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MINUTES OF THE 48th GENERAL ASSEMBLY OF THE EUROPEAN ASSOCIATION FOR THE STUDY OF DIABETES

held in Berlin Messe, Germany, Thursday 4 October 2012 at 18:00

Present:	**Dr. Andrew J.M. Boulton**	**(President)**
	Dr. Fatima Bosch	**(Vice President)**
	Dr. Michael Roden	**(Honorary Treasurer)**
	Dr. Mark Walker	**(Honorary Secretary)**
	Dr. Cees J. Tack	**(Chair, PGEC)**
	Dr.Viktor Jörgens	**(Executive Director)**
	Dr. Monika Grüsser	**(Vice Director)**
	and 49 members	

The President, Dr. Boulton, welcomed everyone to the 48th General Assembly. He asked those present to stand in memory of the following members, who had passed away: Drs. Angelika Bierhaus, Artur Czyzyk, Keith Taylor and Ulrich Vischer.

1. MINUTES 47th GENERAL ASSEMBLY 2011

Since there were no comments, the minutes were approved unanimously and officially signed as a correct record.

2. REPORTS

a) President

The President's report to the members on the activities of EASD was given in the President's Address before the Minkowski Lecture. It is available under:
http://www.easdvirtualmeeting.org/resources/2694

The President reported on the various activities and expressed his thanks to all partners. Dr. Boulton reported that as expected the EASD Annual Meeting this year in Berlin was doing very well and the number of delegates attending had slightly increased. Dr. Boulton thanked all members of the EASD Office and the Executive Committee for their commitment and hard work.

b) Honorary Treasurer

Dr. Roden reported that the income from membership fees had decreased in 2011 and income from registration fees had increased slightly. There was a decrease in donations which was due to the fact that Merck had paid for two Final Programmes (2009 and 2010) in the year 2010. In 2011 only the Final Programme for 2011 was paid for. Donations for courses increased substantially. The total income of the Association was in the same frame as in 2010.

Regarding expenditure, Dr. Roden reported that Travel/ Meeting Expenses included expenses for more than one Meeting. EASD transferred more than 3 million Euro to the Foundation.

Dr. Roden summed up by saying the accounts of EASD remained healthy.

c) Honorary Auditors

The President asked the Honorary Auditor, Dr. Peter Diem for his report. Dr. Diem confirmed that the accounts had been checked carefully by Dr. Luis Gardete-Correia and were in perfect order. Dr. Boulton asked for the vote to accept the accounts.

The Honorary Treasurer was unanimously discharged (38 votes for and 1 abstention).

d) Honorary Secretary

Dr. Walker reported that the Programme Committee for 2013 had been appointed and had met during the Annual Meeting in Berlin. He stated that 2307 abstracts had been received in 2012. The main countries submitting abstracts are the United States, Germany and Japan. He explained that the questionnaire to rate the 47th AM in Lisbon had produced mostly positive feedback, with 15% of those taking part giving a top score of 5 for venue facilities, scientific programme, prize lectures and EASD symposia. The Rising Star Symposia continue to identify promising and innovative young researchers in Europe. He brought his report to a conclusion by mentioning that he was working closely with the EASD Study Groups to develop ideas for future symposia.

Dr. Walker closed his report by thanking all those who had forwarded ideas for the scientific programme and all members of the EASD staff, in particular Ms. H. Goliberzuch and Mrs. M. Toledo, for their outstanding help and support with the organisation of the EASD Annual Meetings.

Dr. Boulton thanked Dr. Walker for his diligence and asked if there were any questions. There were no further comments.

e) Editor-in-Chief, Diabetologia

In the absence of Dr. Juleen Zierath, Editor-in-Chief, Diabetologia, the President reported the following information:
In total, 2,047 articles were submitted to Diabetologia in 2011, compared with 1,971 in 2010. The proportion of papers triaged had increased to 55%. Such a high level of triage was essential given the increased number of submissions. The Impact Factor fell very slightly from 6.973 in 2010 to 6.814 in 2011. He reported that the initiative 'Then and Now', in which historical articles were highlighted to determine what impact they had on the present day, was proving very successful. Another project, 'Up front', which included 5 short articles (access free of charge) of particular interest chosen by the Editor, was started in 2012. He stated that a panel to advise on cases of scientific misconduct had been convened.

Dr. Boulton expressed his thanks to Dr. Zierath in her absence for her co-operation and hard work. Thanks were also expressed to the team in Bristol, especially to Dr. Judy Naylor, and the outgoing associate editors.

f) Chair, Postgraduate Education Committee

Dr. Tack reported on the courses that had taken place in 2011 and early 2012: Baku/Azerbaijan, Jena/Germany, Kiev/Ukraine, Wroclaw/Poland and Kaunas/Lithuania. He added that all courses in 2011 had been very successful and had attracted delegates from many of the countries neighbouring the one where the course was held. Dr. Tack said courses were being planned in Almaty/Kazakhstan in October 2012 and in Lviv/Ukraine in April 2013.

Dr. Tack stated that with an unrestricted educational grant from Lilly EASD would produce a series of educational films aimed at a wider and less focussed audience than the previous series of lectures.

Dr. Boulton thanked Dr. Tack for his co-operation and hard work.

g) Chair, Extra-European Postgraduate Activities

Dr. Czupryniak reported on the joint postgraduate courses with ADA and IDF: Addis Ababa/Ethiopia in November 2011 and Spier/South Africa in August 2012. He stated that the 8th EASD/ADA/IDF course would be held in Sri Lanka in November 2013. He continued to report that the 5th EASD/CDS/Lilly Course had been held in Beijing in May 2012 and that the 6th was already being planned. He added that an agreement had been signed with the Chinese medical education company EMD to organise a series of webcast lectures to be delivered every two months to up to ten hospitals in Southern China and to arrange for one regular conference with EASD experts taking part. He explained that a three-year (2012-2014) partnership with the Gulf Group for the Study of Diabetes had been agreed on. The first course will take place in November 2012. He stated that future plans included another Best of EASD India course and he stated that negotiations were already underway with a new sponsor.

Dr. Czupryniak brought his report to an end by thanking Dr. Boulton for his support and the EASD team for their help in organising the courses.

Dr. Boulton thanked Dr. Czupryniak for his co-operation and hard work.

3. ELECTIONS

Vice President (2012 – 2015)
The election of Dr. Bernard Thorens was unanimously approved with 42 votes.

Council Members (2013 - 2016)
The election of Drs. Amanda Adler, Hans Hauner, Valeriya Lyssenko, Vilma Urbancic-Rovan
was unanimously approved with 42 votes each.

4. STUDY GROUPS

i) Metabolic imaging – proposal from Dr. Roden
The General Assembly was informed of the decision by the Executive Committee and Council to endorse the initiation of this EASD Study Group.
ii) Diabetes and Cancer – proposal from Dr. Andrew Renehan
The General Assembly was informed of the decision by the Executive Committee and Council to endorse the initiation of this EASD Study Group.
iii) NAFLD – proposal from Dr. Amalia Gastaldelli
The General Assembly was informed of the decision by the Executive Committee and Council to endorse the initiation of this EASD Study Group.

5. HONORARY MEMBERSHIP

The nomination of Drs. Peter Bennett, Johnny Ludvigsson and Isabel Valverde was unanimously approved. Dr. Boulton thanked them for their outstanding contribution to diabetes research.

6. ANY OTHER BUSINESS

A request was made for a reduced registration fee for senior EASD members. Dr. Boulton said this would be discussed at the next Executive Committee meeting in January 2013.

The President thanked Dr. Bosch, retiring Vice President, for her friendly collaboration and diligence during her term of office. He also expressed his sincere gratitude to the Local Organising Committee for their outstanding contribution to the organisation of the 48th EASD Annual Meeting. He warmly thanked Dr. Jörgens, Dr. Grüsser and the EASD team in Düsseldorf for their dedicated work.

Dr. Boulton brought the General Assembly to a close at 18:50.

Agenda for the 49th General Assembly of the European Association for the Study of Diabetes

to be held in the Pi i Sunyer Hall, Fira de Barcelona, Barcelona, Spain on Thursday 26 September, 2013 at 18:00

1. Minutes of the 48th General Assembly, Berlin, Germany 2012

2. Reports

a) President	A.J.M. Boulton
b) Honorary Treasurer	M. Roden
c) Honorary Auditors	P. Diem
	L. M. Gardete-Correia
d) Honorary Secretary	M. Walker
e) Editor-in-Chief, Diabetologia	J. Zierath
f) Chair, Postgraduate Education Committee	C. Tack
g) Chair, Extra-European Postgraduate Activities and Secretary PGEC	L. Czupryniak

3. Elections

To be nominated for retiring member(s):

a) Honorary Secretary 2013-2016	Mark Walker
b) Editor-in Chief, Diabetologia 2013-2015	Juleen Zierath
c) Chair, PGEC 2013-2016	Cornelis (Cees) Tack
d) Chair, Extra-European PGEC 2013-2016	Leszek Czupryniak
e) Council Members 2014-2017	Kare Birkeland
	Jonathan Bodansky
	Claire Levy-Marchal
	Peter Nawroth

4. Study Groups

5. Honorary Membership

6. Any other business

49th EASD Annual Meeting of the European Association for the Study of Diabetes

Barcelona, Spain, 23 – 27 September 2013

Abstracts

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PS 030 Beta cell mass expansion and survival
PS 031 ER stress and apoptosis
PS 032 Islet cell toxicity and survival
PS 033 Experimental immunology
PS 034 Clinical immunology
PS 035 Islet transplantation
PS 036 Insulin is not the only islet player
PS 037 Clinical hypoglycaemia
PS 038 Hypoglycaemia mechanism and prevention
PS 039 Insulin resistance improvement
PS 040 Muscle metabolism
PS 041 Insulin resistance biomarkers
PS 042 Weight loss and caloric modulation
PS 043 Beta cell function in man
PS 044 Incretins: secretions and action
PS 045 Insulin secretion and action: using animal models
PS 046 Gut and gut hormones
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PS 118 Non-invasive characterisation of cardiovascular disease
PS 119 Diabetes in childhood

OP 01 Individualising the choice among GLP-1 receptor agonists

1

Glycaemic control and hypoglycaemia in metformin-treated T2DM patients with exenatide BID vs insulin lispro TID added to titrated insulin glargine QD: the 4B trial

B.H.R. Wolffenbuttel[1], M.A. Nauck[2], R. Shaginian[3], J. Malone[4], S. Cleall[5], D. de Vries[3], B. Hoogwerf[3], L. MacConell[6], M. Diamant[7];
[1]Univ of Groningen, Netherlands, [2]Diabeteszentrum Bad Lauterberg, Germany, [3]Eli Lilly & Co, Netherlands, [4]Eli Lilly & Co, Indianapolis, USA, [5]Eli Lilly & Co, Windlesham, UK, [6]Bristol-Myers Squibb, San Diego, USA, [7]VU University Medical Center, Amsterdam, Netherlands.

Background and aims: Addition of prandial to basal insulin is the current strategy when HbA_{1c} remains elevated in pts with T2DM, but is accompanied by weight gain and increased episodes of hypoglycaemia (hypo). Exenatide twice daily (BID), administered with the largest meals of the day, may improve glycaemic control with a lower risk of hypo than prandial insulin. The 4B Trial prospectively compared glycaemic control of Basal insulin glargine (IG) + Exenatide BID Treatment (BET) to Basal IG + Bolus insulin lispro (IL) 3 times daily (TID) Treatment (BBT) in patients (pts) with uncontrolled hyperglycaemia after 12 wks of intensified IG.
Materials and methods: After a 2-wk screening period and 12-wk basal insulin optimization (BIO) phase to titrate IG dose (INITIATE algorithm) to achieve the lowest possible FBG (target 5.6 mmol/L) without hypo, pts unable to achieve HbA_{1c} ≤7% (53 mmol/mol) enrolled in the 30-wk intervention phase comparing BET to BBT. The BET regimen: titrated Basal IG at bedtime plus Exenatide Treatment 5 mcg BID for 4 wks followed by 10 mcg BID. The BBT regimen: titrated Basal IG at bedtime plus Bolus IL Treatment TID titrated based on 4-point self-monitored blood glucose (SMBG) according to a protocol-specified titration rule (pre-meal glucose of <6.1 mmol/L with no hypo). Primary outcome was non-inferiority of ΔHbA_{1c} for BET vs BBT from baseline to 30 wks in the per protocol population (PP). Secondary outcomes compared the BBT and BET regimens on FG, PPG from SMBG profiles, weight, insulin dose, and hypo in the PP population.
Results: 1036 pts were screened and 917 pts (mean: age 60 y, BMI 32 kg/m^2, T2DM duration 12.6 y, HbA_{1c} 8.5% (69 mmol/mol), serum FG 8.3 mmol/L, IG dose 0.45 IU/kg/d, metformin dose 1993 mg/d) entered the BIO phase. 637 pts had $HbA1_c$ >7% (53 mmol/mol) and were randomised 1:1 to BET (n=316, PP n=247) or BBT (n=321, PP n=263) for 30 wk. HbA_{1c} reduction with BET was non-inferior to BBT for mean change in HbA_{1c} from randomisation to wk 30. LS mean HbA_{1c} decreases at wk 30 were similar in both groups. BET was associated with significant weight reduction, -2.4±0.3 kg, compared to BBT, +2.1±0.2 kg (BET vs BBT tx difference -4.6 kg [95% CI, -5.2 to -3.9], *P*<.0001). IL dose at 30 wks in BBT was 0.5±0.4 IU/kg/d. More gastrointestinal events (which were transient) were noted for BET vs BBT: nausea (32% vs 2%), vomiting (12% vs 1%), and diarrhoea (11% vs 5%). BET pts experienced less hypo overall compared to BBT pts except for nocturnal events (events/100-y: minor: 206 vs 522; major: 2 vs 7; non-nocturnal: 64 vs 350; nocturnal: 144 vs 178).
Conclusion: In this 44-wk study, the 4B Trial results support BET as an alternative to BBT in pts with T2DM not at target after intensified basal IG.

Measure	BET	BBT	BET vs BBT	95% CI
IG at randomisation (IU/kg/d)	0.7 (0.3)	0.7 (0.3)	--	--
IG at 30 wk	0.6 (0.3)	0.6 (0.3)	--	--
HbA_{1c} at randomisation (%)	8.3 (1.0)	8.2 (0.9)	--	--
HbA_{1c} at 30 wk	7.2 (0.9)	7.1 (0.8)	0.03 (0.07)	-0.11, 0.18
ΔHbA_{1c}	-1.1 (0.05)[a]	-1.1 (0.05)[a]	-0.04 (0.07)[a]	-0.18, 0.11
FG at randomisation (mmol/L)	7.1 (2.3)	7.0 (2.5)	--	--
FG at 30 wk	6.5 (2.0)	7.2 (2.8)	--	--
Δ FG	-0.46 (0.16)[a]	0.18 (0.15)[a]	-0.64 (0.20)**	-1.05, -0.24
Δ PPG (mmol/L)				
Δ Morning 2h post-meal[b]	-2.6 (0.14)[a]	-2.3 (0.14)[a]	-0.27 (0.18)[a]	-0.63, 0.09
Δ Midday 2h post-meal[b]	-2.2 (0.16)[a]	-3.1 (0.16)[a]	0.93 (0.21)***	0.52, 1.34
Δ Evening 2h post-meal[b]	-2.9 (0.17)[a]	-3.2 (0.17)[a]	0.28 (0.22)[a]	-0.16, 0.72

Values are mean (SD) except as indicated. Shaded: LS mean Δ by MMRM. [a](SE) *P = .002 for BET vs BBT; **P< .0001 for BET vs BBT. [b]From SMBG profiles. Endpoint data is 30 wk, PP.

