rats. We have also shown that PM blocks the chemical modification of proteins during lipid peroxidation reactions *in vitro*, and have identified a number of PM adducts formed in these reactions. In this study we have examined the mechanism of action of PM by characterizing PM adducts in the urine of PM-treated control, obese and diabetic rats.
Materials and Methods: PM (1-2 g/L in drinking water) was administered to groups (n = 10-12) of control and streptozotocin-induced diabetic Sprague-Dawley rats, to lean (+/*fa*) and Zucker obese (*fa/fa*) rats, and to Zucker diabetic fatty (ZDF) rats for 6-7 months. PM derivatives were identified in urine by multiple reaction monitoring LC/MS/MS and quantified by internal standardization with N-(d_{11}-hexanoyl)-PM.
Results: Six PM adducts were detected in urine of PM-treated rats: *N*-formyl-PM (FAPM) which is formed *in vitro* from peroxidizing linoleic (LA) and arachidonic (AA) acids; *N*-hexanoyl-PM (HAPM), derived from the ω-terminus of ω-6 fatty acids; *N*-nonanedioyl-PM (NDAPM), derived from the carboxyl terminus of LA; *N*-pentanedioyl-PM (PDAPM), derived from the carboxyl terminus of AA; and *N*-pyrrolo-PM (PyPM) and *N*-(2-formyl)-pyrrolo-PM (FPyPM), derived from AA. Levels of PM-adducts in urine of PM-treated, diabetic animals were 5-10 fold higher than in PM-treated controls. FAPM was the major adduct present in urine of non-diabetic and streptozotocin-diabetic Sprague-Dawley rats (~25 and 130 nmol/24 hr), while PDAPM, formed on oxidation of arachidonate, was the major adduct in urine of Zucker lean and obese, and ZDF rats (~10, 70, and 100 nmol/24 hr). With the exception of FAPM, all are formed exclusively from intermediates in lipid peroxidation and were also detected in lipid peroxidation reactions with LA and/or AA *in vitro*.
Conclusion: We have identified specific, lipid-derived adducts trapped by the AGE/ALE inhibitor PM *in vitro* and excreted in urine of PM-treated rats. We conclude that PM protects against nephropathy in hyperlipidemic, diabetic and obese animals, both by reducing plasma lipids and by trapping intermediates in advanced lipoxidation reactions. We also propose a mechanism of action of PM involving cleavage of α-dicarbonyl intermediates in AGE/ALE formation.

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Nerve growth facilitates the healing of diabetic wounds by stimulating angiogenesis and inhibiting apoptosis.

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Background and Aims: We recently discovered that neural polypeptide NGF is pro-angiogenic and promotes tissue healing in a mouse model of limb ischemia, thus providing the first evidence of a neural drive for reparative angiogenesis. Since impaired neovascularization compromises wound healing in diabetes, we evaluated if NGF facilitates repair of diabetic wounds by improving endothelial cell (EC) biology.
Materials and Methods: Two full-thickness 4-mm wide skin wounds were produced in the interscapular region of streptozotocin-induced diabetic mice, using a disposable skin punch equipment. Starting immediately after punching and continuing through the following 3 days, NGF (1 μg in 20 μL PBS) was daily applied onto the right-sided ulcer, while an equal volume of vehicle was added onto contralateral wound (n=19 mice). Control mice received PBS on both sides. Wound closure was monitored until 14 days. Histological examination was performed at sacrifice. Satistical analysis was performed by ANOVA.
Results: NFG administration accelerated healing rate of cutaneous wounds ($P<0.001$) and enhanced capillarization by 2.5 and 2.0 times at 3 and 14 days, respectively ($P<0.01$). These effects were associated to a 3-fold increase in EC proliferation rate ($P<0.01$) and 2-fold inhibition of EC apoptosis ($P<0.05$). The NGF low affinity p75 receptor, previously recognized to be pro-apoptotic, was significantly downregulated at EC level following treatment ($P<0.05$). Quantitative RT-PCR analysis documented an 8.9-fold increase in vascular endothelial growth factor (VEGF) expression in NGF-treated wounds ($P<0.05$).
Conclusion: These results indicate that NGF exerts a curative action on diabetic wounds by facilitating vascular regeneration and suppressing apoptosis, possibly through a VEGF-mediated mechanism. These discoveries provide mechanistic explanation for the curative effect of NGF and support application of the polypeptide for the treatment of non-healing diabetic wounds.

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Advanced glycation end products (AGEs) augment production of vascular endothelial growth factor (VEGF) and tumor necrosis factor alpha (TNFα) in human monocyte-derived macrophages.

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Background and Aims: Diabetes-induced changes in immune cell function produce an inflammatory cell phenotype,associated with the upregulation of pro-inflammatory cytokines and downregulation of growth factors from macrophages. It is also becoming evident that diabetes results in the increased expression of angiogenic growth factors, particularly vascular endothelial growth family of proteins (VEGF). Glucose binds to protein amino residues and forms early glycation products such as Schiff base and Amadori products. The final Maillard reaction leading to the production of *advanced glycation end products (AGE*) dependents on plasma glucose concentration. AGE are now thought to contribute to the development of chronic vascular dysfunction, including the complications of diabetes. Macrophages/monocyte receptor for AGE moieties mediates the uptake of AGE-modified proteins by a process that also induces tumor necrosis factor α (TNF α).
Aim: To determine VEGF and TNF production in human monocyte-derived macrophages stimulated with glycated BSA and HSA using different methods of glycation *in vitro*.
Materials and Methods: BSA or HSA were incubated with D-glucose in PBS or sodium phosphate buffer at 37°C, for different time periods. Alternatively, BSA was glycated by adding sodium cyanoborohydride in sodium phosphate buffer. Glyoxylic acid was added to this solution and the mixture was incubated for 24 hours at 37°C, yielding Nε-(carboxymethyl)-lysine (CML)-BSA. Human elutriation-derived macrophages/monocyte were stimulated with glycated BSA or HSA for 24 hours.
Results: VEGF production by monocytes was clearly seen in supernatants from cells stimulated with extensively glycated HSA, relative to HSA incubated in the absence of glucose (411±138pg/ml *versus* 60±11pg/ml; $p<0.001$). TNFα production was also significantly augmented in cultured human monocyte-derived macrophages stimulated with glycated HSA, with the highest level of TNFα production observed at 6 hours. Similarly, TNFα was induced by BSA-CML, although VEGF production was observed only with 9.5mg/ml BSA-CML; $p<0.001$).
Conclusion: Our results suggest an important role for AGE in the stimulation of the development of angiogenesis observed in diabetic complications.
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Glucose intake by normal human subjects induces nuclear factor-κ B (NF-κ B) binding to several NF-κ B binding sites in the Tumor Necrosis Factor (TNF)-α promoter region and transcription of the TNF-α gene.

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Many inflammatory cytokines, chemokines, tissue factor, and matrix metalloproteinases require transcriptional regulation by NF-κB. The promoter region of the TNF-α gene contains several NF-kB binding sites: κB1, κB2, κB2α, κB3. We have previously demonstrated a significant increase in reactive oxygen species (ROS) generation and NF-κB binding using the general NF-κB binding consensus sequence and nuclear fractions from peripheral blood mononuclear cells (MNC) of human subjects given a glucose challenge (75 grams). In the present study we examined whether the ingestion of 75 grams of glucose (dissolved in 300 ml of water) over 10 minutes by six normal subjects induced NF- κB binding to the aforementioned NF-κB binding sites of the TNFα promoter region. Blood samples were obtained prior to and at 1, 2, and 3 hours after glucose intake. MNC were isolated by differential centrifugation. Nuclear fractions were obtained from MNC and NF-κB binding was measured by the electromobility shift assay (EMSA). There was a significant increase in binding activity over the basal for three out of the four NF-κB binding sites: κB1 binding activity at 2 hour (15.03 ± 4.08%, $p<0.05$); κB2 binding activity at 2 hour (122.33 ± 38.30%, $p<0.05$); κB2α binding activity at 2 hour (128.18 ± 33.86%, $p<0.05$). For κB3 binding activity there was no statistically significant change. In addition, we also measured TNF-α mRNA levels utilizing Real Time RT-PCR in MNC from 6 normal subjects who ingested glucose. The level of TNF-α mRNA increased at 1 hour (166.21 ± 138.42%), 2 hour (89.89 ± 108.73%), and at 3 hour (23.11 ± 45.21%). We conclude that 1) glucose induces an increase in NF-κB to 3 out of 4 separate NF-κB binding sites especially NF-κB2 and NF-κB2α in the TNF-α promoter region; 2) glucose induces an increase in TNF-α gene transcription; 3) glucose sets up a comprehensive pro-inflammatory effect at the cellular (MNC) level.

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IGF-I receptors are more expressed than insulin receptors in human coronary artery endothelial cells.

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Background and Aims: Cardiovascular disease is a major cause of morbidity and mortality in persons with diabetes. Endothelial cell dysfunction probably plays an important role in development of diabetic angiophaty. Little is known about the presence and function of insulin-like growth factor-I receptor (IGF-IR) and insulin receptor (IR) in human coronary arterial endothelial cells (HCAEC). Our aim was to characterize IGF-IR and IR in cultured HCAEC.
Material and Methods: HCAEC were cultured according to the manufactures instructions. The mRNA expression of IGF-IR and IR was measured by quantitative real-time polymerase chain reaction analysis. IGF-I and insulin receptor protein was assessed by ligand binding using ^{125}I- insulin and ^{125}I-IGF-I. Phosphorylation of IGF-IR´s by insulin and IGF-I was assed by immunoprecipitation and Western blot analysis.
Results: Our results show that in HCAEC cells IGF-IR are approximately 3 to 5 fold more express than IR ($p< 0.001$). The specific binding of ^{125}I-IGF-I in HCAEC was 1.37±0.19% (mean±SEM) of total ^{125}I-IGF-I added. The concentration needed to give half-maximal displacement, EC_{50}, was

$6.86x10^{-10}$ M for unlabelled IGF-I, $5.42x10^{-8}$ M for glargine and $3.18x10^{-8}$ M for insulin. Glargine and insulin were about 100-fold less potent than IGF-I to displace ^{125}I-IGF-I. The specific binding of ^{125}Insulin was 0.17±0, 03% of total ^{125}I-insulin added. The total specific binding of ^{125}I-insulin was thus 9-fold lower than the specific binding of ^{125}I-IGF-I (p=0.003). Due to the very low specific binding of ^{125}I-insulin it was not possible to calculate EC_{50} values. The β-subunit of the IGF-IR was phosphorylated by IGF-I in a concentration of 10^{-8}M, but not by insulin 10^{-8}M. Insulin in high concentration 10^{-6}M phosphorylated the β-subunit of the IGF-I receptor.
Conclusion: In human coronary arterial endothelial cells IGF-I receptors are 3 to 5 fold more expressed than insulin receptors. IGF-I in low concentration activates its own receptor in the endothelial cells. Our demonstration of IGF-I receptors in endothelial cells suggest that IGF-I is important for regulation of endothelial function.

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Glucose upregulates the autocrine production of VEGE from immune cells of elderly Type 2 diabetic subjects: potential modulatory effect of TGF-b.
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Background and Aims: VEGF is an angiogenic growth factor or cytokine with well identified vascular and neurotrophic effect. Besides to endothelial cells, immune compartment could contribute to VEGF circulating and paracrine secretory pool in diabetic subjects and glucose could be directly involved in this mechanism.
Subjects and Methods: The aim of the study was to determine VEGF together with TGF-b secretions in the supernates of peripheral blood mononuclear cells (PBMC separated by Ficoll Hypaque; Solerte SB et al, JCEM 1999) of 47 elderly (age range 65- 82 yr)(HbA1c range 6.4-8.9%) Type 2 diabetic subjects (EDS group) with hyperinsulinemia and microalbuminuria. PBMC ($7.75x10^6$ cells/mL) were conditioned in vitro with glucose (1,5,10,50 mM/mL/cells) and glucose+IL-2 (50 U/mL/cells). VEGF and TGF-b were determined by high sensitive ELISA (R&D; Systems).
Results: VEGF basal levels were significantly higher in EDS group than in healthy matched controls (377±74 pg/mL vs 265±68 pg/mL,p<0.001). VEGF release from PBMC was dose-dependent increased in EDS group during exposure with glucose (381±80 pg/mL; 479±91 pg/mL; 597±102 pg/mL and 713±142 pg/mL respectively after 1,5,10,50 mM) and this increase was also documented, even if less pronounced, in the Control group (p<0.001 vs EDS). The co-incubation of glucose with IL-2 enforced the release of VEGF from PBMC of both groups. The upregulation of VEGF secretion during exposure with glucose was associated with a concomitant enhancement of TGF-b in the supernates of PBMC (249±58 pg/mL; 296±68 pg/mL; 427±88 pg/mL and 589±111 pg/mL respectively after 1,5,10,50 mM) and VEGF was also significantly correlated with TGF-β levels (p<0.01 for glucose 1 and 5 mM/mL/cells; p<0.001 for glucose 10 and 50 mM/mL/cells).
Conclusions: We suggest that glucose can contribute to increase the availability of VEGF from immune cells in Type 2 EDS by means of juxtacrine regulation of VEGF by TGF-b. Inflammatory burst of cytokines (e.g. IL-2) from immune cells could potentiate this effect. This mechanism could be potentially dangerous in the development of widespread glucose-dependent vascular disease and atherogenesis in Type 2 diabetes mellitus of old age.

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Effects of high glucose on expression of Pigment Epithelium Derived Factor (PEDF), a powerful inhibitor of angiogenesis, in endothelial cells.
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Background and Aims: Endothelial cell migration & proliferation are early steps in angiogenesis, and the extent to which new vessel formation occurs in diabetic microvascular disease reflects a balance between endothelial cell responses to various stimulators (e.g VEGF) and inhibitors (eg PEDF) of angiogenesis. Although glucose affects the regulation of pro-angiogenic growth factors, there is very little information about whether high glucose affects the expression of inhibitors of angiogenesis, especially the major inhibitor, PEDF. Thus, this study measured the effects of high glucose exposure on expression of PEDF (mRNA and protein) in cultured endothelial cells.
Materials and Methods: Human umbilical vein endothelial cells were cultured to confluence in EGM2 media supplemented with 5% FCS. Cells were quiesced for 24h in 1% FCS media and treated with 5mM or 20mM glucose for 24h. Total RNA was extracted by the GITC/phenol/chloroform method and reverse transcribed to cDNA. Semi-quantitative real-time PCR was performed to assess PEDF expression, relative to the control gene TF2D, using Cyber-green fluorescence in an ABI Prism 7700 cycler. Total protein was extracted from treated cells and subjected to SDS-PAGE and immunoblotting using an anti-PEDF antibody.
Results: Endothelial cells exposed to 20mM glucose for 24h had 5-fold higher expression of PEDF mRNA relative to TF2D compared with cells cultured under 5mM glucose conditions. One-tailed t-test indicated a significant difference from control (p=0.00015, n=15). Western blotting showed approximately 2-fold higher PEDF protein expression in cells exposed to high glucose.
Conclusion: PEDF is the major inhibitor of angiogenesis. These results indicate that short-term exposure to high glucose concentrations significantly increases PEDF gene expression in endothelial cells. This may reflect a protective compensatory mechanism which limits the angiogenic response in poorly controlled diabetes. Identifying factors that up-regulate anti-angiogenic pathways deserves further investigation.

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Protein kinase C inhibition reverses high-glucose-induced expression and secretion of vascular endothelial growth factor in human umbilical vein endothelial cells.
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Background and Aims: Vascular endothelial growth factor (VEGF) is a potent angiogenic factor and plays a prominent role in endothelial dysfunction in diabetes. In the previous studies, we have demonstrated that inhibition of protein kinase Cα and β_2 attenuated high-glucose-induced endothelial permeability in human umbilical vein endothelial cells (HUVECs), but it is unclear whether VEGF is implicated in PKC-mediated endothelial permeability. In this study, we investigated whether high glucose increases the expression and secretion of VEGF from HUVECs, and whether PKC inhibition reverses this pathological change.
Material and Methods: 2nd passage of HUVECs were incubated with 5.5 mM glucose or 20.5 mM glucose for 3, 24 and 48 hrs, then the medium and cells were collected for measurement VEGF secretion and protein expression using ELISA and western blotting using specific VEGF antibody, respectively. 30 nM of LY379196 (a selective PKC β inhibitor) or 150 nM of Hypocrellin A (a natural occurring PKC inhibitor) was added to cells pre-incubated with 20.5 mM glucose medium for 48 hrs in another series of experiments, in which concentrations of PKC inhibitors were used in the previous studies. VEGF was detected in both medium and cell as above.
Results: VEGF released into the medium was significantly higher by HUVECs treated with 20.5 mM glucose for 48 hrs compared with the cells incubated with 5.5 mM (131±9% vs control, p<0.05, n=3). VEGF protein expression was also significantly increased in the cells with high glucose (135±5% vs control, p<0.05, n=3). Incubation with LY379196 (30 nM) exhibited a tendency to reduce VEGF release from the cell into the medium compared with non-treated cells but a significant difference has not been achieved (14.33±1.73 vs 18.95±2.26 pg/10^5cells, P=0.064, n=6). 150 nM of Hyprocrellin A has significantly decreased high-glucose- induced secretion of VEGF from HUVECs (12.2±3 pg vs 18.95±2.26 pg/10^5cells, P<0.05, n=6). The results of Western blotting showed that both 30nM of LY379196 and Hyprocrellin A reduced the over-expression of VEGF from 130±14% (compared with 5.5 mM glucose control) to 98.3±20% (P<0.05, n=6), and to 88±18.5% (P<0.05, n=6) respectively.
Conclusions: High glucose concentration increases VEGF secretion and expression in HUVECs, which in turn, at least in part, contribute to PKC-mediated increased endothelial permeability. PKC inhibition reverses high-glucose-induced over secretion and expression of VEGF, indicating production of VEGF in HUVECs may be regulated by PKC pathway.

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Nitric Oxide and Endothelial Dysfunction

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Differential mechanisms of endothelium-dependent vasodilator responses of human resistance arteries in hypertensive and normotensive subjects.

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Background and Aims: Essential hypertension is associated with resistance artery dysfunction and abnormal nitric oxide (NO) mediated endothelium-dependent vasodilation. It has been suggested that the importance of endothelium derived hyperpolarising factor (EDHF) increases as a compensatory response. We hypothesised that essential hypertension in human subjects would be associated with insulin resistance and endothelial dysfunction; with a relative reduction in the importance of NO, and a relative increase in the importance of EDHF.
Materials and Methods: 12 male hypertensive subjects (systolic BP>160 mmHg or diastolic BP>90 mmHg) were recruited along with 12 normotensive, age-matched controls. Insulin sensitivity was assessed by means of an isoglycemic hyperinsulinemic clamp. Resistance arteries were dissected from a gluteal fat biopsy. Vessels were mounted on a wire myograph and a standard normalisation and start-up protocol was followed. Endothelium dependent and independent vasodilation were assessed by generation of cumulative concentration response curves with carbachol (concentration range 10^{-9} to 10^{-5}M) and S-nitroso-N-acetyl-penicillamine (SNAP) (concentration range 10^{-9} to 10^{-5}M) following preconstriction with norepinephrine ($3x10^{-7}$M). To investigate mechanism, carbachol curves were repeated following incubation with either the NO synthase inhibitor NG-nitro-L-arginine (L-NOARG) (10^{-4}M) plus the cyclooxygenase inhibitor indomethacin (10^{-5}M), or the toxins apamin (10^{-7}M) and charybdotoxin (10^{-7}M).
Results: Hypertensive subjects were significantly less insulin sensitive than controls. Maximal endothelium-dependent vasodilator response was impaired in the hypertensive group (35.9%±9.5%) compared with the control group (7.4%±3.5%) ($p<0.05$). There was no impairment of endothelium-independent vasodilation. Incubation with both L-NOARG / indomethacin and toxins (apamin and charybdotoxin) significantly impaired endothelium-dependent vasodilation in both hypertensive and control groups (in both cases $p<0.05$, 2-way ANOVA for repeated measures). Toxins had a significantly greater inhibitory effect on endothelium-dependent vasodilation than L-NOARG / indomethacin in the hypertensive group ($p<0.05$, 2-way ANOVA). Conversely, L-NOARG / indomethacin had a significantly greater inhibitory effect in the control group ($p<0.05$, 2-way ANOVA).
Conclusion: Hypertensive subjects are less insulin sensitive than matched controls. Resistance arteries from hypertensive subjects show impaired endothelium-dependent vasodilator responses. Myographic findings suggest that NO mediated resistance artery vasodilation is of reduced importance in hypertensive subjects compared with controls, and that EDHF is of greater importance in these arteries – perhaps as part of a compensatory mechanism.

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Mechanisms of endothelial dysfunction in diabetic vessels.

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Background and Aims: The acceleration of atherosclerosis in diabetis mellitus results in a higher risk of cardiovescular events as well as mortalities. Growing clues from animal and human researches have showed that special defects of endithelium of diabetic vessel exihibited crucial roles. Tetrahydrobiopterin exihibited remedial effects on endithelium-dependent vasodilation in in vitro studies. Statins have been convinced to upregulate eNOS expression .we aimed at study the effects of high glucose on eNOS as well as its cofactor tetrahydrobiopterin (BH4)and possible effects of Statins on BH4 synthesis in endothelial cells.
Materials and Methods: BAECs (Bovine aortic endothelial cells) from passages 3-10 were used. ENOS protein was analysed by Western Blot , mRNA by RT-PCR. Both nitrite and nitrate were measured by HPLC, based on Griesse reaction, and expressed as NOx produced by BAECs. Superoxide was determined by FACS(fluororecin-activated cell sorter).Both introcellular BH4 levels and GTPCHI activities were analysed by HPLC. The concentrations of D-glucose were set as 5.5mM,12.5mM and 25mM. Mannitol was used as control of osmolarity.
Results: The results were expressed as mean± SD, statistic analysis were caried on by Student-t test, and a statistial significance was accepted when $P < 0.05$. High glucose caused an increase in eNOS protein as well as mRNA expression, but a decrease in NOx produced by eNOS. Superoxide anion was increased by high glucose and could be inhibited by L-NAME,apocinin, and allopurinol, respectively. Activity of NADPH oxidase was also increased by high glucose. Both intracellular BH4 levels and GTPCHI activities were inhibited by high glucose. Statins increased intracellular BH4 levels as well as GTPCHI activities directly.
Conclusion: We conclude here that in case of diabetic vessels, decreased NOx and increased superoxide were resulted from dysfunction of eNOS and increased activity of NADPH oxidase. Inhibition of intracellular BH4 levels as well as GTPCHI activities were the main reasons for eNOS dysfunction leading by high glucose. Statins could restore the activities of GTPCHI and increase intracellular BH4 levels directly.This added more meanings to the pleiority of antiatherosclerotic functions of statins.

the relation between glucose, eNOS and GTPCHI

concentration of glucose (mM)	5.5	12.5	25
eNOS protein (relative dnsity) *p<0,05	100+-15.01	102+-13.16*	130+-32.01*
GTPCHI activity (pmoles/mg protein/h)	82.25+-2.09	75+-3.47*	64.4+-4.25*

*p<0,05

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Endothelial dysfunction iduced by food advanced glycation endproducts (AGE) can be restored by compounds stimulating nitric oxide (NO) metabolism in human subjects.

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Background and Aims: In diabetic subjects, already a single ingestion of AGE-rich food can result in a significant inhibition of the flow-mediated arterial dilation (FMD), an in vivo measure of endothelial dysfunction. Increased AGE blood levels have been related to reduced endothelial NO levels, and this reduction can result in decreased FMD. Aiming to inhibit this food-AGE effect in vivo, we focused on the acute effect of compounds that stimulate NO metabolism.
Materials and Methods: Therefore we studied in 8 subjects (2 patients with type 2, 4 patients with type 1 diabetes and 2 nondiabetic subjects) the acute effect of an AGE-rich beverage (300 ml containing 1.8 million AGE-Units and 4.2µ Mol methylglyoxal derivatives but no carbohydrates or lipids) on the FMD (expressed as % increase in arterial diameter after occlusion compared to the baseline diameter of the brachial artery). FMD was measured by high-resolution ultrasound after an overnight fast at baseline and at 90 and 150 min after taking the AGE-rich drink. Four days later, the same FMD test was repeated in each subject. In addition folic acid (50 mg/d) and ascorbic acid (2 g/d) were given orally 2 and 1 day and 30 min prior to the oral uptake of the AGE-rich beverage. L-arginine (9g) as NO-precursor has been dissolved into the beverage.
Results: In all subjects, FMD was reduced significantly by the AGE-rich drink alone from 5.25±2.30 % at baseline to 3.02±1.52% at 90 min ($p<0.01$). The AGE-induced FMD decrease was more pronounced in smokers than in nonsmokers. Pretreatment with folic acid, ascorbic acid and L-arginine abolished the acute food AGE-effect on the FMD completely: 5.09±1.76% at baseline to 5.20±1.43 % at 90 min ($p=0.80$). Accordingly, the observed FMD changes (-2.23±1.77% at 90 min after food-AGE alone vs. +0.11±1.19% after pretreatment and AGE intake) differed significantly ($p<0.01$). Replacing the AGE-rich drink by 300 ml tapwater resulted in no significant FMD differences between baseline and 90 min values.
Conclusion: Food compounds that stimulate NO metabolism can counteract at higher amounts than in standard food acute food-AGE induced arterial endothelial dysfunction in diabetic and nondiabetic subjects.

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Proinsulin C-peptide increases nitric oxide production through an ERK-dependent transcriptional enhancement of eNOS expression in rat aortic endothelial cells.

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Background and Aims: C-peptide is released from the pancreatic b cell into the circulation along with insulin after the cleavage of proinsulin. Although C-peptide had been considered to be biologically inactive, recent studies have suggested that C-peptide possesses several biological activities including improvement of microvascular functions. In addition, our finding that C-peptide stimulation of mitogen-activated protein kinase (MAPK) cascades activates transcription factors such as CREB in LEII microvascular endothelial cells indicates that C-peptide possibly controls vascular functions by altering endothelial gene expression. To test this hypothesis, we examined effects of C-peptide on nitric oxide (NO) production and endothelial isoform of NO synthase (eNOS) expression, since NO has emerged to be a key signaling molecule in the maintenance of cardiovascular homeostasis.
Materials and Methods: Aortic endothelial cells (RAEC) were isolated from female Wister rats and cultured in DMEM with 20% fetal calf serum, heparin and endothelial growth supplement (Harbor Bioproducts). The identity of the isolated cells as endothelial cells was confirmed by their cobblestone-like shape, von Willebrand factor expression and acetylated LDL uptake. After confluence, the cells were serum-starved for 24 hr and treated with C-peptide (10 nM). NO production was determined by DAF-2 fluorescence dye method and relative amounts of eNOS protein and its mRNA were semi-quantified by Western blot and RT-PCR analyses, respectively.
Results: RAEC treated with C-peptide increased eNOS mRNA expression within 1 hr after the treatment, but failed to alter inducible NOS mRNA levels. The amounts of eNOS protein were also augmented following the increase in its mRNA, but prior treatment of the cells with actinomycin D abolished the rise in the enzyme protein. Basal NO production in the cells treated with C-peptide was doubled at 3 hr after the treatment. However, prior treatment of the cells with the p44/p42 MAPK (ERK) kinase inhibitor, PD98059, abrogated these stimulatory effects of C-peptide accompanied with inhibition of C-peptide-induced ERK activity.
Conclusion: In the aortic endothelial cells, C-peptide augmented eNOS protein and NO production through ERK-dependent increase in eNOS gene transcription. The present findings may explain a mechanism of some of the reported C-peptide actions on vasculature and indicate a pivotal role of C-peptide in vascular homeostasis.

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Impaired skeletal muscle NOS activity in insulin resistant non-diabetic subjects with strong family history of T2DM.

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Background and Aims: Impaired nitric oxide (NO) generation may represent the first step in the development of endothelial dysfunction/atherosclerosis. Previously, we have shown that basal and insulin-stimulated muscle NOS activity is impaired in well-controlled type 2 diabetic subjects and the defect correlates closely with the severity of insulin resistance. Here we sought to determine (NOS) activity and protein content in skeletal muscle of non-diabetic subjects with strong family history of T2DM (FH+) and healthy non-diabetic (C) subjects under basal and insulin-stimulated conditions.
Materials and Methods: Ten C (mean age = 36 ± 4 y, BMI = 26.5 ± 1 kg/m^2, FPG = 89 ± 1 mg/dl, FPI = 6 ±1 μU/ml, HDL 49 ± 2, TG 88 ± 14) and 7 appropriately matched FH+ (38 ± 5 y, 26.2 ± 1 kg/m^2, 92 ± 2 mg/dl, 9 ± 1 μU/ml, 43 ± 3 mg/dl, 119 ± 24 mg/dl) subjects received an 80 mU/m^2/min euglycemic insulin clamp to measure (Rd); vastus lateralis muscle biopsies and measurement of VCAM and ICAM before and after 4 hr of insulin. NOS activity was measured in muscle samples with a labeled L-arginine to citrulline conversion assay.
Results: Rd in FH+ was reduced by ~25% vs. C, (10.5 ± 0.5 vs. 8.0 ± 0.5 mg/(kg*min), $P < 0.05$). Basal NOS activity was comparable between groups (C: 292 ± 77 vs FH+:206 ± 80 pM/min·mg protein, P= NS). In response to physiologic hyperinsulinemia NOS activity increased 3 fold in C after 4 hours (973 ± 275 pM/min·mg protein, $P = 0.05$ vs. basal). In FH+ subjects, insulin failed to stimulated NOS activity at 4 hr (293 ± 98 pM/min·mg protein, P = NS from basal). Basal levels of VCAM (352 ± 14 vs. 353 ± 11 ng/mL, P = NS), and ICAM (170 ± 15 vs. 161 ± 7 ng/ml (P =NS) were not different in FH+ vs. C subjects. Plasma VCAM and ICAM levels did not change during insulin infusion. In FH+ basal NOS activity correlated inversely with FPI levels (r = -0.82, P = 0.05) and trended to correlated inversely with basal VCAM levels (r = -0.60, P = 0.10).
Conclusions: In summary, there is a clear defect in insulin-stimulated NOS activity in muscle in non-diabetic family history positive subjects. This impairment of NOS activity may contribute to the documented reduction in insulin-stimulated muscle blood flow seen in genetically predisposed subjects.

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An early sign of endothelial dysfunction: impairment of the NO/cGMP pathway in the postprandial state in Type 1 diabetes mellitus.

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Background and Aims: Hyperglycaemia initiated increased oxidative stress may contribute to the loss of endothelial nitric oxide bioavailability in diabetes mellitus leading to endothelial dysfunction. Assessment of the postprandial state has gained importance due to postprandial hyperglycaemia being considered as an independent risk factor for cardiovascular disease. The authors assessed the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) pathway in the fasting and postprandial state in type 1 diabetic and matched control subjects.
Materials and Methods: In 20 type 1 diabetic patients (age: 34.1±11.8 years, body mass index: 24.1±6.0 kg/m^2, duration of diabetes: 16±10 years, HbA_{1C}: 8.3±1.9%, [x±SD], 10 without, 10 with late diabetic complications) and 20 matched control subjects (age: 39.7±8.6 years, body mass index: 25.3±4.8 kg/m^2) plasma nitrite/nitrate (fluorometrically), cGMP levels (ELISA), lipid peroxidation end products (TBARS method), glucometabolic and lipid parameters were measured in the fasting state and following a test meal.
Results: In the fasting state cGMP levels were found to be significantly lower in the diabetic group compared to the control group (2.5±1.1 vs. 4.6±2.7 nmol/l, p=0.01). There was no difference in the cGMP levels between the diabetic subgroups. No difference was found between the concentrations of NO stable end products (nitrite/nitrate) between the diabetic and control groups. The patients with late diabetic complications had lower basal nitrite/nitrate levels compared to those without complications (33.9±17.5 vs. 56.5±21.8 μmol/l, p=0.006). A significantly higher level of lipid peroxidation end products (TBARS) was found in diabetic subjects (6.7±2.0 vs. 5.0±1.3 μmol/l, p=0.004). The control subjects responded to the test meal with an increase in the cGMP levels, while in the diabetic groups no change was detected (4.6±2.7 to 5.5±2.5 nmol/l, p=0.02).
Conclusion: The results suggest that in subjects with type 1 diabetes mellitus nitric oxide might already have an impaired ability to induce cGMP production in the fasting state prior to the development of late specific complications or microalbuminuria. Postprandial hyperglycaemia is suggested to interfere with endothelial NO action in both diabetic groups, as shown by the unchanged cGMP levels. The manifestation of diabetic complications was found to be associated with decreased basal nitric oxide generation. The impaired ability of NO to induce changes in the cGMP levels both in fasting and postprandial state may be an early sign of endothelial dysfunction in type 1 diabetes mellitus.