Clinical Trial Registration Number: NCT00960661
Supported by: Eli Lilly, Amylin Pharmaceuticals

2

Sustaining HbA_{1c} control over three years during treatment with exenatide once weekly compared with insulin glargine

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[1]Bristol-Myers Squibb, San Diego, USA, [2]Hopital Brabois, Nancy, France, [3]Internist/Diabetology, Hamburg, Germany, [4]VU University Medical Centre, Amsterdam, Netherlands.

Background and aims: The DURATION 3 study was an open-label, randomised, controlled trial of patients with T2DM comparing the once-weekly fixed-dose formulation of the GLP-1 receptor agonist exenatide (EQW) with titrated insulin glargine (IG) over 3 years. Sustained glucose control is difficult to achieve due to the progressive deterioration of insulin secretion over time requiring therapy escalation. These additional analyses assessed the comparative abilities of EQW vs IG to sustain HbA_{1c} control as defined below.
Materials and methods: Patients were followed through a 26-week core study with an option to continue treatment up to 156 weeks. Dosage for the IG group was determined by the Initiate Insulin by Aggressive Titration and Education (INITIATE) algorithm. HbA_{1c} control was defined as achieving and maintaining an HbA_{1c} ≤7% (53 mmol/mol) after 26 weeks of treatment. Loss of, or failure to achieve, HbA_{1c} control was defined as an HbA_{1c} >7% at two consecutive visits (10 to 12 weeks apart) or an HbA_{1c} >9% at one visit after 26 weeks of treatment.
Results: The intent-to-treat (ITT) population comprised 233 EQW and 223 IG patients. Baseline characteristics (EQW vs IG) included mean age (57 vs 58 years), % male (52 vs 55), mean duration of diabetes (both 8 years), and mean HbA_{1c} (both 8.3%). Within both treatment groups 70% of patients were taking metformin (MET) alone and 30% were taking MET and a sulfonylurea. Mean daily insulin dose increased from baseline 10 IU to 31 IU at Week 26 and 39 IU at Year 3. Of the enrolled participants, 140 EQW and 147 IG patients completed 3 years in their original treatment groups. Within the ITT population, a greater percentage of EQW patients achieved and sustained HbA_{1c} control until last visit (50%) compared with IG patients (43%). Similarly, a higher percentage of EQW 3-year completers (43%) demonstrated HbA_{1c} control through 3 years compared with IG patients (33%). EQW ITT patients had a longer period of HbA_{1c} control. The median time of control was 25.0 months for EQW patients and 16.7 months for IG patients (Kaplan-Meier estimates *P*=0.03 from Wilcoxon test). The ITT population hazard ratio of failure in control over 3 years was 1.4 (IG/EQW, *P*=0.006 from Cox proportional hazard model). Among ITT patients with HbA_{1c} control until last visit the following results were observed. Greater HbA1c reduction was observed in EQW (LS mean = -1.32%) compared with IG (-1.17%; 95% CI difference = -0.34, 0.04, *P*=0.12). EQW patients exhibited less reduction (LS mean = -2.28 mmol/l) in fasting serum glucose compared with IG patients (-3.07; 95% CI difference = 0.03, 1.54, *P*=0.04) and greater weight loss than IG patients (LS mean = -3.44 kg vs +0.70, respectively (95% CI difference = -5.71, -2.56, *P*<.0001). EQW patients demonstrated less hypoglycaemia (event rate per year 1.1) than IG (3.1). EQW patients reported greater nausea and diarrhoea than IG patients with EQW patients reporting 18% nausea and 15% diarrhoea and IG patients reporting 2% nausea and 4% diarrhoea.
Conclusion: Throughout the 3-year trial of EQW vs IG a greater number of EQW patients achieved and sustained HbA_{1c} control with greater weight reduction and less hypoglycaemia compared with IG patients, despite continued insulin up titration.
Clinical Trial Registration Number: NCT00641056

3

Positive effects of liraglutide as adjunct to insulin in type 1 diabetes: glycaemic control and safety in a randomised, double blind, placebo controlled crossover trial

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[1]Endocrinology, University of Sheffield Medical School, UK, [2]Medicine, Medical University of Graz, Austria, [3]Endocrinology, Medical University of Graz, Austria, [4]Novo Nordisk A/S, Søborg, Denmark, [5]Novo Nordisk Pharma Ltd, Tokyo, Japan.

Background and aims: Treatment with a GLP-1 analogue as adjunct to insulin may positively impact glycaemic control and other parameters in type 1 diabetes (T1D). In a placebo controlled trial investigating effects of liraglutide as adjunct to insulin on counter regulatory hormone responses to hypogly-

caemia, we also assessed short term effects on glycaemic control, body weight (BW), hypoglycaemia and overall safety.
Materials and methods: 45 adults with T1D were allocated to 1 of 3 dose groups of liraglutide (0.6, 1.2 and 1.8 mg/day) and placebo as add on to insulin for 4 weeks in a crossover design with a washout period of 2 to 3 weeks. Starting dose of liraglutide was 0.6 mg/day, with weekly dose escalation of 0.6 mg until target dose was reached. Subjects received target dose for a minimum of 2 weeks. Daily insulin dose, change in HbA_{1c} and mean 9 point self measured plasma glucose (SMPG) were measured for the efficacy assessment. Number of hypoglycaemic episodes, adverse events (AEs), vital signs (pulse, blood pressure [BP]) and BW were measured for the safety assessment.
Results: Baseline characteristics were similar between groups: age 34.5±11.2 years, BW 74.1±11.4 kg, BMI 23.9±2.4 kg/m^2, HbA_{1c} 7.6±0.8%, T1D duration 16.6±9.4 years [mean±SD]. There was no difference in daily insulin dose at baseline between groups. At end of treatment, liraglutide at 1.2 and 1.8 mg significantly lowered daily insulin dose (by 27% for 1.2 mg vs placebo and 24% for 1.8 mg vs placebo). No systematic differences in mean SMPG 9 point profile or change in HbA_{1c} were observed. Frequency of hypoglycaemic events was unaffected; no severe events were reported. A small increase in pulse was seen with liraglutide treatment compared to placebo; however this was not statistically significant. No significant treatment differences in systolic and diastolic BP were seen. No AEs were reported as serious. As expected, gastrointestinal (GI) AEs were more frequent with liraglutide, with nausea the main contributor, and few cases of diarrhoea, vomiting or other; no cases were reported as severe. 1 withdrawal (of 3) was due to a treatment related AE (vomiting). Liraglutide induced weight loss of up to 3.3 kg, which was not considered a safety issue.
Conclusion: This trial supports a pharmacological effect of liraglutide in T1D that may contribute to glycaemic control. Besides the known GI side effects, no liraglutide related safety or tolerability issues were identified in this short term trial in T1D. The observed weight loss is of potential clinical benefit. Long term controlled trials are needed to establish the overall clinical benefit of liraglutide and its potential utility in the treatment of T1D.

	Liraglutide 0.6 mg / placebo	ETD or R [95% CI] p-value	Liraglutide 1.2 mg / placebo	ETD [95% CI] p-value	Liraglutide 1.8 mg / placebo	ETD [95% CI] p-value
Daily insulin dose (U) at end of treatment*	42.1 / 47.0	R = 0.90 [0.77 ; 1.04] p = 0.138	32.6 / 44.8	R = 0.73 [0.63 ; 0.85] p <0.001	31.8 / 41.7	R = 0.76 [0.66 ; 0.88] p <0.001
Mean 9-point SMPG profile (mmol/L)	9.95 / 9.34	ETD = -0.39 [-1.30 ; 0.52] p = 0.388	9.02 / 8.59	ETD = 0.43 [-0.50 ; 1.35] p = 0.351	9.76 / 8.85	ETD = 0.91 [-0.01 ; 1.83] p = 0.051
Change[b] in HbA_{1c} (%)	-0.10 / -0.01	ETD = -0.09 [-0.36 ; 0.18] p = 0.504	-0.12 / -0.12	ETD = 0.01 [-0.27 ; 0.28] p = 0.971	-0.04 / -0.02	ETD = -0.02 [-0.29 ; 0.25] p = 0.891
Hypoglycaemic[c] events N (%) E	14 (93.3) 289 / 14 (100) 313	N/A	13 (92.9) 263 / 13 (100) 326	N/A	14 (100) 316 / 15 (100) 295	N/A
GI AEs N (%) E	10 (66.7) 12 / 2 (14.3) 2	N/A	12 (85.7) 24 / 1 (7.7) 2	N/A	13 (92.9) 31 / 3 (20.0) 5	N/A
GI AEs related to nausea N (%) E	8 (53) 8 / 1 (7.1) 1	N/A	11 (78.6) 14 / 1 (7.7) 1	N/A	11 (78.6) 13 / 2 (13.3) 2	N/A
Change[b] in BW (kg)	-1.2 / 0.8	ETD = -2.0 [-3.1 ; -0.9] p < 0.001	-3.3 / 0.4	ETD = -3.7 [-4.9 ; -2.6] p < 0.001	-2.9 / 0.4	ETD = -3.3 [-4.4 ; -2.2] p < 0.001

Note: Unless otherwise stated, data are presented as estimated means. a = end of the 4-week treatment period b = change from baseline to end of treatment. c = assessed according to ADA definition. Abbreviations: AE = adverse event; BW = body weight; CI = confidence interval; E = events; ETD = estimated treatment difference; GI = gastrointestinal; N = number; N/A = not applicable; R = ratio, SMPG = self-measured plasma glucose, U = units.

Clinical Trial Registration Number: NCT01536665
Supported by: Novo Nordisk

4

Efficacy and safety of dulaglutide vs metformin in type 2 diabetes (AWARD-3)

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Background and aims: This Phase 3, randomised, double-blind, parallel-arm, monotherapy study compared the efficacy and safety of 2 doses of dulaglutide (DU), a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist, to metformin (MET) in patients with early type 2 diabetes (mean 2.6 years) treated with diet and exercise alone or in combination with 1 oral antidiabetic medication (low dose for ≥3 months before screening).
Materials and methods: Patients (N=807; mean baseline [BL] age, 55.6 years; HbA_{1c}, 7.6%; weight, 92.3 kg) were randomised to once-weekly DU 1.5 or 0.75 mg or MET 1000 mg twice-daily. Primary hypothesis was DU 1.5 mg is noninferior to MET on HbA_{1c} change from BL to 26 weeks (wks). Key secondary hypotheses were DU 1.5 mg is superior to MET, and DU 0.75 mg is noninferior and/or superior to MET.
Results: Both DU doses were superior to MET at 26 wks and DU 1.5 mg was superior to MET at 52 wks as measured by HbA_{1c} change from BL. Incidence of serious adverse events was 5.2% for DU 1.5 mg, 7.4% for DU 0.75 mg, and 6.0% for MET. The rank order of incidence of gastrointestinal-related adverse events among groups was: DU 1.5 mg >MET >DU 0.75 mg. At 52 wks, documented symptomatic hypoglycaemia (≤3.9 mmol/L) incidence was 6.3%, 5.9%, and 4.9% for DU 1.5 mg, DU 0.75 mg, and MET, respectively (overall p=0.756).
Conclusion: In conclusion, both once-weekly DU doses at 26 wks and DU 1.5 mg at 52 wks demonstrated superior glycaemic control compared to MET and were well tolerated.

Summary of efficacy results at primary and final time points

Primary Time Point (26 Weeks, ITT, LOCF)	**DU 1.5 mg (n=269)**	**DU 0.75 mg (n=270)**	**MET 2000 mg (n=268)**
HbA_{1c} change (%), LS Mean (SE)	-0.78 (0.06)††	-0.71 (0.06)††	-0.56 (0.06)
% of patients with HbA_{1c} <7%	61.5#	62.6#	53.6
Weight change (kg), LS Mean (SE)	-2.29 (0.24)	-1.36 (0.24)#	-2.22 (0.24)
Final Time Point (52 Weeks, ITT, LOCF)			
HbA_{1c} change (%), LS Mean (SE)	-0.70 (0.07)††	-0.55 (0.07)†	-0.51 (0.07)
% of patients with HbA_{1c} <7%	60.0#	53.2	48.3
Weight change (kg), LS Mean (SE)	-1.93 (0.29)	-1.09 (0.29)#	-2.20 (0.29)

Abbreviations: ITT = intent-to-treat; LOCF = last observation carried forward; LS = least squares; mg = milligrams; SE = standard error.
†and †† multiplicity adjusted 1-sided p<0.025 for noninferiority or superiority, respectively, vs MET, for HbA_{1c} change only.
2-sided p<0.05 vs MET.

Clinical Trial Registration Number: NCT01126580
Supported by: Eli Lilly and Company

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HARMONY 3: 104 week efficacy of albiglutide compared to sitagliptin and glimepiride in patients with type 2 diabetes mellitus on metformin

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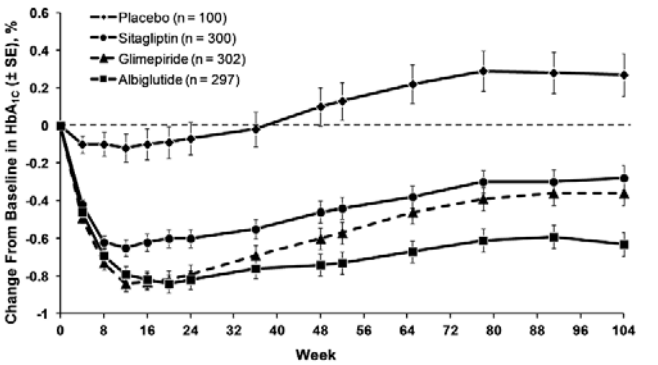
Background and aims: To evaluate the efficacy, tolerability, and safety of once-weekly albiglutide (Albi) compared to placebo (Pbo), sitagliptin (Sita), or glimepiride (Glim) in patients with type 2 diabetes mellitus who had inadequately controlled haemoglobin A_{1c} (HbA_{1c}: 7–10%) on metformin (Met).
Materials and methods: The 3-year trial was designed as a randomised, double-blind, Pbo- and active-controlled, Phase III study. Subjects taking stable Met were randomised to Albi (30 mg), Pbo, Sita (100 mg), or Glim (2 mg). Pa-

tients meeting predefined hyperglycaemia criteria qualified for blinded dose titration (Glim 2 to 4 mg; Albi 30 to 50 mg). Patients were allowed to continue in the study if rescue for persistent hyperglycaemic rescue was required. The primary objective was to evaluate HbA_{1c} change from baseline at Week 104, using an analysis of covariance model adjusting for baseline HbA_{1c},region, prior myocardial infarction history, and age.