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Circadian variation in urinary excretion of sodium and its relation to nitric oxide metabolite and cGMP excretions in Type 1 diabetes mellitus.

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Background and Aims: Several disorders in sodium (Na^+) metabolism, such as an increase in total body exchangeable sodium or altered tubular reabsorption, were found in diabetic patients. Local production of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) play a role in regulation of kidney function influencing the renal vascular tone and sodium handling. The aims of the study were to evaluate the circadian variation in urinary Na+ excretion and to explore the role of NO and cGMP in renal Na+ handling in type 1 diabetes mellitus (DM1).
Materials and Methods: Excretion rates of Na^+, nitrite/nitrate (NOx) and cGMP were measured in 17 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 (I-VI) and one 6-hour overnight (VII) urine speciments were collected during hospitalisation and controlled dietary intake of sodium and nitrate.
Results: The circadian variations in Na+ excretions were different in DM1 and C ($p<0.5$), although they followed a circadian rhythm with a daytime peak ($p<0.001$) in both groups and Na+ excretions per 24-hour were comparable in DM1 and C (150±49 vs 151±40 umol/min). The excretion of NOx was lower in DM1 compared to C (ANOVA-$p<0.01$). The cGMP excretions were comparable in DM1 and C (24h: 417±273 vs 476±223 pmol/min). Circadian variations in NOx and cGMP excretions were not significant. Significant correlations were detected between Na+ and cGMP excretions, r ranged from +0.67 to +0.82 ($p<0.01$) in C, while no relationship has been found in DM1.
Conclusion: DM1 subjects with normal renal haemodynamics displayed an impaired circadian variation in sodium excretion. Reduced local production of NO and the absence of association between cGMP and Na+ excretion in DM1 support the hypothesis that local production of NO and cGMP are involved in altered sodium homeostasis in diabetic kidney. (Supported by IGA MZ CZ grant VZ/CEZ:L17/98:00023001)

Urinary excretion		I	II	III	IV	V	VI	VII
Na^+ (umol/min)	DM1	101±40*	149±79	160±76	178±74	155± 79	163±57**	118±53
	C	146±62	175±101	184±67	166±88	144±75	105±37	82±54
NOx (nmol/min)	DM1	503±235***	512±285**	575±322*	585± 299	580±374*	843± 700	415±385
	C	1007±38	857±389	1110±857	812±409	1054±658	955±582	739±454
cGMP(pmol/min)	DM1	286±142	415±321	417±339	505±504	376±301	614± 483	387±298
	C	464±195	797±212	368±138	445±275	397±89	489±304	450±240

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Determinants of Endothelial Dysfunction in Diabetes Mellitus

1202

Patients with Type 2 diabetes and ischemic heart disease have reduced endothelial function, but similar coronary flow reserve and carotis intima-media thickness as compared to non-diabetic patients with ischemic heart disease.

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Background: Patients with type 2 diabetes mellitus have an increased incidence of ischemic heart disease (IHD) and angiopathy is the major cause of morbidity and mortality in these patients. Endothelial dysfunction is the earliest marker of atherosclerosis and plays a key role in the pathogenesis of diabetic angiopathy. The aim of the present study was to compared patients with IHD +/- diabetes with respect to endothelial function, carotis intima-media thickness and coronary flow reserve.
Methods: In a case control design 16 male patients with type 2 diabetes mellitus and 15 male control patients were studied. All with documented IHD. Brachial artery responses to reactive hyperemia (with increased flow producing endothelium-dependent vasodilation) and to nitroglycerine were measured with a 7.5 MHz linear-array ultrasound transducer. Coronary flow reserve was calculated as the ratio of maximal hyperaemic (induced by adenosine) to resting coronary flow velocity, measured intra coronary from a Doppler flow wire. Carotis intima-media thickness was measured using a 7.5 MHz linear-array ultrasound transducer.
Results: No significant difference in age, body mass index, blood pressure, lipids, urine albumin and ankle-brachial index were found. All patients in the control group had a normal oral glucose tolerance test.

	Diabetic patients with IHD (n = 16)	Control patients with IHD (n = 15)	P value
Endothelium-dependent dilation (%)	1.8 (0.9-5.5)	6.6 (4.8-15)	0.011
GTN- mediated dilation (%)	5.6 (3.2-10)	10 (4.7-15)	NS
Plasma-endothelin-1 (pg/ml)	6.6 (5.9-7.7)	6.0 (5.5-7.6)	NS
Intima-media thickness (mm)	0.76 (0.67-0.95)	0.77 (0.64-1.00)	NS
Coronary flow reserve	2.1 (2.0-2.6)	2.4 (2.2-2.8)	NS
Fasting plasma-insulin (pmol/l)	51.5 (40.0-108)	40.0 (31-43)	0.012
Glycosylated hemoglobin (%)	7.2 (6.6-8.1)	5.7 (5.3-5.9)	< 0.001

Data are median (quartiles), GTN = glyceryl trinitrate

Conclusion: Patients with type 2 diabetes mellitus and IHD have a significant reduced endothelium-dependent dilation compared to non-diabetic patients with IHD, whereas no significant difference in coronary flow reserve and intima-media thickness could be demonstrated between the groups.

1203

Glargine and regular human insulin similarly acutely enhance endothelium-dependent vasodilatation in normal subjects.

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Background and Aims: Human insulin enhances the vasodilatory effect of acetylcholine (ACh), an endothelium-dependent vasodilatator, in normal subjects. Effects of the long-acting insulin analogue insulin glargine on *in vivo* endothelium-dependent and –independent vascular function are unknown. We therefore compared effects of insulin glargine and human regular insulin on endothelium-dependent and –independent vasodilation induced by ACh and sodium nitroprusside (SNP) *in vivo* in normal subjects.
Materials and Methods: Ten normal men (age 33±3 yrs, BMI 23±1 kg/m^2) were studied on two days in a double-blind, randomized, cross-over fashion. In each study, blood flow responses (venous occlusion plethysmography) to intrabrachial artery infusions of ACh and SNP were determined during infusion of saline and intravenously maintained normoglycemic hyperinsulinemic conditions. Hyperinsulinemia (120 min,

infusion rate 1 mU/kg×min) was created by infusing either insulin glargine or human regular insulin.
Results: Endothelium-independent blood flow responses to low (3 µg/min) and high (10 µg/min) doses of SNP were unaffected by both insulin glargine (12.2±0.9 vs. 13.4±1.5 and 19.1±1.4 vs. 19.6±1.7 ml/dl×min, saline vs. insulin, low and high dose) and regular human insulin (11.2±1.1 vs. 12.0±1.7 and 16.8±1.9 vs. 18.4±2.6 ml/dl×min, respectively). In contrast, endothelium-dependent blood flow responses to both low (7.5 µg/min) and high (15 µg/min) doses of ACh increased significantly and similarly by insulin glargine [13.9±1.6 vs. 19.3±2.2 ml/dl×min (saline vs. insulin, +39%, p<0.01) for low dose ACh and 17.3±2.1 vs. 23.2±3.1 ml/dl×min (+34%, p<0.02) for high dose ACh] and regular human insulin [11.5±2.0 vs. 15.8±2.7 ml/dl×min (+38%, p<0.05), and 14.0±2.5 vs. 21.1±3.5 ml/dl×min (+51%, p<0.01).
Conclusion: Insulin glargine and regular human insulin have similar acute stimulatory effects on endothelium-dependent vasodilation in humans.

1204

Acute hyperohomocysteinemia causes endothelial dysfunction in patients with Type 2 diabetes.
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Background and Aims: High plasma levels of homocysteine (Hcy) affect endothelial function in non-diabetic subjects. However, the effect of hyperohomocysteinemia on endothelium-dependent (EDV) and non-endothelium-dependent vasodilation (NEDV) in subjects with type 2 diabetes (DP) is not clearly known. The aim of this study was to examine the effect of acute hyperhomocysteinaemia on endothelial function in DP.
Materials and Methods: We examined the effect of L-methionine (M)-induced hyperhomocysteinemia (0.1g/Kg of M) on EDV and NEDV in 8 DP (age 62.2 ± 7.0 years, diabetes duration 15.6 ± 11.4 years, without macroangiopathy). EDV and NEDV were calculated as the percentage changes of the brachial artery diameter, as compared with the baseline values, after occlusion of the brachial artery and after sublingual administration of 5 mg isosorbide dinitrate, respectively. Measurements were performed in the fasting state and at the 3rd hour after the M load.
Results: M administration resulted in an increase in plasma total Hcy from 11.8 ± 4.6 to 25.7 ± 6.1 µmol/l (P<0.0001). EDV was reduced from 6.8% to 0.0% (P<0.0001) and NEDV was reduced from 15.0% to 1.6% (P=0.001).
Conclusion: It is concluded that acute hyperhomocysteinemia affects both EDV and NEDV in patients with type 2 diabetes. This effect is probably mediated by the adverse metabolic effects of hyperhomocysteinemia.

1205

Endothelium-dependent vasodilation is not impaired by postprandial elevations of plasma insulin in postmenopausal women with and without Type 2 diabetes.
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Background and Aims: The impact of postprandial plasma insulin fluctuations on endothelial function is controversial. We evaluated how meal-induced increases in plasma insulin affect endothelial function in resistance vessels of postmenopausal women.
Methods: We studied 19 postmenopausal women with type 2 diabetes (DM group) and 10 non-diabetic controls (C group). Glycaemic indices and forearm blood flow (FBF) were measured in the fasting state and 3h after a mixed meal (660 kcal, 55% fat). FBF was measured with strain-gauge plethysmography at baseline and during acetylcholine infusion to assess endothelium-dependent vasodilation. ACh induced FBF changes were expressed as the increase above baseline (ΔFBF). Data is presented as mean and SEM.
Results: ΔFBF was lower in the DM group when compared with the C group both in the fasting (10.48±1.30 vs 18.69±2.61 ml/min/dl, p<0.01) and postprandial state (11.74±1.53 vs 18.82±2.50 ml/min/dl, p<0.01). The meal was associated with an increase in FBF at baseline (for C group 2.75±0.37 to 4.04±0.75 ml/min/dl, p<0.005; for DM group 3.27±0.28 to 4.62±0.39 ml/min/dl, p<0.005), but had no effect on ΔFBF (for C group 18.69±2.61 vs 18.82±2.50 ml/min/dl, p=0.77; for DM group 10.48±1.30 vs 11.74±1.53 ml/min/dl, p=0.28). The meal-induced rise in insulin was higher in the DM group (148.16±28.29 vs 95.20±35.89 pmol/L, p<0.05), but meal-induced changes in ΔFBF did not differ between the groups (p=0.40). There was a strong inverse correlation between plasma insulin and ΔFBF both in the fasting (r = -0.637, p<0.0005) and the postprandial state (r = -0.513, p<0.005). The meal-induced rise in plasma inulin was not associated with a change in the form of the relationship between plasma insulin and ΔFBF.
Conclusion: In postmenopausal woman with and without type 2 diabetes, plasma insulin levels are inversely correlated with endothelium-dependent vasodilation (in resistance vessels), but meal-induced elevations of plasma insulin in the postprandial state are not associated with further impairment of endothelium-dependent vasodilation.

1206

Critical acute glucose levels impair vascular reactivity of macro and microcirculation in non-diabetic rabbits.
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Background and Aims: Exposure of endothelial cells and blood vessels in vitro from diabetic and non-diabetic animals to high glucose concentrations (~50 mM), which are not usually observed in diabetic outpatients, decrease the vasodilation mediated by endothelium-derived nitric oxide. The aim of this study was to evaluate the relationship between different levels of glucose in range observed in outpatients with type 2 diabetes (9.1 mM to 15 mM) and those used for diagnosing diabetes (7, 7.8 and 11.1 mM) and the impairment of vascular reactivity of the macro and microcirculation in non-diabetic rabbits.
Materials and Methods: Aortic rings and isolated perfused kidneys from non-diabetic rabbits were acutely exposed (3 h) to normal (5.5 mM - control group) or high (7 - 25 mM) D-glucose.
Results: Kidneys perfused with high glucose (11.1, 15 and 25 mM) but not with 7 mM, had endothelium-dependent (acetylcholine-induced) maximal vasodilation blunted in comparison to control group (respectively 24±3, 24±4 and 16±1 vs 41± 4%, p<0.05). Bradykinin (BK)-induced renal vasodilation was also reduced by high glucose (15 mM), from 21 ± 2 to 15 ± 2% (p< 0.05). Three-hour infusions of mannitol (15 and 25 mM) modified only the maximum vasodilating effect of acetylcholine (ACh) in the renal circulation, with a reduction from 41 ± 4% (control group) to 30 ± 3% and 27 ± 2% (p < 0.05). ACh-induced relaxation was impaired in aortic rings incubated with high glucose (15 and 25 mM) in comparison to control group, (respectively 32 ± 5 and 23±4 vs 51±5%, p< 0.05) but not with 11.1 mM of glucose. BK-induced aortic dilations were also blunted by the high glucose concentrations of 15 and 25 mM from 53 ± 9% to 23 ± 4% and 20 ± 2% respectively. Three-hour incubation periods of the aortic rings with mannitol (15 and 25 mM) did not modify the vasodilating effects of ACh. Finally, endothelium-independent vasodilation induced by sodium nitroprusside was not affected in both systems by high glucose concentrations (15 and 25 mM).
Conclusion: Acute hyperglycemia corresponding to the range observed in patients with type 2 diabetes induces endothelial dysfunction of macro and microcirculation of normal rabbits. It is also noteworthy that aortic endothelium-dependent vasodilation was not affected by glucose levels used to diagnose diabetes while dilation of the renal circulation was significantly reduced by these same glucose concentrations.

1207

Early dysfunction and long-term improvement in endothelial vasomotor function in an angiographically normal artery after an acute coronary syndrome.
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Background: Endothelial dysfunction is an initial event in the development of atherosclerosis and may contribute to the progression of the disease. In previous trials, forearm blood flow measurements or intracoronary testing at the location of the culprit lesion, have shown a high incidence of abnormal endothelium-dependent vasoreactivity in patients (pts) after acute coronary syndrome (ACS).
Objective and Methods: The present study tests the hypothesis that ACS may be associated with endothelial dysfunction far from the responsible lesion. Serial doses of acetylcholine were infused in an angiographically normal coronary artery to assess endothelium-dependent coronary vasomotor response.Endothelium-independent response was studied by intra-coronary injection of molsidomine. These tests were performed before any coronary angioplasty, at least 72 hours after the last symptom and the

withdrawal of vaso-reactive drugs, 43 consecutive male pts (aged 54.3 +/- 1.7 years) with one or two focal lesions were included (dyslipidemia, diabetes and malignant disease were exclusion criteria). All the patients had similar tests 6 months later. At baseline and follow-up a large panel of lipid analyses (total cholesterol, triglyceride, HDL-C, LDL-C, VLDL-C, apoA-1, apoB, apoCIII, apoE, LpA1, Lp(a) were carried out. Inflammatory markers (fibrinogen,hs-CRP) and adhesion molecules (VCAM-1, ICAM) were assessed.

Results: Of the 43 pts, 35 (81.4 %) presented initial abnormal endothelium-dependent vasomotricity. The univariate analysis did not show any significant relationships between baseline vasomotor function and clinical or biological characteristics.At 6 months, a significant improvement was observed in 27 (77.1 %) of the 35 In univariate analysis, predictors of improvement were : HDL-C and apoA1 6-month levels, delta HDL-C (% change between 6 months and baseline) and 6 months hs-CRP value. In multivariate models, HDL-C, apoA1 and delta HDL-C remained statistically significant. A strong inverse relation was found between % arterial diameter change (baseline to 6 months) and 6-month hs-CRP level ($r = -0.67$, $p < 0.0001$).

Conclusion: Patients with ACS have frequent and severe initial endothelial dysfunction in angiographically „normal“ coronary arteries. In most cases, we observe a significant improvement at follow-up, suggesting a possible systemic reaction component in the first few days after ACS. The relationship between improved vasomotor function and HDL-C values shows the need for therapeutic intervention to increase HDL-C and particularly the apoA1 subfraction.

1208

Persistently impaired endothelial function in Type 2 diabetic patients, but not in non-diabetic patients, after acute myocardial infarction.

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Background and Aims: Endothelial dysfunction is an early feature in patients at risk for coronary atherosclerosis and a major cause of morbidity and mortality in diabetes. Also associated with endothelial dysfunction is hyperhomocysteinemia. We sought to determine temporal changes in endothelial function and in plasma levels of total homocysteine (tHcy) and soluble E-selectine (sE-selectine) after acute myocardial infarction in type 2 diabetic patients compared to non-diabetic patients.

Materials and Methods: We compared 20 type 2 diabetics (male=14, female=6, age 65±3) with 25 non-diabetic patients (male=17, female=8, age 61±2) suffering an acute myocardial infarction. Using high-resolution ultrasound, we measured brachial artery responses to reactive hyperemia endothelium-dependent dilatation (EDD) and nitroglycerine-induced dilatation (NID) acute and 60 days at follow up. At the same time plasma tHcy and sE-selectine levels were measured.

Results: In the diabetic group, EDD did not change during the months after the myocardial infarction contrasting a slight but not significant increase in non-diabetics. Two months after the myocardial infarction, the difference in EDD between diabetics and non-diabetics was significant (1.5 ± 0.8 % vs 4.1 ± 0.5 %, $P<0.01$). There was a significant increase in NID in both groups from the time for myocardial infarction to the measurements two months later in diabetics and in non-diabetics (9.7 ± 1.2 % vs 13.2 ± 1.5 %, $P<0.05$ and 7.9 ± 0.8 % vs 12.4 ± 1.0 %, $P<0.001$, respectively). Plasma tHcy and sE-selectine levels did not differ between groups.

Conclusion: These results show a persistent endothelial-dependent dysfunction in type 2 diabetic patients. We suggest that a profound disturbance in the endogenous nitric oxide pathway may be involved to the elevated cardiovascular morbidity in type 2 diabetic patients.

Baseline clinical and biochemical characteristics of study groups.

	Type 2 diabetes	Control subjects
BMI (m^2)	27.9± 0.9	26.0± 0.8
Previous AMI (%)	4 (20)	5 (20)
Smokers (%)	3 (15)	5 (20)
Hypertension (%)	8 (40)	10 (40)
sBP (mmHg)	132± 4	135± 4
dBP (mmHg)	77± 3	77± 3
S-total-cholesterol	5.1± 0.2	5.8± 0.3*
S-LDL (mmol/L)	3.1± 0.2	3.9± 0.2*
S-HDL(mmol/L)	1.1± 0.1	1.3± 0.1*
S-Triglycerdides (mmol/L)	2.5± 0.5	1.7± 0.1

Data are presented as means ± SEM. dBP, diastolic blood pressure; sBP, systolic blood pressure. * P<0.05 diabetics vs control subjects.

Brachial artery measurements at baseline and at 60 days follow-up

	Type 2 diabetics Baseline	60 days	*P-value*	Control subjects Baseline	60 days	*P-value*
Onset (Before EDD) 0.10	3.95±		3.98±	0.95	4.13±	3.87±
Brachial artery end-diastolic diameter (mm)	0.14	0.45	0.17	0.13		
EDD Δ Brachial artery end-diastolic diameter (%)	1.9± 0.6	1.5± 0.8	0.71	3.2± 0.6	4.1± 0.5	0.19
Onset (Before NID)	3.98±	4.00±	0.92	4.15±	3.90±	0.17
Brachial artery end-diastolic diameter (mm)	0.13	0.10		0.16	0.14	
NID (400 µ g Glyceryl trinitrate)	9.7± 1.2	13.2± 1.5	0.02	7.9± 0.8	12.4± 1.0	0.0008
Δ Brachial artery end-diastolic diameter (%)						

EDD=Endothelial-dependent vasodilatation.
NID=Nitroglycerin-induced vasodilatation.
Δ =differences in percent.

1209

Dynamic videocapillaroscopy in Type 2 diabetes mellitus with and without microvascular complications.

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Background and Aims: After induced ischaemia, blood flow velocity increases less and spends more time to get maximum levels among type 2 diabetic patients than among normal individuals, when observed by hand nailfold videocapillaroscopy (HVC). Using capillar transverse segment area as a response parameter we studied vascular response after isquemia by HNV between diabetic patients with and without retinopathy.

Materials and Methods: 15 healthy individuals with negative familial history of diabetes and normal glucose tolerance oral tests (group A), 20 type 2 diabetic patients with serum frutosamine levels under 3 mg% were selected: 10 had normal eye fundus (group B) e 8 had diabetic retinopathy (group C). Smoke, body mass index >35 mg%, arterial hypertension, clinical signs of neuropathy, use of vascular active drugs and serum creatinine levels above 1,5 mg% were exclusion criteria. HVC was done after 30 min rest under controlled temperature (24 to 26o C) using stereoscope microscope attached to videocamera, VCR and TV. Ischaemia/reperfusion test was done in hand fingers where sphigmomanometer was put in the 4th finger, 20 mm Hg above maximum arterial pressure for 1 min and the images, taped for 3 min, were captured each 2 sec during 40 sec and analysed by Pentium II microcomputer (Leica EWS and Scanpro 3 softwares). Capillar transverse segment area (CTSA) was defined by a perpendicular line tangent to internal arch curvature. CTSA maximum increase percent (ICSA%) after ischaemia as well as the time spent to get it (CTSAt) were measured. Statistical comparisons were made through Kruskal Wallis test (significance 5%).

Results: CTSA after rest showed no significant difference among A, B and C groups (371,02 ± 109,57 X 437,15 ± 101,88 X 363,61 ± 108,13µ2 p=0,25). ICSA% was lower among groups B and C when compared to controls, but the difference was not significant (49,85 ± 34,62 and 30,65 24,15 X 60,46 ± 31,53% p=0,053). CTSAt was significantly lower (p=0,0033) among controls (4,83 ± 2,75sec) than groups B (12,6 ± 7,48sec) and C (14,57 8,3 sec). That difference remains among groups A X B and C (p<0,05) but disappears when comparing B X C (p>0,05)

Conclusion: Type 2 diabetic patients with clinical retinopathy have abnormal answer to ischaemia when studied by HVC. That answer is the same observed in patients without retinopathy, even with good recent metabolic control. Those results suggests that vasomotility is precociously altered in type 2 diabetes.

1210

Capillary filtration and peripheral nerve dysfunciton in Zucker rats.
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Background and Aims: We have already provided evidence for an increase in capillary permeability associated with peripheral nerve dysfunction in Wistar rats with streptozotocin-induced diabetes. An increase in capillary filtration is often found in obese subjects. The aim of this study was to look for such microcirculatory and nerve disorders in the insulin resistance Zucker rat.
Materials and Methods: Male fatty control rats and 10 male fatty Zucker rats were investigated. Zucker rats had significantly higher body weight ($p < 0.01$) and slightly higher blood glucose (NS) than control rats. At 6 and 9 months, an in vivo test of capillary filtration of albumin (CFA) was performed with technetium-99-labelled albumin and sensitive and motor conduction velocities were measured. The isotopic test included a venous compression on a hindlimb. Radioactivity was followed externally using a gamma detection device, after removal of venous compression. This allowed to calculate interstitial albumin retention (AR) and to evaluate lymph function by the fast Fourier transform of the radioactivity disappearance curves and the amplitude ratio of the low and high frequency peaks (LF/HF).
Results: In Zucker rats AR was significantly higher than in control rats at 6 months ($17.0 \pm 3.6\%$ vs $1.7 \pm 0.6\%$; $p < 0.001$), and yet higher at 9 months ($24.6 \pm 4.0\%$ vs $5.2 \pm 1.8\%$; $p < 0.001$). LF/HF ratio was significantly higher only at 9 months ($0.94 \pm 0.81\%$ vs $0.18 \pm 0.63\%$; $p < 0.001$). In Zucker rats, motor nerve conduction velocities and the amplitudes of the motor potentials decreased between 6 and 9 months whereas they increased in control rats (maturation). Sensitive conduction velocities and potential amplitudes did not differ between Zucker and control rats.
Conclusion: These data suggest that obesity and insulin resistance induce microcirculatory disorders characterized by an early increase in capillary filtration with secondary saturation of lymph uptake of interstitial albumin, and early functional alterations of motor nerves which may result from endoneural edema.

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Vascular Reactivity in Diabetes

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Lack of relationship between endothelial dysfunction in larger vessels and defects in microvascular hemodynamics in Type 2 diabetic vasculopathy.
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Background and Aims: Both the reduced reactive hyperemia due to microvascular dysfunction and the impairment of endothelium-dependent vasodilatation as a functional marker of atherogenesis have been suggested to be early events in diabetic angiopathy. Later in the course of diabetes endothelium-independent vascular response to glycerol trinitrate indicates a reduced vascular smooth muscle cell responsiveness or a decreased vasodilatation capacity resulting from morphological vessel wall damage. The question that arose was whether or not there is a relationship between microvascular and macrovascular disturbances in type 2 diabetic patients.
Materials and Methods: 63 type 2 diabetic patients and 44 non-diabetic control subjects were investigated. Lumen diameter of the brachial artery was measured by high-resolution ultrasound at rest, during reactive hyperemia, and 4 min after application of 400 µg glycerol trinitrate (GTN) sublingually. Capillary blood cell velocity (CBV) was assessed in the dorsal middle phalangeal area of the left ring finger by laser Doppler anemometry during rest and after release of arterial compression. Flow-mediated dilatation (FMD), GTN-induced vasodilatation and the increase in CBV were expressed as percentage changes related to the baseline conditions.
Results: FMD% ($3.8 \pm 0.8\%$ vs. $6.9 \pm 0.9\%$; $p < 0.05$), GTN% ($5.6 \pm 0.7\%$ vs. $14.9 \pm 1.7\%$; $p < 0.05$) and CBV% ($63 \pm 11\%$ vs. $124 \pm 19\%$; $p < 0.05$) were reduced in the diabetic patients compared to their control subjects. There was a positive correlation between FMD% and GTN% ($r = 0.38$; $p = 0.001$). Neither FMD% nor GTN% correlated with CBV%.
Conclusions: The association of FMD% and GTN%, both markers of atherosclerosis, might have been only weak because endothelial dysfunction precedes a decrease in vasodilatation capacity due to morphological vessel wall damage. The lack of a relationship between FMD% and CBV% lends support to the suggestion that endothelial dysfunction in larger vessels resulting from impaired nitric oxide (NO) release and disturbances in microcirculation, where NO plays a very limited role, develop independently in the course of diabetes.

1212

Impaired microvascular function is associated with raised plasma N-terminal pro-brain natriuretic peptide level in Type 2 diabetic patients.
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Background and Aims: In humans, brain natriuretic peptide (BNP) is produced in myocardium, with the left ventricle being the main site of production in response to volume and pressure overload, promoting natriuresis, diuresis and vasodilation. BNP plasma levels are raised in heart failure. Raised BNP plasma levels have also been described in absence of overt heart failure in type 2 diabetic subjects with microalbuminuria. We assessed in type 2 diabetic subjects the relationship between BNP plasma levels, microalbuminuria and endothelial function.
Materials and Methods: We recruited 61 type 2 diabetic patients (15 women, 46 men, mean age 56 years), and 38 healthy subjects matched for gender and age as control subjects. Endothelial function was assessed from the skin blood flow response, expressed in arbitrary perfusion units (PU), to local administration (iontophoresis) of the endothelium dependent vasodilator acetylcholine (Ach). Plasma N-terminal ProBNP was measured using an immunoassay with a commercial analysis kit (Roche Diagnostic). Albumin/creatinine ratio was calculated from a morning urinary spot.
Results: There was a significant difference in the maximal response to Ach iontophoresis between diabetics [median: 317 PU (CI 2.5%-97.5% 143-536)] and controls [400 PU (207-623)] ($p= 0.007$). In the diabetic patients,

there was a significant inverse relationship between skin blood flow response to Ach and plasma levels of BNP (p=0.017). There was a significant difference in plasma levels of BNP between microalbuminuric diabetics (n=18) [155 pg/ml (15-564)] and normoalbuminuric diabetics (n=38) [40 pg/ml (6-581)] (p<0.001) as well as with controls [54.5 pg/ml (13-171)] (p=0.018). Coronary artery disease was also associated with plasma BNP in diabetic patients [Diabetics with coronary artery disease (n=13), plasma BNP 122 pg/ml (24-614), and diabetics without coronary artery disease (n=43), plasma BNP 42 pg/ml (6-543)] (p=0.0013). When the skin blood flow response to Ach was submitted to multivariate analyses with parameters known to influence microvascular function, plasma level of BNP remained a significant determinant. In the same way, when plasma level of BNP was submitted to multivariate analyses, microalbuminuria was still a significant determinant. BNP was also independently associated with coronary artery disease.
Conclusions: We demonstrate for the first time in this trial that, in type 2 diabetes, increased BNP levels are associated with both microangiopathy and macroangiopathy.

1213

Forearm vasoconstrictor response during sympathetic stimulation is impaired in patients with uncomplicated diabetes mellitus Type 1.
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Background and Aims: An increase in capillary blood flow and pressure is viewed as an important factor in the pathogenesis of microangiopathic complications in diabetes ('haemodynamic hypothesis'). This early vasodilatation might be explained by inhibition of the sympathetic nervous system [SNS] due to chronic hyperglycaemia.
The aim of this study was to compare SNS function and activity between patients with uncomplicated type 1 diabetes Mellitus [DM1] and healthy control subjects [C] using several different methods to assess SNS activity.
Materials and Methods: Fifteen DM1 patients (M:F=11:4; age 29.1 ± 8.2 yr.; DM duration 6.4 ± 3.9 yr.; HbA1c 7.9 ± 1.3%) and thirteen age- and sexmatched healthy volunteers (M:F=9:4; age 23.4 ± 3.4 yr.) participated in the study. On the same day SNS activity was measured biochemically (arterial and venous plasma catecholamines) in conjunction with power spectral analysis and haemodynamic measurements (heart rate [HR], blood pressure [BP], forearm blood flow [FBF]). These parameters were measured both at rest (baseline) and during sympathoneural stimulation (lower body negative pressure [LBNP]). In addition muscle sympathetic nerve activity [MSNA] (microneurography) was performed before (baseline) and during a cold pressor test [CPT]. Finally FBF was measured after local α and β adrenergic blockade to investigate basal sympathetic tone. Forearm vascular resistance [FVR] was calculated by dividing mean arterial pressure by FBF and expressed in arbitrary units (AU).
Results: LBNP-induced vasoconstriction was significantly attenuated in DM1 (FVR from 34 ± 3 to 45 ± 5 AU) compared to C (FVR from 38 ± 5 to 54 ± 3 AU); ΔFVR DM1 vs. C: 12 ± 4 vs. 19 ± 3 AU p<0.05. After α and β adrenergic blockade FVR decreased in both groups to the same extent indicating normal vascular structure. HR, systolic and diastolic BP did not differ between the two groups at baseline or during LBNP and α and β adrenergic blockade. There was no difference in plasma catecholamines (arterial and venous) between the two groups. Microneurography (successful in 13 subjects per group) showed no significant difference in burst frequency at baseline or during CPT in DM1 patients. (CPT DM1 vs C: ΔMsna 4.5 ± 0.7 vs. 5.8 ± 1.3 bursts/100 beats p=ns). Finally, power spectral analyses of systolic BP and RR-interval showed unaltered MF variance in DM1.(SBP MFvar/tot var DM1 vs C: 0.30 ± 0.02 vs. 0.28 ± 0.04; p=ns).
Conclusion: Baseline SNS activity is not decreased in uncomplicated DM1 patients. However, during sympathetic activation DM1 patients exhibit decreased forearm vasoconstrictor response, which possibly contributes to increased intracapillary pressure.