Results: Baseline age was 54.5 years (mean, SD 10.0), BMI 32.6 kg/m^2 (5.5), weight 90.7 kg (19.3), HbA_{1c} 8.1% (0.8), duration of diabetes 6.0 years (4.8). A reduction in HbA_{1c} was seen in all active treatment groups. For the change in HbA_{1c}, Albi was statistically superior compared to Pbo (−0.91%; 95% CI: −1.16, −0.65, p <0.0001), Sita (−0.35%; 95% CI: −0.53, −0.17, p = 0.0001), and Glim (−0.27%; 95% CI: −0.45, −0.09, p = 0.0033). Albi had superior reduction in fasting plasma glucose (FPG) (mmol/l) compared to Pbo (−1.53; 95% CI: −2.16, −0.90 p <0.0001), Sita (−0.86; 95% CI −1.30, −0.41, p = 0.0002) and Glim (−0.56; 95% CI −1.01, −0.12, p = 0.0133). All treatment groups (except for Glim) had modest mean weight loss at 104 weeks (Pbo: −1.00 kg, Sita: −0.86 kg, Glim: 1.17 kg, Albi: −1.21 kg). Weight change (kg) compared to Albi was similar for Pbo (−0.2; 95% CI −1.14, 0.73, p = 0.7) and Sita (−0.4; 95% CI −1.01, 0.31, p = 0.3). Albi weight loss was superior to Glim (−2.4 kg; 95% CI −3.03, −1.71, p <0.0001). Gastrointestinal adverse events through Week 104 with Pbo/Sita/Glim/Albi were nausea, 11%/7%/6%/10%; diarrhoea, 11%/9%/9%/13%; and vomiting, 1%/4%/4%/6%. Injection site reactions occurred in 5% of patients randomised to Pbo, 6% for Sita, 8% for Glim, and 17% for Albi. The incidence of pre-rescue documented (≤3.9 mmol/l) symptomatic hypoglycaemia events was 4% for Pbo, 2% for Sita, 18% for Glim, and 3% for Albi; no severe hypoglycaemia events were reported.

Conclusion: Albiglutide treatment for type 2 diabetes mellitus as add on to Met therapy was durable and superior to Sita and Glim in HbA_{1c} and FPG reduction, superior in weight loss to Glim, and well tolerated up to Week 104.

Figure. Model-Adjusted[1] Change From Baseline in HbA_{1C} Through Week 104 (Intent-to-Treat; Last Observation Carried Forward[2])



[1] Analysis of covariance model adjusted for baseline HbA_{1C}, region, history of prior myocardial infarction, and age category. A prespecified statistical testing procedure was performed for Albi vs Pbo followed by Albi vs active control for noninferiority and subsequent superiority
[2] Last observation prior to study discontinuation or hyperglycaemia rescue

Clinical Trial Registration Number: NCT00838903
Supported by: GlaxoSmithKline

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Therapeutic efficacy of lixisenatide added to basal insulin is greater when FPG is well-controlled

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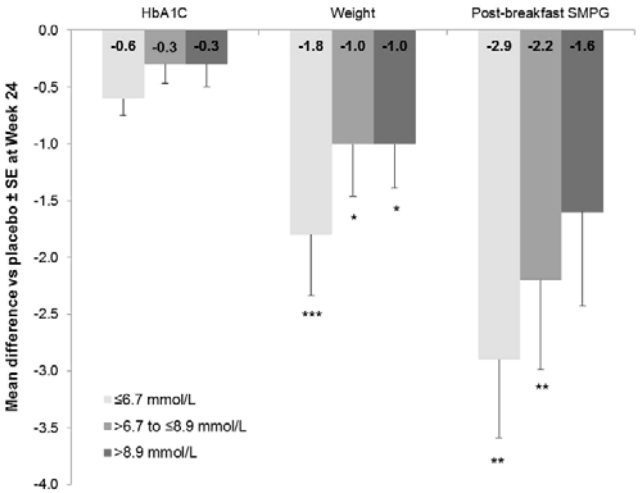
Background and aims: Lixisenatide is a novel once-daily prandial glucagon-like peptide-1 receptor agonist recently approved in Europe for treatment of type 2 diabetes (T2DM) in adults. In the GetGoal-L study, treatment with lixisenatide added to stable basal insulin caused significant reductions in HbA_{1c} levels, postprandial glucose, and body weight compared with placebo. The present *post hoc* analysis of data from this study examined whether the fasting plasma glucose (FPG) level at baseline was related to the ability of added lixisenatide to control hyperglycaemia.

Materials and methods: GetGoal-L was a double-blind, placebo-controlled study with a mean treatment period of 24 weeks, in which 496 patients with T2DM and inadequate glucose control on a stable dose of basal insulin ± metformin were randomised to lixisenatide 20 µg once daily or placebo. The current analysis was based on patients who were included in the HbA_{1c} change from baseline analysis at Week 24. Patients were subdivided according to baseline levels of FPG as group 1 (G1): FPG ≤6.7 mmol/L (120 mg/dL); group 2 (G2): FPG >6.7 to ≤ 8.9 mmol/L (160 mg/dL); and group 3 (G3): FPG >8.9 mmol/L. Efficacy parameters included HbA_{1c}, weight, 7-point self-measured blood glucose (SMBG) profiles and basal insulin dose.

Results: Clinical characteristics at baseline were similar between lixisenatide and placebo within each group. Mean duration of diabetes (years) with lixisenatide vs placebo was, respectively, G1=13.9 vs 14.8, G2=11.6 vs 11.8 and G3=12.1 vs 10.8. Mean duration of basal insulin treatment (years) with lixisenatide vs placebo was, respectively, G1=4.0 vs. 4.4; G2=2.4 vs 2.8 and G3=2.7 vs 2.5 years. Mean BMI (kg/m^2) in the lixisenatide group vs placebo was, respectively, G1=31.5 vs 31.8; G2=32.1 vs 33.0 and G3=32.6 vs 32.8. Mean HbA_{1c} (%) for lixisenatide vs placebo was G1=8.1 vs 8.1, G2=8.4 vs 8.4 and G3=8.7 vs 8.7. Adding lixisenatide reduced HbA_{1c}, weight, and SMBG post-breakfast in all subgroups, but participants with baseline FPG <6.7 mmol/L showed greater placebo-adjusted reductions than those in the two other groups (Figure 1). The placebo-corrected mean dose change in basal insulin (± standard error) from baseline to endpoint was G1=-4.8 (2.5), G2=-6.0 (2.5) and G3=-0.7 (2.6), respectively.

Conclusion: Adding lixisenatide to basal insulin improved glycaemic control and the effect was most pronounced in patients with controlled FPG. These findings are consistent with lixisenatide's known effect on prandial glycaemic control.

Figure. Placebo-adjusted mean changes in HbA_{1c}, body weight, and post-breakfast SMBG from baseline to endpoint (Week 24) according to baseline FPG.



*p<0.05; **p<0.01; ***p=0.001. SMPG=self-monitored plasma glucose; SE=standard error.

Clinical Trial Registration Number: NCT00715624
Supported by: Sanofi

OP 02 Predictors of cardiovascular disease

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Risk factors for stroke and subtypes of stroke in patients with type 1 diabetes

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Background and aims: Type 1 diabetes is associated with a markedly increased risk of stroke. Risk factors for stroke and different subtypes of stroke in type 1 diabetes are, however, poorly studied. Thus, the aim was to determine the risk factors for stroke and its subtypes cerebral infarction, lacunar infarction, and cerebral hemorrhage in a large study population of patients with type 1 diabetes.
Materials and methods: A total of 4,083 patients with type 1 diabetes from the nationwide multicenter FinnDiane (Finnish Diabetic Nephropathy) Study, without a history of stroke at baseline, and available follow-up data on stroke were included. Strokes during follow-up were identified from FinnDiane follow-up visit questionnaires, medical files, death certificates, and national hospital discharge records. All strokes were classified by stroke neurologists based on medical files and brain imaging. At baseline, mean age of the patients was 37.4 ± 11.8 years, duration of diabetes 21.3 ± 12.1 years, 51% were males, 20% had diabetic nephropathy (DN), 32% had severe diabetic retinopathy (SDR), 46% had a history of smoking, and 36% had anti-hypertensive treatment (AHT). Cox proportional hazards analyses with forward stepwise variable entry and removal were performed in order to determine the risk factors for the different end-points.
Results: During 9.0 ± 2.7 years (36,680 person-years) of follow-up, 149 (3.6%) patients suffered an incident stroke. The independent risk factors for all strokes, cerebral infarction, lacunar infarction, and cerebral hemorrhage are presented in the table below. Sex, age at onset of diabetes, insulin dose per kg body weight, coronary heart disease, current smoking, diastolic blood pressure, waist circumference, high sensitive C-reactive protein, LDL-cholesterol, HDL-cholesterol, triglycerides, and anticoagulative medication were not independent risk factors for any of the end-points studied.
Conclusion: In this largest study so far on risk factors for stroke in type 1 diabetes, we were able to show that in patients with type 1 diabetes, the microvascular diabetic complications, as well as poor glycemic control and insulin resistance, are associated with an increased risk for stroke. The traditional risk factors for stroke (smoking, hypertension, and age/duration of diabetes) were also risk factors in type 1 diabetes. For the different subtypes of stroke, the risk factors were similar, except lower BMI that was associated with an increased risk of cerebral hemorrhage.

	All Strokes n=149	Cerebral Infarction n=105	Lacunar Infarction n=58	Cerebral Hemorrhage n=44
Duration of diabetes (years)	1.04 (1.02-1.06)	1.06 (1.04-1.08)	1.05 (1.02-1.08)	NS
DN (yes/no)	2.28 (1.46-3.54)	2.81 (1.75-4.51)	2.72 (1.45-5.10)	2.63 (1.13-6.15)
SDR (yes/no)	1.90 (1.15-3.13)	NS	NS	2.91 (1.15-7.38)
HbA_{1c} (mmol/mol)	1.17 (1.04-1.32)	1.23 (1.06-1.41)	1.22 (1.01-1.47)	NS
SBP (mmHg)	1.02 (1.01-1.03)	1.02 (1.01-1.03)	1.02 (1.00-1.03)	1.02 (1.01-1.04)
AHT (yes/no)	2.52 (1.46-4.35)*	2.98 (1.56-5.71)*	3.44 (1.47-8.03)*	NS
History of smoking (yes/no)	1.58 (1.10-2.29)	1.93 (1.23-3.02)	NS	NS
BMI (kg/m^2)	-	-	-	0.88 (0.80-0.97)
eGDR ($mg*kg^{-1}*min^{-1}$)	0.83 (0.75-0.92)†	0.78 (0.69-0.88)†	0.76 (0.65-0.88)†	NS
MS (yes/no)	NS	NS	1.86 (1.03-3.35)≠	NS

Data are presented as HR with 95% CI. *separate model with systolic blood pressure (SBP) and diastolic blood pressure (DBP) excluded, †separate model with HbA_{1c}, SBP, and DBP excluded, ≠separate model with waist circumference, SBP, and DBP excluded. eGDR=estimated glucose disposal rate, MS=metabolic syndrome.

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ProBNP strongly predicts future macrovascular events independently from the baseline coronary artery disease state in patients with and without type 2 diabetes

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Background and aims: The power of pro-B-type natriuretic peptide (proBNP) to predict cardiovascular events is unclear, in particular in patients with type 2 diabetes (T2DM).
Materials and methods: We therefore aimed at investigating whether proBNP predicts major cardiovascular events in patients with T2DM as well as in subjects without diabetes in a large cohort of patients characterized by coronary angiography at baseline. We prospectively recorded major cardiovascular events in a cohort of 718 consecutive patients undergoing coronary angiography for the evaluation of established or suspected stable CAD over 3.2±1.2 years.
Results: Overall, the incidence of cardiovascular events was higher among patients with T2DM than among subjects who did not have diabetes. Cardiovascular risk increased significantly over tertiles of proBNP both among patients with diabetes (6.3%, 24.1%, and 32.4%,; p = 0.004) and among non-diabetic subjects (11.5%, 11.4%, and 21.1%; p = 0.012). Also as a continuous variable, baseline proBNP proved strongly predictive of major cardiovascular events both among patients with T2DM and among non-diabetic subjects (standardized adjusted HRs = 1.40 [1.12 - 1.74], p = 0.003 and 1.19 [1.06 - 1.33], p = 0.003, respectively) after adjustment for age, gender, BMI, LDL cholesterol, HDL cholesterol, hypertension, and smoking. Additional adjustment for the angiographically determined baseline presence of CAD did not significantly attenuate these results.
Conclusion: We conclude that proBNP both among patients with type 2 diabetes and among non-diabetic subjects strongly predicts future macrovascular events independently from the baseline coronary artery disease state.

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Biomarkers for prediction of CVD in type 2 diabetes

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Background and aims: Improving prediction of cardiovascular disease (CVD) in type 2 diabetes (T2D) is important for tailoring therapy and also for risk stratification into clinical trials. Here we examined a wide range of 42 potential serum biomarkers for incident CVD in a combined analysis of data from five European cohorts.
Materials and methods: The study included T2D patients from 5 cohorts: Go-DARTS (n=1204), the Scania Diabetes Registry (n=666), MONICA/KORA (n=308), IMPROVE (n=94) and 60-year Old Stockholm Study (n=46). Baseline samples from incident cases of major CVD (MI or stroke) and an equal number of age and diabetes duration stratum-matched controls free of CVD at end of follow up were analysed. 9 markers were analysed by standard ELISAs or on automated Roche systems (Elecsys 2010 and Cobas Integra) and 33 were measured on a Luminex platform. Candidate biomarkers were chosen based on a literature mining search tool, previous reports of association with CVD in diabetic or non-diabetic cohorts or based on pathways reported as implicated in CVD. Data analysis was by logistic regression using 50-fold cross validation with forward selection and by 50-fold cross validated backward selection with LASSO penalty. Clinical covariates measured at baseline included in models were age, sex, diabetes duration, BMI, height, blood pressure, smoking, LDL, HDL, Triglycerides, HbA1c, eGFR, study centre, and medication (including antihypertensive, aspirin, lipid-lowering agent and insulin use).
Results: Of 42 biomarkers analysed 16 were retained as improving the prediction of CVD beyond the clinical covariates. N-terminal pro B-type Natriuretic Peptide (NT-proBNP), Apolipoprotein CIII (ApoCIII), soluble RAGE

(sRAGE), high sensitivity Troponin T (hsTnT), IL-6 and IL-15 were the most strongly associated with CVD (see table for ORs). However the increment in the area under the ROC curve was slight; from 0.67 for a model with clinical covariates only to 0.73 for a model including all clinical covariates and 16 associated biomarkers.
Conclusion: We confirm the association of NT-proBNP and hsTnT with incident CVD in T2D. IL-6 is known to be associated with incident CVD in general populations and we confirm that finding now in T2D. We also report a novel association of IL-15 with incident CVD in T2D. The inverse associations between ApoCIII and sRAGE with incident CVD are unexpected and need further investigation. The improvement in prediction of CVD with these 16 biomarkers in this cross validation study indicates that validation of these markers is now warranted.