1214

Forearm skin microvascular reactivity, IGF-1, and IGFBP-1 in healthy subjects with and without heredity for Type 2 diabetes.
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Background and Aims: An impaired skin microvascular reactivity has been demonstrated in healthy subjects with heredity for type 2 diabetes, which might be an early marker for vascular disease. Low levels of insulin-like growth factor binding protein-1 (IGFBP-1) have been related to decreased insulin sensitivity and cardiovascular disease. The aim of the present study was to investigate associations between skin microvascular reactivity, IGF-1, and IGFBP-1 in healthy subjects with and without heredity for type 2 diabetes.
Materials and Methods: Thirtyseven male subjects, free of medication, non-smokers, and with normal oral glucose tolerance tests were investigated. Twentyone of the subjects had (Group A), and 16 had no heredity for type 2 diabetes (Group B). The two groups were matched for age and body mass index. Forearm skin microvascular reactivity was investigated by laser Doppler fluxmetry (AU) before and after iontophoresis of endothelial- (acetylcholine; Ach) and non-endothelial- (sodium nitroprusside; SNP) dependent substances. Plasma levels of IGF-1 and IGFBP-1 were investigated by RIA.
Results: Group A showed impaired (p<0.03) skin microvascular responses (Ach: 2.5±1.2 AU; SNP: 3.0±1.6 AU), higher levels of IGF-1 (184±38 µg/L p<0.03), while IGFBP-1 was not significantly different (20±14 µg/L; p=0.22), as compared to Group B (Ach: 4.0±1.9 AU; SNP: 4.4±1.6 AU; IGF–1: 157±20 µg/L; IGFBP–1: 23±11 µg/L). So far the analyses of IGF-1 and IGFBP-1 have been performed in only 24 of the subjects, i e 13 in Group A and 11 in Group B. In Group B, significant correlations were observed between the maximal microvascular responses to Ach (r=0.75; p<0.01), SNP (r=0.64; p=0.02), and IGFBP-1, respectively. In Group A, a non-significant correlation was seen between the maximal microvascular response to Ach and IGFBP-1 (r=0.57; p=0.07), while no correlation was seen between the maximal response to SNP and IGFBP-1. No significant differences were seen between the two groups regarding systolic and diastolic arm blood pressures, puls rate and blood lipids.
Conclusion: The preliminary results of the present study indicate that healthy subjects with heredity for type 2 diabetes have higher levels of IGF-1 than healthy subjects without heredity. Furthermore, only the group without diabetes heredity demonstrated significant correlations between skin microvascular reactivity and IGFBP-1 levels. Hence, the present data suggest that IGF-1 and IGFBP-1 may influence skin microvascular reactivity, e g by changes in smooth muscle cell function of the vessel walls.

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Time course for development of functional skin microangiopathy in patients with Type 1 diabetes.
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Background and Aims: An impaired skin microvascular reactivity has been shown in patients with type 1 diabetes, but it is not known when in the course of diabetes these disturbances occurs. In the present study, the skin microcirculation in patients with type 1 diabetes was investigated longitudinally from onset of diabetes.
Materials and Methods: Fifteen patients (nine male) with type 1 diabetes and a mean ± SD age of 28 ± 8 years at onset of diabetes were investigated. The capillary blood cell velocity (CBV) in the nailfold of dig IV (left hand) was investigated by videophotometric capillaroscopy (12 patients), and the total skin microcirculation in the same area by laser Doppler fluxmetry (LDF) (15 patients) 1, 2, 3 and 7-12 years after the debut of diabetes. CBV (mm/s) and LDF (AU) were studied during rest, and following a 1-min arterial occlusion (200 mmHg) at the proximal phalanx of the digit. Peak and time to peak CBV and LDF, respectively, were determined during postocclusive hyperaemia. Thirty-two healthy controls (19 male) with a mean age of 43 ± 11 years were investigated by LDF 2-4 times during 9 years.
Results: Resting LDF and time to peak LDF (tpLDF) did not differ between patients (n=15) and controls during the first three investigations, while tpLDF was significantly (p<0.01) prolonged 7-12 years after onset of

diabetes. TpLDF in controls was not significantly changed during the study period. Peak LDF during postocclusive hyperaemia was not significantly different as compared to the healthy controls and did not change during the investigation period. Percentage increase of CBV during postocclusive hyperaemia (n=12) decreased significantly 7 years after onset of diabetes as compared to the first investigation (188 ± 132 and 119 ± 142, respectively; p=0.04). A non significant (p=0.08) tendency to longer time to peak CBV was seen 7 years after onset of diabetes (21 ± 26 s), as compared to the first investigation (8 ± 3 s). Arm and finger blood pressures, HbA1c and skin temperature were not significantly changed during the study period.
Conclusion: The results of the present study indicate that functional disturbances in skin microcirculation of fingers are not present until at least three years after onset of type 1 diabetes, while a majority of the patients have developed disturbances after 7-12 years.

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Vascular reactivity and insulin resistance in Type 2 diabetic patients.
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Background and Aims: Endothelial dysfunction and insulin resistance co-segregate in several disease states and might share common pathogenetic bases. Whether insulin resistance and abnormal endothelial are linked also in type 2 diabetic subjects is unknown.
Materials and Methods: Ninety-one type 2 diabetic patients were studied in 3 centres after a 4-week washout (from antidiabetic drugs) period. Equipment and procedures were carefully standardised. Insulin sensitivity was measured using the euglycaemic (6.0 mM) hyperinsulinaemic (40 mU/min/m^2) clamp technique. The forearm blood flow responses to graded intra-arterial infusions of acetylcholine (Ach) and sodium nitroprusside (SNP) were measured by strain-gauge plethysmography; all traces were read centrally and blind to the clinical parameters. Routine biochemistry was assayed centrally.
Results: Patients were divided into quartiles of insulin resistance (IR). Their mean (±SD) values of insulin-stimulated glucose uptake (M) were: 46.7±7.5, 31.7±4.1, 23.2±1.9, and 15±3.2 µmol/min/kgffm (p<0.0001 by ANOVA). The four groups had similar gender distribution (F/M = 4/19, 4/18, 7/16, and 3/20), age (56±10, 59±7, 56±8, and 60±10 yrs), fasting plasma glucose (10.1±2.1 mM, 9.3±2.4, 10.3±2.2 and 11.0±2.4, p=0.123), HbA1c (7.9±1.1, 7.4±1.1, 7.8±1.0 and 8.2±1.3, p=0.144), and similar LDL-/HDL- cholesterol ratio (2.7±0.9, 2.7±0.8, 2.9±0.8, and 2.8±0.6 p=0.850). Body mass index (27.0±2.5, 27.3±3.3, 28.8±3.7, and 30.1±3.4 kg/m2, p=0.005) and prevalence of hypertension (17, 41, 35, and 61%, p=0.020) significantly decreased through IR quartiles. Maximal blood flow percent increase above baseline in response to both Ach (758±326, 543±362, 662±488, and 325±230%, p=0.004) and SNP (707±414, 585±299, 592±210, and 425±111%, p=0.028) were progressively lower in the four IR groups. When the maximal Ach response was normalised by the maximal SNP response (to control for smooth muscle cell reactivity), a tendency to a progressive decrease across IR quartiles was still observed (1.39±0.92, 1.04±0.67, 1.14±0.77 and 0.85±0.47, p=0.148). In the whole group, regression analysis indicated that insulin resistance was inversely related to both the Ach/SNP (r=0.25, p=0.027) and the SNP responses (r=0.27, p=0.014). Adjusting for BMI and hypertension did not attenuate the strength of these associations (partial r= 0.25 and 0.24, respectively).
Conclusion: In type 2 diabetic patients insulin resistance is associated with both endothelial and non-endothelial vascular dysfunction of peripheral resistance arterioles. The link between the metabolic and the vascular defects is not explained by obesity or hypertension.

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Strong relationship between postprandial glucose concentrations and impaired vasoreactivity in patients with Type 2 diabetes.
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Background and Aims: Postprandial hyperglycemia is considered an important risk factor for cardiovascular disease. Patients with type 2 diabetes are characterised by impaired vasoreactivity and impaired postprandial clearance of glucose. Glucose and other factors such as insulin, blood pressure and heart rate may influence postprandial vasoreactivity. We therefore investigated whether glucose independently predicts vasoreactivity after a glucose challenge in both healthy volunteers and patients with type 2 diabetes.
Materials and Methods: We evaluated the effect of an oral glucose challenge (75 g) on flow-mediated vasodilation (FMD) of the brachial artery (Wall-track system with a 10 MHz ultrasound probe) in twenty healthy volunteers and twenty well-controlled metformin-treated patients with type 2 diabetes who did not use vasoactive therapy (HbA1c 6.6±0.9%). FMD, insulin- and glucose concentrations, blood pressure and heart rate were assessed at baseline and at 1, 2, 3 and 4 hours after the glucose challenge.
Results: Patients with type 2 diabetes had impaired vasoreactivity compared to healthy volunteers at baseline (FMD 4.7±2.3% (mean±SD) vs. 8.4±2.4% respectively, p<0.001). A glucose challenge significantly impaired FMD by approximately 47% in both healthy volunteers and patients with type 2 diabetes (FMD from 8.4±2.4% to 4.5±2.4% (at t=1), p<0.05 and from 4.7±1.9% to 2.4±1.2% (at t=2), p<0.05, respectively) and FMD returned to baseline levels at t=4. Glucose- and insulin concentration significantly increased at 1, 2 and 3 hours post-challenge and returned to baseline levels at t=4 in both groups. In healthy volunteers there was no correlation between FMD and glucose (see table). In patients with type 2 diabetes, post-challenge FMD was significantly inversely correlated to post challenge glucose (r = - 0.46, p<0.001), but not to post challenge insulin.
This relationship was still present after correction for other postprandial factors.
Conclusion: Glucose is an important determinant for vasoreactivity after an oral glucose challenge in patients with type 2 diabetes. Reducing postprandial hyperglycemia could therefore have beneficial effects on vasoreactivity and could improve cardiovascular outcome

Healthy Volunteers (n=20)

Glucose	**4.8±0.3**	**7.8±1.0**	**6.2±1.1**	**5.9±0.8**	**5.1±0.6**	**3.9±0.6**	**4.1±0.3**	mmol/l
Insulin	**5.2±3.5**	**60.0±31.1**	**40.2±16.7**	**31.0±11.3**	**16.8±11.8**	**4.9±2.1**	**2.8±1.0**	mU/ml
FMD	**8.4±2.4**		**5.3±2.9**		**4.5±2.4**	**6.6±2.4**	**7.8±2.4**	%

Diabetes Patients (n=20)

Glucose	**7.2±1.5**	**10.8±1.9**	**13.7±2.5**	**14.2±3.4**	**13.8±3.2**	**11.0±3.2**	**8.0±3.0**	mmol/l
Insulin	**8.9±7.9**	**23.3±18.6**	**30.3±20.2**	**33.4±25.6**	**28.4±19.9**	**20.6±13.0**	**10.9±7.4**	mU/ml
FMD	**4.7±1.9**		**2.4±1.2**		**3.3±2.0**	**3.2±1.7**	**5.1±2.1**	%
HbA1C	**6.6±0.9**							%
	0	**0.5**	**1**	**1.5**	**2**	**3**	**4**	Time (h.)

Mean±SD, At t = 0 : glucose load (75 g)

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In Type 2 diabetes therapy of insulin resistance ameliorates endothelial dysfunction independent of glucose control.
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Background: Insulin resistance (IR), an important feature of type 2 diabetes (NIDDM), is an independent risk factor for arteriosclerosis and cardiovascular mortality. However the mechanism by which IR brings about arteriosclerosis is not known. A potential link could be the impairment of function of the endothelial layer. Therefore we asked if therapy of IR can improve endothelial function.
Materials and Methods: We performed a double-blind cross-over trial in 12 patients with newly detected NIDDM. They were assigned to a treatment with either rosiglitazone 4mg or nateglinide 60 mg twice daily for 12 weeks in random order. Forearm blood flow (FBF) was measured after each treatment period using strain gauge venous occlusion plethysmography. IR was assessed by euglycemic clamp technique.
Results: We performed dose response studies of acetylcholine (ACh) with and without exogenous insulin infused into the brachial artery. ACh-response was significantly increased after rosiglitazone treatment (maximum FBF 12.8±1.3 vs. 8.8±1.3 ml/100ml after rosiglitazone and nateglinide, respectively, P<0.05). The response could be further increased by co-infusion of exogenous insulin in the rosiglitazone group (maximum FBF 15.9±1.2, P<0.05). Blockade of endogenous nitric oxide (NO) -synthase by N-monomethyl-L-arginine-acetate largely prevented the increased vasodilation after rosiglitazone with and without exogenous insulin. IR improved by 60% after rosiglitazone treatment (Mc-value 3.7±0.3 vs. 2.3±0.3 mg/(kg min) after rosiglitazone and nateglinide,

respectively, P<0.01). There was a significant correlation between insulin sensitivity and blood flow response (R=0.51, P<0.01). Glycemic control was not different between both treatments (HbA1c 6.1±0.1 % vs. 6.1±0.1 % and fasting blood glucose 6.4±0.3 vs. 7.0±0.4 mmol/l after rosiglitazone and nateglinide, respectively).
Conclusion: Rosiglitazone improved endothelial function in NIDDM. This improvement was related to insulin sensitization, but it was independent of glycemic control. The improvement of endothelial function was largely mediated by NO.

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The polymorphism of platelet glycoprotein receptor GP IIIa and its relationship with diabetic retinopathy in patients with Type 2 diabetes mellitus.
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Background and Aims: Recent studies show that platelets may play important role in the development of diabetic microangiopathy. The glycoprotein complex (GP) GPIIb/IIIa is a fibrinogen receptor on platelets. Platelet-membrane glycoproteins are highly polymorphic. One of these polymorphisms is in GP IIIa glycoprotein. The GP IIIa polypeptide is polymorphic due to a single base change at position 1565 resulting in either proline (PlA1) or leucine (PlA2) at position 33 in the protein. This polymorphism in GPIIIa receptor (PlA1/2) may play important role in a platelet aggregation and adhesion. It has been recently reported that the PlA2 variant may be strongly associated with the risk of acute coronary syndromes, particularly in younger persons. The aim of the present studies was to determine the relationship between the PlA1/2 polymorphism and susceptibility to diabetic retinopathy.
Materials and Methods: We have examined 75 patients with type 2 diabetes, 30 without diabetic retinopathy (DR) and 45 without it and 123 controls for PlA1/2 polymorphism. There were no significant differences between the duration of diabetes (p=0.103, 9.8±5.5 years in patients without retinopathy and 11.5±3.95 in patients with retinopaty), age (p=0.619, 58.2 vs. 59.4 years), level of HbA1c (8.3% vs. 9.2%, p=0.103) and sex distribution (p=0.39) in both groups. Genomic DNA was isolated from peripheral blood leukocytes from all patients and controls. For genotyping of the PlA 1/2 polymorphism, a specific DNA fragment that contains this polymorphism was amplified by polymerase chain reaction (PCR) using specific primers. Amplified PCR products of GP IIIa gene were digested with 1.6 U Nci I endonuclease at 37ºC for 16 hours. Digested fragments were analyzed by 2% agarose gel electrophoresis.
Results: Distribution of the PlA genotypes in the group of patients without DR was (A1/1=26, A1/2=4 and A2/A2=0), in the group of patients with DR (A1/1=39, A1/2=5 and A2/A2=1) and in the healthy controls (A1/1=93, A1/2=27 and A2/A2=3).We found no differences in the distribution of the PlA1/2 genotypes or alleles in both groups of patients (p=0.69) and controls (p=0.17).
Conclusion: These results suggest that this polymorphism does not contribute to the development of diabetic retinopathy.

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Common polymorphisms of the human glyoxalase-1 gene and pro-thrombotic factors.
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Background and Aims: Advanced Glycation Endproducts (AGE) accumulate at an accelerated rate in diabetes mellitus and induce changes in the vascular endothelium that may contribute to micro-and macrovascular disease. The interaction of AGE with cell-associated binding proteins induces expression of pro-thrombotic and pro-inflammatory molecules. Methylglyoxal (MG), which forms MG-derived AGE the primary intracellular AGE induced by hyperglycaemia, is elevated in diabetic subjects with vascular disease. Detoxification of MG occurs through the glyoxalase system incorporating glyoxalase-1 and glyoxalase-2. Overexpression of glyoxalase-1 in endothelial cells prevents hyperglycaemia induced AGEs formation. Perturbations of the glyoxalase-1 gene (GLO1) may result in vulnerability to vascular complications through alterations in pro-thrombotic factors due to AGE interactions. To investigate this, we screened the coding region and 1kb of the 5' untranslated region of GLO1 for polymorphisms and determined their relationship to prothrombotic markers in 519 healthy individuals.
Materials and Methods: GLO1 was screened using Polymerase Chain Reaction and Temperature Modulated Heteroduplex Analysis. Biallelic

variation was confirmed using an ABI 310 automated sequencer and studied by Restriction Fragment Length Polymorphism.
Results: Common single nucleotide polymorphisms (SNP) were identified at positions –8 (c to t) and 20202 (c to a, Ala111Glu) from the translation start site and studied in 535 healthy subjects from 89 families. Data for 18 family members was not available; genotype frequencies of the remaining 519 for position 20202 and –8 were in Hardy-Weinberg equilibrium and linkage disequilibrium (cc=105, ca=266, aa=148; cc=126, ct=279, tt=114; D=27% respectively). A significant association was found between SNP at position 20202 and PAI-1 antigen levels and –8 and Factor XIII complex when analysed for pro-thrombotic markers by ANOVA (factor VII coagulation activity, PAI-1 antigen level, fibrinogen, tPa and Factor XIII complex)(p=0.001 and p=0.042 respectively). When adjusted for pedigree using SOLAR, a significant association remained between the SNP at position 20202 and log PAI-1 levels and was estimated to account for 1.3% of its variance (p=0.04). When adjusted for confounding factors (age, sex, smoking status, insulin resistance (HOMA), 4G/5G PAI-1 SNP) the association proved to be non-significant (p=0.1).
Conclusion: In healthy individuals the common SNP at position –8 and 20202 in human GLO1 are associated with pro-thrombotic markers. This association is weakened when adjusted for confounding factors, but suggests that a differential ability of glyoxalase-1 to reduce the formation and subsequent interaction of AGE may have a role in the structural and functional manifestations diabetic vascular disease.

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The risk for venous thromboembolism is markedly elevated in diabetes patients.
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Background and Aims: Diabetes mellitus is associated with several alterations in coagulation and fibrinolysis, that may lead to a thrombogenic propensity but it is still not known whether these changes in fact cause increased risk of venous thromboembolism (VTE).
Methods: In a retrospective study we evaluated the medical records of all 302 adult patients (M/F 151/151), who were admitted to the Umea University Hospital with verified deep venous thrombosis or pulmonary embolism during the years 1997-1999. The patients were classified as diabetic (D, n=56) and nondiabetic (ND, n=246) according to previously or newly diagnosed diabetes as defined by the 1998 WHO criteria. The total number of diagnosed diabetics in different age groups in the catchment area were obtained from computerized registries of patients in the primary health care centres and the Umea University Hospital and this was related to background data from the population registry.
Results: The incidence of VTE increased with age in both D and ND. D patients were significantly older than ND (71.1±12.9 and 65.8±16.8 (mean±SD) yrs, respectively, p=0.010) and they more often had coronary heart disease, hypertension and congestive heart failure (p<0.05), whereas the sex distribution did not differ. The annual incidence rate of VTE among D in the population was 431/100000 (95 % CI 375-496) and in ND 78/100000 (68-88) and the risk ratio for D vs ND was 5.57 (4.17-7.43). Both pulmonary embolism and deep venous thrombosis were more common in D but pulmonary embolism was relatively more frequent (37.5% vs 22.3% of all VTE in D and ND, respectively, p=0.018). The annual VTE incidence was elevated in both type 1 (704/100000; 314-1566) and type 2 D (412/100000; 312-544). The overall Standardized morbidity ratio was 2.27 (1.75-2.95), i.e. D patients were more prone to VTE even after adjustment for age differences.
Conclusions: These results suggest that the age-adjusted risk for venous thromboembolism is more than 2-fold higher among diabetic patients as compared to the nondiabetic background population.

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Insulin enhanced thrombin-activatable fibrinolysis inhibitor expression through PI3 kinase/Akt pathway.
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Background and Aims: Thrombin-activatable fibrinolysis inhibitor (TAFI) is a glycoprotein, linking coagulation and fibrinolysis. We have reported that plasma TAFI levels were elevated in the patients with type 2 diabetes mellitus and that it was significantly correlated with insulin resistance and the visceral fat area of the patients. Furthermore, TAFI was expressed and secreted from adipocytes. However, only a limited study has elucidated about the regulation of TAFI expression in adipocytes. We hypothesized that TAFI was an important causative factor of hypofibrinolysis in patients with obesity and insulin resistance and that insulin was a potent modulator of the gene expression of TAFI, such as other adipocytekines. To evaluate this hypothesis, we examined the enhanced expression of TAFI by insulin and analyzed its molecular mechanism in 3T3-L1 adipocytes.
Materials and Methods: We used 3T3-L1 cells to study gene expression of TAFI in adipocytes. Before treatment, cells were well differentiated into adipocytes and serum starved over night. Cells were pretreated with or without various inhibitors (phosphatidylinositol 3-kinase (PI3 kinase) inhbitor wortmannin, MEK-1 inhibitor PD98059 and protein kinase C inhibitor GF109203X) for 1 h and then incubated with insulin for 3 h. Total RNA was extracted from the cells and the expression levels of TAFI mRNA was examined by RT-PCR. Furthermore, in order to selectively turn on the Akt kinase cascade, 3T3-L1 was stably transfected with a tamoxifen regulatable Akt-1 construct (MER-Akt). Cells were differentiated into adipocytes and treated with tamoxifen for 3 h and then observed TAFI mRNA.
Results: The expression of TAFI mRNA was induced by insulin in 3T3-L1 adipocytes. Wortmannin inhibited insulin-induced expression but PD98059 and GF109203X had no effects. These data suggested that the gene expression of TAFI was enhanced by insulin and regulated by PI3 kinase signaling pathway. In MER-Akt expressing 3T3-L1 adipocytes, tamoxifen activated Akt and induced the expression of TAFI mRNA to a similar extent by insulin. Taken together, TAFI was induced expression by insulin through PI3 kinase/Akt pathway in 3T3-L1 adipocytes.
Conclusion: The expression of TAFI was enhanced by insulin and the activation of Akt alone is sufficient to induce the TAFI expression in 3T3-L1 adipocytes. It is supposed that plasma TAFI levels are regulated at least in part by transcription levels in adipose tissues of patients with obesity or insulin resistance. The elevated plasma TAFI may contribute to the increased risk of atherothrombotic disease.

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Thiazolidinedione-mediated inhibition of PAI-1 expression in C11-STH human vascular endothelial cells: a possible mechanism for rosiglitazone and pioglitazone-mediated attenuation of atherosclerosis in the metabolic syndrome.
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Background and Aims: Accelerated atherosclerosis (AS) is a part of the metabolic syndrome (MS). Vascular endothelial cell (EC) dysfunction is associated with the progression of AS. PAI-1 secreted from vascular EC's is a marker of EC function. PAI-1 is increased in plasma of patients with the MS and correlates with the progression of AS. PAI-1 mRNA and protein is increased in response to insulin and tumour necrosis factor a (TNFa), and may be causal in accelerated AS identified in MS.
The thiazolidinediones (TZD) rosiglitazone (RG) and pioglitazone (PG) modulate their effects via activation of the nuclear receptor peroxisome proliferator-activated receptor (PPAR) gamma. The effects of TZD's and other PPAR gamma agonists on PAI-1 expression in vascular EC's remain controversial with reports of both induction and inhibition of PAI-1 expression.
Our study aims to clarify this uncertainty and assess the effects of these agents on events surrounding PPAR gamma activation.
Methods: RNA extraction and Northern Blot Analysis
RNA (10 ug) extracted from C11-STH cells was subjected to gel electrophoresis and Northern blotting. Hybridisation was performed using the 1.1 kb Pst-1 fragment of PAI-1 and the 1.1 kb Pst 1 fragment of GAPDH.
PAI-1 Protein
Conditioned medium from C11-STH cells was removed and stored at -20oC. The concentration of PAI-1 protein was measured by enzyme linked immunosorbent assay using kits from Biopool (TintElize, Umea, Sweden).
Nuclear Protein Preparation and Electrophoretic Mobility Shift Assay (EMSA)
5-10ug of nuclear protein (NP) from C11-STH cells were mixed with a 32P radiolabelled oligonucleotide (OGN) harbouring the PPAR gamma consensus binding sequence (5'-CAAAACTaggtcaAAAAGGTCA-3') and loaded onto a 5 % acrylamide gel, electrophoresed for 2 hours, dried and subjected to autoradiography.
Results: We have demonstrated that RG and PG inhibit both TNFa and insulin-mediated induction of PAI-1 mRNA and protein expression in C11-STH cells at 10 micromolar over 16 hours. Neither RG nor PG inhibited

basal PAI-1 expression. NP's extracted from C11-STH cells demonstrated specific binding to an OGN harbouring a PPAR gamma consensus binding sequence.
Conclusions: The TZD's RG and PG attenuate PAI-1 expression in stimulated C11-STH cells. C11-STH cells express NP's capable of binding to an OGN harbouring a consensus PPAR gamma binding motif suggesting that the regulation of PAI-1 gene expression by TZD's may occur through this nuclear receptor. We hypothesize that inhibition of stimulated PAI-1 expression partly accounts for the postulated benefits of these agents in vascular protection in subjects with the MS.

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IRS-1 mediates platelet inhibition by insulin.
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Background and Aims: Hyperresponsive platelets are partly responsible for the athero-thrombotic state in diabetes mellitus. Platelet agonists induce activation mainly through the heterotrimeric G-protein Gq and at the same time lower the level of the inhibitor cAMP through the G-protein Gi. Since insulin is known to attenuate platelet functions by interfering with mechanisms that reduce the level of cAMP during platelet stimulation, we investigated whether this mechanism was mediated via Gi.
Materials and Methods: Isolated, washed platelets were incubated with 0.01-100 nmol/l insulin under euglycemic conditions and the mobilization of calcium ions by ADP (10 µmol/l) and alpha-thrombin (0.25 U/ml) was measured together with levels of cAMP, tyrosine phosphorylation of Gi alpha2 and involvement of IRS-1. Platelet aggregation was measured in platelet-rich plasma.
Results: Upon platelet activation, insulin dose-dependently inhibited calcium mobilization (17% at 0.5 nmol/l and 30% at 100 nmol/l, $P<0.01$) and partially abolished the decrease in cAMP accumulation that accompanies thrombin-induced calcium mobilization ($P<0.01$). Inhibition by insulin is transient and optimal after 5 (ADP) and 10 (alpha-thrombin) minutes incubation (resp. 1 and 100 nmol/l). L-NMMA (100 µmol/l) did not antagonize the cAMP stabilizing effects of insulin. Insulin induced a transient dose-dependent increase of the tyrosine phosphorylation of Gi alpha2, being maximal after 2-5 minutes incubation (1 nmol/l insulin). Within this time period IRS-1 co-precipitated with Gi alpha2 upon incubation with 1 nmol/l insulin. Dissociation of IRS-1 from Gi alpha2 was delayed when incubated with 100 nmol/l insulin. These findings suggest that insulin interferes with the activation of Gi alpha2. Epinephrine is known to activate platelets via Gi2/Gz and to inhibit the activity of IRS-1. Indeed, epinephrine abolished the effects of insulin on calcium mobilization, cAMP, aggregation and tyrosine phosphorylation of Gi alpha2.
Conclusion: Insulin inhibits platelet functions by interfering with the mechanisms that reduce the level of cAMP during platelet stimulation via tyrosine phosphorylation of Gi alpha2 by IRS-1.

PS Education 1
Teaching the Teachers

1225

Taking diabetes to school - a survey of school nurse knowledge of general diabetes management and insulin pump therapy: implications for diabetes educators.
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Background and Aims: Diabetes is one of the most common chronic conditions in school-age children, affecting 151,000 children and adolescents in the United States (US). Insulin pump therapy is becoming increasingly more popular in the US with this age group. While children are at school, teachers and school nurses are the primary caretakers and therefore play a key role in the treatment of diabetes during the school day. The purpose of this survey was to determine the level of knowledge about diabetes mellitus and insulin pump therapy in school nurses. Results of the survey can assist diabetes educators in the development of educational programs and materials to educate school nurses and teachers about general diabetes management issues and insulin pump therapy in school-age children.
Materials and Methods: School nurses attending a 2 day regional continuing education symposium on various topics were surveyed to determine their general diabetes knowledge and basic insulin pump therapy knowledge. The questionnaire was distributed prior to a general session presentation on "Update on Diabetes Management during the School Day" and a breakout session on "The Basics of Insulin Pump Therapy". Questionnaires consisted of 24 multiple-choice questions on general knowledge and insulin pump therapy. The general knowledge section included questions about the following four topics; treatment of hypoglycemia, sick-day management, insulin action, and exercise. The insulin pump section included questions about general management and safety issues associated with pump therapy, and troubleshooting pump issues during the school day.
Results: All of the 153 symposium attendees received questionnaires. One hundred questionnaires were returned. Over 40% of the school nurses could not identify symptoms of diabetic ketoacidosis (DKA) and basic action of rapid-acting insulin. Additionally, 23% did not know that exercise could lower blood glucose levels. Twenty-one percent of the survey respondents either left the insulin pump question section blank of stated on the questionnaire that they lacked the lacked the knowledge to complete the section. Of the 79 respondents that answered the questions, 34% did not know how fast DKA could develop if the infusion of insulin was interrupted or how to respond to more than two unexplained blood glucose levels over 250mg/dl and 35% did not know what action to take if the infusion set was accidentally disconnected.
Conclusion: The results suggest that school nurses are poorly informed about insulin pump therapy and many lacked general diabetes knowledge about sick day management, insulin action and effects of physical activity on blood glucose levels. Diabetes educators can use this information to create continuing education programs for school nurses. The program content should include information about general diabetes management topics in the school setting to minimize the risk of short-term complications, such as hypo and hyperglycemia. With the increased use of insulin pump therapy in pediatric populations to achieve more desirable blood glucose control as well as to accommodate flexibility in food choices and physical activity, school nurses need to be better equipped to provide the continuity of care during the school day.

1226

A new tool to evaluate the change of HCP's professional personality in therapeutic patient education.
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Background and Aims: **T**herapeutic **P**atient **E**ducation (**TPE**) is an approach which forces HCPs to reconsider their professional identity so as to improve their relationship with the patient. Five years ago we implemented a 3-year Diploma (DIFEP) on **TPE** run by Geneva University Medical School and have developed the concept of professional personality to better understand the change HCPs go through during the training. In the field of evaluation of continuing education programmes, few tools take into account the precise characteristics of such a programme. We have therefore

created a tool using specific criteria to show the change of participants' professional personality during that training.
Materials & Methods: A group of 9 experts (3 doctors, 2 nurses, 2 psychologists, 1 specialist in adult education, 1 anthropologist), held 3 meetings. They were chosen according to their profession and their status in the DIFEP (2 **TPE** graduates, 2 teachers, 2 programme creators and 3 coordinators). Led by a specialist in continuing education, the exchanges clarified the focus of the evaluation and the requisite criteria. This tool is tested on current Diploma participants in order to validate these criteria. After this test, 3 semi-directive discussions led by a social sciences specialist (held before the programme, end of the first year, end of the programme, and 1 year after termination) should allow us to understand the manner in which participants have benefited from this training.
Results: The group of experts agreed on the concept "*change of the professional personality*" as being the parameter which could show the impact of the DIFEP training. This modification can be defined by 4 criteria describing the evolution of the participant towards: **1)** his **identity**, *e.g. ability to step back*, *to analyse one's actions, assertiveness;* **2)** the **others**, *respecting the differences, working in an interdisciplinary setting, ability to build in conjunction with others (professionals and patients);* **3)** his **environment**, *finding his own place, analysing and intervening in the working environment, innovating;* **4)** his **training**, *taking his place within a group of trainees, ability to question himself, understanding the process of learning.*
Conclusions: The impact of continuing education programmes for HCPs on **TPE** must continuously be assessed in order that they remain accurate and pertinent. Such an evaluation must not only consist of taking into account the participants' degree of satisfaction and/or practical skills, it must also test numerous criteria reflecting the evolution of the professional personality. This evolution is necessary for HCPs to really modify their values, attitudes and behaviour towards patients. Therefore, the participation of the **TPE** graduate during the process of creating such a tool is essential.