Logistic regression model adjusted for all clinical covariates and all selected biomarkers		
Normalised Biomarker	Odds Ratio per standard deviation of normalised biomarker (95% CI)	Wald p-value
N-terminal proB-type Natriuretic Peptide	1.72 (1.49, 1.99)	<0.001
Apolipoprotein C-III	1.26 (1.10, 1.43)	<0.001
sRAGE	0.82 (0.73, 0.91)	<0.001
High Sensitivity Troponin T	0.85 (0.77, 0.94)	0.002
Interleukin-6	1.16 (1.05, 1.29)	0.004
Interleukin-15	1.17 (1.06, 1.29)	0.001

Supported by: SUMMIT Consortium IMI-2008/115006

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Metabolic profiles improve the prediction of cardiovascular events in elderly patients
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Background and aims: One of the most important determinants of cardiovascular health is the age's person, therefore the management of cardiovascular diseases (CVD) in elderly people entails great challenge. However, why age is such a critical component of CVD etiology remains unclear. A possible explanation of vascular senescence process is the mitochondrial damage and dysfunction. We hypothesized that metabolomic profiling would identify biomarkers predicting major cardiovascular events (MACEs) in elderly people, improving the clinical standard cardiovascular risk factors.
Materials and methods: Targeted mass spectrometry-based profiling of 49 metabolites was performed in a group of very old participants (n=67, mean age=78±3 years) with a high rate of previous CVD (68%). Principal component analysis and Cox proportional hazards regression modeling were used to evaluate the relation between the metabolite factors and recurring MACEs. We tested discrimination ability and reclassification of clinical and metabolomic models
Results: At follow-up (median=3.5 years), 17 MACEs occurred (5 cardiovascular deaths, 1 nonfatal myocardial infarction, 7 nonfatal strokes and 4 peripheral artery surgeries) (incidence=7.3% person-years). Metabolite factor 1, composed by medium- and long-chain acylcarnitines, and factor 7 (alanine) were independently associated with MACEs, after adjustment for clinical CV covariates [HR=1.77 (95%CI=1.11-2.81, p=0.016) and HR=2.18 (95%CI=1.17-4.07, p=0.014), respectively]. However, only factor 1 significantly increases the prediction accuracy of the Framingham Recurring-Coronary-Heart-Disease-Score, with a significant improvement in discrimination (integrated discrimination improvement=7%, p=0.01) and correctly reclassifying 41% of events and 37% of non-events resulting in a cNRI=0.79 (p=0.005)
Conclusion: Ageing mitochondrial dysfunction evaluated by metabolomic profiling is associated with MACEs, independently of standard predictors.

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Low concentrations of testosterone predict acute myocardial infarction in men with type 2 diabetes mellitus
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Background and aims: Low testosterone concentrations in men have been associated to diabetes, hypertension and CVD-mortality and all cause mortality. We aimed to investigate the associations between endogenous testosterone and acute myocardial infarction (AMI) in men with type 2 diabetes mellitus (T2DM).
Materials and methods: The study was conducted in 1109 subjects ≥40 years of age who participated in a population survey conducted in a rural area of southern Sweden with baseline examinations in 1993-94. Information about smoking habits and physical activities was assembled using validated questionnaires. BMI, waist hip ratio (WHR) and blood pressure measured with a sphygmomanometer in the supine position after 5 min rest were obtained. Total-, LDL-and HDL-cholesterol and triglycerides were measured after overnight fasting. Total testosterone, estradiol and sex hormone-binding globulin (SHBG) analyses were obtained in 99% of cases. A diagnosis of T2DM was based on one of three criteria: a) A known history of T2DM, b) Two consecutive fasting blood glucose ≥ 6,6 mmol/L and c) Subjects with fasting blood glucose >5,5 had an OGTT test and T2DM was defined if two consecutive 2h blood glucose were >11.0mmol/L. Individual patient information on the AMI events in this observational cohort study was ascertained by record linkage with national inpatient and mortality registers from baseline through 2012. IBM-SPSS Statistics version 20 was used for the analyses. Hazard ratios (95% Confidence Intervals) were examined, with adjustment for risk factors in Cox proportional hazard models stratified on sex and T2DM (present or not).
Results: The prevalence of T2DM at baseline was 10% in men and 7,5% in women. During follow-up of 13,8y±5,5y, there were 89 AMI events in men (74 in diabetics and 15 in non diabetics) and 72 in women (61 in non diabetics and 11 in diabetics). In the age adjusted Cox models there was a significant inverse association between AMI morbidity and testosterone in men with T2DM (HR=0,86 CI(0,75-0,98)). There was no significant association in men without diabetes or in women regardless of T2DM. The association remained significant with adjustment for SHBG and oestradiol (HR=0,84 CI(0,72-0,99)). Finally, in the model including WHR, systolic blood pressure, total cholesterol and active smoking the association remained significant (HR=0,83 CI(0,7-0,99)). There was no significant association between AMI and SHBG or estradiol in men or in women, with or without T2DM.
Conclusion: Low concentrations of testosterone predicted AMI in diabetic men in this cohort independent of other risk factors. Whether testosterone replacement therapy could reduce the risk of AMI in men with T2DM remains to be investigated.
Supported by: SI and FoU VGR

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Soluble C5b-9, a marker for terminal complement activation, is not associated with markers of atherosclerosis or prevalent cardiovascular disease: The CODAM study
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Background and aims: The complement system has been implicated in the development of both type 2 diabetes (DM2) and cardiovascular disease (CVD). Complement is a complex protein network that can be activated via different enzymatic pathways, which all converge on the terminal pathway. Soluble C5b-9 (sC5b-9) is a product of terminal complement activation and the most downstream factor in the cascade. In DM2 patients with acute myocardial infarction, sC5b-9 at admission predicted a poor outcome. However, there are no studies on the role of sC5b-9 in the development of cardiometabolic disease. Therefore, we investigated the associations of sC5b-9 with

markers of atherosclerosis [carotid intima-media thickness (cIMT), ankle-arm blood pressure index (AAIx)] and prevalent CVD in a cohort with a high prevalence of DM2.
Materials and methods: We conducted cross-sectional analyses among 548 individuals (61% men, mean±SD age 59.4±6.9 years, 22% with impaired glucose metabolism (IGM) and 26% with DM2) from the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) Study. Prevalent CVD was defined on the basis of self-reports, signs of myocardial infarction on an ECG and/or an AAIx<0.9; cIMT was measured by ultrasonography and AAIx by Doppler ultrasound. SC5b-9 was measured with a commercial ELISA (intra-assay CV 5.1%, inter-assay CV 11.8%). Associations between sC5b-9 and study outcomes were analysed with multiple logistic (for prevalent CVD) or linear regression (for cIMT and AAIx) analyses. All analyses were adjusted first for age, sex and glucose metabolism status (minimal model), and furthermore for BMI, waist, blood lipids, blood pressure, eGFR, physical activity, smoking and use of medication.
Results: The prevalence of CVD was 28%, cIMT was 0.77±0.16 mm, AAIx was 1.09±0.12 and sC5b-9 was 113±34 ng/ml. Plasma sC5b-9 (per 1 SD increase, 1SD=34 ng/ml) was not associated with markers of atherosclerosis or CVD, neither in the minimal model [cIMT: β=-0.004, (95%CI: -0.017; 0.009); AAIx: β=0.002 (-0.008; 0.012); CVD: OR=0.99 (0.82; 1.21)] nor in the fully adjusted model [cIMT: β=-0.009, (95%CI: -0.021; 0.004); AAIx: β=0.004 (-0.006; 0.014); CVD: OR=0.97 [0.78; 1.20)].
Conclusion: In the CODAM study population, activation of the terminal complement pathway, as represented by systemic concentrations of sC5b-9, was not associated with markers of atherosclerosis or prevalent CVD. It remains to be determined whether other aspects of terminal complement activation may be involved in the aetiology of CVD.
Clinical Trial Registration Number: NHS2010B194

OP 03 Biomarkers and risk scores, out with the old, in with the new?

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Calcium concentration predicts future development of diabetes and impaired glucose tolerance: the Insulin Resistance Atherosclerosis study
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Background and aims: Serum calcium concentration and calcium supplementation have been associated with cardiovascular mortality. Calcium concentration has also been associated with impaired glucose tolerance (IGT) independently of the confounding effect of age, obesity, or vitamin D concentration. Accordingly, we aimed to examine the relationship between total calcium concentration and risk of future development of IGT and diabetes.
Materials and methods: Incident diabetes and incident IGT were ascertained in 863 and 723 participants in the Insulin Resistance Atherosclerosis Study after a 5-year follow-up period using the 2003 American Diabetes Association criteria. Insulin sensitivity index (S_I) and acute insulin response (AIR) were directly measured and glomerular filtration rate was estimated (eGFR) using the Modification of Diet in Renal Disease. Logistic regression analysis was used to assess the relationship between calcium concentration and incident diabetes (or incident IGT).
Results: The relationship between incident diabetes and calcium concentration modeled by a smooth function was statistically significant (p = 0.020), but was not linear (Wald test, p = 0.040). Therefore, risk of developing diabetes and IGT were assessed by categories of calcium concentration (Table). Calcium concentration ≥2.5 mmol/L was associated with increased risk of incident diabetes and incident IGT. As a continuous variable, calcium concentration was an independent predictor of incident diabetes (OR x 1 SD, 1.34 [95% CI: 1.07 - 1.68]) after adjusting for age, sex, race/ethnicity, family history of diabetes, BMI, plasma glucose levels, S_I, AIR, eGFR, and diuretic drugs.
Conclusion: Calcium concentration predicts the development of type 2 diabetes. Calcium concentration may play a role in the diabetes disease process independent of measured glucose, insulin secretion, and insulin resistance.

Odds of developing diabetes or IGT by categories of calcium concentration

Adjustment Models	<2.13 mmol/L	2.13 - 2.24 mmol/L	2.25 - 2.37 mmol/L	2.38 - 2.49 mmol/L	≥2.50 mmol/L
A. Incident diabetes as the dependent variable					
n	84	320	327	98	34
Basic model: Age, sex, ethnicity, and clinic	1.24 (0.64 - 2.42)	Referent	1.27 (0.81 - 1.98)	1.84 (1.01 - 3.34) *	2.78 (1.22 - 6.36) *
Basic model + BMI, family history, fasting and 2-h glucose, S_I, AIR, eGFR, and diuretic drugs	0.92 (0.40 - 2.09)	Referent	1.18 (0.69 - 2.01)	1.94 (0.96 - 3.91)	3.15 (1.14 - 8.73) *
B. Incident IGT as the dependent variable					
n	70	277	275	77	24
Basic model: Age, sex, ethnicity, and clinic	1.27 (0.71 - 2.27)	Referent	1.52 (1.04 - 1.22) *	1.48 (0.85 - 2.58)	3.10 (1.29 - 7.45) *
Basic model + BMI, family history, fasting and 2-h glucose, S_I, AIR, eGFR, and diuretic drugs	1.23 (0.62 - 2.42)	Referent	1.82 (1.18 - 2.83) †	1.54 (0.81 - 2.95)	3.03 (1.15 - 7.98) *

* p <0.05; † p <0.01

Supported by: NHLBI and NCRR GCRC

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Visceral adiposity predicts incident diabetes more strongly in South Asians than in Europeans or African Caribbeans, independent of insulin resistance and other fat depots

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Background and aims: South Asians and African Caribbeans have higher risks of diabetes than Europeans, and different distributions of ectopic fat. The role of individual fat depots in determining diabetes and whether this differs by ethnicity is unclear.
Materials and methods: We used data from a UK cohort, the Southall And Brent Revisited (SABRE) study, comprising 2197 Europeans, 1355 South Asians and 650 African Caribbeans at baseline. Baseline (1988-1991, mean age 52±7) and follow-up (2008-2011, mean age 70±6) measurements included fasting blood and anthropometry. Incident diabetes was identified from primary care record review, self-report (plus anti-diabetic medication), glycaemia exceeding 1999 WHO diagnostic thresholds, hospital statistics and death certificates.Surface anthropometry cannot distinguish between abdominal visceral (VAT) and subcutaneous (SAT) adipose tissue. We therefore used CT to derive ethnic specific equations for VAT and SAT and applied these to baseline measures. We summed subscapular and suprailiac skinfolds to assess truncal adiposity, and triceps, thigh and suprapatellar skinfolds for peripheral subcutaneous fat. Predictive models for incident diabetes by ethnicity were fitted with each ectopic depot, and each measure of glycaemia/insulin resistance, individually and in combination.
Results: There were ethnic differences in baseline VAT, which was greatest in South Asians, then Europeans, then African Caribbeans (means 149 ±70 cm^2, p<0.001, 120±76cm^2 and 100 ±64 cm^2, p=0.0006). Diabetes was more prevalent at follow-up in South Asians (33%) and African Caribbeans (30%), compared to Europeans (14%). Baseline VAT, of all the fat measures, was one of the strongest predictors of incident diabetes in all ethnicities. After adjustment for other baseline fat measures, the association between VAT and diabetes remained in South Asians and Europeans but not African Caribbeans. Adjustment for any measure of glycaemia/insulin resistance did not wholly account for the association between VAT and diabetes in any ethnic group. When fasting insulin was added to a model including all ectopic fat depots, the association between VAT and incident diabetes remained only in South Asians (standardised OR 2.09, vs. 1.28 in Europeans, and 1.05 in African Caribbeans).
Conclusion: VAT was one of the strongest predictors of diabetes in all ethnicities. This association remained independent of other fat depots and of measures of glycaemia/insulin resistance in South Asians, but not in Europeans or African Caribbeans. Our findings suggest that underlying mechanisms governing the association between VAT and diabetes differ by ethnicity, and VAT plays a particularly important role in the aetiology of diabetes in South Asians.

Risk of incident diabetes (OR) in association with VAT, by ethnicity

1 SD increase in VAT *Covariates*	European, n=1356			South Asian, n=842			African Caribbean, n=335		
	OR	95%CI	p	OR	95%CI	p	OR	95% CI	p
age, sex	2.16	1.82, 2.57	<0.001	2.09	1.71, 2.56	<0.001	2.27	1.61, 3.20	<0.001
age, sex, baseline SAT, truncal skinfolds, peripheral skinfolds, BMI	1.65	1.12, 2.43	0.01	2.13	1.43, 3.17	<0.001	1.21	0.63, 2.30	0.57
age, sex, baseline SAT, truncal skinfolds, peripheral skinfolds, BMI, baseline fasting insulin	1.28	0.71, 2.28	0.41	2.09	1.17, 3.75	0.01	1.05	0.41, 2.70	0.91

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A simple self-assessment score for predicting the risk of non-alcoholic fatty liver disease

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Background and aims: Although non-alcoholic fatty liver disease (NAFLD) is a prevalent and rapidly increasing disease worldwide associated with the escalated risk of morbidity and mortality, few screening models are available to evaluate the risk of NAFLD. Considering the clinical impact of NAFLD on public health, we developed and validated a self-assessment score for presence of NAFLD in the general populations and compared it to other screening methods.
Materials and methods: The development cohort included 15676 subjects (8313 men and 7363 women) who visited National Health Insurance Service Ilsan Hospital in year 2008-2010. Abdominal ultrasound and biochemical analyses were performed for the regular health check-up. After excluding patients with excessive alcohol consumption (>140g/week for men and 70g/week for women) or existing liver diseases and thyroid disorders, logistic regression analysis was conducted to determine predictors of NAFLD and derive risk score models. We validated our models and compared with other existing methods using an external dataset (n = 66868).
Results: In the self-assessment simple models, age, sex, waist circumference, BMI, history of diabetes and dyslipidemia, alcohol intake, physical activity and menopausal status were independently associated with NAFLD. We calculated a NAFLD self-assessment score (range, 0-15), and a cut-point of 8 or higher defined 58% or 45% of men or women as high-risk for NAFLD and yielded a sensitivity of 80% or 87%, specificity of 67% or 72%, positive predictive value of 72% or 56%, and negative predictive value of 76% or 93% (area under the curve [AUC]=0.82 or 0.87) in men or women, respectively. Comparable results were obtained in the validation dataset.
Conclusion: This self-assessment score may be useful for identifying individuals at high-risk for NAFLD. Further studies are needed to evaluate the utility and feasibility of this score in various settings.