1227

Distance delivery of education in diabetes care for Primary Care Organisations.
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Background and Aims: The Certificate in Diabetes Care has been a University course for many years. To meet demand, a new method of „Distance Delivery" was introduced using Primary Care Organisations (PCOs). Two health professionals from a PCO attend training at the University, then return home and deliver the Certificate course to GPs, nurses and other professionals in their own area. Local teachers are recruited from all sectors of care. Quality is assured by University staff. The Certificate course comprises 5 taught days, three pieces of written course work, a written examination and a research project.
The aim of this study was to evaluate effectiveness, feasibility and acceptability of this innovative method of delivering diabetes care education.
Materials and Methods: Evaluation was by 2 questionnaires, the first on day 1 of the Certificate course, and the second with the final assessed work, usually 9 months later.
Results: Over 569 participants completed both questionnaires, from 21 completed courses. Knowledge of 68 diabetes-related topics increased ($P<0.001$). A mean of 4 changes per practice, made or planned by the end of the course, were reported, including tighter targeting of BP, HbA1c and BMI; more footcare; and better recall systems. The final levels of knowledge and the number of changes in practice were similar for all subgroups of participants (size of practice, teaching practice, having special diabetes clinics, having a diabetes register). Thus, the course is effective for all health care professionals. 97% of participants would recommend the course to others. The changes reported on Distance Delivered courses matched those from courses led by staff of the University in content and frequency.
Conclusion: This innovative method of Distance Delivery of university level diabetes education for health care professionals, is effective, feasible, and acceptable. It is an appropriate way to implement the necessary education in Diabetes Care to meet national requirements. Educated health care professionals can deliver effective education, support and advice to people living with diabetes.

1228

Diabetes literacy and its determinants in diabetic patients attending a new endocrinology centre in northern India.
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Background and Aims: Diabetes literacy or diabetes education is a relatively unknown concept in northern India. There are few diabetologists, and even fewer educators to serve a rapidly increasing, ill-informed population of diabetics. This study was performed in a new endocrine center offering counseling services to a population that had no prior exposure to diabetes education. The objectives were to assess the diabetes literacy of patients attending a new endocrine clinic, to ascertain the factors determining diabetes literacy, and to assess its effect on glycemic control.
Research Design and Methods: 170 consecutive diabetic patients with a minimum follow-up of 30 days at our center, including at least 2 consultations with a trained diabetes educator, were administered a pre-tested questionnaire by a resident doctor. Patients scored the quality of knowledge shared with them in 15 domains as 2 [good], 1 [average] or zero [poor], thus giving a total Diabetes Literacy Score [maximum possible score 30]. Scores were analyzed for correlation with various variables including glycemic control. This was assessed by measuring the mean of 3 fasting blood glucose levels. Statistical significance was measured by t test.
Results: 94 males [66 urban, 28 rural; 6 illiterate] and 76 females [60 urban, 16 rural; 18 illiterate] completed the questionnaire. Of these, 58 males and 60 females enjoyed adequate glycemic control. The average literacy score was 17.92 (59.73 %). The best scores were obtained in the domains of diet (81.17 %), tablet compliance (74.12%) and insulin technique (71.76%), while the poorest score related to counseling for sexual dysfunction (23.52%).
There was no correlation of literacy score with age, gender, educational status, duration of diabetes or family income. Scores correlated strongly with duration of follow-up (19.32 in persons with follow-up > 6 months vs. 16.17 in those with follow-up < 6 months; $p< 0.01$) and with glycemic control (19.16 in adequately controlled vs 16.03 in inadequately controlled subjects; $p <0.01$). There was significant correlation with positive family history of diabetes (19.10 vs. 17.17; $p = 0.05$)
Conclusions: Regular counseling contributed to improved glycemic control in diabetic patients, irrespective of age, gender, socioeconomic background, educational status or duration of disease. Diabetes literacy was better in patients with longer follow-up at our center, in persons with adequate glycemic control, and in those with a positive family history of diabetes. The study also pointed out lacunae in our counseling services, e.g., education regarding sexual dysfunction.

1229

Diabetes education: results of a global survey.
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Background and Aims: The International Diabetes Federation (IDF) Diabetes Education Consultative Section's (DECS) efforts are targeted to address the needs of three audiences: the person with diabetes and those affected by diabetes, the health care professionals responsible for providing diabetes care and the public. The aim of this project was to capture education practices that apply to each of these audiences through a global survey.
Materials and Methods: DECS members designed the survey that was sent to IDF member associations via mail and electronically. Global surveys representing 57 countries and the 7 IDF regions were examined for 122 diabetes healthcare professionals (24.6% physicians, 12.3% nurses, 7.0% educators, 3.5% pharmacists, 2.6% dietitians, 15.8% diabetes association professional, 17.5% academia, 16.7% other) for calendar year 2002.
Results: When asked who provides education in your country, all regions identified physicians as the primary education resource except in the Eastern Mediterranean and Middle East, which reported pharmacists as the primary resource. Two to three day courses were most frequently reported as the mechanism used to train educators; however, Africa and South East Asia reported that there was very limited or no educator training available. All respondents reported that patients accept the role of diabetes educators and all were aware of published studies validating the importance of education. Teaching tools are reported to be available except in Africa, but all regions supported the need for computerized resources and networking opportunities. The respondents expressed interest in an international summit addressing the global needs and problems related to diabetes

education and identified the IDF as the primary resource for support in training. Although all countries said that patients have access to diabetes education, barriers were reported in all countries. The most frequently reported barriers were financial, limited access, lack of knowledge and education resources. Training and advocacy efforts directed toward health ministers and public awareness were the mechanisms most often identified as a means to address these barriers.
Conclusions: Despite limitations of the survey method, consistent themes were reported that need to be addressed as the world incidence of diabetes grows. For prevention and treatment of diabetes to be successful through education initiatives, governments and local, national and international health associations need to organize efforts to promote the training, financial support, access and public awareness of diabetes education.

1230

Insulin injections of elderly patients with Type-2 diabetes mellitus and impaired cognitive function after participation in a structured geriatric treatment and teaching programme for insulin therapy.
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Background and Aims: Special didactic strategies have to be considered in a geriatric treatment and teaching programme (TTP), because cognitive function of elderly patients is often impaired (Diab Stoffw. 2000; 9:227-233). In 1999 a specialised TTP for patients with type-2 diabetes mellitus and insulin therapy was developed from Schiel et al. (Braun et al. Diabetologia 2001; 44: A251), the Dicof-TTP.
Materials and Methods: 57 patients with type-2 diabetes mellitus older than 54 years, who admitted hospital for participation in a TTP for insulin therapy and had an impaired cognitive function (IQ<=90 points) were included in the trial. They were either randomized to the standard TTP of Berger et al. (n=31) or to the geriatric Dicof-TTP (n=26). Immediately after participation in the TTP and 6 months later the ability for correct insulin injection was assessed using a standardized „ Handling-Test". Patients` characteristics are shown in Table 1.
Table 1. Patient`s characteristics at randomisation
*(*HbA1c: Diamat® , NR:4,4-5,9%]*

	Standard TTP	Dicof TTP	p-value
Number of patients	31	26	
Age (Years)	67,6± 9,1	70,0± 8,5	0,32
Diabetes duration (years)	9,5 (0,04-35,4)	10,9 (0,14-24,5)	0,76
HbA1c (%)*	10,4± 2,1	10,4± 1,5	0,95
Cognitive function (IQ-Points)	81,3± 5,1	79,4± 6,3	0,22

Results: Patients participated in the geriatric DicoF-TTP showed clearly better results in the „Handling-Test" six months after TTP (Table 2).

Table 2. Results Handling-Test

	Dicof-TTP (n=26)		**Standard-TTP(n=31)**	
	After TTP	**6 months later**	**After TTP**	**6 months later**
When to inject insulin?	26/100%	25/96,2%	31/100%	29/93,5%
How long do you wait with meal after you have injected insulin?	26/100%	**24/92,3%**	30/96,8%	**20/64,5%*#**
Pen shaken?	22/84,6%	**24/92,3%**	29/93,5%	**21/67,7%*#**
Insulin dose correct ?	22/84,6%	22/84,6%	29/93,5%	27/87,1%
Abdomen wrinkle made before insulin injection?	23/88,5%	**22/84,6%**	27/87,1%	**15/48,4%*#**
Injection correct?	25/96,2%	23/88,5%	30/96,8%	**24/77,4%#**
Place of insulin injection correct?	25/96,2%	23/88,5%	27/87,1%	24/77,4%
Waiting after insulin injection correct ?	22/84,6%	**23/88,5%**	27/87,1%	**16/51,6%*#**

Number/% of patients, who managed tasks correctly *p<0,05 DicoF vs. Standard Group; #p<0,05 Standard Group after TTP vs. 6 months later

HbA1c-decrease and avoidance of acute comlpications were comparable in both groups, concerning diabetes-self-management the Dicof-TTP was more effective (Braun et al. Diabetologia 2001;44: A251).
Conclusion: Adequate structured treatment and teaching programmes like the geriatric Dicof-TTP for insulin therapy, which improves outcome quality considerably, should be available in routine care to meet the geriatric therapeutic goals independence and acceptance of treatment.

PS Education 2

Rehabilitation, Foot Care - Visual Improvement

1231

Referring patients with prolonged self-management difficulties to diabetes rehabilitation.
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Background and Aims: Diabetes self-management is demanding and a considerable number of patients have difficulties to sustain adequate self-management, showing in poor control and/ or psychosocial problems. We developed a Multidisciplinary Intensive Education Program (MIEP) for patients with prolonged self-management difficulties. At one year follow-up, MIEP turned out to be effective in improving HbA1c and health related quality of life (HR-QoL) . The aim of this study is to determine how potential participants of MIEP can be detected and whether referral to MIEP is efficient.
Materials and Methods: 114 participants of MIEP were compared with 231 non-referred consecutive patients at the diabetes outpatient clinic of the University Hospital Groningen. For 201 outpatients, their physicians assessed whether they considered them appropriate for MIEP. We measured HbA1c, self-reported (severe) hypoglycemia, HR-QoL (Rand-36, sub-scales: mental health, vitality, social and physical functioning) and problem areas in diabetes (PAID, sub-scales: negative emotions, treatment problems, food-related problems and lack of social support).
Results: Out of 201 outpatients, the physicians indicated 54 patients to be potential candidates for MIEP. Logistic regression showed that this physicians' judgement was predicted by respectively age (lower), HbA1c (higher) and social functioning (lower). ANOVA analyses with Bonferroni post-hoc test were applied to compare the three groups of patients (114 *participants*, 54 outpatient *potential participants* and 147 outpatient *non-participants*). Both participants and potential participants were younger (44 vs. 49 years), had higher HbA1c (8.44 and 8.84 vs. 7.67) and had worse social functioning. Moreover, participants scored worse on vitality and mental health and on all 4 sub-scales of the PAID compared to non-participants, whereas potential and non-participants did not differ on these variables.
Conclusion: With respect to referral to MIEP, physicians primarily focus on HbA1c and to some extent on social functioning and younger patients are more likely to be referred to diabetes rehabilitation. Besides HbA1c and social functioning, participants of MIEP, who are selected by several members of the multidisciplinary rehabilitation team (physician, diabetes nurse specialist, dietician and psychologist) showed a much wider variety of diabetes-related (psychological) difficulties. These findings might indicate that psychological problems are underdiagnosed in diabetes treatment resulting in erroneously not referring to additional care. The current system of 3 or 4 10-minutes check-ups a year offers unsatisfactory opportunities to detect these problems; a more integrated screening method has been advised, in which the diabetic nurse specialist could play a more central role.

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Long-term effects of an interval education program for rehabilitation of diabetics with manual occupations.
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Background and Aims: The education of insulin treated diabetic patients working as craftsmen or in industrial occupations has to be more intensive due to a higher risk of hypoglycemias and a more difficult blood glucose control. Therefore, a novel interval education and training program was developed in a specialized rehabilitation clinic for diabetes including a basic education over 2 weeks followed by 2 booster education periods over 1 week each after 6 and 12 months.
Patients and Methods: Among 105 patients with intensified conventional insulin therapy who had completed the whole program, 58 subjects could

be evaluated 3 years after the start of the first education by means of a mailed questionaire received from their house doctors. Data of these 58 individuals (type 1: 86%, type 2: 14%, male: 74%, mean age: 26.6 years, mean diabetes duration: 10.9 years) were compared between the beginning of the first education (T1), the end of the second booster education (T4) and the 3-year follow up (T5).
Results: The mean weekly number of blood glucose self monitoring increased from 20.83 (T1) to 27.76 (T4) and 32.63 (T5) (P<0.001). The average HbA1C value (normal 4.3-6.1%) decreased from 8.26% (T1) to 6.84% (T4) and 6.82% (T5) (P<0.001). The rate of severe hypoglycemias per year and patient slightly raised from 0.069 to 0.103 during the first year of the program and returned to the basic level after the second year (P=ns). The mean duration of hospitalisation per year and patient due to diabetes or complications was reduced from 5.7 to 1.8 and 1.0 days during the first and third year of the program (P<0.01). In parallel, the average absence from work due to diabetes morbidity fell from 35.6 to 11.6 and 11.9 days (P< 0.001).
Conclusion: Despite frequently difficult working and social conditions, insulin treated diabetic patients with manual occupations can achieve a very good quality of metabolic control by means of a novel interval education and teaching program designed for their special requirements.

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Long-term effects of diabetes rehabilitation for patients with prolonged self-management difficulties.
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Background and Aims: In the Northern part of the Netherlands, diabetes patients who underbenefit from regular care showing in prolonged poor glycemic control and/ or psychosocial problems, can be referred to a Multidisciplinary Intensive Education Programme (MIEP). MIEP is a 10-week (and 2 booster sessions) outpatient rehabilitation programme based on the empowerment approach. The aim of this study is to determine long-term effects and determinants of effect.
Materials and Methods: Ninety participants (age 47 (SD 14); diabetes duration 12 (SD 10 years); 45 % male) were measured at baseline (T=0), 3 (T=1) and 12 months follow-up (T=2). We measured glycemic control (HbA1c), health related quality of life (HR-QoL: Rand-36, sub-scales mental health, vitality, social and physical functioning; disease-specific DQOL, sub-scales satisfaction with treatment and impact of diabetes) and indicators of empowerment (health locus of control and coping). 231 non-referred outpatients served as controls.
Results: Both at T=1 and T=2 HbA1c improved compared to T=0, although initial improvement was partly lost (M at T=0 = 8.3 SD 1.1; M at T=1 = 7.8 SD 1.1; M at T=2 = 8.0 SD 1.1). At T=1, men and women improved about equally, but women consolidated improved HbA1c, whereas men relapsed. Patients improved in several HR-QoL domains (mental health, vitality, social functioning and satisfaction with treatment), without relapse at T=2. At vitality and social functioning, patients even further improved between T=1 and T=2. Patients with worse baseline functioning improved most in HR-QoL. After MIEP, patients became more empowered (both at T=1 and T=2), which explained additional variance in HR-QoL improvement. At T=2, participants did not differ anymore from average, non-referred diabetes patients from the outpatient clinic.
Conclusion: MIEP has benefical one year follow-up effects on HbA1c, HR-QoL and empowerment, especially for patients with poor status at baseline. MIEP holds promise for patients with prolonged self-management difficulties, for whom other forms of care have proven to be inadequate.

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Rehabilitation of Type 1 diabetic patients: the experience of rehabilitation diabetologic centre in Belarus.
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Background and Aims: To estimate the influence of rehabilitation course on the metabolic control, psychological status, diabetes-associated behaviour, frequency of acute complications and the duration of hospitalisation in patients with diabetes mellitus type1 (DM1).
Materials and Methods: Study group consists of 34 DM1 patients (mean age 27,0±1,1, mean DM1 duration - 12,0±1,3 years (21f, 13m). Patients received the 16 days rehabilitation course in Republican Rehabilitation Diabetologic Centre (RRDC). The course included insulintherapy correction, psychodiagnostics (interview; self-estimation of a psychological condition on parameters of status of health, activity and mood; estimation of situation and personal anxiety by Spilberger) with the subsequent psychocorrection (individual assistance, groups training), physical exercises program, self-monitoring education by special program. Rehabilitation measures effectiveness estimated by dynamics of fasting and postprandial glycemia, daily insulin dose, psychological status parameters, diabetes-related behaviour, acute complications incidence (hypoglycemia, ketoacidosis), hospitalisation duration at 1 year follow-up.
Results: By the end of rehabilitation course decrease of fasting glucose from 11,5±0,4 to 8,6±0,3 mmol/l (p<0,001) and postprandial glycemia from 10,2±0,3 to 7,9±0,2 mmol/l (p<0,001); daily insulin dose from 0,74±0,03 to 0,65±0,02 U/kg (p<0,05), improvement of psychological status from 4,9±0,1 to 5,6±0,1 points (p<0,001); activity from 5,0±0,1 to 5,7±0,1 points (p<0,001); mood from 5,4±0,1 to 5,8±0,1 points (p<0,01). Situate anxiety decreased from 36,0±0,9 to 32,0±1,0 points (p<0,01), personality anxiety from 37,0±1,0 to 34,0±1,0 points (P<0,05). One year after rehabilitation 47% of patients perform self-monitoring vs 31% prior the rehabilitation; insulin dose correction depending on glycemia - 100% vs 47% (p<0,01), on food carbohydrates content 89% vs 32% (p<0,01), on physical activity - 89% vs 21% (p<0,001). Frequency of heavy hypoglycemia decreased from 0,59±0,17 to 0 times per year per person (p<0,01), frequency of ketoacidosis - from 0,41±0,14 to 0,23±0,10 times per year per person. Emergency hospitalisation duration decreased from 5,5±2,8 to 2,9±1,6 days per year per person, planned - from 3,1±1,1 to 1,2±0,9 days per year per person.
Conclusion: Complex rehabilitation of DM1 patients including self-monitoring education, physical exercises, psychocorrection improves treatment effectiveness that is confirmed by decrease of acute complications frequency and hospitalisation duration. The created rehabilitation scheme can be recommended for the implementation.

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Factors affecting foot-care knowledge and foot-care behaviors in Korean diabetic patients.
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Background and Aims: Foot complications are one of the major health problems in diabetic patients, increasing the incidence of amputation and subsequent disabilities. We performed this study to identify factors affecting foot-care knowledge and foot-care behaviors in Korean diabetic patients.
Materials and Methods: The patients with diabetes mellitus attending a university hospital (n = 193) were enrolled during a 1 month period. A clinical nurse specialist conducted patient interviews and foot examinations. The interview was based on the questionnaires dealing with various aspects of foot-care knowledge and behavior.
Results: Among the socio-demographic variables, sex and age affected the foot-care knowledge and behaviors: Women showed significantly higher level of foot-care knowledge (p=0.039) and foot-care behaviors (p=0.002). Young people showed higher level of foot-care knowledge than old people (p=0.001). Patients having had higher education showed higher level of foot-care behaviors (p=0.006). Having had a foot-care education affected foot-care knowledge (p<0.001) and foot-care behaviors (p<0.001). Foot-care knowledge and foot care behaviors were significantly inter-related (r=0.375, p<0.001). Nevertheless, in patients having had previous foot injury, the level of foot-care behavior was higher (p = 0.010) than those without previous foot injury, even though foot-care knowledge level was not different.
Conclusion: This study reemphasized the importance of foot-care education especially in men and old patients. Emphasis should be made on the foot-care behavior skills rather than the foot-care knowledge. By comprehensive foot-care education, more patients with diabetes will perform effective foot-care, and save their foot from amputation.

1236

Evaluation of a structured foot education programme for patients with diabetes mellitus at risk for foot complications.

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Background and Aims: To determine the effects of a structured foot education programme for patients with diabetes and an increased risk for foot complications.
Materials and Methods: The study population consisted of 101 patients with type 1 (n=8) or type 2 (n=93) diabetes (M-73, F-28), the duration of diabetes was 15,29±9,88; at mean age 62,9±8,4 years. History of ulcers (n=52), of wich (n=7) were not yet healed. Diabetic neuropathy had (n=97) patients, peripheral vascular disease (n=30), osteoarthropathy (n=4). 12 had a minor amputation, one had a major amputation. The vibration threshhold was 1,50±1,58 right, 1,56±1,63 left. The foot education programme for patients with diabetes took place as part of the „Barfuß“ (barefoot) project of the German Association of Diabetes Counsellors (VDBD). This project consisted of initial structured further training of diabetes educators according to the guidelines of the international consensus on the diabetic foot. Follow-up took place with 95 patients (6 drop out: data not available) at 7,47±2,40 months subsequent to completion of the education programme. The programme was evaluated by measuring objective outcome parameters and questionnaires on foot knowledge, selfcare, satisfaction and burdens of diabetes and due to foot problems, and Quality of Life with Diabetes (LQD-R2).
Results: According to the rating scales on foot condition, the parameter skin care had improved at follow-up (p=0,001). The parameters callus (p=0,017) and nail care (p=0,023) showed significant improvement. 6 months after education there were significant changes in knowledge (p=0,032) and reported selfcare (p=0,0001). The questionnaire on burdens and satisfaction with the feet showed significant changes on the factors „appreciation“ and „defence“ (p=0,0001). The questionnaire on Quality of Life with Diabetes showed increase in satisfaction (p=0,005).
Conclusion: We conclude that patient-centred, empowerment-oriented education programmes can lead to positive behavioural changes and attitudes.

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Development of therapeutic diabetes education standards for the visually impaired.

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Background and Aims: A previous study of the status of visually impaired diabetics in Poland revealed poor knowledge about diabetes, lack of adaptive techniques and skills, improper health behaviours and poor metabolic control of diabetes – all deficiencies that could be helped by improvements in education. Therefore, the aim of the current study was to develop and implement standards of therapeutic diabetes education for visually impaired persons with diabetes (VIP), with the goal of improving both general adaptation and metabolic outcomes.
Materials and Methods: An Adaptive Teaching Program (ATP) for VIP was developed and pilot tested. Materials included a program manual and sets of adaptive teaching tools to be used to consistently implement the ATP. The ATP was tested with 93 VIP-s (mean: age 54.9± 14.4, diabetes duration 21.1± 9.8, duration of visual impairment 11.6± 9.3) at six centres throughout Poland.
Results: As we presented previously, the ATP improved both the metabolic control and independence of participating VIP. Draft Standards for Therapeutic Education and Rehabilitation of VIP-s based on the ATP were agreed upon by an interdisciplinary Consensus Conference held at the close of the pilot test period. The final version of the standards will be presented. It includes the following topics: main problems and needs of VIP-s, terminology, strategies and principles of therapeutic education, methods of adaptive teaching, and medical issues. The latter addresses quality assurance, and the training of diabetes educators and rehabilitation instructors to use the Standards. The outcomes and recommendations of this project also inspired development of the first Polish Voice Module for Accu-Check® Active for VIP-s which will be presented in separate presentation.
Conclusion: Visually impaired persons with diabetes are capable of caring for themselves when they are provided with appropriate adaptive diabetes self-management education, tools, and techniques. Regional consensus standards may serve diabetes educators and rehabilitation instructors by helping them identify their own competencies and VIP-s needs, develop program content, facilitate ongoing quality improvement, and guide prioritisation of resources.

PS Education 3

Health Beliefs, Psycho-Social Motivation

1238

Identifying the relationship of health beliefs and complication reduction practices of Chinese individuals with Type 2 diabetes.

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Background and Aims: There is a high prevalence of diabetes complications among individauals with diabetes in Malaysia. Health beliefs of individuals with diabetes have been suggested as one of the factors that infulence health behavior, that contributes to development of diabetes complications. This study aimed to identify the relationship of health beliefs and compliaction reduction practices among Chinese individuals with Type 2 Diabetes Mellitus in Malaysia.
Materials and Methods: Using the Health Beleif Model framework, a correlation study was undertaken with 128 Chinese subjects of both genders (63 males, 65 females) with Type 2 diabetes age range 40 to 82, mean 60.5 +/- 8.42 from a government urban hospital and four governemt rural health centers. The data was collected using a 60-item questionnaire read to each subjects by the investigator. Respsonses were recorded by the investigator using a 5 point Likert scale. Data was analysed using descriptive statistics, Spearmen rank order correlation coefficient and Mann Whitney U test.
Results: The literacy level of the subjects was low. Seventy-eight percent had less than 6 years of education. Seventy-two percent of the subjects were aware of diabetes complications and their risk factors. However, they did not perceive diabetes as a serious disease nor perceive themselves as being susceptibile to diabetes complications. Thus very few practiced complication prevention measures. There was no statistical significance in health beleifs between urban and rural settings and genders ($p>0.05$). There was significant correlations betwee complication preventive behavior and perceived severity $r = 0.396 (p<0.05)$ and perceived susceptibility $r = 0.237 (p<0.05)$; education level of the subjects and perceived severity $r = 0.379 (p<0,05)$, perceived susceptibility $r = 0.342 (p<0.05)$ and complication reduction behavior $r = 0.359 (p<0.05)$, There was a negative correlation between subjects' age with perceived severity $r = -0.14 (p<0.05)$, perceived susceptibility $r = -0.293 (p<0.05)$ and complication reduction behavior $r = -0.16 (p0.05)$.
Conclusion: In conclusion, the findings suggest that the poor complication preventive behavior among the subjects was due to lack of perceived seriousness of diabetes and lack of perceived susceptibility to diabets complications that could be due to low literacy, lack of diabetes knowledge, denial and lack of symptoms.

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Assessing the health beliefs, attitudes and knowledge of urban and rural diabetic patients in India - a qualitative study.

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Objective: To look at the health beliefs, attitudes and knowledge of urban and rural diabetic patients in India. Setting: The town of Jamnagar in the state of Gujarat, India, and the surrounding villages.Participants: 80 patients. 40 from Jamnagar presenting at the out-patient department of the diabetic and general out patient clinics of MP Shah Hospital. 40 from the surrounding villages, either presenting at MP Shah Hospital or in the diabetic camp, in the village of Ravalsa.
Methods: Qualitative semi-structured interview, pile sorting exercises and structured vignette, a relatively new method designed to validate the researchers understanding of primary level culture. In designing the questionnaire the aim was to validate the results of the study using triangulation – using data obtained from one method and comparing it with data obtained from another.
Results: Wide inter-patient variations of knowledge. Often dependant on own experiences. General good knowledge of the need for diet, exercise, and cessation of smoking. Poor knowledge of the causes of diabetes and complications of long term disease. Prevalence of 52.5 % (95% CI 0.37 to 0.67) of long term diabetic complications in urban patients vs. 15% (95% CI 0.04 to 0.26) in rural patients. Poor compliance and knowledge of the need for regular follow up in villages**.** 83% (95% CI 0.76 to 0.92) of patients were non smokers. Literacy of 65% (95% CI, 0.50 to 0.79) in urban patients, and 45% (95% CI 0.29 to 0.60) in rural patients.
Conclusions: The results showed there is wide variety of thoughts, beliefs, attitudes in the diabetic patients in both rural and urban clinics. Most patients had their knowledge dictated by their own personal experiences. Few are given any formal education regarding lifestyle, diet and blood sugar control. Consultations are brief and rushed, with little opportunity to discuss diabetic control. Friends, family and talking to other patients during clinics are the main sources of information. Patients are generally well aware of the need for good diet, exercise and the need to stop smoking. Nearly all the patients had type II, NIDDM. Diet seemed to be one of the most important issues for the patients, when asked about their diabetes. Little awareness of the need for regular glucose monitoring in rural patients and long term complications of poor diabetic control in both groups.

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Influence of non-verbal intellect grade (non-v.IG) on the level of knowledges and compensation of diabetes.

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Background and Aims: To estimate the level of knowledges and the state of diabetes compensation in patients with different parameters of non-verbal IG in dynamics of the 1 year observation.
Materials and Methods: 47 patients with type 1 diabetes mellitus were included in our investigation. Patients were divided into 2 groups. The 1st group consisted of 22 patients (13 females and 9 males) with a high and mean non-v.IG (109.36±11.98%), their mean age was 28.32±5.49 years. Mean disease duration was 8.23±6.03 years. The 2nd group consisted of 25 patients (16 females and 9 males) whose mean age was 26.08±11.31 years and disease duration - 13.12±8.86 years with a low non-v.IG (75.14±9.93%). The training course was conducted according to 5-day-cycle (18 hrs). The training was conducted for patients of the 1 st and 2nd group: 1 time per 6 months with the aim to control and appreciate. Patients had the 2 training courses a year. This training was conducted taking into account individual peculiarities of each patient. There was the more prolonged training (24 hrs) in the 2nd group. The level of knowledges in accordance with a standard program where a number of the correct answers was expressed as % from total number of questions under testing. Compensation status was evaluated in the beginning and in 9 months after training with constant glycemia correction and 3 month HbA1c assessment (using the analyzer DCA 2000 Bayer). Non-v.IG was investigated according to standard test. Statistical analysis of nonparametrical systems was performed by criterion of Wilcoxson for conjugated pairs (program Statistika).
Results: Changes in parameters of the level of knowledges and HbA1c in dynamics of 9 months in patients with diabetes mellitus with different indices of non-v.IG ($P<0.05$) are shown in table.

	Level of knowledges before training (%)	Level of knowledges after training (%)	HbA1c before training months	HbA1c in 9
1st group	64.5±21.5	98.5±7.5	10.28±1.81	5.86±0.67
2nd group	59.5±14.0	74.5±5.8	11.04±1.7	7.91±1.16

Conclusion: Data obtained are indicative of the improved level of knowledges and compensation status of diabetes after double training courses taking into account that these parameters were much more better in the group with high Non-v.IG. Non-v.IG influences on the training efficiency and it has to be taking into account in the differential approach to training groups.

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Is it possible to modify anxiety level and what effect has it on the feeling of life satisfaction in parents of diabetic children using different treatment methods?

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Background and Aims: It is a well-known fact that any chronic disease affects not only a patient's life in many aspects: psychological, social and professional but also the deterioration of life quality is observed in his family. Diabetes is one of diseases where higher anxiety level is measured in parents of children suffering from it. New methods of treatment not only aim at improving medical care but also promise improvement in the quality of life. The aim of this study was to examine anxiety level in parents of

diabetic children and adolescents with IDDM using different treatment methods (multiple daily injections - MDI versus pump system - CSII) and if it has the effect on their feeling of life satisfaction.
Materials and Methods: The data was collected in 2002. Subjects were randomly asked to fill in the State-Trait Anxiety Inventory (STAI) measuring anxiety as a state and a trait and the 5-point quality of life questionnaire (58 questions concerning different aspect of life, including satisfaction with treatment) during the routine control visit at the Outpatient Diabetic Clinic for Children and Adolescents. N parents: 119 (58 of children CSII and 61 of children MDI; Children age ; Mean = 10.6). The level of HbA1c was also measured. Other variables (parent's sex, child's sex, diabetes duration) were also controlled.
Results: There was no significant difference between anxiety level measured as a state and a trait (Mean = 43.64 State vs. 43.35 Trait). There was no effect of treatment method, hemoglobin level (Mean = 7.92, STD=1.43), age of the child and diabetes duration (Mean = 5 years, STD=3.4)on anxiety level (both as a state and a trait) or satisfaction with life (Mean = 139.45 CSII and 147.44 MDI) in parents of diabetic children using different treatment methods. Life satisfaction is related to anxiety level, both as state ($F=24.46$, $df=2$, $p<0.001$) and as trait ($F=62.19$, $df=2$, $p<0.001$). The relationship between anxiety level and life satisfaction was detected, both as a state ($R=0.425$, $p<0.001$) and a trait ($R=0.598$, $p<0.001$). The higher anxiety level there is the lower life satisfaction, depending both as a state (Life satisfaction = 92.988+1.156xState, $p<0.001$) and as a trait (Life satisfaction = 65.589 + 1.798 x Trait, $p<0.001$)
Conclusion: Results indicate anxiety level is not modified in parents of diabetic children. Surprisingly, the treatment method or hemoglobin level don't have any effect either on anxiety level or life satisfaction, as we expected and as many studies show. Parents who experience more anxiety feel less satisfied with their life. It may have a great effect on the relations in the family, particularly the parent-child relationship. More analysis is required to establish factors underlying such results but they can have great implications for further treatment, especially psychological care.

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Motivational and psychological factors associated with control of Type 2 diabetes mellitus (T2DM) and cardiac risk factors (CRFs).
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Background and Aims: Prevention and management of coronary artery disease (CAD) in individuals with T2DM require control of blood glucose and CRFs - blood pressure (BP), body mass index (BMI), waist circumference (WC), physical activity, and low- (LDL-C) and high-density (HDL-C) lipoprotein levels. The influence of both motivational and psychological factors that may contribute to control of multiple CRFs has not been widely explored. The purpose of this cross-sectional study was to determine the association of factors identified in self-determination theory, along with psychological factors, with control of T2DM and CRFs.
Materials and Methods: Older adults (n=59) with T2DM, who were enrolled in a screening trial, the Detection of Ischemia in Asymptomatic Diabetics (DIAD) Study, completed the following questionnaires to assess: autonomous versus controlled motivation (Treatment Self-Regulation Questionnaire [TSRQ]); autonomy support (Modified Health Care Climate Questionnaire [HCCQ]); competence (Perceived Competence for Diabetes Scale [PCDS]); anxiety (Crown-Crisp Anxiety Index [CCAI]); depressive symptoms (Center for Epidemiologic Studies-Depression [CES-D]); and hostility (Cook-Medley Hostility Index [CMHI]).
Results: The mean age was 62 ± 7 years (T2DM duration, 7.8 ± 6.5 years); 48% were female. Control of T2DM and CRFs was frequently suboptimal. Spearman correlation coefficients (r_s) revealed significant associations between: 1) HbA1c and PCDS ($r_s = -.28$) and anxiety ($r_s = .30$); 2) HDL-C and anxiety ($r_s = -.38$) and hostility ($r_s = -.24$); 3) physical activity and PCDS ($r_s = .61$), TSRQ ($r_s = .28$), and anxiety ($r_s = -.36$); and 4) BMI/WC and CES-D ($r_s = .30$), PCDS ($r_s = -.52$) and TSRQ ($r_s = -.29$) scores. Control of LDL-C and BP were not associated with any of the factors under study.
Conclusions: Potentially important relationships between these motivational and psychological factors and control of T2DM and CRFs are suggested. These factors merit further attention in intervention studies aimed at improving DM management and reducing CAD risk.

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Effects of regular health education on cardiovascular risk factors in Chinese Type-2 diabetic patients: a one-year prospective randomized study.
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Background and Aims: It has been suggested that regular reinforcement is an important part on the management of diabetic patients. We aimed to assess the effect of regular diabetic health education on their cardiovascular risk factors in Chinese type-2 diabetic patients.
Materials and Methods: This is a 1-year prospective randomized study. 178 type-2 diabetic subjects were recruited from 3 regional diabetic centers in Hong Kong. All of them were followed up every 3-monthly by a physician of their corresponding center. Ninety of them received additional reinforcement with diabetic health education by a trained nurse after the doctor's consultation. The same nurse provided same level of education to all the subjects. The other 88 subjects received same medical care except the nursing reinforcement. The outcome measures included glycemic control (fasting plasma glucose, HbA_{1c}), body mass index, waist circumference (WC), blood pressure (BP) and lipid profiles, which were assessed before and 1-year after education.
Results: Of the 90 cases undergone regular health education, 44 (48.9%) were men and 46 (51.1%) were women. The mean age was 55.0 ± 9.0 years (median 55.0 years, range 34-72 years). The 88 controls were age and sex matched: mean age 56.1 ± 10.2 years, p: NS; men to women ratio 34:55, p: NS. At the end of the study, patients undergone education had reduction in their WC (86.3 ± 10.2 to 84.1 ± 9.3 cm, $p<0.001$), diastolic BP (79 ± 10 to 76 ± 11 mmHg, $p<0.01$), HbA_{1c} (8.6 ± 1.6 to $8.1 \pm 1.5\%$, $p<0.01$), total cholesterol (TC) (5.7 ± 0.8 to 5.0 ± 0.9 mmol/L, $p<0.001$) and low-density lipoprotein cholesterol (LDL) (3.6 ± 0.7 to 3.1 ± 0.9 mmol/L, $p<0.001$) levels. Control subjects had reduction in their TC (5.7 ± 0.9 to 5.0 ± 0.9 mmol/L, $p<0.001$) and LDL (3.6 ± 0.7 to 3.0 ± 0.8 mmol/L, $p<0.001$) levels. Other cardiovascular risk factors were not significantly changed in the controls. Addition of drugs and/or dosage increment of the 3 major medications were similar between the 2 groups (anti-diabetic drugs, lipid lowering agents and anti-hypertensive agents).
Conclusion: Regular reinforcement with diabetic health education is useful. It helps to better control some of the cardiovascular risk factors such as obesity, BP, glycemic status and lipid profile in Chinese type-2 diabetic patients.

PS Health Care Organisation 1:
Improving the Process of Care (I)

1244

Establishment of blood glucose monitoring system using an internet.
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Background and Aims: The internet is used world wide as a communication tool. To improve the quality of diabetes control, we undertook to investigate the effectiveness of an **I**nternet-based **B**lood **G**lucose **M**onitoring **S**ystem (IBGMS) on changes in HbA_{1c} levels.
Materials and Methods: We conducted a randomized clinical trial involving 110 patients who visited to outpatients clinic at the Kangnam St. Mary's Hospital for 3 months. The study subjects were treated with IBGMS for 12 weeks and the control group received the usual outpatient management over the same period. HbA_{1c} and other laboratory tests were performed at baseline and at the study close.
Results: At baseline no significant differences were found between the two groups with respect to age, sex, diabetes duration, BMI, blood pressure, HbA_{1c} and other laboratory data. In the follow up test, the study group showed a significant reduction in HbA_{1c} by 7.1% (0.54% absolute, P=0.001), while the control group showed a greater HbA_{1c} increase (P=0.054). Moreover, there was a remarkable reduction of HbA_{1c} by 11.1% (0.92% absolute, P <0.001) in the patients with $HbA_{1c} \geq 7.0\%$ in the study group and those with HbA_{1c} <7.0% maintained a good level of HbA_{1c} of 6.32% at the study close.
Conclusion: This new IBGMS resulted in a significant reduction of HbA_{1c} during the study period. We propose that this IBDMS be used as a new method of diabetes control.

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Telemedicine-based diabetes disease management program to support out-patient diabetes care.
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Background and Aims: Diabetes and its complications belongs to one of the most important health care problems our days. Beside increasing prevalence and decreasing age at onset of the disease, deficiencies in the management of out-patients contribute to major public and individual health care problems. In contrast to the demands of evidence-based guidelines, diabetes care systems very often lack continuity for out-patients as well as support by patient centred management programs utilising state of the art knowledge. The aim of this study is to design a telemedicine-based diabetes disease management program (DDMP) which provides permanent support in terms of metabolic management and prognosis on an individual basis to any out-patient and physician on any place over 24 hours of the day.
Materials and Methods: To establish a telemedicine-based communication network a novel diabetes service center (DCC) has been created. The DCC comprises two main components, namely the interactive advisory program KADIS® (Karlsburger Diabetes Management System) and the telemedicine communication system TeleDIAB®. KADIS® is an evidence-based advisory program, which allows to predict effects of therapeutic measures on the 24-hour blood glucose profile on an individualised basis. In addition, a prognosis module allows both identification of risk constellations within the monitored out-patient data and prediction of worsening of the disease to initiate appropriate interventions. To guaranty the permanent access to the remote DCC which is running the KADIS® program, the communication network TeleDIAB® has been established providing the transmission of data, information, and advice between out-patients, physicians, and KADIS® of the remote DCC. TeleDIAB® comprises three main components: Patient Unit, Physician`s Medical Workstation and the DCC.
Results: The DCC assists *patients* for 24 hours the day (i) with individually suited advice independent of their actual location, (ii) in self-management of metabolic control as well as supports *physicians* (iii) with analysed out-patient data including patient related recommendations according to the current medical knowledge, and in addition (iv) realises the communication between the partners within the health care network. The feasibility and efficacy of the telemedicine-based diabetes disease management program has been verified in a pilot study by comparing two groups of randomly selected patients treated with (28 patients) and without (11 patients) KADIS®-based support. During a 21-month period KADIS®-supported advice, the HbA1c decreased from 9.0 ± 1.1 % continuously to 6.4 ± 0.8 % and reduced average per patient costs by 3.00 per day in contrast to 7.75 ± 1.5 % and no cost reduction in the group without KADIS®-support (p<0.01).
Conclusion: The results obtained so far support the hypothesis, that telemedicine-based diabetes disease management networks in combination with an evidence-based interactive advisory program may provide an efficient tool to improve significantly efficacy of out-patient diabetes care.

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The use of a multi-access service for the diabetes management allows better glycaemic control in insulin treated diabetic patients.
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Background and Aims: Multi -Access Services for Diabetes Management (M2DM) system, funded by the European Comission, is an integrated platform of information technologies based on Web technology. It has been designed to provide medical assistance to diabetic patients and, among other utilities, the system allows the patient to send self-monitoring data (glycemic values, insulin doses, diet changes, exercise data) stored in the reflectometer by using a modem. The physician reviews the monitoring data and proposes further adjustments of the treatment. Furthermore, the system is provided by an e-mail service.Objective- To evaluate the impact of using M2DM on glycemic control in a group of insulin treated patients.
Materials and Methods: 28 (4 type 2) diabetic patients were randomly assigned to use M2DM system or to follow their medical planned visits as usual. Two patients rejected to continue in the study and have been excluded of the analysis.
Results: - Baseline characteristics did not differ between M2DM group (N=14, age 36±14 y, 8 men, BMI= 22.7±1.9 kg/m2, age at diagnosis 24.5±9 y, 50% insulin pump) and control group (N=12, age 47±2 y, 4 men, BMI 23±2.2 kg/m2 , age at dignosis 24.5±12 y, 50% insulin pump). During the study period, M2DM group patients sent a median of 803 glucose values (533-1511), 733 insulin values (386-1107) and 8 e-mails (0-23). After 6 months, a significant decrease of HbA1c was observed in the M2DM but not in the control group:(see Table 1; *p=0,01) Two patients from the M2DM group withdrew the study after the 6-month evaluation.
Final HbA1c change correlated inversely with the number of e-mails (p<0.05) and tended to correlate with the number of glucose values sent (p=0.08).
Conclusion: The use of M2DM system for the management of patients with diabetes mellitus seems to be a useful tool for glycemic control improvement in insulin treated diabetic patients.

Table 1

	Baseline	6 months	12 months
M2DM group	8,1 (6,1-11)	7,2 (4,7-8,7)*	7,2 (4,7-8,7)
Control group	7,8 (6-9,2)	7,3 (6-11)	7,9 (6,8-9,2)

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Clinical evaluation of a multi-access service for the management of diabetic patients with insulin therapy (M2DM project).

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Background and Aims: Recent advances in Information and Communication Technology allow new models of diabetes management, which provide assistance to patients regardless of their distance from Health care providers. The M2DM project, funded by the European Commission, has the specific aim to investigate the potential of novel telemedicine services in Diabetes management.
Materials and Methods: A multi-access system based on the integration of Web Internet access, telephone access through Interactive Voice response systems, and the use of palmtops and smart modems for data downloading has been implemented. A multicenter (Italy, Spain and Germany) randomised controlled study has been realised with clinical, organizational, economic, usability and users' satisfaction outcomes. We present the preliminary results of available clinical data after a 12 month usage of the system.
Results: 62 diabetic patients with insulin therapy used the system (55 type1 diabetes mellitus (DM), 7 type 2 DM, 49 adult, 13 pediatric, mean age 35 years SD 16,2 years, 37 males, 25 females). They transferred a total of 40595 blood glucose (BG) readings and 15497 Insulin dosages (ID) (avg. 12.7 BG and 4.8 ID per patient and week). Every three months clinical visits were held and HbA1c was tested. During the first three months 2,3% of a total of 13510 BG readings were below 50 mg/dl, 16,6% were higher than 250 mg/dl. During the last three months of the study period only 1,6% were below and 19,5% were above the threshold values. Both the reduction in hypoglycemias ($p<0,003$) as well as the increase of hyperglycemias ($p<0.001$) were statistically significant. Mean BG levels decrased from 177,6 mg/dl (SD 81,3) to 174,2 mg/dl (SD 73,2), median BG decreased from 165,4 mg/dl to 155,0 mg/dl, but there was no statistical significance. HbA1c levels showed a significant decrease from initially 8.15% (SD 1.85) to 7.4% (SD1.33) ($p<0.001$), the reduction in variance was significant, too ($p<0.034$).
Conclusion: A telemedicine based Management of Diabetes mellitus leads to a significant improvement of HbA1c levels and reduces the rate of hypoglycemias. It is an excellent tool to improve the metabolic situation of diabetic patients with insulin therapy. A complete analysis of organizational, economic, usability and users' satisfaction outcomes plus the results of the control group will be available soon.

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Educating the future workforce for diabetes management.

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Background and Aims: A constant challenge for all health services is to make the best use of limited resources available. Within this remit, chronic disease patient education is recognised as having the potential to bring real benefits to people. However, questions about sustaining its long-term impact remain unanswered. To find answers to this question, a RCT of an empowering education programme was undertaken with 89 patients. Findings from this work highlighted the need for an interdisciplinary team approach to patient education and clearly demonstrated the need for interprofessional education (IPE). A second study was therefore undertaken to research the feasibility of implementing IPE at pre-registration levels. This paper aims to show how the work from these two studies was used to inform the development of a working plan for IPE and the relevance of this plan to future diabetes management.
Materials and Methods: A theoretically constructed educational intervention was developed specifically for people with type 2 diabetes. Clinical, behavioural and psychological outcomes were measured at 6 and 12 months and the relationship between these and participants' perspectives of the intervention and its effects were assessed through 10 focus group interviews. The second study included a systematic review of the IPE research literature, alongside focus group interviews with a purposive sample of 34 participants from clinical and academic environments, and from people with chronic diseases. The findings from these two methods were integrated to inform educational practice.
Results: The patients' intervention was psychologically effective over the long term ($p=0.01$), and there were significant differences in self-monitoring behaviours ($p=0.002$), but clinical effectiveness had only short-term significance ($p=0.005$). Whilst positive improvements in diet and exercise behaviour were noted over the long-term, these did not reach significance (diet, $p=0.20$; exercise, $p=0.26$). The focus group data showed how intervention had empowered participants and helped them to enter the behaviour change cycle. Relationships with and between health care professionals were found to be critical to the long-term results of these outcomes. The systematic review of the IPE research literature and the focus group interviews demonstrated the advantages of this form of education. In particular, it highlighted its role in the creation of professional identification and its potential for breaking down traditional barriers to team working. IPE, as a precursor to inter-professional working, was therefore shown to be essential for meeting the requirements of patients with complex needs, such as those who have diabetes.
Conclusions: The results from this work have now been used to inform pre-registration IPE at the University of Liverpool. Relating educational planning to 'real life' clinical practice has raised the profile of IPE to students, practitioners and academics alike and this has enhanced its development in the learning environment.

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The role industry in national diabetes programs: big bad wolf or good Samaritan?

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Background: The pharmaceutical industry (Industry) is frequently criticised in the international press and the medical literature for the manner in which it sometimes pursues its commercial interests. However, Industry is an important stakeholder in health care and some pharmaceutical companies are increasingly amenable to contributing to population health improvement over and above their primary role as a supplier of therapeutics and diagnostics. For example, this case study describes an international project initiated in 2001 by one pharmaceutical company to promote and enhance national diabetes programs (NDP) through its international affiliate companies.
Aim: The aim of the project was to provide Novo Nordisk's international affiliate companies with frameworks and tools to encourage their involvement in enhancing NDPs in countries where they already exist and supporting their development in countries which did not currently have an NDP.
Methods and Materials: The project was predicated on:
- conducting a series of international workshops to inform, train and focus relevant staff on NDP
- the development of an electronic diabetes situation analysis and review tool
- the development of a national diabetes "toolbox" covering various aspects of NDP

41/60 international affiliates were represented at the workshops prior to which all 41 completed a baseline situation analysis of diabetes in their country. All 41 participating affiliates made a plan for a national program their company could undertake in its home country. These addressed a range of issues from community awareness and early detection to aged care programs.
Results: At 6 month follow up 31/41 had completed the review tool and 35/41 had further developed and/or commenced implementation of these plans. Significantly more affiliates reported forming partnerships with other agencies or organisations and had increased support for national diabetes organisations and health professional training within their countries. In addition several affiliates had embarked on joint diabetes projects in partnership with government and/or professional diabetes organisations eg i) China – comprehensive diabetes actions plans in conjunction with government ii) Russia – mobile diabetes complications screening with government and senior clinicians and iii) Australia – developing guidelines for care of diabetes in the elderly with a diabetes health professionals organisation.

Conclusion: It is clear from this example that Industry can play an important role in supporting and adding value to the development and implementation of national diabetes programs and is well situated to contribute to the global effort to promote a co-ordinated and comprehensive approach to population health activities aimed at reducing the burden of diabetes.

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Pharmacy-based diabetes management programme in Portugal: preliminary results of a pilot intervention.

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Background and Aims: To test the implementation and evaluation methodology of a pharmacy-based diabetes management programme, following the concepts of Disease Management and Pharmaceutical Care, in Portuguese pharmacies and to assess the impact of the programme.
Materials and Methods: Prospective intervention in 31 community pharmacies selected according to pre-defined criteria. Type 2 diabetic patients selected by pharmacists according to inclusion criteria. Pharmacists received specific training to follow diabetic patients and to document patient data and care provided. This training addressed disease-specific topics, pharmacotherapy and the process to provide pharmaceutical care (20 hrs./person).
Results: 143 patients initially enrolled, out of which 127 on follow up for more than 6 months (as of 31-12-2002). The average patient is female, mean age of 63.5 years, ≤ 4 years of education. The health centre is the most usual setting for medical care (65%) and the general practitioner is the most common doctor seen (73%). Average time spent by the pharmacist was 19 min. at initial visit and 20 min. at follow up visits. A total of 1614 follow up visits was documented (58/pharmacy, 13/patient). During the same period, 270 medical appointments were reported. Pharmacists performed 4625 measurements (average 165/pharmacy, 36/patient), out of which 1640 capillary blood glucose (BG) and 1548 blood pressure (BP). It was observed a decrease in fasting BG values (of 10 mg/dL), in postprandial BG values (of 13.5 mg/dL) and in both systolic (of 8 mmHg) and diastolic BP values (of 4 mmHg). The % of patients with postprandial BG < 180 mg/dL, BP < 130/80 mmHg and BP < 140/90 mmHg increased from 30% to 35%, 12% to 24% and 39% to 46.5%, respectively. Statistic significance still being assessed. Patients were requested to bring HbA1c values, since this is not performed in pharmacies. However, records were scarce. Pharmacists documented 471 drug-related problems (DRPs) and 1638 interventions. The most common DRP categories were dosage (44%) and lack of drug/compliance problems (27%). Counselling represented 72% of pharmacists interventions. A direct contact to the prescriber or a referral was reported for 15% of interventions. Prescribers accepted 35% pharmacists direct recommendations and modified drug therapy in 6.5% of either pharmacists direct recommendations or referrals.
Conclusion: Although these findings are still preliminary, they seem to suggest a more structured pharmacist's performance and improvements in clinical outcomes through collaboration with prescribers. Further findings will be presented at the congress. These results have been used to adjust the model for expanding the programme in Portuguese pharmacies in 2003.

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Lack of relationship between quality of life, glycaemic control and knowledge about diabetes in Type 2 diabetic patients: necessity of improvement in the educational approach.

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Background and Aims: To study the relationship between variables describing diabetes characteristics, knowledge concerning the disease, know-how about treatment and self-monitoring, effects of diabetes on social, occupational, family life and quality of life among 136 type 2 diabetic patients hospitalized for education sessions in a health care network: REDIAB Touraine.
Materials and Methods: Knowledge and know-how were assessed by specific questionnaires. Personal records were filled up for each patient. The Nottingham Health Profile scale in its French version assessed the quality of life. Principal component analysis was performed to bring out typologies of patients according to the relationship between variables.
Results: 40% of the total variance was explained by three principal components, corresponding to three typologies:
Patients with degenerative complications, glucose levels instability, treated with insulin and suffering from frequent hypoglycemia;
Patients with an internal health locus of control, a good knowledge of diabetes and its treatment and an apparent good compliance about advices on diet and physical activity;
Patients with an impaired quality of life, social isolation, no acceptation of the chronic disease and poor glucose control.
Neither fair glucose control nor quality of life was related to the knowledge on the various aspects of diabetic disease.
Conclusion: Education on diabetes knowledge seems insufficient to improve glyucose control in type 2 diabetic patients. During education sessions, it is mandatory to give priority to the management of hypoglycaemia, to work on diabetes acceptation and to give moral support in order to improve quality of life and increase the impact of our educational actions.

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Patient adherence improves glycemic control.

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Background and Aims: It is generally thought that patient engagement is critical to achieving diabetes goals. However, there has been little quantitative assessment of the glycemic impact of different measures of adherence, and some studies have failed to show a benefit of adherence to measures such as frequency of home glucose monitoring. To study the influence of alternative measures of patient behavior, we evaluated the effect of appointment-keeping and medication adherence on HbA1c (A1c) levels after one year of management.
Materials and Methods: We studied 1,560 patients with type 2 diabetes who presented for a new intake visit to the Grady Diabetes Clinic in Atlanta between 1991 and 2001, and returned for a follow-up visit and A1c after 1 year of care. Appointment-keeping was assessed according to the number of scheduled intervening visits that were kept, and medication adherence was expressed as the percent of visits for which self-reported diabetes medication use was exactly as recommended at the preceding visit.
Results: The patients had average age 55 years, BMI 32 kg/m2, diabetes duration 4.6 years, and baseline A1c 9.1%; 90% were African-American, and 63% were female. All had been scheduled for 6 follow-up visits within the first 6 months and at least 1 visit every 3 months thereafter - a minimum of 7 intervening visits. After 1 year, the patients had an average of 5 intervening visits. Those who kept more appointments had lower A1c levels after 12 months of care: 7.6% with 6-7 intervening visits vs. 9.7% with no intervening visits (p=0.0001 for trend). Medication adherence averaged 89%, and better adherence was associated with lower A1c levels after 12 months of care: 7.8% with 76-100% adherence, vs. 10.0% with 0-25% adherence (p=0.0001). After adjusting for age, gender, race, BMI, diabetes duration, initial A1c, and diabetes therapy in multivariate linear regression analysis, the benefits of appointment-keeping and medication adherence remained significant and contributed independently; the A1c after 12 months of care was 0.12% lower for every additional intervening appointment that was kept (p=0.0001), and 0.34% lower for each quartile of better medication adherence (p=0.0009).
Conclusion: Keeping more appointments and taking diabetes medications as directed led to substantial improvements in A1c even in a group of subjects who generally returned as scheduled and took their medications as recommended. Efforts to enhance glycemic outcomes should include emphasis on these simple but critically important aspects of patient adherence.

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Use of insulin in Type 2 patients with microvascular complications.

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Background and Aims: Recently published evidence based procedures in the German disease management programme (DMP) for type 2 diabetes are strongly demanding a detailed and structured glucose lowering therapy

strategy for patients with failing therapy targets and/or any registered endpoint like stroke, CAD or MI. The use of insulin is considered to be an advantageous approach in patients with microvascular complications. Therefore a cross-sectional analysis in a quality management diabetes register was performed to gain further knowledge about the consequences of a possible implementation of these strategies.
Materials and Methods: A web based documentation system for quality management in diabetes care was used for data acquisition between January 2002 and December 2002. Datasets (n=4.207) of documented type 2 patients in Austria were taken for analysis in a cross-sectional approach. The criteria for data analysis were: HbA1C, stroke, myocardial infarction and CAD. Since the published DMP programme did not describe in full detail the criteria for appropriate therapy targets, the analysis was made on the assumption of three possible definitions of failing the targets: HbA1C>9, HbA1C>9,5 and HbA1C>10. The chosen thresholds should demonstrate possible consequences on diabetes care in a health system.
Results: The baseline characteristic of the analysed population (n=4.207) was (mean/±SD): 45.6% male, BMI (29.36/± 5.79), HbA1C (7.8%/± 1.86%), diabetes duration (7.9/±8.1), age (66.3/±11.8). Of n=4.207 patients between 538 and 738 would be eligible for insulin as a preferred strategy depending on the threshold level of HbA1C used. The following table gives detailed results. The second column in the table shows the percentage of patients eligible for a therapy change from any other glucose lowering strategy to insulin according to the suggestions of the DMP. The three rows show the dependency on the threshold of HbA1C used.
Conclusion: A strategy change towards insulin would determine a widespread implementation of type 2 patient education programmes for insulin users. Depending on defined targets of glucose lowering strategies there is the need for education capacities of at least 70.000 type 2 patients in Austria. When implementing DMP strategies like the above-named a health system has to ensure the required structures for achieving the desired results.

n=4207	%	BMI (SD)	HbA1C (SD)	Duration (SD)	Age (SD)
CAD, MI, stroke, HbA1C>9	17.54	29.18 (5.17)	9.24 (2.2)	9.49 (8.7)	66.6 (12.3)
CAD, MI, stroke, HbA1C>9.5	15.24	28.99 (5.12)	9.23 (2.4)	9.46 (8.8)	66.9 (12.3)
CAD, MI, stroke, HbA1C>10	13.86	28.96 (5.08)	9.17 (2.5)	9.49 (8.9)	66.9 (12.2)

PS Health Care Organisation 2:
Improving the Process of Care (II)

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Interventions to prevent identified health risks in patients with Type 2 diabetes need to be improved.
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Background and Aims: To assess the management of type 2 diabetic patients enrolled in a French preferred-provider organisation (PPO).
Materials and Methods: We conducted an internal audit of REVEDIAB, a PPO in Essonne and Val de Marne in the south east of Paris, which provides to health profesionnals special training for type 2 diabetes management and facilities for patient's education. The physicians were asked to collect data on monitoring, treatment and complications of patients, to assess leading risks and to describe interventions for each patient during the past year.
Results: from April to September 2002, 47 physicians (42 GPs, 4 endocrinologists and 1 cardiologist) evaluated the management of 452 patients. Mean age of patients was 63.6 ± 11 yrs, duration of diabetes was 9.3 ± 5 yrs. Compliance with monitoring was: 97.5% for HbA1c 90.1% for serum creatinine, 87.4% for total cholesterol, 84.8% for triglycerides, 77.3% for funduscopy, 75.2% for HDL cholesterol, 74.5% for ECG, 73.7% for microalbuminuria. Prescriptions consisted of diet alone for 5.7% patients, oral hypoglycaemic agents (OHA) for 76.5%, insulin alone or combined with OHA for 17.9%. Anti-hypertensive agents were prescribed to 69.8 % patients, lipid-lowering drugs and low dose aspirin to 47.2% and 36.8% respectively. 51.2% of patients performed SMBG. 26.6% of patients had HbA1c $\leq$6.5%, 41.6% had an HbA1c from 6.6% to 7.9%, and 31.8% had an HbA1c $\geq$8%. 24.4% of patients had a BP >140/80 mmHg. 32.1% had a LDL >1.30 g/l. Complications were nephropathy (mostly microalbuminuria) for 32.8% of patients, coronary heart disease for 16.6%, retinopathy for 15.9%, peripheral vascular disease for 7.9%, cerebrovascular disease for 3.8% and 2.3% had a foot ulceration. The leading risks assessed by physicians were high risk of cardio-vascular disease (CVD) for 37.3% of patients, diabetes uncontrolled for 33.3%, obesity for 32.2%, lack of motivation for 20.8%, active smoking for 8.2%, high risk of foot ulceration for 6.7%, visual impairment for 5.1% and proteinuria for 3.8%. Intervention for patients with high risk of CVD were: cardiologist's visit (68%), exercice test (48%) and 54.3% were treated by low dose aspirin; if a coronary heart disease was present, 76.9% were treated with low dose aspirin, 64.1% by beta-blockers, 59% with ACE inhibitors, 56.4% with statins and 25.6% with these 4 drugs combined. Of patients whose diabetes was uncontrolled, 55.4% had an intervention to improve glycemic control. Of patients with obesity, 29.4% attended a dietician and 59.8% had metformin as first line OHA. Of patients with high risk of foot lesion, 22.6% attended a podiatrist. Of patients with proteinuria 16.7% attended a nephrologist. Of patients with active smoking, 11% attended a tobaccologist.
Conclusion: The monitoring of type 2 diabetic patients by these highly motivated physicians was close to guidelines. However, interventions and treatments to prevent identified health risks were insufficiently undertaken, resulting in an undue risk of complications.

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The ENTRED Study demonstrates improvements in diabetes care process indicators among people treated with oral antidiabetic agents, France, 1998-2001.
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Background and Aims: The ENTRED study aims at describing and monitoring the health status and health care quality of people treated for diabetes in France, based on a national random sample.
Materials and Methods: In the administrative medical database of the national health insurance system that covers all employees in France, 10,000 adults were randomly selected among those reimbursed of insulin and/or oral antidiabetic treatment between October-December 2001. People reimbursed of insulin treatement(20%[19-21]) were excluded from the

present analysis that was based on medical claims of 7 987 people in 2001. Results from the ENTRED study were compared to those of an exhaustive audit conducted on the same database, with a similar methodology, in 1998 (n=875,247).
Results: Mean age of people treated for diabetes with oral antidiabetic treatment was 65 years and H/F sex-ratio 1.1, and did not change between 1998 and 2001. The table compares the % of people who were reimbursed of various diabetes care process indicators in 1998 and 2001.
Conclusion: Major national improvements have been observed, in particular with regards to HbA1c and lipid monitoring, following a national diabetes campaign led by the health insurance system and professional associations. Further follow-up of diabetes care process indicators is being pursued on ENTRED 2002-2003 data. The ENTRED study will also measure potential improvements in biological and health outcome indicators through patients and provider questionnaires.

Diabetes care process indicators	1998 audit (%)	ENTRED 2001 (%[95%CI])
Last 3 months Metformin treatment	50	55 [54-56]
Antidiabetic agent combination	39	41 [40-42]
Hypolipidemic treatment	38	41 [40-42]
Cardiovascular treatment	71	71 [70-72]
Last 6 months ≥1 HBA1c	41	64 [63-66]
Yearly ≥ 1 Lipid measurement	57	65 [65-67]
≥ 1 Microalbuminuria measurement	11	16 [15-17]
≥ 1 ECG	28	29 [28-30]
≥ 1 Ophthalmology visit	39	42 [41-43]
≥ 1 Endocrinology visit	5.5	6.2 [5.7-6.7]
100% fee coverage (should be requested by providers for each diabetic patient to the insurance system)	70	73 [72-74]

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Quality assurance in diabetes care: Long-term results of a national diabetes centre.
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Background and Aims: Ten years ago our team joined the common European DiabCare project established for continuous quality and efficacy evaluation of diabetes care. The aim of the present study was to reveal any improvement of process (in terms of completeness) or outcome measures by the application of this quality control tool during a 7-year period (1994-2000).
Patients and Methods: Altogether 2692 annual patient records were available for the whole period (patients with Type 2 diabetes [T2DM]: 1784; age: 62±10 [±SD] years; diabetes duration: 11.5±8.4 years; 42% on insulin therapy; patients with Type 1 diabetes [T1DM]: 764; age: 37±12; duration: 15.6±11.4). Trend- and correlation-analyses were used as statistical tools. First and last year data are given in the results section.
Results: Frequency of yearly at least one HbA1c measurements (79-99%; P<0.0001) and also their values (T1DM: 7.9-7.2%; P<0.0001; T2DM: on oral drugs [OAD]: 7.7-6.8%; p<0.0001; on insulin therapy [ins]: 8.2-7.0%; p<0.0001) both improved during the analysed period. Similarly an amelioration was observed in the measured systolic and diastolic blood pressure values in all groups (T1DM: 125-121/75-73 mmHg; P=0.06 and 0.05 respectively; T2DM [OAD]: 139-127/81-71 mmHg; P<0.0001 in both cases; T2DM [ins]: 133-128/78-72 mmHg; P<0.0001 in both cases). A significant decrease in total cholesterol (5.5-4.9 mmol/l) and triglyceride levels (2.0-1.8 mmol/l) could also be revealed. Frequency of yearly microalbuminuria measurements (32-71%; p<0.0001) improved, too, and this tendency was also observed in the frequency of eye- (85-95%; p<0.0001) and foot-examinations (67-95%; p<0.0001).
Conclusions: Results from the analysed period indicate an improvement in the quality of care, confimed by the continuous use of quality assurance tools: both in completeness and outcome. This amelioration was most pronounced in T2DM [OAD], a group of patients poorly controlled generally. Changes in guidelines and their implementation could also be reflected in better outcomes. The different patient characteristics in the different years or the methodical shifts might also effect our results.