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Investigating the validity of the UKPDS outcomes equations in current clinical practice

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Background and aims: The UKPDS outcomes equations are routinely used to assess the benefits associated with glucose control in contemporary type 2 diabetes mellitus (T2DM) cohorts escalating to 2nd or 3rd line therapy. Their validity has been questioned because they were derived from a cohort of newly diagnosed T2DM subjects and diabetes management practices have evolved considerably since the UKPDS reported. The objective of this study was to re-calibrate the equations to contemporary healthcare data to better understand their continued relevance to assess the benefits of glucose control in T2DM.
Materials and methods: We utilized data from The Health Improvement Network (THIN) database over the period 1/1/2004 to 31/12/2009. In contrast to the UKPDS newly diagnosed population we selected patients either aged > 64 years with HbA1c >7.5% and diabetes duration >10 years or aged >55 years with at least 1 established cardiovascular risk factor. Weibull survival equations were fitted to the following endpoints; myocardial infarction (MI), stroke, congestive heart failure (CHF), ischemic heart disease (IHD), amputation, blindness and end-stage renal disease (ESRD) using R. Missing data was modelled using multiple imputation.
Results: Data were available on 68,990 T2DM subjects meeting the inclusion criteria with mean age 66.1 years, 46% female, 8.8 years duration of diabetes; with mean body mass index (29.7m/kg^2), HbA1c (8.0%), systolic blood pressure (147.5mmHg), total cholesterol (5.1mmol/l) and HDL cholesterol (1.3mmol/l). Log hazards (standard error) associated with unit changes HbA1c were 0.097(0.011) for MI, 0.084 (0.008) for CHF, 0.051 (0.009) for

stroke, 0.112(0.02) for blindness, 0.043 (0.007) for ESRD and 0.225 (0.017) for amputation. These estimates were statistically consistent (at the 95% level) with the original UKPDS log hazards except for IHD (non-significant in the THIN database) and ESRD (non-significant in UKPDS).
Conclusion: This study demonstrates that, in general, the UKPDS outcomes equations retain their validity for assessing the relationship between HbA1c and macrovascular and microvascular complications in current T2DM subjects. It is likely that use of UKPDS equations will overestimate the incidence of IHD and underestimate the incidence of ESRD.
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Skin collagen advanced glycation endproducts (AGEs) as prospective markers of sub-clinical cardiovascular disease (CVD) outcomes in type 1 diabetes
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The DCCT/EDIC previously reported that skin collagen intrinsic fluorescence and AGEs had long-term associations with the incidence of microvascular diseases in type 1 diabetes. However, the association between AGE and CVD events has not been explored in EDIC. Other studies have reported cross-sectional but not prospective associations between noninvasive skin auto/intrinsic fluorescence and CVD outcomes in diabetes. In this report we investigated the long-term association of collagen-linked fluorescence at 370/440nm (CLF) in collagen digest, a novel acid-labile fluorescent AGE named LW-1, and a panel of 9 other AGEs measured in skin collagen digest from skin biopsies (n=216) obtained near DCCT closeout (1993), on subclinical CVD outcomes. CLF was associated with the mean coronary artery calcium (CAC) at EDIC Yr 7-9 (n=187), and risk of CAC>0 and CAC>100 (each P<0.002). These associations remained significant even after false discovery rate (FDR) adjustment for multiple tests (each P<0.03). Adjustment for mean DCCT A1c weakened the association with CAC>0 but not with CAC >100 or mean CAC. Change of Intimal-Media Thickness (IMT) from EDIC Year 1 to 6 (n=127) was predicted by higher methylglyoxal hydroimidazolones (MG-H1), LW-1, and pentosidine even after adjustment for A1c (each P<0.022). Likewise, cardiac MRI left ventricular (LV) mass at EDIC Yr 15-16 (n=142) was associated with higher LW-1 and CLF with or without adjustment for A1c (each P<0.035). In contrast, DCCT mean A1c was positively associated with risk of CAC, but nullified after adjustment for CLF or furosine. IMT and MRI endpoints were not associated with DCCT or EDIC mean A1c in this sub-cohort. These results point to the unique ability of CLF and selected tissue AGEs to predict future risk of subclinical CVD, and a likely role for glucose-mediated matrix crosslinking in LV mass and IMT. They also suggest that tissue AGEs might more reliably predict subclinical CVD risk than HbA1c.
Clinical Trial Registration Number: NCT00360893; NCT00360815
Supported by: NIH, NIDDK

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Assessment of serum ghrelin as a biomarker of HNF1A-MODY
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Background and aims: Ghrelin is a non-glycosylated hormone composed of 28 amino acids. It is present in the serum in two major molecular forms - desacyl and acylated ones. Ghrelin expression by the digestive tract epithelium is regulated by the hepatocyte nuclear factor 1A (HNF1A), a transcription factor whose mutations cause one of the forms of maturity onset diabetes of the young (MODY). As shown in an animal model, ghrelin expression is increased by approximately five-fold in homozygous HNF1A knock-out mice (Hnf-/-) as compared to a wild type. Furthermore, this is followed by its five-fold higher concentration in serum with a subsequent reduction in insulin secretion. The relationship between ghrelin and HNF1A gene mutations in humans has not yet been evaluated. In this study, we assessed ghrelin level in various forms of diabetes and evaluated it as a potential biomarker for HNF1A MODY.
Materials and methods: We examined 47 diabetic HNF1A gene mutation carriers, 55 type 2 diabetes (T2DM) subjects, 42 type 1 diabetes (T1DM) patients and 31 glucokinase (GCK) gene mutation carriers with diabetes. Blood specimens for the ghrelin measurement were taken in the fasting state. Ghrelin was measured using a commercially available EIA kit with polyclonal antibodies against the C-terminal fragment of its both forms (total ghrelin). Samples were diluted prior the analysis. Comparisons of ghrelin concentrations were performed with the t-test and linear regression. Ghrelin concentration was used as a dependent variable and group, BMI, HbA1c, age at examination were covariates in the multiple analysis. To control the extent of degradation of the analyte, we also included length of storage, defined as the time between blood collection and assay determination, as a covariate.
Results: Mean ghrelin concentration in HNF1A-MODY subjects was 0.77 ng/ml (SD=0.33 ng/ml). It was twofold higher than in T2DM group (0.39±1.6 ng/ml; p<0.001) and by 50% higher than in the T1DM group (0.49±0.19 ng/ml; p<0.001). No significant differences were found between HNF1A and GCK MODY (0.7±0.2 ng/ml; p=0.32). The difference between HNF1A-MODY and T1DM remained significant (p<0.001) after adjusting for the relevant confounders and HNF1A mutation carrier status was the only independent variable associated with ghrelin in the multivariate model. The discriminative accuracy as expressed by the area under the curve (AUC) of serum ghrelin between T1DM and HNF1A-MODY was 0.74 (95%CI: 0.63; 0.84; p<0.001) with the corresponding sensitivity and specificity of 74% and 66.7%. The difference between HNF1A-MODY and T2DM was not significant (p=0.18) after adjusting for age and BMI.
Conclusion: Serum ghrelin level is lower in HNF1A-MODY than in both common polygenic forms of diabetes. It may be a valuable complementary marker for clinical differential diagnosis between HNF1A-MODY subjects and T1DM patients although, its discriminative accuracy is too low to allow use as a sole screening test. For T2DM, it appears that differences in clinical characteristics contributed to the observed difference against HNF1A-MODY in ghrelin levels.
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OP 04 Beta cell genes and type 2 diabetes

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The P300/CBP-associated factor (PCAF) is a histone acetyl transferase that controls beta cell function

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Background and aims: Pancreatic β-cells control insulin secretion through a fine-tuned process. Dysfunctions of this particular cell type are at the origin of pathological conditions, such as Diabetes Mellitus and cancer. A common component of the two type of diabetes, i.e. immune type 1 and non-immune type 2 (T2D), is a decrease of β-cell mass, finally resulting in hyperglycemia and its subsequent deleterious consequences. Therefore, a complete understanding of the factors and mechanisms responsible for β-cell maintenance is of high importance and could be of interest for the treatment of diabetes. We and others have demonstrated that cell cycle regulators, in particular E2F1, CDK4 (and its repressor INK4) and pRb are key regulators of glucose and lipid metabolism through their role in white adipose tissue, muscle and β-cells. The histone acetyl transferase PCAF has been shown to physically interact and up-regulate E2F1 activity through acetylation. Moreover, PCAF has been associated to the regulation of a metabolic gene program in response to insulin, suggesting potential functions for this transcriptional coregulator in the control of metabolic pathways.
Materials and methods: In this project, we used global PCAF KO mice, as well as isolated Pcaf +/+ and -/- islets. Islet structure was analyzed by classical histochemistry, and immunofluorescence. Ultra-structural analysis was performed by electron microscopy. Gene expression analysis was performed by qRT-PCR, and global gene expression by illumina DNA chips. Analysis was performed using the Ingenuity Pathway analysis software.
Results: We show here that Pcaf-/- mice are hyperglycemic in fed conditions and are resistant to diet-induced obesity. Moreover, these mice are glucose-intolerant under chow or high-fat diet due to decreased insulin secretion after a glucose load. Using isolated islets, we further demonstrated by static incubation experiments that Pcaf-/- β-cells were unable to secrete insulin in response to glucose. Electron microscopy analysis of isolated islets from Pcaf+/+ and -/- mice showed defects in insulin maturation and/or crystalization process, suggesting that PCAF could play a role in both maturation and secretion of insulin. Key genes involved in insulin crystalization/maturation processes were shown to be down-regulated upon Pcaf genetic inactivation, reinforcing the key role for this lysine acetyltransferase in the control of β-cell functions. Interestingly, GCN5 and P300, which are closely related to PCAF, could not compensate for PCAF deficiency, suggesting a β-cell specific function for PCAF. Moreover, Ingenuity pathway analysis of global gene expression from Pcaf +/+ and -/- isolated islets revealed a PDX-1 and HNF1a gene regulatory network dependent on PCAF. Finally, genetic analysis (using the dataset of DIAGRAM) revealed that two frequent single nucleotide polymorphisms (rs12639214 and rs12639078) were associated with T2D risk ($P < 5 \times 10\text{-}3$) in the Kat2b gene, encoding PCAF.
Conclusion: In summary we provide evidence that the PCAF acetyl transferase is involved in glucose homeostasis and could play a key role in type 2 diabetes physiopathology. The molecular mechanisms linking PCAF to β-cell functions are under investigations.
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Exploration of novel genes contributing to pathogenesis of type 2 diabetes in human pancreatic islets

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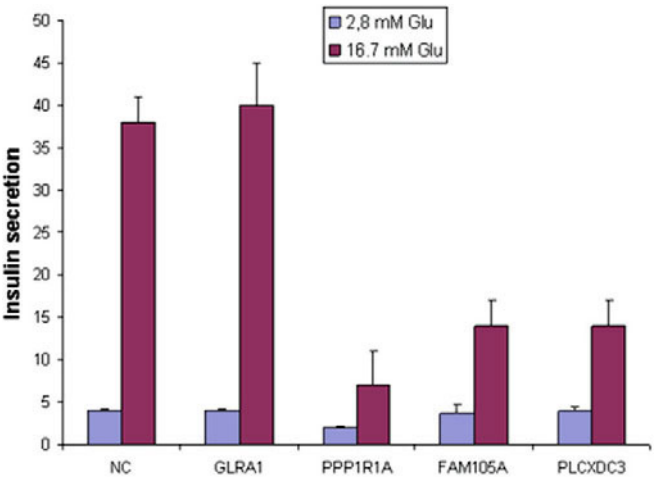
Background and aims: Normal glucose homeostasis is characterized by low HbA1c level and appropriate insulin secretion. Hence, gene expression signatures associated with these two phenotypes could be essential for islet function. To address this question, we have identified genes whose expression in human islets correlate with normal/high insulin secretion and low HbA1c level, followed by functional studies could be an interesting approach to uncover novel genes involve in the pathophysiology of T2D.
Materials and methods: Global a spearman's correlation test was used to correlate microarray gene expression data from human pancreatic islets (90 donors) with HbA1c level and insulin secretion from the same donors. The correlated genes were prioritized based on their correlation p-value and genes showing the strongest correlation were selected for further studies. This included silencing of their expression using siRNA in clonal rat β-cell line (INS-1 831/13). Transfection efficiency was determined by qRT-PCR and secreted insulin with the Coat-A-Count Insulin radioimmunoassay kit.
Results: Expression of 518 genes showed strong positive correlation with insulin secretion and inverse correlation with HbA1C ($p < 0.5$). Among the top 10 ranked genes (GLR1A, ATP2A3, MCF2L2, PTEN, PPP1R1A, SYT13, PLXCD3, ENO2, FAM105A, PFKFB2) several genes (ATP2A3, PTEN, SYT13 and PFKFB2) were previously shown to be involved in insulin secretion and islet function. Also, expression of these 10 genes was down-regulated in diabetic compared to non-diabetic islets. siRNA silencing of GLRA1, PPP1R1A, PLXCD3 and FAM105 in INS-1 cells showed high knockdown efficiency (70-90%). Insulin secretion in response to 2.8 mM and 16.7 mM glucose 72 hr after siRNA transfection showed a significant reduction ($p < 0.5$) for PPP1R1A, FAM105A and PLCDX3 compared to the negative control, whereas, silencing of GLRA1 had no influence on insulin secretion (Fig 1). Analysis of the insulin content in transfected cells did not show any difference compared to the siRNA negative control suggesting a defect in the secretory machinery rather than in insulin production.
Conclusion: By searching for genes whose expression in human islets was associated with low HbA1c and normal insulin secretion we identified and functionally confirmed PPP1R1A, FAM105A and PLCDX3 as potential regulators of islet function in man.

Figure 1: Gene knockdown of PPP1R1A, FAM105A and PLCXD3 in INS-1 cells resulted in reduced insulin secretion compared to negative control, whereas, silencing of GLRA1 showed no effect.



Supported by: The Swedish Research Council

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Paternal allelic mutation at the Kcnq1 locus reduces pancreatic beta cell mass via epigenetic modification of Cdkn1c

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Background and aims: In a large-scale SNP analysis of Japanese type 2 diabetes patients, a SNP in the *Kcnq1* gene on chromosome 11 was reported as a significant risk factor associated with the development of type 2 diabetes. The

Kcnq1 gene is known as an imprinting gene, and non-coding RNA *Kcnq1ot1* is expressed from the *Kcnq1* genomic region and regulates the expression of neighboring genes located adjacent to the *Kcnq1* gene via epigenetic modification. However, the association of *Kcnq1* mutation with the development of type 2 diabetes mellitus has not been shown yet. Then, we analyzed the effect of a mutation at the *Kcnq1* locus on the pancreatic β-cells to clarify the development mechanism of type 2 diabetes mellitus.
Materials and methods: We generated *Kcnq1* heterozygous knockout mice in which exon 2 of *Kcnq1* on chromosome 7 has been replaced by a neomycin resistance gene.To analyze the effect of imprinting, *Kcnq1* heterozygous mice were classified into 3 groups: wild-type mice group (WT); mice with mutations in the alleles inherited from one's mother (MH); and mice with mutations in the alleles inherited from one's father (PH).
Results: An analysis of unclassified *Kcnq1* heterozygous mice showed that the mutation did not affect insulin secretion and glucose tolerance. However, pancreatic β cell mass both at birth and at 24 weeks of age was significantly reduced in PH mice compared with WT or MH mice. Further, the expression level of *Kcnq1ot1* was significantly decreased and the expression level of *Cdkn1c* was significantly increased in the islets of PH. Then, we examined the extent of H3K27 trimethylation at the *Cdkn1c* promoter region in islets of PH, MH, and WT mice by chromatin immunoprecipitation (ChIP) analysis. The level of H3K27 trimethylation was significantly reduced in only PH mice.
Conclusion: These results suggest that disruption of *Kcnq1* results in reduced *Kcnq1ot1* expression as well as increased expression of *Cdkn1c* only when the mutation is on the paternal allele. Furthermore, histone modification at the *Cdkn1c* promoter region in pancreatic islets was found to contribute to this phenomenon. We have now shown that the regulation of gene imprinting at the *Kcnq1* locus was strongly associated with the modulation of pancreatic β-cell mass in the development of type 2 diabetes mellitus.

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Hypoxia alters the expression of the type 2 diabetes-associated Zn^{2+} transporter Slc30a8/ZnT8 and cytosolic Zn^{2+} levels in human and rodent islets

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Background and aims: Hypoxic damage often leads to the loss of functional islets during islet isolation and transplantation, and may also contribute to beta cell failure in Type 2 Diabetes (T2DM). It is not known whether susceptibility of beta cells to hypoxia might be genetically modulated. A single nucleotide polymorphism, rs13266634 in the SLC30A8 gene, is associated with an increased T2DM risk and reduced activity of the pancreatic beta cell-enriched granular zinc transporter ZnT8. We have previously observed decreased expression of *slc30a8* and reduced cytosolic free zinc $\{(Zn^{2+})_{cyt}\}$ in hypoxic mouse islets. We asked whether such changes can also be seen in rat and human islets and what the consequences of this reduced activity may be.
Materials and methods: Isolated murine, rat, and human islets as well as dispersed cells were exposed to hypoxia or normoxia (1% vs. 21% O_2) for 24 hours. For expression analysis, mRNA was isolated and quantified by qRT-PCR. $(Zn^{2+})_{cyt}$ in dispersed islet cells was measured using the FRET-based Zn^{2+} probe eCALWY-4, which was delivered to cells by adenoviral transfection. Cell death was measured by calcein / propidium iodide staining.
Results: Levels of *SLC30A8* mRNA were sharply (>50%) reduced after exposure to hypoxia of human (0.45 ± 0.06 (SE) vs. 1.00 ± 0.15, p<0.01) and rat (0.48 ± 0.05 (SE) vs 1.00 ± 0.02, p<0.001) pancreatic islets. $(Zn^{2+})_{cyt}$ was reduced in human islet cells (0.67 ± 0.13 vs. 1.00 ± 0.14, p<0.05). Likewise ZnT8 protein levels were lowered in dispersed murine pancreatic islets following hypoxia exposure as demonstrated by quantitative immunofluorescence imaging (0.63 ± 0.08 vs. 1.00 ± 0.08, p<0.01). Since we also observed a clear up-regulation of mRNAs encoding the zinc binding metallothioneins 1 and 2 in all species after hypoxia (for human islets: 6.87 ± 1.55 vs. 1.00 ± 0.17 for *MT-1*, 7.47 ± 1.02 vs. 1.00 ± 0.21 for *MT-2,* p<0.05), we asked whether altered metalloprotein abundance might be responsible for the changes in *SLC30A8* expression. Arguing against this view, there was no difference in the expression of the transporter in islets of mice lacking Mt-1 and Mt-2 (129S7/SvEvBrd/$Mt1^{tm1Bri}Mt2^{tm1Bri}$/J). On the other hand, islets from global ZnT8 null mice displayed significantly reduced expression of *MT-1* (0.47 ± 0.04 vs. 1.00 ± 0.02, p<0.01) and *MT-2* (0.44 ± 0.05 vs. 1.00 ± 0.10, p<0.01) in hypoxia, pointing to an impaired response to hypoxia of these islets. The importance of altered metallothionein regulation in hypoxic responses was also demonstrated by the fact that islets from mice null for Mt-1 and Mt-2 exhibited significantly increased cell death after hypoxia (12.7 ± 0.4 % vs. 5.7 ± 0.9 % cell death, p<0.01).
Conclusion: The present study demonstrates that ZnT8, variants of which are strongly associated with T2DM across human populations, is an important modulator of the response of pancreatic beta cells from different species to hypoxia. Lowered cytosolic Zn^{2+} levels and impaired MT1/2 induction at low oxygen tensions may thus contribute to the deleterious effects of *SLC30A8* risk alleles on insulin secretion.
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IGF-2 expression in beta cells induces susceptibility to develop diabetes

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Background and aims: The human insulin like growth factor-II (IGF-2) is located in the insulin (INS) gene region, and this locus is associated with type 1 diabetes (IDDM2). Genome-wide association (GWA) studies have reported an association between IGF-2 mRNA-binding protein 2 (IGF2BP2) with type 2 diabetes. Moreover, we have shown that local expression of IGF-2 specifically in β cells of transgenic mice (Tg-IGF2), leads to a pre-diabetes state with mild hyperglycemia, increased β-cell mass, hyperinsulinemia and altered glucose and insulin tolerance tests. Here, we use Tg-IGF2 mice to study the effect of IGF-2 and insulin signaling pathway on pancreatic islets and its implication in β cell functionality.
Materials and methods: Gene expression profile analysis in islets from IGF-2 transgenic mice was performed using GeneChip™ Affimetrix™ Technology. Islets and INS-1 cells were treated with adenoviral vectors expressing IGF-2 or recombinant IGF-2 protein. IFN-β/IGF-2 double transgenic mice were obtained and phenotyped. Treatment with streptozotocin was performed in Tg-IGF2 mice.
Results: In this work, we report that expression of IGF-2 in β-cells of transgenic mice led to β cell dysfunction and reticulum endoplasmic (ER) stress causing islet dysfunction. Data from gene expression profile of islets from Tg-IGF2 mice showed a reduction in Glut2, GcK and insulin gene expression in β cells, probably due to a reduction in the transcription factors that regulate their expression, such as Pdx1 and HNF3β. These alterations could explain the impaired insulin secretion in Tg-IGF2 mice. In addition, transgenic islets presented expression changes in genes involved in β-cell ER stress, such as SERCA, Xbp1, CHOP, and ER dilatations. In vitro studies, using adenovirus-mediated IGF-2 expression in wild type islets and islets treated with recombinant IGF2 protein, proved the direct effect of IGF-2 in β cell this functionality. IGF-2 effects were in part mediated via the insulin/IGF1 receptor pathway. Furthermore, transgenic islets showed hyperexpression of molecules involved in the immune response, such as MHC class I and II. Thus, local expression of IGF-2 in Tg-IGF2 islets might lead islets to be more sensitive to immune attack and β-cell damage. To determine whether IGF-2 overexpression in β cells could predispose transgenic mice to develop overt diabetes, we treated Tg-IGF2 mice with very low doses of streptozotocin (STZ) that do not affect control mice. After treatment, only transgenic mice became diabetic. Tg-IGF2 mice were also crossed with IFN-β transgenic mice, a mouse model that shows important lymphocytic infiltration in islets. IFN-β/IGF-2 double transgenic mice developed diabetes spontaneously during the first two months of age, whereas the single Tg-IFNβ and Tg-IGF2 mice remained normoglycemic. Therefore, Tg-IGF2 islets were more sensitive to immune attack and β-cell death.
Conclusion: These results indicate that local IGF-2 expression in islets might lead to β cell dysfunction. As a consequence, IGF-2 expressing islets are more sensitive to the immune attack and β-cell damage and trigger onset of diabetes.

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Carriers of loss-of-function mutations in EXT display impaired pancreatic beta cell reserve due to smaller pancreas volume

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Background and aims: A genome wide association study identified exostosin 2 (EXT2) as a novel risk factor for the development of type 2 diabetes mellitus. As EXT genes, involved in the chain elongation step of heparan sulfate (or HSPG) biosynthesis, are intricately involved in organ development, we hypothesized that mutations in these genes might affect pancreatic islet mass and insulin secretion capacity. Here we used a translational approach to study the effect of EXT mutations on pancreatic development, insulin secretion and glucose metabolism in mice and humans with heterozygous EXT mutations causing hereditary multiple exostoses (HME).
Materials and methods: In HME subjects and family-based non-carriers (similar age, sex, and BMI) we performed oral glucose tolerance tests (OGTTs) for insulin resistance, hyperglycemic clamps for insulin secretion capacity and abdominal MRI to assess pancreas volume. In heterozygous EXT1 or EXT2 mice we also performed OGTTs, insulin tolerance tests and harvested each mouse pancreas for extraction of islets (insulin secretion) and immunohistochemistry (beta cell mass).
Results: No differences in oral glucose tolerance and insulin resistance were found in mice and humans with EXT mutations compared to controls. No effects on insulin signalling were found in isolated islets challenged with hyperglycaemia. However, glucose stimulated insulin secretion (hyperglycaemic clamp) showed that HME subjects had a significantly altered glucose induced first-phase insulin secretion (GSIS) (IAUC carriers vs controls: 0.72 [0.46-1.16] vs. 1.53 [0.69-3.36] nmol•l-1•min-1, P=0.046) as well as an impaired beta cell reserve (upon arginine bolus)(IAUC carriers vs controls: 7,14 [4,22-10,95] vs. 10,32 [7,91-12,70] nmol•l-1•min-1 p=0.041). In line with these finding, Magnetic Resonance Imaging showed a significantly smaller pancreas volume in HME subjects compared to controls (74 [63-86] vs.87 [82-105] cm3 p=0,016).
Conclusion: Carriers of loss-of-function mutations in EXT showed impaired GSIS without insulin resistance due to reduced functional beta cell mass and decreased anatomic pancreas volume. Our data provide evidence that heparansulfates are important for normal beta-cell function in humans.

OP 05 New frontiers in autoantibody epidemiology

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N-terminally truncated GAD discriminates progression in GAD antibody positive relatives of patients with type 1 diabetes

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Background and aims: Autoantibodies to glutamate decarboxylase (GADA) are sensitive markers of type 1 diabetes (T1D), but diabetes antibody and islet autoantibody standardization program (DASP/IASP) workshops have shown that GADA assay specificity varies with assay format. IASP-2012 included a comparison between GADA assays using radiolabelled full-length human GAD65 (1-585) or N-terminally truncated GAD65 (96-585), which suggested that removal of the first 95 amino acids improved assay specificity. We aimed to determine whether a GAD65 (96-585) based assay could discriminate risk of progression in GADA positive first-degree relatives of patients with T1D (FDRs).
Materials and methods: Samples from 278 FDRs (134 male) participating in the Bart's-Oxford family study of T1D previously identified as GADA positive with a local radiobinding assay were re-assayed with the harmonized standard GADA assay using 35S-methionine labelled GAD generated by in vitro transcription/translation with plasmids encoding (1) full-length GAD65 (1-585) or (2) N-terminally truncated GAD65 (96-585). Median age at first sample was 31 years (range 1 to 57 years) and median follow-up from first sample was 13.2 years (range 0.2 to 27 years). Diabetes was ascertained by annual questionnaire. Assay thresholds were set at the 97.5th percentile of 222 healthy schoolchildren.
Results: Of 278 FDRs previously found positive for GADA, 255 were positive on re-assay using 35S-GAD65 (1-585), 204 with 35S-GAD65 (96-585) and 193 with both labels. Of 66 FDRs who progressed to diabetes, 63 were positive for GADA (1-585) and 61 positive for GADA (96-585). Kaplan-Meier survival analysis showed that because of their higher specificity, GADA (96-585) stratified risk of progression to diabetes in FDRs positive for GADA (1-585) (Figure 1, p<0.001).
Conclusion: Assays using N-terminally truncated GAD65 (96-585) offer improved specificity for diabetes with minimal loss of sensitivity. These findings confirm the lack of association between diabetes and GADA epitopes in the N-terminal region and suggest that GADA assays using GAD65 (96-585) should be adopted for diabetes screening.

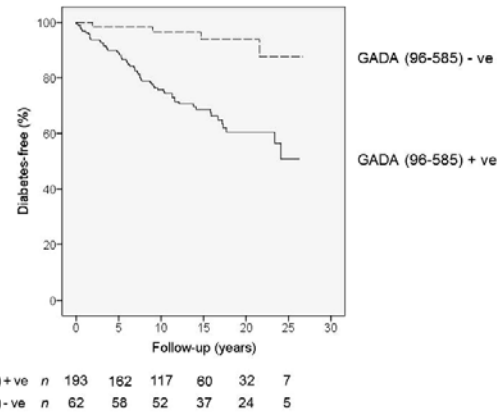

Figure 1. Kaplan–Meier survival analysis; progression to diabetes in 255 relatives of patients with type 1 diabetes found GAD autoantibody (GADA) positive using 35S-labelled full-length GAD65 (1-585), according to positivity with an assay using GAD65 (96-585) ($P < 0{\cdot}001$). The number of diabetes-free relatives remaining at each time-point is given below the axis.

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PTPN2 rs45450798 polymorphism is associated with accelerated progression of beta cell autoimmunity to clinical type 1 diabetes

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Background and aims: Natural history studies on type 1 diabetes (T1D) associated autoimmunity provide an opportunity to identify the phase where each genetic and/or environmental factor exerts its effect in follow-up cohorts of infants at increased risk for T1D. PTPN2 is one of the recently identified non-HLA genes affecting disease susceptibility and the encoded molecule has been observed to modulate beta cell apoptosis. We now set out to characterize the role of a T1D-risk associated polymorphism rs45450798 in the PTPN2 gene in various phases of the autoimmune process leading to T1D.
Materials and methods: The study subjects were participants in the prospective DIPP study and carried T1D-risk associated HLA II genotypes. The study cohort included 521 case subjects who during the follow-up turned positive for at least one biochemically-defined autoantibody (insulin autoantibodies, antibodies against glutamic acid decarboxylase -65 and/or antibodies against islet antigen -2) and 989 control subjects who remained autoantibody negative. The median follow-up time for the appearance of beta cell autoimmunity was 10.0 years (range 0.85-17.5 years) and median follow-up time for the development of T1D after the appearance of beta cell autoimmunity was 4.4 years (range 0.0-15.7 years). During the follow-up 269 case subjects were diagnosed with T1D. PTPN2 rs45450798 polymorphism was genotyped using the Sequenom platform.
Results: The PTPN2 rs45450798 polymorphism showed a borderline association with progression to T1D in the current cohort (p=0.055 comparing the CC, CG and GG genotypes, Cox regression analysis). No effect of the polymorphism was observed on the appearance of the first beta cell autoantibody (p=0.151). In contrast, a strong effect of the polymorphism on the progression rate of already-established beta cell autoimmunity was detected (p=0.001 comparing the CC, CG and GG genotypes, adjusted for seroconversion age and the presence of the high-risk HLA DR3/DR4 genotype). Among subjects homozygous for the minor C allele associated with T1D risk, 13 of 16 (81.2%) progressed to clinical T1D during the follow-up compared to 253 of 498 (50.8%) of subjects with the CG or GG genotype (p=0.000319, HR 2.820, 95% CI 1.603-4.959).
Conclusion: The PTPN2 rs45450798 polymorphism seems to affect the rate of beta cell destruction after the initiation of beta cell autoimmunity but not the initiation of beta cell autoimmunity. PTPN2 has previously been shown to modulate the activation of a specific pro-apoptotic pathway in beta cells. This is in line with the apoptosis of beta cells induced by CD8+ cytotoxic T cells dominating the insulitis in the late phase of the disease process. These findings provide new insights into the factors controlling the destruction rate of beta cells and provide one tool to identify rapid progressors in trials aimed at halting the ongoing beta cell destruction leading to clinical T1D.