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Managing poorly controlled diabetes in the community: a step-up care model.
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Background and Aims: Much of our primary care delivery system has been designed for acute rather than chronic care. Studies have shown that structured care, organized along the lines of a chronic care model, improves patient outcomes. Various organisations have utilised the strategy of enhancing primary care to provide step-up diabetes care within their community, usually by incorporating the use of specialists. Since July 2002, our community health centre has piloted a Family Physician (FP)-run diabetes clinic to better manage our patients with poor glycaemic control. It involves redesigning our current care delivery system to utilise our existing staff in the form of a diabetic care team that comprises FPs, dedicated Community Nurse Educators (CNEs) and our part-time dietitian. Additional features include a disease registry, a regular recall and review system, decision support in case records with prompts for regular preventive screening, and access to a diabetologist to discuss difficult cases. Our initial enrolment only included patients with HbA1c ≥ 9.5%. To assess the effectiveness of this clinic, we performed an evaluation after its initial months of operation.
Materials and Methods: We conducted a retrospective cohort study of the first 63 patients registered with our diabetes clinic. We excluded 8 patients from our analysis: 3 had been referred to the hospital for management of complications identified during their first visit, 4 were newly diagnosed diabetics, and 1 had incomplete data. The glycaemic status (based on HbA1c values) of the remaining 55 patients were compared using paired T-test: 6 months prior to enrolment, upon enrolment into the diabetes clinic, and up to the point of this analysis.
Results: The mean age of the cohort was 63.2 ± 9.2 years, with mean duration of diabetes of 12.4 ± 7.0 years. Majority was female (58%) and Chinese (82%). There was a significant deterioration of the glycaemic status of this cohort whilst under general care, 6 months prior to enrolment from a mean HbA1c of 10.0 ± 1.5 % to 10.8 ± 1.3 % (p=0.001). After a median of 3 visits or mean follow-up duration of 3.3 ± 1.3 months with our step-up diabetes clinic, the average patient in this clinic improved by 1.4 %, with a mean HbA1c of 9.4 ± 1.7 % (p=0.000).
Conclusion: Our initial evaluation of our Family Physician (FP)-run diabetes clinic shows promising results in the management of people with long-standing poorly controlled diabetes in the community.

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Differences in diabetes care among south Asians in Blackburn, Northwest England.
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Background and Aims: Type 2 diabetes has a very high prevalence in migrant South Asian populations. Most UK studies have found that South Asians with diabetes have poorer quality of care as judged by process measures and intermediate outcomes. Studies investigating variations in provision of care by ethnicity have mostly been single cross-sectional studies. This prospective study aimed to compare baseline levels and changes over time in the measurement, and levels, of HBa1c, systolic blood pressure (SBP) and cholesterol between South Asian and non-Asian patients with diabetes; and to determine if differences persist after adjusting for socio-economic status (SES).
Materials and Methods: Blackburn is a district in Northwest England with a population of 137,000, of whom over 26,000 (19%) are South Asian - mainly of Indian or Pakistani descent. Using a population-based diabetes information system (DIS) we describe changes in the measurement and mean levels of HbA1c, SBP and cholesterol between 1995 and 2001 for South Asian and non-Asian patients.
Results: In 1995 there were 1034 South Asian and 4200 non-Asian patients with diabetes aged between 25 and 84 years on the DIS. Non-Asian patients were more likely to be older (61 yrs v 54 yrs; p<0.001), male (55% v 52%; p<0.03), managed solely in primary care (61% v 56%; p<0.003), and have type 1 DM (20% vs 6%; p<0.001) than Asian patients. After adjusting for age, sex, place of management and type of diabetes, there were no statistically significant differences in the proportion of Asian and Non Asian patients who had their cholesterol, HbA1c and SBP recorded in 1995 but Asians had lower cholesterol, lower SBP and higher HbA1c levels than non-Asians.

Between 1995 and 2001 there were similar increases in the measurement of these intermediate outcomes, except for cholesterol, with measurement of cholesterol increasing more rapidly among non-Asian patients (2.6% vs 2.0% average annual increase, p=0.007). By 2001, Asian patients were less likely to have a recorded measurement for cholesterol (Absolute difference 2.5%, p=0.042), for HbA1c (4.8%, p<0.001), and for SBP (3.9%, p=0.004). The reduced likelihood of receiving care remained after adjusting for SES. Average levels of cholesterol and SBP fell sharply over the study period but the falls were greatest among Non Asians (cholesterol, p<0.001; SBP, p<0.001). Similarly, an increase occurred in Hb1Ac with the greater increase among Asians (Average annual increase: 0.15% vs 0.22%, p<0.001). By 2001 these changes led to similar cholesterol levels in both groups whilst Asian patients had substantial worse levels of HbA1c (difference 0.50% (0.38 to 0.62)) and SBP (difference 4.8 mmHg (6.7 to 2.9)).
Conclusion: In 1995 there were no differences in the recorded measurement of clinical care between Asian and non-Asian patients with diabetes. However, by 2001 Asian patients had substantially poorer quality of care. Glycaemic control was substantially poorer but SBP better in Asian patients with diabetes at the beginning and end of the study period. These differences in process and intermediate outcomes of care were not due to variations in SES.

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Are managed clinical networks associated with improved clinical outcomes?
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Background and Aims: Diabetes is associated with excessive morbidity and mortality yet evidence demonstrates tight glycaemic, blood pressure and cholesterol control improves clinical outcome. NHS Tayside established a Diabetes Managed Clinical Network (MCN) in 1999 to deliver population based diabetes care.
Materials and Methods: The MCN implemented multi-faceted interventions and evaluated indicators of quality care (SIGN), as part of a clinical governance and multidisciplinary education strategy. This involved determining service priorities, developing a web-based, clinical record (DARTS/SCI-DC), regional guidelines (SIGN based), increasing patient involvement, 'on-line' audit, certificated diabetes module, diabetes forums x 3, out-reach seminars and biennial conference. We evaluated process indicators/outcomes in 2001/2002.
Results: All hospitals and 72 Practices collaborated - 300+ staff attended education initiatives, 65 completed the certificated course. Every Practice and diabetes clinic has access to the clinical management system, automated audit, out-reach seminars, access to and knowledge of guidelines. 2001/2002 data respectively indicates improvement: *Population* 9694 (2.5%) / 11 216 (2.9% prevalence), *HbA1c testing* 91% / 93.1%, *HbA1c (average)* 8.1% / 7.7%, *BP testing* 74% / 85%, *BP (average)* 140/79 mmHg / 140/77 mmHg, *Cholesterol testing* 71% / 79%, *Retinal Screening* 63% / 68.3%.
Conclusion: MCNs promote professional education, multidisciplinary working and are associated with improved clinical outcomes.

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An educational model for diabetes care and prevention in primary care.
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Background and Aims: Diabetes constitutes a rapidly growing worldwide health problem. It is a potential source of severe individual and public consequences. In order to avoid extensive individual suffering and public economic expense, the prevention and care of diabetes must be properly directed and timed and effectively implemented. In Finland, under the umbrella of the Development Programme for the Prevention and Care of Diabetes in Finland 2000–2010 (DEHKO) an educational model for enhancing diabetes prevention and care was developed and tested in co-operation with a primary health care centre in the municipality of Lempäälä (population 17100).
The model was developed to match the needs of the health care centre identified by the personnel. The aim was to activate the personnel, to reinforce the existing knowledge and skills and the ability to apply them in a clinical setting, to identify and modify the attitudes and to promote the status, organisation and routines of diabetes prevention and care. The different professions in primary health care must be empowered to be able as teams to adequately support the self-care and quality of life of people living with diabetes. We are aiming at a model which can be tailored according the varying needs of health care facilities.
Materials and Methods: The model comprised: 1) lectures or „forums" for the entire personnel to update and dispense the essential knowledge and to negotiate common practices. 2) patient education training programme for nursing professionals in order to develop tools for supporting positive changes in health behaviours. 3) task groups to identify problems in diabetes care and create, implement and document practical solutions for them. The tutors of these task groups were supervised by an experienced group-work facilitator. Most of the lectures were prepared and presented by local professionals and experts. Finally, the whole personnel took part in a „Diabetes Day" where new practices were reviewed and a mutual understanding upon their implementation pursued.
Results: The participation rate in the different domains of the model was impressive, 42-69 % for forums, and the qualitative feedback was decidedly positive. The nurses were encouraged to work with groups and the number of educational groups increased. The number of referrals foot care increased from 32 in 2001 to 102 in 2002 and there was a considerable increase (21 %) in the number of relevant laboratory tests, too. These results, however, cannot directly and solely be attributed to the education, given the other diabetes relevant activities taking place during the process.
Conclusion: The model was successfully developed and implemented. It achieved the main goals. The prevention and care of diabetes was put on the agenda, the status of these activities was promoted and the self-confidence and the mutual trust of the personnel was enhanced.

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Short-term lifestyle intervention is beneficial in insulin-treated Type 2 diabetic patients in primary healthcare in Finland: the KASDIA Study.
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Background and Aims: DCCT, UKPDS and the Kumamoto study have provided evidence for benefits of intensive insulin therapy on patients with diabetes mellitus. However, insulin therapy has only moderate effectiveness in actual clinical practice, partly due to real and perceived concerns among both patients and physicians. Lifestyle interventions are effective in preventing or delaying incidence of T2D. The purpose of this study was to assess effectiveness of a brief lifestyle intervention on glycemic control in a population-based cohort of insulin-treated T2Ds in a Finnish primary care setting in 1999-2000.
Materials and Methods: In Kangasala primary health centre in September 1999, among a total of 706 (4.2% prevalence) diabetic patients 619 had T2D, of whom 562 were in general practitioner care in 16,615 population of 30-69 years of age. There were 100 T2Ds who were on insulin alone or in combination treatment. A total of 63 insulin-treated T2Ds took part in a brief lifestyle intervention programme aimed at reducing levels of risk factors for cardiovascular disease. The participants were systematically (by birth dates) allocated to individual (IND; n=33) or group intervention (GR; n=30), whereas non-participants (NON; n=37) received only conventional care. All T2D cohorts were followed in the electronic Finstar patient registry from baseline (September 1999) to 2-year follow-up.
Results: At baseline, the GR and IND groups were well balanced whereas the NON had relatively more males, a larger insulin plus sulphonylurea combination treatment group, and larger mean insulin dose. Daily insulin doses increased by 13 U/d (95% CI: 4 to 22) in NON, and 10 U/d (5 to 15) in participants (GR~IND). However, glycemic control (HbA1c) did not increase in NON (0.2%; -0.3% to 0.7%) but increased in participants (-0.6%; -0.8% to –0.4%). Body mass index (kg/m2) increased only slightly in all groups.
Conclusion: In primary health care in Finland, brief lifestyle intervention in insulin-treated T2D patient self-management education improved HbA1c levels at short-term (3-6 months) and 2-year follow-up. Daily insulin doses increased only moderately, and without clinically significant increase in body mass index at the same time. Our results encourage to increase activities in primary health care that utilise lifestyle interventions even in established T2D patient groups such as insulin-treated patients.

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Assessment of stages diabetes management as a diabetes quality improvement project implemented in Curitiba, Brazil.

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Background: Staged Diabetes Management (SDM), a practice management program which uses evidence-based algorithms and treat-to-target approach has been implemented at Hospital Nossa Senhora das Graças, Curitiba, Brazil as a diabetes model of care that could benefit patients, providers and the health care system. SDM was customized to reflect current practice in Brazil, translated into Portuguese and the entire diabetes care team was trained on its use. We set out to evaluate the effectiveness of SDM in improving metabolic outcomes and standards of care in patients seen at our hospital outpatient diabetes clinic.
Methods: Since the program started in 1998, diabetes care has been delivered for 523 patients with 310 patients currently active in the program. The present study was conducted to evaluate a subset of 84 patients from this population who have completed 1 year follow-up; (36F, 48M), age 59.5+11.3 y/o, with type 2 diabetes diagnosed for 7.3+5.7 years. Data was collected during regular clinic visits to assess metabolic control measured by HbA1c, fasting glucose, random capillary glucose, blood pressure, lipid profile, and treatment provided. Self-management education including nutrition and physical activity was assessed during educator visits asking about frequency and duration of exercise and patients were encouraged change lifestyle by the dietitian.
Results: At the initial visit 46.4% of the patients had HbA1c >8.0%, after 1 year 28.8% had HbA1c>8%. Average HbA1c decreased from 7.8+1.4% at initial visit to 7.1+1.2% at 1 year (p=0.00006). Initial fasting glucose was 177.9+61.4mg/dl and decreased to 156.7+65.8 mg/dl (p=0.009), systolic blood pressure was 146+22mmHg and decreased significantly to 138+20mmHg (p=0.01), diastolic blood pressure did not change. A lipid profile was done in 100% of patients with no significant change noted. All patients received a well-documented foot exam including a risk assessment as well as monitoring for nephropathy and eye exam yearly. 34,5% of patients had microalbuminuria before starting program. Change in diabetes treatment at baseline and 1 year is on the table.At baseline 71% of patients were sedentary; 58.3% changed lifestyle exercising more than three times a week at one year.
Conclusion: SDM implementation improved metabolic and blood pressure control with standards of care (foot exam, eye exam, screening for nephropathy, annual lipid profile) being done in all patients. Metabolic outcomes were improved due to patients being switched to the most appropriate therapy based on SDM algorithms.

Treatment	Diet %	S.U. %	Metformin %	Oral Combination %	Insulin %	Insulin + Oral %
Baseline	26.0	41.0	3.6	16.6	7.1	4.7
1 year	4.8	8.3	23.8	19.0	10.7	33.3

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Establishing international standards of care and education: staged diabetes management.

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Background and Aims: Ninety-five percent of the estimated 300 million individuals with diabetes worldwide are treated by primary care physicians and allied health professionals. We set out more than a decade ago to improve their approach to diabetes care and education using a systematic, evidence-based program called Staged Diabetes Management (SDM). In this paper we summarize the results to date.
Materials and Methods: SDM, using clinical pathways customized to each community, was implemented in 15 countries. In each site standards of care and education were developed to assure the highest quality of performance. Clinical pathways for the detection and treatment of each type of diabetes and insulin resistance-related disorders were modified in collaboration with local experts to reflect the limited resources of many of the countries. Following translation into the regional language, an implementation and dissemination plan was established. Baseline and follow-up data were collected and reported.
Results: Changes to health care and education processes and structures occurred in each site. Structural changes included: (1) the training of nurse educators; (2) establishment of multi-disciplinary care and education teams; (3) construction of prevention and care clinics; and, (4) the establishment of model diabetes centers in both the public and private sectors . Process changes consisted of : (1) establishment of one common standard of practice; (2) agreement on the criteria for detection of diabetes (fasting plasma glucose > 126 mg/dL (7 mmol/L) and/or casual plasma glucose > 200 mg/dL (11.1 mmol/L)); (3) criteria for initiation and change in therapies (e.g. in type 2 diabetes fasting plasma glucose > 350 mg/dL (19.4 mmol/L) recommends initiation of insulin); (4) acceptance of treat to target for all metabolic diseases (e.g., diabetes: <7% HbA1c, HTN: <130/80 mmHg) ; (5) establishment of a patient education program with training of appropriate health professionals and patient educators; (6) surveillance for complications including foot examination and screening for microalbuminuria. Outcome data reported from various sites show: (1) significant ($p<0.05$) improvement in primary care management of diabetes as reflected in HbA1c (with an average decrease of two percentage points); (2) significant ($p< 0.001$) increase in surveillance for such complications as renal, neurological and macrovascular disease with change ranging from 10 to 80%; (3) significant ($p<0.0001$) decrease in the amputation rate (from 40/1000 to 4/1000); (4) significant ($p<0.0001$) decrease in adverse perinatal outcome in women with GDM (average reduction in macrosomia for 75%).
Conclusion: Over the past decade more than 38,000 health professionals have been trained in SDM in Japan, Turkey, Brazil, Pakistan, Mexico, Singapore, France, Germany, Poland, Thailand, Taiwan, Malaysia, Republic of Korea, Russia and United States. The program has resulted in a profound change in the way these health professionals care for and educate more than one million individuals with diabetes. Adoption of comprehensive care has also led to the establishment and expansion of the role of diabetes educators in many of these countries.

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Quality of control of risk factors in Type 2 diabetic patients in the Moscow region.

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Background and Aims: To evaluate the quality of control of major modifiable risk factors in Type 2 diabetic patients in Moscow and the Moscow region.
Materials and Methods: 299 Type 2 patients were randomly selected from 97 practices of primary care internists and endocrinologists. They were 60 males and 239 females, their mean age was 60.4 ± 10.1 (range, 41-83) yrs and duration of diabetes 9.2 ± 6.7 (range, 1 to 32) yrs. All patients were assessed for HbA1c (upper normal limit 6.2%), fasting cholesterol and triglycerides, BMI, blood pressure and smoking status, as well as antidiabetic, antihypertensive, lipid-lowering and aspirin medication.
Results: 62/299 (20.7%) of patients were on diet only, 159/299 (53.1%) on oral agents, 49/299 (16.4%) on insulin and 29/299 (9.7%) on combined insulin + oral agents therapy. Mean (± SD) HbA1c was 9.2± 1.8% (range, 5.3 to 14.7%). Among patients < 65 years of age only 21/193 (10/9%) had an HbA1c level below 7%. The HbA1c levels were not significantly different between the four antidiabetic treatment groups. Mean BMI was 31.3± 5.6 kg/m2, with 10.6% of the whole group being normalweight, 34.9% overweight and the rest 54.5% being obese. Among 267 patients with arterial hypertension, 79.4% had their sitting blood pressure ≥140/90 mmHG, and only 6% had blood pressure below 130/85 mmHg. Antihypertensive medications had been administered to 233/267 (87.3%) of hypertensive patients. There was no difference in blood pressure values in patients who had been and had not been administered antihypertensive treatment. Fasting hypertriglyceridemia was found in 40.8% of patients, hypercholesterolemia in 64.3%, both - in 31.4%; none of patients with elevated lipids was taking lipid-lowering agents. Only 13% of patients were current smokers. Low dose aspirin therapy had been administered to 16 out of 110 (< 15%) of patients with coronary heart disease. Proportion of patients, in whom all 5 surrogate treatment goals (HbA1c, lipids, blood pressure control, BMI and non-smoking) were reached, was as follows: all 5 goals - 2.3%, 4 goals - 8%, 3 goals 17.5%, 2 goals 36.5%, 1 goal 31.2%, none - 4.6%. There were no significant differences in any of the parameters studied between patients who had been followed with their diabetes by primary care endocrinologists or those who had been followed by primary care internists.
Conclusion: Quality control of major modifiable risk factors for diabetic micro- and macroangiopathy and achievement of surrogate treatment goals in Type 2 diabetic patients of the Moscow and Moscow regions are unsatisfactory. Possible reasons for absence of difference between treatment groups and between management provided by endocrinologists or internists will be discussed.

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Improved patient outcomes through regular monitoring at GP diabetes clinics.

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Background and Aims: During 1996 to 1998 a Diabetes Integrated Care Pilot Project was held in the Macleay Hastings Valleys. Population of Macleay Hastings Valleys was 83,760 (1996 census) and 22% of the population were over 65 years of age compared to the New South Wales (NSW) average of 11.9%. The Aboriginal Population was 6.8% compared with the NSW state average of 1.7%. The current population is 90,000 (2001 census) and 27% of the population are over 65 years of age with the NSW State average being 17%.
During the Pilot project diabetes educators held clinics in GP surgeries. Following the success of the Pilot project the clinics were continued with one diabetes educator holding clinics in GP surgeries. Aims of the Project were to:
- Ensure all people with diabetes in the Macleay Hastings Valleys had access to regular complication screening and appropriate management.
- Develop a system to measure the health outcomes of people with diabetesin the Macleay Hastings Health District.
These aims are the same for the current project.
Material and Methods: A diabetes educator conducts regular clinics in GP surgeries and collects data and performs physical examinations which include measurement of height , weight , blood pressure and foot examination. Patients are selected by the GP and 77% are over 60 years of age. During the period from January 2000 to December 2002 approximately 400 people with diabetes were seen at these clinics.
Results: HbA1c , lipids and microalbumin results of patients with diabetes have improved during the current program.

Statistics

Pilot 1996-1998	HbA1c <7.5% 29.4%	Cholesterol <5.5 mmol/L 31%	Trigs <2.0mmol/L 26.9%	HDL >1.0mmol/L 21.8%	Microalbumin <30mg/l 22.3%
Current clinics 2000-2002	HbA1c <7.0% 60%	Cholesterol <4.0mmol/L 35%	Trigs <2.0mmol/L 54%	HDL > 1.0mmol/L 80%	Microalbumin <20 mg/L 78%

Conclusion: Health outcomes of people in the Macleay Hastings Valleys have improved due to regular monitoring at clinics in GP rooms.

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Report on the first two years of the IDF Child Sponsorship Program.

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Background and Aims: The diagnosis of a child with diabetes places a great financial burden on many families in developing countries. Costs can reach 600 USD per year, and so many cannot afford adequate insulin, syringes, needles, and blood glucose monitoring equipment. These children become chronically unwell. They often rapidly develop devastating complications, and frequently die. A sponsorship program has been established by the IDF Consultative Section for Childhood and Adolescent Diabetes to help meet this need.
Methods: Diabetes Australia - NSW provides office space and other assistance, and industry has assisted with funds for infrastructure,. Advertisements were made, with the permission of all State Diabetes Australia branches, in the national diabetes magazine. Sponsors commit to regularly contribute to the program.
70% of funds collected are sent overseas, and 30% are reserved for administration. Diabetes centres in developing countries are assessed as potential recipients. Before funds are sent, a Memorandum of Understanding is signed, with a defined reporting framework and financial trail. The centre identifies the priority needs and the children most in need of support. Children supported in the program are medically audited. We calculate that around 700 sponsor equivalents are needed for the program to be sustainable in the long term.
Results: The program commenced in late 2000. By the end of 2002, USD 85,400 had been raised. We have around 150 regular sponsors in Australia and other countries, plus there are a number of sponsors in the Netherlands, and we have also received assistance from a Norwegian branch association. The program was successfully implemented in three pilot countries - the Philippines, Fiji, and Papua New Guinea, and has now expanded to include centres in India and Romania. We are examining the possibility of assisting in four other countries. Funds have been used to purchase insulin, syringes and needles, blood glucose monitoring equipment and supplies, HbA1c testing, travelling costs to clinics, and patient education. Financial auditing and clinical reporting are proceeding well.
Conclusion: The IDF Child Sponsorship Program has been successfully established.

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The epidemiology and impact of hypoglycaemia in an Australian population: evaluation by postal survey.

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Background and Aims: The epidemiology of the manifestations of hypoglycaemia among patients with diabetes are not well understood. In addition to direct symptoms, hypoglycaemia adversely affects people in many ways, causing fear and anxiety. Consequently, sufferers adopt avoidance strategies to prevent hypoglycaemia. The aim of this study was to evaluate the epidemiology and impact of hypoglycaemia in an Australian population.
Materials and Methods: A postal survey was mailed to subjects identified through the local Diabetes Australia group, and 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 symptomatic, nocturnal and severe. Events were reported for a 6-month period.
Results: 740 patients responded to the survey. Of the respondents, 77% had Type 1 diabetes and there was a 51:49 female:male ratio (mean age 48 and 55 years, respectively). The overall mean frequency of hypoglycaemia was 22 events per person over 6 months (Table). In a multivariate analysis, the frequency of hypoglycaemia was directly and independently associated with reduced health-related quality of life (QoL; $p < 0.05$). In people who experienced severe or nocturnal events, 50% reduced their insulin dose to prevent hypoglycaemia. Other preventative behaviours included eating more (76%), reducing physical exercise (26%) and taking sick leave (10%). On average, it took 2 days and 1.3 days to recover fully from a severe and a nocturnal event, respectively.

Hypoglycaemic events per person over 6 months by type and age group

Type of event	<45 years	45–60 years	>60 years	All
Severe	0.9	1.8	0.9	1.2
Nocturnal	6.8	4.6	2.9	4.8
Symptomatic	24.8	16.5	9.2	17.2
All events	30.2	21.4	11.9	21.5

Conclusion: Hypoglycaemia is a frequent adverse event of existing diabetes treatments, particularly in younger Type 1 patients. Since hypoglycaemic events directly affect lifestyle and can indirectly compromise metabolic control through decreased insulin dosing, it is likely that new therapies, which could provide glycaemic control with reduced hypoglycaemia, would confer significant improvements in QoL.

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Outcome of diabetes patients attending a hospital diabetes clinic.

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Background and Aims: Although the prevalence of diabetes mellitus is increasing in Hong Kong, we have little data on diabetes care delivery. The study aims to investigate the outcomes and the pattern of diabetes care in a hospital diabetes clinic.
Materials and Methods: Two hundred diabetes patients newly admitted to the diabetes clinic of Tung Wah Eastern Hospital in 1996-1997 were randomly selected. Their clinical and diabetes care data were collected from medical record for up to two years.
Results: Of 196 patients eligible for study, their age were 60.3+/-10.5 years, 54.1% were male, duration of DM was 4.6+/-6.0 years and the majority (98.5%) had type 2 DM. At the end of two years, there were decreases of HBA1c of 1.1% (8.5+/-2.2 to 7.4+/-1.3%, $p<0.001$), fasting glucose of 1.1mmol/L (9.1+/-2.9 to 8.0+/-2.3 mmol/L, $p<0.001$), cholesterol of 0.4 mmol/L (5.6+/-1.3 to 5.2+/-0.9 mmol/L, $p<0.001$) and triglycerides of 0.2mmol/L (2.0+/-1.5 to 1.8+/-1.7 mmol/L, $p=0.067$). Blood pressure showed no change, body weight increased 1.3kg (63.9+/-11.5 to 65.2+/-13 kg) and 40 (20.6%) patients developed new diabetic complications. During the two years, nine (4.6%) patients defaulted follow up and six (3.1%) patients died; each patient attended the doctor 6.3+/-2.9 times and 11(5.7%) patients had been admitted into hospital. Of the remaining 180 patients, 141 (78.3%) patients had their condition stable enough for referral to primary care doctors, among which, 102(56.7%) were recruited into the Shared Care Programme.
Conclusions: Hospital diabetes clinic provides satisfactory metabolic control to diabetic patients but there is need of more attention to the control of blood pressure, lipids and body weight. Majority of diabetes patients can have their diabetic condition stabilized and referred to primary care doctors under the Shared Care Programme.

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The economic costs of diabetes mellitus foot syndrome.

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Background and Aims: The diabetic foot is associated with a heavy financial burden thet has not been previously addressed prospectively among Nigerians. This study set out to determine the direct financial costs of diabetic foot ulceration as part of assessing the disease burden of diabetes mellitus.
Materials and Methods: This was a prospective study in which 20 patients admitted to the medical wards of a Teaching hospital were studied. The direct economic cost attributable to diabetes mellitus foot syndrome (DMFS) were determined by costing the „units of service“ and the period covered.
Results: Seventeen and three type 2 and type 1 DM patients respectively were studied. The mean (range) of their hospital stay was 60 (4-176) days. Surgical procedures were carried out in 10 (50%) of them. The mean (range) of the costs incurred by the patients was NGN93,256:7k (NGN40,700.00k-NGN580,746:00k). Drugs and bed costs accounted for > than 50% of the total costs incurred. There was no statistically significant difference between the mean costs incurred by the males and the females. However in subjects wityh type 2 DM, the mean costs incurred from drugs, surgery and other miscellaneous causes were statistically significantly higher than in those patients with type 1 DM.
Conclusion: Foot ulcers in patients with diabetes mellitus as reflected in the enormity of the direct costs in this report remain a major concern from the economic standpoint. These costs which are ill-afforded by these patients could be reduced to the barest minimum by forestalling the development of diabetes mellitus foot syndrome. The risk factors for the development of diabetic foot ulcers in Nigeria need to be looked into in order to put in place measures to bring about a reduction in the disease burden.

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Indirect cost of diabetes in Tunisian diabetic population.

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The aim of the study is to evaluate indirect cost and quality of life in Tunisian diabetic population .Our study evaluate annual loss time for diabetes care: hospital admission, consultation,investigation,self monitoring,education sessions in 200 diabetics,mean age 46.2±17.6yrs(82 type1, 109 type2 and 9 gestational diabetes).The diabetes duration was 10.7±7.6yrs.The first study group(n=92)have nocomplicated diabetes,the second(n=108)have moderate or severe complications:32 % hypertension,10 %coronaropathy,25 % retinopathy and 5 %nephropathy. Our result indicate that global annual loss time was 27.5 days for the first group, the major loss time was related to hospital admission(43%)and medical visit(27%)and 59.5 days for diabetics with moderate complications,the major loss time was related to feet(60%)and cardiovascular(27%)complications.The global annual loss time increase to 156.5 days in patients presented severe complications,the major loss time was related to renal disease(64%)and feet complications(17%). These complications affect highly intangible costs and diabetic quality of life. The loss year working by premature retirement was 7.33±3.5 yrs, mean annual sickness absence was 20 days in 49 %, loss job was observed in 16 %, anxiety in 36%, social dysfoction in 24 % and severe depression in 18.5 %.
Conclusion: Indirect costs of diabetes and quality of life in peopele with diabetes were highly affected by complications.These need effective interventions as early detection, prevention and treatment of diabetes and its complications, education and an effective manage of outside hospital patients offering them easily access to different medical care requirement.

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Insulin supply in Bangladesh.
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Background and Aims: Insulin is a life saving drug for all cases of type-1 diabetes mellitus and for some cases of type-2 Diabetes mellitus. It is therefore essential that health services providers have a clear idea about the yearly requirement of Insulin and to ensure uninterrupted supply of Insulin.
Materials and Methods: Data regarding population of Bangladesh was obtained from statistical pocket book of Bangladesh. Prevalence of diabetes mellitus was obtained from publication of Dr. M.A. Sayeed at al. Quantity of insulin sale was obtained from the record of insulin importers of International Agencies of Bangladesh, ACI Bangladesh and BIRDEM.
Results: From the data it was found that population of Bangladesh is 125.5 million and that there are about 22,80,000 diabetics in Bangladesh. Average insulin requirement is 18 units per patient per day and 10% of all diabetics need insulin. Daily insulin requirement for all patients is therefore- 2,28,000 X 18 = 41,04,000 = 4.1 million unit. Thus in one year insulin requirement will be 4.1 X 365 ie 1496 millions units of insulin for whole of Bangladesh. Insulin is imported from NOVO Nordisk of Denmark and Eli Lilly of France. Total insulin sale was 547 million units in the year 2001; of this animal insulin was 120M units and human insulin was 427M Units. Both 40 IU/ml and 100 IU/ml insulin was sold and the ratio between then was 222:315. Regular insulin, Insulatard insulin, Premix Insulin, were used.
Conclusion: In Bangladesh the projected requirement of insulin is 1496 million units per year. Actual sale was 547 million units. The difference can be explained by the fact that more than half of diabetics do not know that they have diabetes mellitus. Known diabetic patient often do not take adequate amount of insulin because of fear of pain, lack of education, lack of motivation and lack of affordability.

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Cost-effectiveness analysis of intensive blood glucose control in patients with Type 2 diabetes in Bangladesh.
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Background and Aims: Diabetes with coronary heart disease (CHD) is increasing at an alarming rate. Cost of uncontrolled and complicated diabetic CHD patients are increasing at the same rate. Studies regarding the prevalence of diabetic cardiopathy and its cost burden in developing countries are relatively rare. The study aimed to estimate and compare the cost of conventional versus intensive blood glucose control in patients with type 2 diabetes. 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: Thirty diabetic patients with cardiopathy attending the Cardiology Outpatient Dept, BIRDEM were selected randomly and interviewed in March 2002 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 cost of accompanying person(s)] from consumer's point of view. A comparison was made between patients undergone intensive glycemic control (Group 1) and others (Group 2). Incremental cost-effectiveness has been calculated for patients with type 2 diabetes (mean age 52 years). The comparison was made between the cost for conventional (primarily diet) glucose control versus intensive control with a sulphonylurea or insulin. The incremental cost per event-free year gained within the trial period was calculated.
Results: The cost analysis in 30 patients showed that the total cost of treatment was US$ 13,308.16 with an average of US$ 443.60 per patient. On comparing the both groups, it was found that the cost difference was US$ 6657.74. The incremental cost of intensive management was $178 ($95 to $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 1.19 (0.79 to 1.81) years. The incremental cost per event-free year gained was $356 (costs and effects discounted at 6% a year) and $198 (costs discounted at 6% a year and effects not discounted).
Conclusion: Intensive blood glucose control in patients with type 2 diabetes significantly increased treatment costs but substantially reduced the cost of complications and increased the time free of complications. The prevalence of cardiopathy of diabetic patients is almost equivalent to that in developed countries which indicates that comprehensive care can reduce the burden of cardiopathy of diabetic patients even in a developing country. Further monitoring and advanced studies is needed to examine different ways in which an intensive blood glucose control policy can be translated into standard practice and the role of new drugs can be calculated.