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Relation between beta cell autoimmunity and levels of allergen-specific IgEs in young Finnish and Estonian children

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Background and aims: The Th1/Th2 paradigm assumes that an active autoimmune process prevents or suppresses allergen-specific IgE responses and vice versa. We set out to test this assumption in young Finnish and Estonian children.
Materials and methods: Population based cohorts of 3-year old children (52.4% males) in Finland (n=1530) and Estonia (n=1642) were tested for signs of type 1 diabetes (T1D) associated autoimmunity (autoantibodies against insulin [IAA], glutamate decarboxylase [GADA], islet antigen 2 [IA-2A], and zinc transporter 8 [ZnT8A]) and IgE-mediated allergic sensitization (total IgE and allergen-specific IgEs to cat, dog, egg, cow's milk, peanut, timothy, birch, and dust mite [D. pteronyssinus]). These children were also genotyped for HLA-conferred T1D-susceptibility.
Results: Although Finnish children carried high and moderate HLA risk genotypes more frequently than Estonian children (14.0% vs. 7.6%, p<0.001), the proportion of seroconverted children (2.6% vs. 3.0%, p=0.53) and the distributions of combinations and numbers of positive autoantibodies were comparable (p=0.55 and p=0.18, respectively) in these populations. Total IgE level was higher in Estonian children (median 33.1 vs. 24.7 kU/l, p<0.001), while allergen-specific sensitization (sIgE≥0.35 kU/l) was more common in Finnish children (35.2% vs. 27.2%, p<0.001). Levels of total and allergen-specific IgEs were similar in children with and without autoantibodies, except for timothy, which was higher in IAA-negative children (p=0.02). IAA levels were lower in children positive for cow's milk IgE (p<0.001), whereas the levels of other autoantibodies were comparable in children with and without other positive sIgEs. In the whole cohort we observed weak inverse correlations between IA-2A levels and several allergen-specific IgEs (cat [rS= -0.05, p=0.01], dog [rS= -0.06, p=0.001], peanut [rS= -0.06, p<0.001], birch [rS= -0.04, p=0.01], timothy [rS= -0.07, p<0.001], and dust mite [rS= -0.11, p<0.001].
Conclusion: Positivity for diabetes-associated autoantibodies does not prevent allergen-specific IgE-sensitizations or the other way around. The inverse associations between allergen-specific IgEs and IA-2A titers, IA2A being a marker of an advanced autoimmune process, suggest that advanced beta-cell autoimmunity may reduce allergic sensitization to certain extent.
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Incidence of type 1 diabetes in Sweden 2007-2011: reassessing the spring harvest theory

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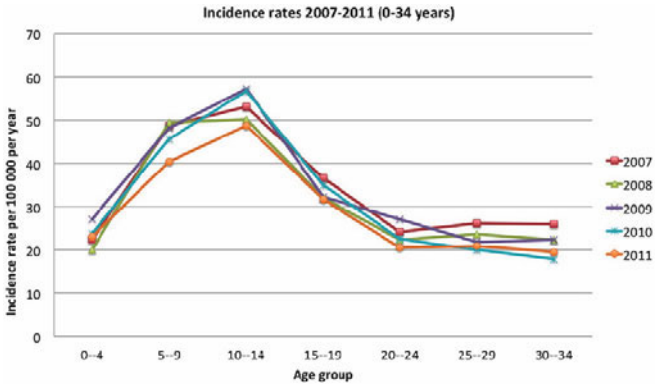
Background and aims: International multicentre studies report an annual 2.5-3.5 % increase in the incidence of type 1 diabetes (T1DM) among individuals aged 0-14 years. The steepest increase has been noted in the age group 0-4 years and it appears that onset of T1DM has shifted to younger ages. It has been postulated that the increasing incidence in the age-group 0-14 years is due to onset at a younger age and thus mirrored by a reciprocal decrease among individuals older than 14 years (i.e. spring harvest theory). Studies investigating the latter age group have been less standardized and results have been contradictory. It is unknown whether overall cumulative incidence has increased or whether disease onset has merely shifted to younger ages. Previous reports from Sweden, based on two different incidence registers, are in accordance with the spring harvest theory. We aimed to assess the reliability of these reports and attempt to estimate the incidence by other procedures.
Materials and methods: Capture-recapture methodology was used to assess level of ascertainment in the Diabetes Incidence Study in Sweden (DISS) register, which has been used to calculate incidence trends for individuals aged 15-34 years. New estimations of the incidence was done by using the Drug Prescription Register started in 2005. All subjects who collect prescribed insulin for the first time (no insulin collected the previous year), with exclusion of individuals who also obtained oral antidiabetics and females with <3 prescriptions, were counted from 2005 to 2012.
Results: DISS had 55 % level of ascertainment. For the same age-group we calculated, via our proxy for diagnosis, a three times higher incidence than previously reported with no temporal trend during 2007-2011. We recaptured 97 % of the individuals from the Drug Prescription Register in the National Diabetes Register, where 93% had a clinical classification of T1DM. Males showed consistently higher incidence rates than females. The highest incidence rate was noted for males 10-14 years which reached 64.2 per 100 000

per year in 2009. The average age-specific rates per 100 000 per year were 23.3, 46.5, 53.1, 33.5, 23.4, 22.5 and 21.7 at ages 0-4, 5-9, 10-14, 15-19, 20-24, 25-29 and 30-34 years, respectively (2007-2011; graph). Our incidence rates were comparable with figures from the Swedish Childhood Diabetes Register (individuals 0-14 years), which has outstanding level of ascertainment. We did not observe any significant temporal trend regarding age at onset (0-34 years).

Conclusion: This study questions previous reports from Sweden and contradict the postulated spring harvest theory.



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Enterovirus RNA in longitudinal blood samples from children at the highest genetic risk of type 1 diabetes, and the development of islet autoimmunity: the MIDIA study

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Background and aims: Enterovirus is suspected to participate in the pathogenesis of islet autoimmunity leading to type 1 diabetes, but only a few longitudinal molecular studies are available, and their results are conflicting. There is a considerable heterogeneity among case-control studies nested within large prospective cohorts as to the association of enterovirus RNA in blood to islet autoimmunity, and positive findings seem to be limited to the Finnish population. Our aim was to investigate such an association in a Norwegian high-risk cohort study, which represents another Nordic population that largely shares the climate, socioeconomic conditions, and lifestyle with the Finns.

Materials and methods: Children with a single HLA-DQ-DR genotype, conferring the highest genetic risk for type 1 diabetes, formed the Norwegian birth cohort MIDIA ("Environmental Triggers of Type 1 Diabetes"). Their blood samples taken at 3, 6, 9, 12 months, and then annually, were tested for autoantibodies to insulin, GAD65, and IA-2 as markers of islet autoimmunity. Among 911 children at risk, 48 cases developed positivity for two or more autoantibodies confirmed in subsequent samples by October 2011, and were included into the present analysis. Two controls from the cohort were matched to each case by the follow-up time, date of birth and county of residence. Enterovirus was tested in RNA obtained from frozen cell packs after removal of plasma, using a meticulously controlled reverse transcriptase TaqMan real-time PCR. The assay was similar to the previous works from Finland, whereas the RNA came from cells rather than serum.

Results: Enterovirus RNA was observed in 15% of the 778 tested blood samples. Positivity for enterovirus in blood was not associated with the appearance of islet autoantibodies, as demonstrated in a logistic regression model with random intercept: OR = 1.01, 95%CI 0.58 - 1.79, p=0.96 for the enterovirus positivity in the sample with first islet autoantibodies or in preceding samples. Similarly, no significant association was found in the time window just prior to developing autoantibodies, or in samples with high levels of enterovirus RNA.

Conclusion: We observed no link between enterovirus RNA in blood and the development of islet autoimmunity in a large Norwegian cohort with homogeneously high genetic risk. Of note, the detection method and the RNA source yielded high enterovirus positivity rates, rendering our study higher power than some of other works. Our negative finding does not exclude an existence of a diabetogenic virus genotype responsible for only a small proportion of infections - genotyping of enteroviruses in blood is therefore warranted, although it is technically extremely difficult.

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NPY minor autoantibodies in newly diagnosed type 1 diabetes patients

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Background and aims: Neuropeptide Y (NPY) was suggested as a minor autoantigen in a selective screening of new autoantigens targeted in Type 1 Diabetes (T1D). Nearly 9% of newly diagnosed T1D patients were found to have autoantibodies against NPY (NPYA). NPY is a neurotransmitter expressed in the central and peripheral nervous system implicated in vasoconstriction, insulin secretion and in regulation of food intake. A single nucleotide polymorphism (SNP) at rs16139 (T1128C) identified an amino acid substitution from Leucine (L) to Proline (P) (L7P) associated with atherosclerosis, impaired glucose tolerance and a three- to four-fold increased risk for Type 2 diabetes (T2D). We aimed to determine 1) the influence of NPY-L (Leucine) and NPY-P (Proline) autoantibodies on the diagnostic sensitivity of T1D, 2) the association of NPY-LA and NPY-PA with other islet autoantibodies such as GADA, IAA, IA-2A and the ZnT8A variants, and 3) the association of NPY-LA and NPY-PA to HLA-DQ genotypes in newly diagnosed T1D patients in the Skåne study.

Materials and methods: Newly diagnosed T1D patients (n=675; median age 10.2 years) from six pediatric clinical centers in Skåne, South of Sweden, were recruited between 1996 and 2005. Serum samples at diagnosis were analyzed for autoantibodies against NPY-L and NPY-P in a Radiobinding assay as well as against insulin, GAD65, IA-2 and ZnT8. The patients were genotyped for HLA-DQ genotypes. Control samples were obtained from 398 healthy blood donors in Skåne during 2004.

Results: The frequency of positive T1D patients was 17% (n=115) for NPY-LA ($p<0.001$) and 24% (n=161) for NPY-PA ($p<0.001$) as compared to 1% (n=4) of the controls. Our results showed that 2.1% (n=14) of the T1D patients were positive only for the NPY-LA compared to 0.5% (n=2) of controls while 9% (n=61) of the T1D patients were single positive for the NPY-PA variant compared to the controls who were negative for this variant. By including the NPY-LA and NPY-PA to the other major autoantibodies the autoantibody negative T1D patients were reduced from 5.5% (n=37) to 4.8% (n=32). The median levels of the NPY-LA were higher in both T1D patients (31 U/mL; $p<0.001$) and the controls (23 U/mL; $p<0.001$) compared to the levels of NPY-PA (26 U/mL; $p<0.001$) in T1D patients and the controls (16 U/mL; $p<0.001$). The levels of the two NPYA variants were strongly correlated ($R2=0.631$; $p<0.001$). The majority of the T1D patients with NPY-LA (49.6%; $p=0.005$) and NPY-PA (52.8%; $p<0.001$) were diagnosed between the age of 10 and 14 years. Neither of the NPYA variants appeared with any of the GADA, IAA, IA-2A nor the ZnT8A, although the NPY-PA variant showed a slight agreement with ZnT8W (kappa 0.07, $p=0.030$) and GADA (kappa 0.06, $p=0.040$). There were no associations observed between the NPYA positive patients and the HLA-DQ genotypes in the patients.

Conclusion: We conclude that NPY is a minor autoantigen in newly diagnosed T1D patients and therefore, autoantibodies against NPY should be further investigated in the autoimmune disease of T1D. By including the NPYA the diagnostic sensitivity of T1D was increased from 94.5% to 95.2% which may suggest that NPYA could be important at T1D diagnosis.

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OP 06 Molecular players in adipogenesis

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The novel adipokine WISP2 regulates adipogenesis and is a canonical Wnt ligand

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Background and aims: Adipose precursor cell commitment and differentiation are regulated by the canonical WNT and BMP4 signalling pathways. We have recently shown that these pathways cross-talk via WISP2 (WNT1-inducible signalling pathway protein 2), a novel adipokine which regulates PPARγ activation as an intracellular protein by inhibiting the effect of BMP4 to promote the nuclear targeting of the PPARγ transcriptional activator ZNF423. However, WISP2 is also a secreted protein and we here focus on its mechanisms of action as an extracellular protein.
Materials and methods: The experimental studies were performed with 3T3-L1 cells and NIH-3T3 cells. The cells were treated with recombinant WISP2 or Wnt3a; transfected with siRNA or full-length or truncated WISP2. The latter plasmid had a deleted N-terminal signal peptide sequence to prevent WISP2 secretion. mRNA, protein expression levels and different markers of adipogenesis were measured.
Results: We have previously shown that intracellular/cytosolic WISP2 regulates adipogenic commitment of precursor cells in response to BMP4. We here compared the effect of full-length vs secreted WISP2 and expressed both a full-length and a truncated molecule lacking the signal sequence and which, thus, was not secreted. We also compared the effects of adding rWISP2 to the cells to those of Wnt3a, a well-established canonical WNT activator. Wnt3a is able to de-differentiate mature adipose cells by inhibiting PPARγ and, remarkably, we also found that WISP2 did the same measured as significant inhibition of both C/EBPα and PPARγ as well as several PPARγ-regulated genes including GLUT4, adiponectin, aP2 and LPL. These results suggest that WISP2 may also activate canonical WNT. To further examine this, we measured expression and phosphorylation of down-stream molecules for WNT activation and found that both Wnt3a and WISP2 increased pS-LRP 5/6 as well as β-catenin serine phosphorylation, a marker for nuclear targeting, as well as total β-catenin expression. Axin, the rate-limiting protein for β-catenin degradation in the degradation complex, was also significantly down-regulated by both ligands. In line with this, we also found increased nuclear β-catenin levels in the presence of rWISP2 as well as activation of the nuclear receptor for β-catenin in a Tcf/Lef reporter assay (234%, $p< 0.05$) in NIH-3T3 cells. Importantly, we found that full-length, but not intracellular (truncated and not secreted), WISP2 increases β-catenin serine phosphorylation.
Conclusion: These results show that the secreted adipokine WISP2, which is highly expressed in mesenchymal stem cells and early precursor cells, is a regulator of adipogenesis and PPARγ activation as a canonical WNT ligand, thereby keeping the cells in an undifferentiated state. Our previous studies have shown that it also retains the PPARγ transcriptional activator ZNF423 in the cytosol and that this complex is dissociated by BMP4 thereby initiating the first steps of adipogenic commitment and PPARγ activation. However, full differentiation also requires inhibition of secreted WISP2. Thus, WISP2 plays a key role in the regulation of adipogenesis by both regulating precursor cell commitment in response to BMP4 as well as the subsequent differentiation and PPARγ activation as a WNT ligand.