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Collaboration between IDF and Rotary Foundation to expand insulin availability: Bolivia the initial model.
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Background and Aims: Insulin, a WHO essential drug is not universally available. The IDF Insulin Committee and IDF Adopt a Child have explored creative ways to increase insulin availability. Impediments have included reliable systems of care and documentation of recipient accountability. Utilization of non governmental orgaizations is an attractive model to improve donor response.
Materials and Methods: Rotary Foundation, with nearly worldwide presence has a program of matching grants where Rotary Districts in a donor country and a recipient country collaborate in a project. Rotary uses this method because local Rotarians have knowledge of need and can offer fiscal oversight. Monies are raised locally in the donor and recipient Rotary districts. Funds are then matched by Rotary Foundation. This process effectively doubles local fundraising. IDF has invested considerable energy in developing the Adopt a Child Programme. Operations manuals with full fiscal accountability are available. Using IDF and Rotary contacts we identified Bolivia as a country with willing IDF members and Rotary members. Collaboration using the IDF Adopt a Child Model Programme yielded a grant to Rotary Foundation.
Results: We were successful in obtaining USD$106,000 for the project. This process required many exchanges of information and ideas between IDF, Rotary districts and members as well as Rotary Foundation. We sought full Rotary Foundation Board review in order to vet the model and maximize potential for future funding. Insulin suppliers and meter suppliers collaborated in making insulin available at sustainable prices. Children receive insulin and supplies through IDF sponsored clinics. The IDF model requires careful attention to both disease outcomes and fiscal accountability.
Conclusion: This project demonstrates the potential to utilize the vast Rotary network in collaboration with IDF member organizations to institute programs designed to help alleviate insulin shortages. This report should serve to alert IDF Member Organizations of the potential to collaborate with Rotary International. The IDF Insulin Committee stands ready to aid in the institution of such projects.

PS Health Care Organisation 4:
Improving Patients' Outcomes (II)

1274

Are different clinical practice guidelines associated with regional differences in diabetes care?

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Background and Aims: Treatment guidelines for diabetes and cardiovascular disease differ between Europe and North America. The 2nd Joint Task Force of European and Other Societies on Coronary Prevention recommended target blood pressure (BP) levels < 140/90 mmHg and LDL cholesterol (LDL) levels < 3.00 mmol/l (116 mg/dl). The American Diabetes Association recommends BP levels < 130/80 mmHg and LDL < 2.59 mmol/l (100 mg/dl).
Materials and Methods: To evaluate behavioural risk factors, treatment, and intermediate health outcomes in Europe and North America, we analysed baseline data from ADOPT, a global, randomised, controlled clinical trial designed to compare the efficacy and safety of initial monotherapies in patients with type 2 diabetes (T2D).
Results:

	Europe (n = 2,074)	North America (n = 2,219)	*P*-value
Age (years)	58	55	< 0.0001
Male (%)	61	55	< 0.0001
White (%)	97	79	< 0.0001
Treated for hypertension (%)	48.3 48	7 0.0107*	
Treated for dyslipidaemia (%)	3.1	3.7	0.0389*
BMI (kg/m^2)	30.8	33.0	< 0.0001*
Systolic BP (mmHg)	137	129	< 0.0001*
Diastolic BP (mmHg)	81	79	< 0.0001*
LDL (mmol/l)	3.16	2.90	< 0.0001*
Smokers (%)	16.4	13.9	0.0019*

**P*-value adjusted for age, gender, and race

Conclusions: After adjusting for age, gender, and race, T2D subjects in Europe were leaner, more likely to smoke, and less likely to be treated for hypertension and dyslipidaemia than T2D subjects in North America. Systolic BP, diastolic BP, and LDL were significantly lower in North America. There are important differences in behavioural risk factors and cardiovascular risk factor treatment and control between Europe and North America that may reflect the influence of different clinical practice guidelines.

1275

Population based measurement of quality of diabetes care with 59000 HbA1c values in the state of Thuringia/Germany.

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Background and Aims: There is insufficient knowledge about the outcome quality of diabetes therapy in primary care in Thuringia and elsewhere. The „diabetes map" is an experimental epidemiological method which assesses the outcome of type 2 diabetes care in a broad area. The map displays the distribution of HbA1c as an indicator of metabolic control of type 2 diabetes patients over a defined period. The method provides information about regional differences at one point of time but also over a period of time (assessing the outcome of specific public health care interventions, medications etc.).
Materials and Methods: Collection of all HbA1c-values (1.1.2002 – 31.3.2002) of Thuringian patients in medical laboratories who worked for Thuringian general practitioners (GP). Each HbA1c-value was identified by the postal code of the GP who ordered the test. Each HbA1c-value was adjusted by a standardization procedure (calculation of relative HbA1c [relative HbA1c = absolute HbA1c / mean normal of healthy subjects (2 SD upper + lower limit) / 2)]). The Free State of Thuringia (2.421.871 inhabitants) consists of 23 districts which were adopted for the diabetes map. Personal data concerning patients or physicians were not allocated.
Results: 20 participating laboratories collected 74.936 HbA1c-values, from which 59.702 values belong to the state of Thuringia: Mean HbA1c of whole Thuringia was 6.75 % (mean normal of healthy subjects 5 %). 3.0 % of all HbA1c-values were increased two times or more. The percentage of two times or more increased HbA1c-values in the 23 Thuringian territories varied more than 100% (Schmalkalden-Meiningen 1,8%, Suhl 4,8%).
Conclusion: The number of HbA1c-values and participating medical laboratories has increased threefold since the first diabetes map in 1997. The quality of metabolic control of type 2 diabetes patients in primary care in Thuringia is better than supposed, provided the method proves to be sufficiently valid. Clinically relevant differences in the percentage of patients who need immediate intervention (relative HbA1c >2.0) suggest that there are differences in the quality of primary care in particular Thuringian districts. A comprehensive and unselected HbA1c-map was created at very low costs since it is a by-product in medical laboratories. The Thuringian HbA1c-mapping will be performed on an annual basis for a further evaluation of the introduced method.

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Effectiveness of screening techniques for diabetes in general practice.

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Background and Aims: The systematic screening of high-risk groups for diabetes is now recommended. We aimed to assess the effectiveness of different screening techniques for diabetes in General Practice.
Materials and Methods: All patients aged 30 to 64 registered with a UK General Practice, with known BMI ≥30 (n =287, prevalence 6.6%) were randomised to three Groups: a) receiving a postal invitation for screening (n=96), b) opportunistic screening - primary care medical records were clearly marked, recommending an invitation for screening at the next consultation (n=96); c) Control (n = 95). Each intervention group was further divided to receive: a) fasting venous blood glucose measurement or b) random venous blood glucose measurement. Additionally, all patients in this age range of known Asian origin (n=32, prevalence 0.74%) were similarly randomised.
Results: In the obese cohort, there were no significant differences in age or sex between the 5 sub-groups, 10 patients (3.5%) died or left list during Study Period, Primary Care consultation rate (12/12 period) for opportunistic group averaged 5.1 (range = 0 – 18). Screening uptake in opportunistic groups was higher than postal invitation groups (64% v 53%), across all groups uptake was higher in random than fasting measurement (64% v 52%), 58% of the intervention group and 18% of controls underwent glucose measurement during the year. 1.6% of the intervention group (n = 3) and 3.2% of controls (n = 3) were diagnosed with diabetes, there was a significant difference between intervention and control groups for the diagnosis of impaired glucose tolerance and impaired fasting glycaemia, though numbers were small (5 v 1), random was as effective as fasting glucose measurement in finding abnormalities and there was no difference between fasting and random groups in the number of confirmatory tests required. In the cohort of Asian patients, uptake for screening was extremely low (3/16 = 18.75%)
Conclusion: Opportunistic screening using random blood glucose measurement may be a more effective method of screening than systematic postal recall and fasting blood glucose measurement in encouraging uptake. Screening is perhaps more effective at identifying pre-diabetes states than diabetes. Traditional approaches are not particularly successful in encouraging uptake for screening in Asian patients. A larger trial is needed to validate these pilot data.

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Undiagnosed impaired glucose homeostasis associated to metabolic syndrome (EGIR) on high-risk Spanish population.

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Background and Aims: In order to estimate the prevalence and cardiovascular risk of the metabolic syndrome (MS) associated to undiagnosed impaired glucose metabolism a multicenter (10 primary health-care centers, 250,000 inhabitants), cross-sectional study was carried out.
Materials and Methods: Undiagnosed subjects aged>40 y. with one or more risk factors for developing diabetes (obesity, family history, hyperglycaemic agents or previous unclassified glucose abnormalities) were screened and diagnosed by means of a standardized 75 g oral glucose tolerance test to measure fasting and 2h plasma glucose. An assessment of the cardiovascular risk using Framingham Hearth Study reference based on

sex, age, body mass index (BMI), smoking, glucose metabolism, blood pressure and lipid profile was also performed.
Results: Of the 580 diagnosed subjects, 330 (56.9%) were female, mean age 58.1 years and BMI 31.2 kg.m^{-2}, 292 (50,3%) with only one risk factor and 288 (49.7%) with two or more risk factors, 91 (15.7%) were active smokers, 278 (47.9%) had hypertension and 342 (59%) obesity. A total of 345 (59.5%) presented undiagnosed glucose abnormalities: 158 (27.2%) type 2 diabetes, 92 (15,8%) impaired glucose tolerance (IGT), 46 (7.9%) impaired fasting glucose (IFG) and 49 (8.4%) IGT plus IFG. Regarding impaired glucose metabolism, 203 (58.8%) individuals fulfilled the EGIR criteria as for MS, with a mean cardiovascular risk of 15.7±9.2 compared to a 8.5±6.7 for those with normal glucose metabolism (p<0.001). Odds ratio for the MS referred to new diagnosed diabetics was 17.5 (5.5-55.3) if they associated hypertension and 27.6 (8.7-87.9) when they associated obesity.
Conclusion: An intensive screening focused on a high-risk population for developing type 2 diabetes evidenced not only an increase in diabetes prevalence but also the phenotypic features of the Metabolic Syndrome and an overt association to cardiovascular risk.

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Prevalence of metabolic syndrome (EGIR) related to cardiovascular disease among diabetic Spanish individuals.
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Background and Aims: The prevalence of the metabolic syndrome (MS), according the WHO criteria modified by the EGIR (European Group for the Study of Insulin Resistance), in type 2 diabetic individuals compared to non-diabetic subjects and its cardiovascular risk impact are still unknown in Spain.
Materials and Methods: In order to estimate these epidemiological data, a multicenter (10 primary health-care centers) cross-sectional study involving a random age-stratified sample (15-74 y) was carried out. From January until December 2001 the morphometric profile, risk factors including evidence of microalbuminuria and confirmed cardiovascular events were recorded. Furthermore, an assessment of global cardiovascular risk using Framingham Hearth Study reference based on sex, age, body mass index (BMI), smoking, glucose tolerance (fasting plasma glucose and HbA1c), blood pressure and lipid profile was also performed.
Results: Of the 2,222 diagnosed subjects, 1,181 (53.2%) were female, mean age 49.8±18.4 years, 452 (20.3%) were active smokers, 702 (31.6%) had hypertension, 499 (22.4%) dislipemia, and 234 (10.5%) had type 2 diabetes. Regarding diabetic individuals, 140 (59.8%) fulfilled the EGIR criteria as for MS, with a mean cardiovascular risk of 19.9±7.3 compared to a 9±6.8 for individuals without MS (p<0.001). The prevalence of cardiovascular disease ranged from 5.3% for non-diabetic subjects to 26.5% for the diabetic ones, and accounted for a 27.8% in the MS group (p<0.001). Odds ratio for cardiovascular events associated to each MS factor was 1.81 (1.26-2.61).
Conclusion: The increasing prevalence of the Metabolic Syndrome among diabetic Spanish population and its strong association to cardiovascular disease suggests an improvement of the resources focused on primary prevention.

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Cohort study of corporate employees during routine examinations.
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Background: The study was designed to detect and treat early subjects with „boundary type“ (IGT and IFG) and clinically defined diabetes mellitus (DM) among corporate employees who underwent routine yearly medical examinations during 2000 and 2001.
Method: An oral glucose tolerance test (OGTT) subject group was selected with an initial screening using fasting plasma glucose (FPG) (≥ 110 mg/dl and < 140mg/dl) and glycoalbumin (GA) (>15.6%) values. A total of 503 subjects out of 12,929 males were included into the OGTT group (335 of 6,416 in 2000 and 168 of 6,513 in 2001). There were 53 males tested in both years. Age, body mass index (BMI), plasma glucose (FPG, 1-h PG and 2-h PG), hemoglobin A1c (HbA1c), GA and immunoreactive insulin (IRI) (0-h and 1-h) were used as analysis items.
Results: (1) Based on OGTT results, the 503 males were classified into normal glucose tolerance (NGT) 207 (41.2%), IFG 58 (11.5%), IGT 136 (27.0%) and DM 102 (20.3%) groups using criteria set forth by the Japan Diabetes Society (1999). Further, the 207 males with NGT were divided into 2 groups of 124 subjects with 1-h PG < 160 mg/dl and 83 subjects with 1-h PG ≥ 160 mg/dl. **(2)** In terms of insulin secretion, the IRI value of the NGT group with 1-h PG ≥ 160 was significantly higher than those in the NGT group with 1-h PG < 160 mg/dl (P < 0.05). The IRI of the IGT group was significantly lower than that of the IFG group (P < 0.01). Two subjects in the NGT group displayed high IRI (0-h) values in comparison to the Japanese IRI (0-h) average and possessed HOMA-R indexes of 7.68 and 4.43. These data suggest both patients to be insulin resistant. **(3)** On follow-up of 53 males, the classification of 28 males was unchanged, while 10 males were changed from DM to a better criteria and 15 males were moved from NGT, IGT and IFG to DM. **(4)** The DM prevalence rate was estimated to be 4.3% in 2000 and 3.6% in 2001 based on the first screening by FPG and GA. Based on FPG and HbA1c, the rates were 3.9% in 2000 and 3.3% in 2001. The DM prevalence rate in each age range was 1.1 - 1.3% for those 31-40 years of age, 4.4 - 5.0% for those 41-50 years of age, and 8.4 - 8.5% for those 51-60 years of age.
Conclusion: The importance of measuring IRI to determine the status of insulin secretion was reaffirmed. To lower the DM prevalence rate, strict treatment management is required for not only DM subjects, but also for IGT, IFG and NGT subjects.

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A retrospective analysis from a hospital data base in France: the paradox of very obese patients in a Type 2 diabetes population.
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Background and Aims: Type-2 diabetes is a common risk factor for micro- and macro-vascular complications. In general population, obesity is associated with high risk of related-disease. In a type-2 diabetes population, we examined retrospectively the impact of BMI status on the prevalence of CVD as myocardial infarction and angina pectoris and on the type of prevention.
Material and Methods: We analysed data from a cohort of type-2 diabetes patients with a mean follow-up of 3 years. The cohort (n=2056) was stratified in 4 BMI groups calculated on the highest measured body weight: 18.5-24.9 kg/m^2 (normal), 25-29.9 kg/m^2 (overweight), 30-34.9 kg/m^2 (obese) and over 35 kg/m^2 (very obese). Demography and prevalence of CVD were analyzed in each BMI group compare to the „normal“ group.
Results: Demography and profile of the cohort is described below (statistical tests versus „normal“ group):

N=2056	Normal	Overweight	Obese	Very obese
Group size: n, (%)	355,(17)	795,(39)	589,(29)	317,(15)
Mean age (years)	59.8	61.1	60.2	58
Gender (% female)	35.2	34	48.2*	65.9*
Mean diabetes duration (years)	13.5	13	12.2*	11.2*

* p<0.05

After adjustment on age, gender, diabetes duration, familial cardiovascular history, and on the presence of other coronary risk factors (low HDL cholesterol, hypertriglyceridemia, high systolic blood pressure, tobacco history and albuminuria over 300mg/24h), multivariate odds ratio were calculated as follows.

N=2056 BMI type	Odds ratio estimates for CVD among type-2 diabetes according to BMI	p-value
Normal	1	-
Overweight	1.7	0.014
Obese	1.87	0.006
Very obese	1.68	0.051

Prevalence of ECG-exercice test were 15%, 25% (p<0.05), 25% (p<0.05) and 16.4% (p>0.05), in normal, overweight, obese and very obese group respectively.
Ratios „prevalence of coronarography/prevalence of myocardial infarction“ were 0.26 (1.4/5.38), 0.37 (3.4/9.03), 0.55 (3.9/7.02) and 0.9 (4.1/4.6) in normal, overweight, obese and very obese group respectively. It showed

„very obese“ group underwent heavier and more efficient prevention of CVD.
Conclusion: We confirm obesity (BMI above 25 kg/m^2) is positively and independently associated with risk of CVD in a type-2 diabetes population. We observe less prevalence of CVD in „very obese“ group despite we adjust our analysis on several confounding factors. As an explanation to this phenomenon, the „very obese“ group benefits of more intensive care for prevention of CVD in comparison with others groups. This intensive management may have a greater economic impact on health care system.

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Effect of long-term response to glycaemic control treatment on diabetic blindness, end-stage renal disease and amputation in a Type 2 diabetes population: an analysis using the diabetes mellitus model.
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Background and Aims: Glycaemic control therapies differ in the degree to which they can lower HbA_{1c} over time and, consequently, influence long-term microvascular outcomes. Evaluation of the long-term effects of these therapies requires clinical studies of 10 years' duration or longer. In lieu of such data, computer simulation models can be used to predict the long-term effects of treatment. This analysis investigated the effect of improved glycaemic control on severe late diabetic complications, specifically blindness, end-stage renal disease (ESRD) and amputation, in a simulated population of patients with Type 2 diabetes.
Materials and Methods: The diabetes mellitus model (DMM) was used to simulate a population of patients with Type 2 diabetes. Simulations were performed for a cohort of 100,000 patients, targeting an HbA_{1c} of 7.5%. Baseline characteristics were: mean age at diabetes onset 52 ± 5 years; mean duration of diabetes 13 ± 3 years; male:female ratio 48:52; body mass index (BMI) 28 ± 5 kg/m^2; HbA_{1c} 8.5 ± 0.5%. Three scenarios for responsiveness to treatment with regard to HbA_{1c} targets were assumed: 1) in 10 years, HbA_{1c} increases by 2%; 2) in 10 years, HbA_{1c} increases by 1%; 3) mean HbA_{1c} over 10 years is at the target level. For the three scenarios, the risk of developing blindness, ESRD and amputation were calculated individually for each patient and cumulated to 10-year incidence rates.
Results: Risk reductions of 11% for blindness, 14% for ESRD and 4% for amputation were observed for scenario 2 versus scenario 1. Risk reductions for scenario 3 versus scenario 1 were 30% for blindness, 35% for ESRD and 14% for amputation. Thus, 3.4 events/10,000 patient–years of blindness, 0.5 events/10,000 patient–years of ESRD and 1.3 events/10,000 patient–years of amputation would be avoided with scenario 2 versus scenario 1. Furthermore, 9.4 events/10,000 patient–years of blindness, 1.2 events/10,000 patient–years of ESRD and 5.3 events/10,000 patient–years of amputation would be avoided with scenario 3 versus scenario 1.
Conclusion: These results demonstrate that even small improvements in the long-term response to glycaemic control treatment can reduce the risk of developing diabetic blindness or ESRD and the risk of amputation.

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Simulation of micro- and macrovascular outcomes in a Type 2 diabetes population using the diabetes mellitus model: effect of improved glycaemic control.
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Background and Aims: In patients with Type 2 diabetes, inadequate glycaemic control can lead to micro- and macrovascular complications. Since glycaemic control therapies differ in the degree to which they lower HbA_{1c} over time, it is important to evaluate the long-term effects of these therapies to establish those most likely to delay the development of complications. However, clinical studies of 10 years' duration or longer are required to obtain such data. In lieu of such studies, computer simulation models can be used to predict the long-term effects of treatment.
Materials and Methods: This analysis investigated the effect of improved glycaemic control on the cumulative 10-year incidence rates of diabetic non-proliferative retinopathy (NPRP), clinical neuropathy (DCCT definition), diabetic foot syndrome and myocardial infarction, in a patient population of Type 2 diabetes, using the diabetes mellitus model (DMM). Two cohorts (100,000 patients each) were simulated. Baseline characteristics were: mean age at diagnosis of diabetes 50 ± 5 years; mean duration of diabetes 5 ± 3 years; HbA_{1c} 7.5 ± 0.5%. In a first analysis, targets for HbA_{1c} were set to 7.5% (Cohort 1) and 6.5% (Cohort 2); increase of HbA_{1c} over simulation time was 2% in both cohorts. In a second analysis, improved responsiveness to glycaemic control treatment was assumed for Cohort 2 with an increase of HbA_{1c} over simulation time of 1%.
Results: In the first analysis, risk reductions of 5% for NPRP, 27% for clinical neuropathy, 13% for diabetic foot syndrome and 15% for myocardial infarction were observed for Cohort 2 versus Cohort 1. Thus, 3.2 events/1000 patient–years of NPRP, 6.8 events/1000 patient–years of clinical neuropathy, 1.9 events/1,000 patient–years of diabetic foot syndrome and 1.3 events/1000 patient-years of myocardial infarction would be avoided with tighter glycaemic control targets (HbA_{1c} of 6.5% versus 7.5%). In the second analysis, reductions for Cohort 1 versus Cohort 2 were 7% for NPRP, 34% for clinical neuropathy, 17% for diabetic foot syndrome and 19% for myocardial infarction. Thus, 3.9 events/1000 patient–years of NPRP, 8.5 events/1000 patient–years of clinical neuropathy, 2.4 events/1000 patient–years of diabetic foot syndrome and 1.7 events/1000 patient–years of myocardial infarction would be avoided with tighter glycaemic control targets (HbA_{1c} of 6.5% vs 7.5%) and improved response to treatment (1% HbA_{1c} increase versus 2% HbA_{1c} increase).
Conclusion: These results demonstrate that tight glycaemic control targets and improvements in the long-term response to glycaemic control treatment can reduce the risk of developing micro- and macrovascular outcomes in patients with Type 2 diabetes.

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DiabData: diabetes data warehouses progressively integrated through a common interface.
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Background and Aims: Due to increased life expectancy, increased frequency of diagnosis, and lifestyle changes, the prevalence of diabetes in Europe is expected to increase by 50% over the next 15 years. Decision makers need concise, reliable information about the current background situation of diabetes care at local, national, regional or European levels. What is available at most medical organisations is current data only. Data often are fragmented in separate operational systems such as accounting or payroll so that different managers make decisions from incomplete knowledge bases. The aim of this work was to undertake research to create a diabetes data resource of aggregated data collected routinely by clinicians across Black Sea region.
Materials and Methods: The "Black Sea Tele Diab - diabetes computer system and communication network for Black Sea region (BSTD)" Program began in 1997 and is developing as a strong regional network for data collection. We have developed the BSTD system as an electronic patient record system based on the WHO/Europe dataset and international standards for data security that allows data collection is integrated into clinical work as a necessary part of patient care, and is not viewed as an extra administrative requirement. Following beta testing the BSTD was released without charge on Internet in 2001 as a non-commercial contribution to continuing improves the diabetes care. This report is based upon the data that has been extracted out of diabetes information systems from 24 health care agencies mainly hospital diabetes service around the Black Sea area during the years 1997-2002.
Results: 25231 patients recorded has enabled much to be learnt about the trends in follow-up data from 1997 through 2002 for risk factors of complications associated with diabetes. Glycosylated hemogloblin, foot exam, and microalbuminuria (all done at least once annually) show an improvement in number of persons with diabetes having these tests done. Glycosylated hemoglobin tests increased from 4 percent in 1996 to 18 percent in 2002 and foot exams done increased from 42 percent in 1996 to 68 percent in 2002. Microalbuminuria tests also show an increase from 11 percent to 24 percent in number of persons having the test between 1999 and 2002. The DiabData warehouse not only offers improved information but also makes it easy for decision makers to obtain it that should be used to identify good and bad practice. Foot exam example, presented as hard factual evidence: 17% of Type 1 and 25% of Type 2 patients have neuropathy in BS area, which is significantly higher than 7% of Type 1 and 6.5 of Type 2 have neuropathy, reported by the UKDIABS; 68% of annual patient summaries contain a foot record in BS area, which is significantly higher than the 50.5% reported in the UKDIABS Study; among them 10% of Type 1 and 19% of Type 2 have impaired vibration threshold while 11% of Type 1 and 14% of Type 2 have impaired vibration threshold reported in the UKDIABS study.
Conclusion: This work demonstrate effective implementation of modern information and communication technology to diabetes in Black Sea area for helping to define the research problem of understanding the background situation of diabetes care, focusing on diabetes complications aiming to decreasing diabetes mortality and diabetes morbidity.

PS Health Care Organisation 5:
Diabetes in Primary Care

1284

Long-term quality of diabetes control in patients with diabetes mellitus after treatment in a rural hospital without highly specialised diabetes unit.

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Aims: It was the main goal of the prospective trial to evaluate long-term outcome in respect of quality of diabetes control, the incidence of acute complications and the prevalence of long-term complications in all patients with type 1 and type 2 diabetes mellitus after admission to a rural hospital without a diabetes unit.
Patients and methods: During the period of 1 year all patients (n=101) with type 1 and type 2 diabetes mellitus were studied admitted to the district hospital of Weißenfels, Germany, because of poor quality of metabolic control. One year after hospital demission 74/101 patients (73.3%) were re-examined. Up to this time 13/101 patients (12.9%) died, of 13/101 patients (12.9%) we were not able to track the current address and one patient (0,9%) refused to take part. None of the patients examined was under regular treatment at a diabetes center.
Results: At the time of re-examination HbA1c (normal range 4.4-5.9%, HPLC, Diamat®) in patients with type 1 (n=9) was 7.26± 1.80%, in type 2 without insulin therapy (n=19) it was 6.64± 1.24% and in patients with type 2 diabetes and insulin therapy (n=46) it was 8.19± 1.70%. 6/9 patients with type 1 (60%), 16/19 patients with type 2 without insulin therapy (84.2%) and 13/46 patients with type 2 diabetes and insulin therapy (28.3%) had HbA1c-values within the therapeutic goal (7.2%). The Table shows the other parameters representing quality of diabetes control, blood pressure, the incidence of acute complications and the prevalence of diabetes long-term complications.
Conclusions: Good quality of diabetes control can even reached in a district hospital without a specialised diabetes unit. However, comparing the results with the goals of therapy and the results reported from a nationwide quality circle of diabetes hospitals („Working group of structured diabetes therapy“ of the German Diabetes Association) lacks in therapy are overt in respect of the incidence of acute complications and the quality of blood pressure control. Here an improvement of therapy is mandatory. Following acute recompensation patients with diabetes mellitus and insulin therapy should participate in a structured treatment and teaching programme. Patients with intensifed insulin therapy should admitted to a diabetologist.

Table: Diabetes therapy, metabolic control and complications.

	Type 1 (n=9)	Type 2 without insulin (n=20)	Type 2 with insulin (n=45)
Age (years)	41,5± 12,5	69,3± 14,4	69,6± 10,2
Diabetes duration (years)	17,3± 16,2	8,6± 7,5	14,8± 9,1
Body-mass index (kg/m^2)	27,3± 5,3	26,9± 4,6	28,2± 4,8
Insulin dosage I.U./kg bd wt	0,63± 0,29	/	0,51± 0,20
Therapy with OAD (n pat.[%])	1 (11,1%)	13 (65,0%)	16 (35,6%)
Blood glucose self-monitoring in n patients (%)	8 (88,8%)	0	28 (62%)
Systolic blood pressure (mmHg)	140,0± 16,0	153,0± 25,5	165,0± 29,4
Diastolic blood pressure (mmHg)	83,7± 3,8	87,0± 14,2	88,8± 11,3
Incidence of severe hypoglycaeämia	0,4	0	0,04
Amputations (n pat. [%])	1 (11,1%)	0	1 (2,2%)
Blindness (n pat. [%])	0	0	2 (4,4%)

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Staged diabetes management improves diabetes specific measures in a large group practice.

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Background and Aims: Despite the prevalence and costs associated with managing diabetes, more than half of the two million patients with diabetes treated in managed care organizations in the United States do not meet clinical standards, which have been shown to prevent the complications of diabetes. The aim of this study was to improve diabetes specific measures in a large multi-group practice through the implementation of Staged Diabetes Management (SDM).
Materials and Methods: SDM, a systematic, evidence-based approach to the detection and treatment of diabetes and associated complications, was implemented as a strategic quality improvement initiative in a large group practice. A total of 191 of the 198 primary care physicians from 16 Internal Medicine and Family Practice clinics received a comprehensive six hour SDM training program, which included the pathophysiology of diabetes, a review of treatment options, development and customization of guidelines, case studies on diabetes management, skills training, appropriate documentation and a written implementation plan. The implementation plan included strategies to overcome any structural or process obstacles that would prevent implementation of SDM.
Results: Prior to SDM training, data was collected from a random sample of 545 patient charts representing 18,000 individuals treated for diabetes at 16 clinics. Six months after SDM training, 580 patient charts were randomly selected to evaluate diabetes-specific measures. Post-SDM training, a significant improvement occurred in documentation for A1C, the percentage of individuals with A1C <8%, the number of annual eye examinations and annual screening for nephropathy.
Conclusion: A systematic process for implementing clinical guidelines in a large primary care group practice significantly increased diabetes sentinel measures. As a result, the clinic was able to achieve national recognition for meeting all quality standards of care currently recommended by the American Diabetes Association..

Table 1.

Measure	Pre-SDM N=545	Post - SDM N=580	p-value
% A1C Documented (median and range)	94 (87 to 100)	100 (90 to 100)	p=0.0451
% A1C < 8.0%	63.4 ± 8.9	74.6 ± 7.9	p<0.001 **%**
Blood Pressure Documented (median and range)	100 (94 to 100)	100 (98 to 100)	ns
% Blood Pressure <140/90 mmHg	61.5 ± 9.9	64.6 ± 9.4	ns
% Eye Exam Documented	43.4 ± 13.2	66.7 ± 18.7	p<0.001
% Nephropathy Screening Documented	65.5± 15.1	77.4 ± 18.8	p<0.001

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Public health model compared to community-based model in the Navajo nation: staged diabetes management.

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Background and Aims: The Navajo Nation comprises 255,543 members or 10% of all American Indian people. Of these, 56% live below the US poverty level. Type 2 diabetes affects over one-fifth (51,000) of the adult population. An additional 54,000 to 95,000 adults are undiagnosed or have impaired glucose homeostasis. Approximately 3% of adolescents have impaired glucose tolerance. We sought to understand the differences in implementation of an innovative primary care approach to diabetes in two Navajo communities where different models of health care delivery exist: (1) a Public Health Model staffed and operated under the auspices of the United States Public Health Service, Indian Health Service; and, (2) a Community Based Model staffed and operated by a private, non-profit health care center under the auspices of the local Presbyterian church.
Methods: Staged Diabetes Management (SDM), a primary care, evidence-based approach for the rapid selection of appropriate therapy, was implemented in both programs. Baseline physiological, metabolic and health care delivery data were collected from annual audits. Program planning included community representatives as well as health care professionals. SDM was customized by the health professionals serving in these sites to reflect the culture and resources unique to these communities.
Results: Two years following program implementation data showed significant improvement (p <0.05) in glycemic control in the Public Health

Model with some (not significant) deterioration in the Community Based Model. Both groups had significant ($p<0.05$) improvement in foot examination, diet and exercise education and use of SMBG. Reliance on diabetes therapies followed a similar trend in both models. They differed only with respect to utilization of insulin, with the Public Health Model increasing use of insulin and the Community Based Model decreasing use of insulin.

Comparison of Public Health with Community-Based Approach to Diabetes Care

	Public Health Pre-SDM (n=192)	Public Health Post SDM	Community Based Pre-SDM (N=84)	Community Based Post SDM
HbA1c <7%	15%	33%	30%	21%
BP < 130/85 mmHg	35%	73%	78%	60%
LDL < 100 mg/dL	17%	16% 45%	50%	
Foot Exam	49%	62%	23%	58%
Nutrition Education	36%	55%	33%	77%
Exercise Education	64%	71%	31%	71%
SMBG	49%	100%	17%	39%
Nutrition Only	13%	14%	6%	0%
Oral Agent Monotherapy	24%	29%	48%	23%
Combination Oral Agents	22%	34%	20%	46%
Insulin Alone and in Combination	33%	22%	25%	30%

Conclusions: In the two year duration of the project, 167 medical professionals were trained, as well as 158 community members. The estimated number of patients affected by the program is 9000. For these individuals, improvement in both health care processes and health outcomes were similar whether treated in the Public Health or Community Based Model. The data demonstrate that using SDM, primary care community-based programs, which have fewer resources, can achieve a level of care similar to public health programs.

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Implementation of a systematic approach to diabetes in primary care in Bahia, Brazil improves metabolic outcomes.

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Background: Centro de Diabetes e Endocrinolgia do Estado da Bahia (CEDEBA) is a public health supported diabetes center located in Salvador, Bahia, Brazil. Epidemiologic studies from Bahia show 7.8% of individuals over age 30 have diabetes and is the state with the 4th highest prevalence of diabetes in Brazil. Over the past 5 years, CEDEBA has developed and implemented a diabetes care program for the municipalities of Bahia. This program consists of training local health care teams on prevention, diagnosis and treatment of type 2 diabetes and its chronic complications using an evidence-based diabetes program, Staged Diabetes Management (SDM) adapted to our public health system. From the positive results obtained from SDM implementation at CEDEBA, we set out to study the implementation of SDM through an outreach program targeted at primary care in Bahia. Projecto de Interiorizacao da Atencao ao Diabetico No Estado da Bahia (PRODIBA) was undertaken by our team including endocrinologists, nurses, and a social worker.
Methods: Two communities were identified to participate in PRODIBA from April 2000 to December 2002. Lauro de Freitas (LF) was trained in SDM and had significant support of the political and medical community for implementation. Conceicao do Coite (CC) was not trained in SDM, however a standard diabetes training program was delivered in CC as part of the study. One hundred patients from each community were identified; in LF they were randomly selected from the diabetes registry in the health unit and CC were randomly selected following a diabetes screening and health fair. Patients in each group were >30 years with diabetes diagnosis >5 years. Patients in both communities received free diabetes medications. Data was collected during routine follow-up including HbA1c, random blood glucose, cholesterol, blood pressure and foot evaluation.
Results: Significant improvement in metabolic outcomes and blood pressure were noted in LF whereas in CC there was worsening of both measures. In LF there was a 22% decrease in average random blood glucose (243.19 mg/dl to 187.55 mg/dl; $p<0.01$) and 14.9% reduction in average HbA1c (8.90% to 7.58%; $p<0.0001$) during the study. In LF, 57.7% of the patients at the end of the study had blood pressure below 135/85 mmHg. In CC there was an increase in average random blood glucose (215.8mg/dl to 237.9 mg/dl) and HbA1c (8.06% to 8.17%). There were no changes in either community in BMI or cholesterol due to the lack of dietitians and lipid medications.
Conclusions: Training health care teams on diabetes and providing free hypoglycemic medications is important, but is not the main factor in improving metabolic outcomes. Training, along with implementation of SDM algorithms used by the health care team (LF) was a key factor in achieving the improvement in metabolic and blood pressure outcomes noted.

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Primary care management of Type 2 diabetes in the New Zealand Maori population.

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Background and Aims: The Maori, the indigenous population of New Zealand, suffer from a high prevalence of diabetes of about 8%, associated with high morbidity and mortality rates. An annual diabetes screening (ADS) programme was launched in 1998 in a rural general practice in Northland, New Zealand, offering free screening for diabetic risk factors to all registered diabetic patients. This practice is fully computerised and has 9597 registered patients (64% Maori, 34% European). The aims of this study were to assess the uptake of the ADS programme, the proportion of patients whose diabetic risk factors were monitored, the proportion of patients achieving target values, and recommended pharmacological therapies consequently instituted.
Materials and Methods: All type 2 diabetic patients aged 18 years or more were identified from the practice's list of diabetic patients, and data was retrieved from the computer database regarding demographic details, screening for diabetic risk factors, the results achieved from the screening, and the pharmacological therapies instituted.
Results: A sample of 336 patients was identified (76% Maori, 21% European). The introduction of the ADS programme increased screening rates for the diabetic risk factors shown in the table, as compared to pateints from the same practice who had not been screened by the ADS tool but who had had at least two appointments with a GP during 2001 ($p < 0.001$). Uptake of the ADS was 59% amongst Maori and 63% amongst Europeans. The proportion of patients failing to achieve target values amongst the Maori significantly exceeded those of European patients on several parameters (Table).
Conclusion: Introduction of an ADS programme was highly successful in improving screening for diabetic risk factors in patients who attended, with moderate uptake amongst both Maori and Europeans. However, the proportion of patients failing to achieve target values remained high, especially among the Maori. Thus reduction of complications requires more intensive management of identified risk factors.
Several factors may need addressing to rectify this situation. Firstly, new approaches to improve implementation of agreed protocols for risk factor management need to be developed and implemented. Secondly, increasing access to medications limited by both tight eligibility criteria set by government policy, such as statins, and restriction of prescription to diabetes specialists (with limited access in rural New Zealand). Thirdly, ADS will need to be combined with culturally sensitive patient education strategies to improve compliance with recommended medical therapies and improve lifestyle and dietary habits.

Clinical parameter (target value, recommended treatment for patients failing to achieving target values)	Number of patients screened within the preceding 15 months (n = 336)	Proportion of patients achieving target values (p value for Chi-square test for Maori compared to European patients)	Proportion of patients failing to achieve target values who were prescribed recommended medication within the preceding 24 months
HbA_{1c} (<7%)	294 (87.5%)	33.3% (<0.001)	
Weight (kg)	249 (74.1%)		
Body mass index (<25 kg/m², biguanide)	187 (55.7%)	7.8%(0.001)	64.9%
Blood pressure (systolic <140 mmHg and diastolic <80 mmHg, >1 antihypertensive medication)	306 (91.1%)	36.7%	41.6%
Total cholesterol (<5 mmol/l, lipid lowering therapy)	264 (78.6%)	27.7% (0.04)	12.7%
Urinary albumin:creatinine ratio (<3, angiotensin-converting enzyme inhibitors)	248 (73.8%)	41.0% (<0.001)	71.2%
Foot examination	215 (64.0%)		

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Enhancing the effectiveness of diabetes treatment at primary-care level on a semi-rural area by active involvement of the diabetes specialist's team.

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Background and Aims: Current recommendations call for interdisciplinary teamwork to optimize treatment and outcome of patients with diabetes. The Western Negev Mobile Diabetes Care Program (WNMDCP) is a mobile clinic that operates at primary care facilities of the WN, a semi-rural area distant from the diabetes centers. We had recently shown a dramatic decrease of 1.8% in HbA1c levels in a primary care population of poorly controlled patients, using the WNMDCP team as advisors to the primary care facility.
The aims of this study were: 1- To enhance the effectiveness of the primary-care team treatment of patients with diabetes. 2- To reduce HbA1c levels in the entire patient population of the clinic.
Methods: A cooperative project of the primary care team and the WNMDCP. Goals and a structured educational and therapeutic flowchart were established jointly. Patients were divided into 3 categories according to HbA1c level: well controlled (WC; <7.0%), fairly well controlled (FWC; 7-9%), poorly controlled (PC; >9%). Each group operates from a specifically structured educational and therapeutic plan. Each group is jointly evaluated by both teams at regular intervals and additional interventions are adopted as required by each group's protocol.
Results: The study started during the year 2001. We present baseline (BL) data and HbA1c results of the first year (YR1) of intervention. 244 of 442 patients are female, mean age: 66 years, BMI 30.8±5.9 kg.m2; FPG: 9.1±3.5 Mmol. HbA1c results for BL and YR1 were: Whole group: 8.3 ±1.9% and 7.4 ± 2.5%; p<0.0001; WC: 6.3 ± 0.5% and 6.6 ± 1.5%; p=NS; FWC: 8.1 ± 0.6% and 7.7 ± 2.0 %; p=0.05; PC: 10.7 ± 1.3% and 9.1 ± 2.2%; p<0.000001, respectively. Distribution among HbA1c categories at BL and YR1 was: WC: 33% and 37%; FWC: 35% and 38%; PC: 32% and 24% respectively; p<0.05.
Conclusion: We achieved a significant 0.9% HbA1c reduction for the whole population of patients with diabetes of the primary care clinic. Cooperation between primary care and diabetes specialist teams has shown to be an effective method for improving metabolic control of patients with diabetes at the semi-rural primary care setup. These are results of the first year of intervention of an ongoing project that will include other metabolic and clinic parameters in the future.

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Impact of educational actions on diabetes care management in a hospital - primary care network dedicated to diabetes: Diacommunication.

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Background: Since 1997 the Diacommunication network brings together 40 GPs, 15 nurses, 5 hospital diabetologists, 2 private diabetologists, 3 cardiologists, 3 ophtalmologists and 2 podiatrists. Its main objectives are to ensure permanence and continuity of diabetes care, global patient management and assure a better coordination of available medical care. To update knowledge of diabetes care in primary care workers, education have been done since 1999, alternatively by specialists, podiatrists and nurses. Each session are followed with a reappropriation exercice through the working out of internal « guidelines » in accordance with current scientific guidelines.
Aim: To estimate the impact of educational actions concerning diabetes management, led in a hospital – primary care network, on quality and efficiency of diabetes care in the field.
Methods:A global estimation of the impact of educational actions concerning diabetes management has been done annually by members since 1999. It uses a diabetes assessment card mentioning the key points of diabetes care practice from the DIABCARE card. One assessment card is filled up each year for each patient consulting a Diacommunication member.Data were collected in 2000 and 2001 while the network members attend 32 hours diabetes education.Analysis compare data between these two years in order to estimate the evolution of local diabetes care practice.
Results: 300 cards were filled up for 2000 and 2001. General characteristics of diabetic patients are the same over the 2 years (age, mean period in years since diagnostic, first treatments prescribed).Main raisons for consulting are medical supervision (77 %) and diabetes imbalance (10 %) in 2000 against respectively (82 %)and (3 %) in 2001.. Diabetes self – monitoring is done by (16 %) in 2000 and (33 %) 2001. HbA1c is performed in 82 % in 2000 versus 93 % in 2001.µalbuminuria 61%in 2000 versus 69% 2001. 83% patients consulted an ophtalmologis in 2000 versus 87 % 2001 ; podiatrist examination 64 % had one in 2000 and 75 % in 2001. An oral antidiabetic bitherapy was prescribed in 23 % in 2000 versus 28 % in 2001 while only 1 % tritherapy in 2000 but 3 % in 2001. Associated treatments for complications increased too especially antihypertensive (from 65 % to 74 %).
Conclusion: We can conclude that continuing education on diabetes care of different primary care workers brought together in a hopital –primary care network, has a very positive impact on global diabetes management.

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Improving glucose control and cardiovascular risk in general practice Type 2 diabetes.

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Background and Aims: The UKPDS showed that improved glucose control reduced microvascular complications in type 2 diabetes with a borderline significant reduction in cardiovascular (CVS) disease. The aim of this study was to evaluate changes in CVS risk factors and calculated cardiac risk in a 1 year program of glucose optimisation.
Materials and Methods: Patients with type 2 diabetes, aged 40-75 years, with an HbA_{1c} of 6.4-10% on diet or oral monotherapy, were recruited from 7 practices. Basal glucose was optimised with a biguanide, thiazolidinedione or sulphonylurea and prandial glucose targeted with a meglitinide, alpha-glucosidase inhibitor or rapid-acting insulin. Therapy was adjusted aiming for capillary plasma glucose values fasting <6mmol/L and prandial <8mmol/L. Standardised guidelines for CVS risk modification were provided and risk factors assessed at onset and 1 year with the Joint Heart Guidelines.
Results: 60 patients were recruited, 65% male, with mean (SD) age 61.0 (8.2) years, BMI 30.5 (4.9) kg/m², HbA_{1c} 7.5 (0.9)% and median (IQR) diabetes duration 3 (1-5) years. A mean overall 0.8% HbA_{1c} reduction was achieved. Significant reductions in systolic blood pressure (144-139mmHg p=0.05), diastolic blood pressure (84-79mmHg p=0.02), triglyceride (2.0-1.7 p=0.001) and smoking were seen. Calculated CVS risk decreased from 19.3% to 16.8% over the year and cerebrovascular risk reduced from 7.2% to 6.8% with intention-to-treat analyses. No significant differences between therapy groups was seen.
Conclusion: Cardiovascular risk can be successfully reduced in a 1 year program of glucose optimisation in general practice. Significant reductions in blood pressure, triglyceride and smoking are seen.

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Low persistence among Type 2 diabetic patients on metformin or sulphonylurea monotherapy in a UK General Practice Database.

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Background and Aims: Patient compliance and persistence with medications are key factors influencing treatment outcomes of chronic diseases such as diabetes. Few studies have evaluated persistence rate of oral hypoglycemic agents (OHA) used as monotherapy among patients with type 2 diabetes.
Materials and Methods: The UK MediPlus database of general practice was used to identify adult patients (age ≥ 18 years) with a type 2 diabetes diagnosis between December 1996 and November 2001. A minimum of 18-month data (including a 6-month pre-index and a 1-year post-index follow-up period) were required for study inclusion. The index date was defined as the date when the first OHA was prescribed. Newly treated patients were identified as those who did not receive any OHA during the 6-month pre-index period. Patients starting with a single drug regimen with either metformin (MF) or sulphonylurea (SU) were included. Persistence was defined as the number of days of continuous therapy during the post-index period. Therapy interruption was defined as any changes to the initial regimen (excluding dose changes) or a discontinuation of therapy (i.e., the initial therapy was not refilled within 30 days from the end of the preceding prescription). Persistence curves were estimated by the Kaplan-Meier method and the factors affecting persistence were estimated by Cox regression. A hazard ratio (HR) greater than 1 means less persistent.
Results: Of the 5,064 patients (mean age: 65.5± 13 years; female: 45.5%) included in the study, 38.6% started with MF monotherapy and 61.4% received SU monotherapy. One-year persistence was achieved in 40.4% of patients (22.6% with a maximum 15-day refilling gap, and 53.5% with a 45-day gap). There was no significant difference in the one-year persistence between patients on MF (41.3%) or SU (39.8%). Factors associated with low one-year persistence included: female (HR: 1.087, $p = 0.0241$), referred to diabetes specialist (HR: 5.627, $p < 0.0001$), developed dyslipidemia (HR: 5.679, $p < 0.0001$), hypertension (HR: 5.642, $p < 0.0001$), and other circulatory system diseases (HR: 3.274, $p = 0.0556$) during the 1-year follow-up period.
Conclusion: In a large general practice database, one-year persistence was low among type 2 diabetic patients who started with a single drug regimen of MF or SU. Female patients, patients referred to a diabetes specialist, or who had other cardiovascular risk factors (hypertension or dyslipidemia) were less likely to stay on their initial OHA therapy.

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Pharmaco-epidemiological and financial analysis of hypoglycaemic agents utilization in Ravenna area.

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Background and Aims: To identify clinical and financial patterns in the pharma-coutilization of diabetes treatment a diabetes long-term register was created by using record linkage techniques. A claims database, collecting all purchased drugs, was used to perform a retrospective analysis for each subject registered with the Local Health Unit (LHU) of Ravenna, a northern Italian city.
Materials and Methods: Starting from January 1st, 1996 to December 31st, 2000, each subject who had received at least two prescriptions for hyglycaemic drugs (ATC code A10) during the same calendar year was defined as diabetic patient. According to each year, diabetic patients were classified as old or new users if they had been/had not been defined as diabetics in preceding years. Subjects who had been prescribed for hyglycaemic drugs once a year or who had never been prescribed for hyglycaemic agents were defined as possible-diabetic and non-diabetic patients, respectively.
Results: In 1996, a total of 357,285 subjects were registered with the LHU of Ravenna including 7,948 diabetic patients (2.2%), 3,257 possible-diabetic patients (0.9%), and 346,080 non-diabetic patients (96.9%). The number of subjects prescribed for hyglycaemic agents raised to 11,983 in 2000, with an increase of 50.8% respect to 1996. The percentage of old users was observed to be rising year by year (2.17% in 1997, 2.47% in 1998, 2.74% in 1999, and 2.93% in 2000) while that of new users broadly constant. In 1996, overall cost for pharmacotherapy accounted to 807117.56. Consistently with the rising number of diabetic subjects, the overall cost for diabetes treatment amounted to 1550736.89 in 2000, with an increase of 92.1% with respect to 1996. Annual overall expenditure increased in each year (12.1% in 1997, 17.5% in 1998, 13.7% in 1999 and 28.3% in 2000).
Conclusion: An appropriate use of claims data may offer a powerful tool, providing information which would otherwise not be available. Moreover this data source represents a relatively inexpensive way to analyze the clinical government of chronic diseases in a non-experimental setting and to obtain results applicable to real world.

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Direct costs of care in Germany for diabetic children and adolescents in the early course after onset.

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Background and Aims: Prospective cost-of-illness study to evaluate in detail diabetes-related direct costs in Germany of the care for diabetic children and adolescents in the early course after onset from the perspective of the statutory health insurers.
Materials and Methods: Based on a population-based incidence study (part of EURODIAB ACE), 573 diabetic subjects <15 years of age at onset (51% male, mean age 8.3 (SD3.8) years) were followed for up to 2 years (mean 1.2, SD 0.3) after onset. Diabetes-related hospitalization and ambulatory care, insulin and self-control regimen, and sociodemographic variables were ascertained using clinical documentation and families' self-reports. Costs per patient-year were estimated based on the prices in the

year 2000. Using multivariate regression, the association between costs and families' socioeconomic status was evaluated, adjusting for age and sex.
Results: The total cost per patient-year was Eur7,069 (SD 3,190), including hospitalization at onset. Onset hospitalization accounted for the majority of the costs (Eur 4,908, SD 2,492). Within post-onset costs, 36% were attributable to self-control of blood glucose, 32% to hospitalization, 15% to insulin, and 12% to ambulatory care. Total costs and costs for hospitalization were significantly higher in children from families with lower compared to highest educated parents and in children from non-German families ($p<0.01$).
Conclusion: Among the direct medical costs of childhood diabetes in the early course after onset, the greatest economic burden was due to hospitalization, in particular at onset. Unexpectedly, self-control of blood glucose accounted for the majority of the post-onset costs. Costs were considerably higher in lower educated and in non-German families. It is recommended to evaluate the cost-effectiveness of outpatient programs targeting children from families with lower social status.

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Predicted costs and outcomes from reduced vibration detection in people with diabetes in the UK.
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Background and Aims: The ability to perceive vibration (vibration detection) has been shown to be a good predictor of long-term complications among patients with diabetic peripheral neuropathy (DPN). The vibration perception threshold (VPT) is defined as the lowest voltage at which vibration can be detected. The aim of the study was to estimate the predicted complications and costs for the NHS associated with reduced vibration detection (VPT≥25V) estimated using a quantitative sensory testing device.
Materials and Methods: A Markov model of DPN progression was constructed for a hypothetical cohort of people with DPN. The model was run over a ten-year period using Monte Carlo simulations to estimate disease progression, predicted costs, number of ulcers and amputations, duration of ulceration, life-years and quality-adjusted life-years (QALYs) according to vibration detection levels. Rates of foot ulceration and amputation, the probability of healing, and health state utility scores were identified by undertaking a focused literature search. The cost data used in the model were derived from a concurrent cost of illness study.
Results: Discounted over ten-years, the average individual with reduced vibration detection incurs approximately 3.3 times more direct medical costs for foot ulcers and amputations (£1,531 vs. £457), yields 0.19 fewer QALYs, and lives for approximately 2 months shorter than an average individual with normal vibration detection (VPT<25V). The long-term complications of DPN (foot ulcers and amputations) experienced by the population with reduced vibration detection will cost the NHS approximately £292m (discounted) over the next ten years.
Conclusion: Effective prevention and treatment of foot ulceration and amputation is time-consuming and expensive. If at-risk individuals with reduced vibration detection could be identified and targeted for intervention, valuable healthcare resources could be saved and improved health outcomes should result. For example, if all individuals in the UK with diabetes and reduced vibration detection were identified and their risk of ulceration and amputation reduced to levels experienced by those with normal vibration detection, this would save the NHS approximately £204m in direct medical costs, and save 29,000 life-years and 36,000 QALYs (discounted) over the next ten years.

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Cost of long-term outcomes and cost reduction with improved glycaemic control using the diabetes mellitus model (DMM).
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Background and Aims: The increasing prevalence of diabetes, especially Type 2, will raise the economic burden on healthcare systems through direct costs of long-term complications, such as blindness and amputation. The objective of this analysis was to estimate the direct costs of diabetic blindness and amputation and to identify potential cost reductions due to improved glycaemic control in patients with Type 1 and Type 2 diabetes in the US.
Materials and Methods: The analysis was based on the results of a simulation model, the epidemiological diabetes mellitus model (DMM) and published DCCT cost data from 1999 (in US$). DMM analyses were performed for two simulated patient cohorts, both representing the general diabetes population in the US ('Diabetes in America' data) at the start of simulation. Simulation 1 assumed that 50% of the population would show improved glycaemic control to <7% (ADA-recommended guidelines) over 10 years. Simulation 2 assumed 'normal' glycaemic control (i.e. HbA_{1c} >7%, as defined by the 'Diabetes in America' data). Mean HbA_{1c} per simulation group and the associated reduction in number of patient–years with complications and related costs were calculated for the two cohorts.
Results: There were relative risk reductions of 22% and 36% for amputation and blindness, respectively, and reductions in the mean costs of complication per 1000 patients after 10 years for amputation and blindness of 22% and 37%, respectively, for the cohort from Simulation 1 versus the cohort from Simulation 2. Thus, improved glycaemic control to ADA guidelines resulted in savings of 850 years of amputation and 2063 years of blindness per 1000 diabetes patients over 10 years of treatment. Taking mean costs per patient–year into account, this equates to total cost per complication reductions of 6103 US$/1000 patients after 10 years (minimum 6500; maximum 6100 US$).
Conclusion: Reduction of HbA_{1c} to near-normal values causes a marked decrease in late diabetes-related complications, such as blindness and amputation, and results in cost reductions and reduced event rates.

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Intensive lifestyle changes or metformin in subjects with impaired glucose tolerance: modelling the long-term health economic implications of the diabetes prevention program in the Australian, French and Swiss settings.
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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 58% and 31%, respectively, versus control (standard lifestyle advice only) in subjects with impaired glucose tolerance (IGT). We have assessed the long-term cost-effectiveness of DPP interventions in Australia, France and Switzerland.
Materials and Methods: A Markov model simulated 3 states: "IGT", "type 2 diabetes", and "dead", using probabilities from the DPP and published data. Published country-specific direct costs were used throughout.
Results: Both interventions improved life expectancy versus control: overall incremental improvements in life expectancy were 0.35 and 0.91 years for metformin and ILC, respectively. Metformin was associated with both increased life expectancy and cost savings versus control in France and Switzerland, but was more expensive in Australia, where a modest increase in cost was observed. ILC was associated with increased life expectancy and reduced costs in Australia, with modestly increased costs in France and Switzerland. Both interventions were highly cost-effective (cost/life year gained versus control < EUR 50,000) in all countries. 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 the intensive lifestyle changes in the DPP.
Conclusions: Metformin and ILC were either cost saving or highly cost-effective in all 3 countries. The initial cost of pharmacological or lifestyle-based intervention in prediabetic individuals should not deter healthcare systems from implementing diabetes prevention programs.

1298

Long-term projection of the costs of the diabetes prevention program in the USA using the CORE diabetes prevention model: intensive lifestyle changes and metformin are both cost-effective.

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Background and Aims: The 3-year costs of implementing the Diabetes Prevention Program (DPP) have recently been published. We used a validated simulation model to project the 3-year costs to patient lifetimes to calculate the costs/life-year gained of intensive lifestyle changes (ILC) or metformin (MET) versus placebo in overweight patients with impaired glucose tolerance (IGT).
Materials and Methods: A Markov computer model simulated 3 states: "IGT", "type 2 diabetes", and "dead". Annual transition probabilities between states for each treatment arm were derived from the DPP, published data on mortality of IGT patients and type 2 diabetes patients versus the normoglycemic population derived from the National Health and Nutrition Examination Survey (NHANES), and national mortality statistics. The long-term effects of delaying the onset of diabetes with ILC or MET on life expectancy were calculated for a cohort of patients with baseline characteristics similar to the DPP population. USA-specific direct costs of implementing the DPP and costs for the states IGT and type 2 diabetes were derived from recent publications. Years free of diabetes, life expectancy and lifetime costs per patient were calculated for each treatment arm of the DPP. Incremental cost-effectiveness ratios were calculated in terms of costs per life-year gained (C/LYG) for ILC versus placebo and MET versus placebo. Costs and life expectancy were discounted at a rate of 3% per annum. Extensive sensitivity analysis was performed to identify parameters with important impacts on outcomes.
Results: Both ILC and MET increased the number of years free of type 2 diabetes, improved life expectancy and were highly cost-effective compared to control.

Treatment Arm	Years Free of Diabetes	Life Expectancy (years) [Non-Discounted (Discounted)]	Total Lifetime Costs per Patient ($)	C/LYG versus Control ($)
Control	8.1	23.8 (16.5)	80,045	comparator
ILC	14.4	24.6 (16.9)	84,652	11,518
MET	10.6	24.1 (16.7)	84,253	21,040

Outcomes were most sensitive to the probabilities of developing diabetes in each treatment arm, the duration of effect of interventions (compliance and drop-outs), the relative risk of mortality for the state of diabetes compared to IGT and the costs of implementing the DPP interventions.
Conclusions: Interventions that delay the onset of type 2 diabetes in overweight IGT patients may lead to important improvements in LE. Despite the increase in costs of implementing the DPP interventions, both ILC and MET were highly cost-effective (C/LYG < $ 28,000) when judged by current international health economic standards.

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Cost-effectiveness in Germany of rosiglitazone-metformin combination in Type 2 diabetes.

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Background and Aims: Current guidelines in Germany recommend use of Rosiglitazone (RSG) in combination with Metformin for treatment of obese patients with Type 2 diabetes when Metformin monotherapy is no longer effective in maintaining glycaemic control. We assess the cost-effectiveness of this strategy compared to combination therapy with Glibenclamide.
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 (incl. test strips), rehabilitation, physiotherapy, foot care and sick leave. The model was used to simulate treatment histories for a mixed incident cohort of 1000 obese patients (BMI≥30). Following failure of glycaemic control with Metformin alone, combination therapy adding RSG was compared to adding Glibenclamide. The threshold for switching therapies was 7% HbA1c. In line with national guidelines, costs were discounted at 5% pa.
Results: The model predicts that adding RSG (4mg titrated to 8mg daily) to Metformin produces better glycaemic control in most patients, and extends viability of combination therapy by at least 7 years before requiring insulin.

Metformin+RSG vs Metformin+Glibenclamide	*Obese*
Mean delay before starting insulin (years)	7.0
Life years gained / 1000 patients	173
QALYs gained / 1000 patients	274
Cost per life-year gained (after 20 years): undiscounted	9730Euro
discounted	6464Euro
Cost per QALY gained (after 20 years): undiscounted	4606Euro
discounted	3055Euro

The extra life-years estimated in a mixed cohort of newly diagnosed patients are conservative as some progress too rapidly to insulin to be eligible for combination therapy. Additional gains in QALYs arise from fewer or delayed complications, and improved quality of life while insulin treatment is avoided. Net cost increases are modest since additional costs of RSG are partly offset by savings from delaying insulin therapy.
Conclusions: Use of RSG in combination with Metformin to improve glycaemic control and delay use of insulin is highly cost-effective in Germany when compared to Metformin + Glibenclamide.

1300

A cost-effectiveness evaluation of adding rosiglitazone versus sulphonylurea to metformin in a US obese Type 2 diabetes population.

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Background and Aims: To compare the cost-effectiveness of adding rosiglitazone 8 mg versus maximal dose sulphonylurea to metformin for obese patients failing to control HbA_{1c} on metformin monotherapy.
Materials and Methods: DiDACT is an established economic model of the long-term complications of type 2 diabetes. The model follows a cohort of 1,000 patients (410 male and 590 female) through multiple stages of microvascular and macrovascular disease. Inpatient, outpatient, and medication costs are included. Failure of glycaemic control was defined as $HbA_{1c} \geq 8.0\%$. The cohort has BMIs of 38 and 43 kg/m^2 for males and females as per 2000 NHIS data using the Center for Disease Control's definition for obesity. Population demographics were taken from National Health and Nutrition Examination Survey III. Costs and outcomes were discounted at 3% per annum.
Results: For males, adding rosiglitazone was estimated to increase total quality of life years (QALYs) by 94 and increase total life years by 53.5, compared with adding sulphonylurea. For females, adding rosiglitazone was estimated to increase total QALYs by 140 and increase total life years by 62. Costs per additional QALY were $38,838 in males and $39,539 in females.

Treatment BMI	Life Years per 1,000 patients	QALYs per 1,000 patients	Lifetime cost per 1,000 patients
MET + RSG BMI ≥ 31	2,535	7,590	$142.0M
1 MET + SU BMI ≥ 31	12,420	7,355	$132.8M

Conclusions: The cost-effectiveness of a rosiglitazone plus metformin combination is comparable with other regularly prescribed interventions (such as statins in the cardiovascular area). These results illustrate that adding rosiglitazone to metformin may lead to long-term benefits in obese patients.

1301

The health care costs of diabetic peripheral neuropathy in the United Kingdom.

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Background and Aims: Diabetic peripheral neuropathy (DPN) is one of three common diabetic microvascular complications, the others being diabetic retinopathy and diabetic nephropathy. DPN can result in foot ulceration and amputation, which incur relatively high and long-term costs. The aim of this study was to quantify the annual direct medical costs of DPN among people with type 1 and type 2 diabetes to the National Health Service in the UK.
Materials and Methods: A prevalence-based cost of illness model was used to estimate the numbers of people with diabetes in the UK having DPN and neuropathic foot ulcers (with no deep infection or accompanied by cellulitis or osteomyelitis) at a given point in time. This model was augmented with an incidence-based model that included toe, foot or leg amputations during a year. Rates of complications were derived from clinical databases and previously published studies. Resource use and unit costs were obtained from drug tariffs and hospital episodes data and augmented with clinical opinion. All costs were estimated in 2001 British pounds. In a sensitivity analysis, we varied the rates of complications to assess the robustness of the cost estimates to plausible variations in the point estimates of complication rates.
Results: The total annual costs of DPN among people with diabetes in the UK were £35m (type 1 diabetes), £217m (type 2 diabetes) and £252m (type 1 and type 2 diabetes). After allowing for plausible variations in complication rates, the costs were between £16m-£61m (type 1 diabetes), £98m-£455m (type 2 diabetes) and £114m-£516m (type 1 and type 2 diabetes).
Conclusions: In the UK, up to 17% of current NHS expenditure on diabetes can be attributed to the management of DPN and its complications. Interventions that successfully treat DPN to prevent or delay its long-term complications will save substantial costs to health care payers.

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The health care costs of diabetic nephropathy in the United Kingdom and the United States.

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Background and Aims: Diabetic nephropathy (DN) is a common diabetic microvascular complication. DN can result in end-stage renal disease (ESRD) necessitating long-term dialysis or kidney transplantation. These complications are associated with relatively high health care costs. The aim of this study was to quantify and compare the rates and annual direct medical costs incurred by health care payers in managing DN in the UK and the USA.
Materials and Methods: A prevalence-based cost of illness model was used to estimate the numbers of people with DN (microalbuminuria, overt nephropathy or ESRD) or a previous kidney transplant at a given point in time. This model was augmented with an incidence-based model that included the numbers of new kidney transplants during a year. Rates of complications were derived from clinical databases and previously published studies. Resource use in the management of DN and kidney transplants was estimated with the aid of clinical opinion. Unit costs were obtained from drug tariffs, hospital episodes data and previous studies. All costs were estimated in 2001 currencies. The ranges in costs were estimated from a sensitivity analysis in which we varied the point estimates of complication rates between plausible limits.
Results: In the UK, the total annual costs to the National Health Service of managing DN were £152m (type 1 diabetes, range: £125m-£230m), £614m (type 2 diabetes, range: £532m-£927m) and £765m (all diabetes, range: £657m-£1.2b). In the USA, the total annual medical costs incurred by all payers in managing DN were $1.9b (type 1 diabetes, range: $1.0b-$2.8b [£0.7b-£1.8b]), $15.0b (type 2 diabetes, range: $7.6b-$22.4b [£5.0b-£14.7b]) and $16.8b (all diabetes, range: $8.5b-$25.2b [£5.6b-£16.6b]).
Conclusion: The total annual cost of DN is 13 times greater in the USA than in the UK. When accounting for the substantially higher number of people at risk, treatment costs are estimated to be 40% greater in the USA. The total cost per person with DN and/or a kidney transplant is £1,758 in the UK and $3,735 (£2,457) in the USA. Interventions that successfully treat DN to prevent or delay its long-term complications will save substantial costs in both health care systems.