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BMPs regulate glucose uptake in 3T3L1 adipocytes in an insulin independent manner

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Background and aims: With the increasing number of patients with pronounced insulin resistance and type 2 diabetes, the identification of insulin-independent mechanisms regulating glucose uptake is of great interest. Bone morphogenetic proteins (BMPs) are cytokines of the transforming growth factor beta superfamily. They are involved in several cellular processes like proliferation and migration of precursor cells but also in differentiation, maintenance and apoptosis. Furthermore, recent studies suggested an impact of BMPs during adipogenesis. To unravel a putative regulatory role of BMPs during glucose homeostasis in adipose tissue we investigated if stimulation with BMP-2 or BMP-6 influences the glucose uptake in 3T3L1 adipocytes and which related factors are regulated by an activated BMP-signaling cascade.
Materials and methods: Fully differentiated 3T3-L1 adipocytes where short/long term stimulated either with Insulin, BMP-2, BMP-6, or combinations thereof. Afterwards, H3-2-deoxyglucose uptake was compared to unstimulated cells. For immunofluorescence based visualization of BMP-mediated GLUT-4 translocation we used a stable 3T3L1 cell line expressing a cMYC-GLUT4-GFP fusion protein. Alterations in the expression of factors related to GLUT-4 translocation and BMP-signaling after stimulation for 2 to 24 hours where measured by Real Time PCR and Western Blot.
Results: Activation of the BMP-signaling cascade by BMP-2 but particularly by BMP-6 leads to an up to 4-fold increase of glucose uptake in 3T3L1 adipocytes compared to controls ($p<0.005$). A combined long term BMP-6/short term insulin treatment had a synergistic effect with up to 6-fold and 2-fold higher uptake rates compared to controls ($p<0.01$) and BMP-6 or insulin treated cells ($p<0.05$), respectively. The increase in glucose uptake was mediated by translocation of GLUT-4 vesicles to the cell surface. On mRNA level PPAR gamma expression was significantly increased already after 2 hours of BMP-6 stimulation, which suggests direct BMP-mediated transcriptional control by binding of Smad-complexes to the PPAR gamma 2 promoter. The mRNA expression of insulin receptor and leptin decreased continuously in long term BMP-6 stimulated cells. Expressions of BMP-6 bound BMP receptors ALK 3, BMPRII and ActRIIa decreased under BMP-6 stimulation after 8 and 18 hours suggesting a negative feedback mechanism after activation of these receptors.
Conclusion: Glucose uptake in 3T3L1 adipocytes is increased by BMP-stimulation especially after long term BMP-6 treatment. The effect is mediated by GLUT-4 vesicle translocation to the cell surface. Combined BMP-6/Insulin treatment acts synergistically to increase glucose uptake rates to a maximum level compared to BMP-6 or insulin treatment alone, which suggest distinct mechanisms of action during regulation of glucose uptake. We hypothesize a BMP-dependent functional interaction of the BMP- and Insulin-signaling pathways during regulation of glucose homeostasis, in which BMP-6 acts as insulin sensitizer.
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SHORT syndrome with partial lipodystrophy due to impaired PI 3-kinase signalling

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Background and aims: Phosphatidylinositol (PI) 3-kinase signaling regulates fundamental cellular processes such as metabolism, proliferation and survival. A central component in this pathway is the p85α regulatory subunit, encoded by PIK3R1.
Materials and methods: We studied two independent families with autosomal dominantly inherited short stature, facial dysmorphism, low body mass index (BMI), Rieger anomaly, teething delay (SHORT syndrome), diabetes and partial lipodystrophy. We used whole-exome sequencing, micro array genotyping, structural modeling and functional studies including insulin signaling in vitro, as well as PI kinase assays in the investigations.
Results: Using whole-exome sequencing, we identified a heterozygous PIK3R1 mutation (R649W) in the two unrelated families with SHORT syndrome and partial lipodystrophy. This mutation led to impaired interaction of p85α with IRS-1 and reduced AKT-mediated insulin signaling in patient fibroblasts and reconstituted pik3r1 knockout preadipocytes.
Conclusion: Normal PI 3-kinase activity is critical for adipose differentiation and insulin signaling, the mutated PIK3R1 therefore provides a unique link between lipodystrophy, growth and insulin signaling.
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The role of microRNA 107 in the regulation of adipogenesis

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Background and aims: MicroRNAs (miRNAs) are a family of small, endogenously expressed, non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. Several miRNAs were reported to be expressed in adipocytes and seem to play a role in the regulation of adipogenesis even with potential impact on adipogenesis dysfunctions. In this study we investigated the effect from miRNAs on human adipogenesis by inhibition of the miRNA processing. Furthermore, we searched for miRNAs that are differentially regulated during human adipocyte differentiation and their putative target genes.
Materials and methods: *In vitro* experiments were performed in human Simpson-Golabi-Behmel syndrome (SGBS) cells, a cell model for human adipogenesis. MiRNA processing was inhibited by knock down of Drosha and Dicer, key enzymes of the processing machinery. Effects on adipogenesis were measured by Oilred staining, counting of differentiated cells and expression analysis of *PPARg*. Regulated microRNAs were identified using Illumina technology based miRNA sequencing. Results were validated by real time PCR.
Results: A knock down of Drosha and/or Dicer significantly reduced fat accumulation to 42±6.8 % and *PPARg* expression by trend to 58±26 %. Sequencing experiments identified 7 miRNAs at least 2.5 fold induced and 12 miRNAs at least 0.4 fold repressed during adipogenesis. MiR107 was 2.5 induced at d12 of differentiation. We could identify Progranulin as a putative target of MiR107. Therefore, *Progranulin* is inversely regulated compared to miR107 during SGBS adipogenesis. Ectopic expression of miR107 in preadipocytes mediated a reduction of Progranulin and inhibits adipogenesis. Furthermore, a knock down of *Progranulin* leads to a significantly reduced fat accumulation to 73±11% (P=0.05), which is accompanied by a decreased *PPARg* (P<0.001) and *aP2* (P<0.001) mRNA expression.
Conclusion: Inhibition of miRNA processing reduced human adipocyte differentiation capacity. We identified 19 miRNAs differential regulated during human adipogenesis and showed that Progranulin is a target of the upregulated miR107. Our results support a regulatory function of miR107 and Progranulin during adipogenesis.

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Regulation of PPARγ activity by a novel serine phophorylation

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Background and aims: The mammalian ste20 kinase (MST) pathway, originally identified as hippo pathway in Drosophila, plays an important role in the regulation of apoptosis and cell cycle control. Previously, we showed that MST2 activates PPARγ and induces adipocyte differentiation. To further characterize this phenomenon, we sought to identify and analyze the phosphorylation of PPARγ by MST2 kinase.
Materials and methods: We performed a mass spectrometric analysis of PPARγ protein which was co-expressed with MST2. We employed various protein analysis methods and Oil red staining to analyze the effect of the phosphorylation in HEK293 cells and 3T3-L1 adipocytes.
Results: Using mass spectrometric analysis, we detected a novel Ser phosphorylation site in PPARγ protein that was co-expressed with MST2. Mutation of Ser to Ala (SA) resulted in a significant decrease in transcription activity of PPARγ compared to WT. Interaction of PPARγ with PGC1β, one of the various PPARγ coactivators, was increased by rosiglitazone. However, SA mutant showed significantly inhibited interaction with PGC1β, indicating the identified Ser phosphorylation may be important for the binding of PPARγ to its coactivators. SA mutation significantly decreased PPARγ-induced adipocyte differentiation of 3T3-L1 cells. Finally, a rabbit pSer-specific antiserum was prepared and detected a strong phosphorylation in PPARγ-WT but not in SA.
Conclusion: In this report, we present a novel Ser phosphorylation site in PPARγ and propose that this phosphorylation may be a novel regulatory mechanism of PPARγ activation by MST pathway.

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The transcription factor Prep1 impairs adipose tissue differentiation

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Background and aims: Adipose tissue is crucial for maintaining energy and metabolic homeostasis. Adipose tissue functionality is closely related to the adipocytes differentiation state, which is regulated by several transcriptional factors. Prep1 is an homeodomain transcription factor belonging to the TALE proteins, which plays an important role in hematopoiesis, organogenesis and development. Previous studies have indicated that Prep1 hypomorphic ($Prep1^{i/+}$) mice, which express only 55-57% of protein, have a complex metabolic phenotype with at least two relevant features. One is the presence of smaller but otherwise normally structured islets with reduced fasting and post-loading plasma insulin levels. The second is increased insulin sensitivity in skeletal muscle and in liver which is accompanied by protection from streptozotocin-induced diabetes. In this study we have focused our attention on the role of Prep1 on the regulation of adipocytes differentiation and on the adipose tissue functionality.
Methods and results: To understand the possible role of Prep1 in adipose tissue we have evaluated the expression of some markers of adipocytes differentiation, including PPARγ, C/EBPα, GLUT4 and aP2/FABP4 in Prep1 hypomorphic and WT mice. Western blot and qRT-PCR experiments show a significant increase of C/EBPα, GLUT4 and aP2/FABP4 protein and mRNA expression in adipose tissue of Prep1 hypomorphic mice, while PPARγ does not change. In addition, insulin- stimulated glucose-uptake is increased in adipocytes isolated from adipose tissue of $Prep1^{i/+}$ mice compared to the adipocytes from WT mice. To further study the function of Prep1 on adipocytes differentiation, we have analyzed Prep1 expression during different steps of adipogenesis in 3T3-L1 murine fibroblasts. Levels of Prep1 are progressively reduced during the conversion from preadipocytes to adipocytes 3T3-L1. Moreover, adipocytes 3T3-L1 stable transfected with Prep1 cDNA show reduced lipid accumulation, after Oil Red-O staining, and reduced mRNA and protein expression of the adipogenic markers C/EBPα, GLUT4 and aP2/FABP4.
Conclusion: All together these data suggest that Prep1 regulates adipocytes differentiation giving a rationale to investigate Prep1 as possible new therapeutic agents in preventing adipose tissue dysfunctions.

OP 07 Type 1 diabetes: lifetime impacts

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A comparison of type 1 diabetes phenotype in a young multi-ethnic urban population

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Background and aims: Type 1 diabetes (T1DM) is a heterogenous condition, presenting with variable phenotypes. The South Asian (SA) and African Caribbean (AC) population in the UK have a higher proportion of type 2 diabetes than the White European (WE) population, but the incidence of T1DM in these groups approaches that of European populations. Whilst studies have demonstrated phenotypic differences in type 2 diabetes (T2DM) between ethnic groups, such as a lower BMI in SA individuals and higher HbA1c in AC individuals, very few studies have assessed the phenotypic differences in a multiethnic T1DM population.
Materials and methods: A cross-sectional retrospective study of subjects with T1DM diagnosed below 35 years of age, from WE, AC or SA ancestry was undertaken from an electronic database in a large multiethnic London diabetes clinic. Age at diagnosis, duration of diabetes, BMI, HbA1c, microalbuminuria, blood pressure, and lipid profiles were compared between ethnic groups. A sub-analysis of subjects diagnosed between 16-35 years was additionally undertaken to assess the same parameters in young-adults diagnosed with T1DM.
Results: 642 individuals diagnosed with T1DM before 35 years of age were identified over a 10-year period. 88% of the population was WE, with 6% each from SA and AC populations. Table 1 shows the differences in measured parameters between each ethnic group with significance. Comparing the WE group to the AC group; BMI was not significantly different, however duration of diabetes was significantly longer with associated younger age at diagnosis in the WE group, HbA1c and microalbuminuria were also lower than in the AC group. Comparing the WE group to the SA group; HDL and systolic BP were significantly higher in the WE group. On comparison of the AC group to the SA group; HbA1c and systolic BP were significantly higher in the AC group. Sub-analysis of those diagnosed 16-35 years (n=346), revealed similar differences in HbA1c, microalbuminuria and BP, but HDL levels were additionally higher in the WE group compared to the AC group and duration of diabetes was similar across all groups.
Conclusion: There are significant differences in the biochemical and clinical attributes of people with T1DM between ethnic groups, with differences in HbA1c, microalbuminuria and blood pressure for the same BMI . In this cohort the changes were most marked when comparing the WE to the AC group, the latter demonstrating poorer control with evidence of microvascular complications and higher BP despite a significantly shorter duration of diabetes and similar BMI. HDL levels were lower in the adult-onset AC group, but other differences persisted despite a similar duration of diabetes. Only some of these differences however, were observed comparing SA individuals to the WE group. These results suggest marked heterogeneity of T1DM between ethnic groups, which may reflect differing underlying pathophysiologies or variations in management. Further study is required to delineate the factors that underlie these differences.

Differences in measured parameters between ethnic groups, diagnosed <35years

Parameters median(IQR)	White European (WE)	African Caribbean (AC)	South Asian (SA)	Significant
Age at diagnosis (years)	16.7 (10.1-23.3)	19.4 (12.8-26.1)	19.1 (12.2-25.9)	
Duration (years)	27.0 (15.6-38.4)*	20.7 (14.1-27.3)*	23.0 (13.2-32.8)	P<0.05
BMI (kg/m2)	25.0 (22.3-27.7)	25.7 (22.5-28.9)	25.3 (22.2-28.5)	P<0.05
Systolic BP (mm/Hg)	130 (119-141)*	135 (121-149)**	122 (112-133)*/**	P<0.05
Diastolic BP (mm/Hg)	75 (69-81)	80 (72-88)*	73 (67-79)*	P<0.05
HbA1c (%)	8.0 (7.1-8.9)*	9.1 (7.6-10.7)*/**	8.3 (7.5-9.2)*/**	P<0.05
Microalbuminuria (mg/mmol)	1.2 (-0.5-3.0)*	3.7 (-44.5-51.9)*	1.2 (-1.4-3.8)	P<0.05
Total Cholesterol (mmol/L)	4.50 (3.90-5.10)	4.40 (3.90-4.90)	4.00 (3.2-4.8)	
HDL (mmol/L)	1.49 (1.21-1.77)*	1.25 (0.95-1.56)	1.30 (1.47-1.14)*	P<0.05
Triglyceride (mmol/L)	0.93 (0.59-1.28)	0.99 (0.58-1.40)	1.07 (0.76-1.39)	

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An international collaboration to compare glycaemic control among people with type 1 diabetes

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Background and aims: Optimising glycaemic control in type 1 diabetes has been shown to reduce microvascular complications. Most guidelines recommend a target HbA1c of 6.5 to 7.5% (48 to 58 mmol/mol). Our aim was to investigate whether comparison of HbA1c of populations with type 1 diabetes in different healthcare systems is feasible as a first step in informing approaches to improving glycaemic control.
Materials and methods: Through open invitation, and with appropriate permissions data were obtained for 142,260 children and/or adults with type 1 diabetes defined using the best available information for each country from 12 countries in total (Austria, Denmark, Germany, Italy, Latvia, New Zealand, Norway, Scotland, Slovenia, Sweden, Ukraine, United States with data from Australia, England, Finland, France and Northern Ireland anticipated) for the previous 12 to 24 months from both population-based registers and clinic-based databases. Data were analysed where available for more than 100 people in each age stratum (<15 years, 15-29 years, 30+ years). The sample size by country therefore varied from 340 to 27,904. Median HbA1c and interquartile range (IQR) were estimated from aggregated data within age strata. Proportions in each population with above target HbA1c (defined as HbA1c ≥7.5% (≥58mmol/mol)) were compared in the same age strata.
Results: Estimated median HbA1c was 7.75% (IQR 7.25% to 8.75%) in individuals <15 years (n=29673), 8.25% (7.25% to 9.55%) in individuals between 15 and 29 years (n=36812) and 8.25% (7.25% to 8.75%) in individuals 30+ years (n=73622). The proportion with HbA1c above target varied between countries from 53.6% to 84.3% for people <15 years of age (n = 29,727), from 50.4% to 86.7% in people of 15 to 29 years (n = 36,812) and from 45.5% to 79.1% in those 30+ years (n = 73,629).
Conclusion: Substantial variations in glycaemic control among people with type 1 diabetes exist between countries. Further work is required to investigate the roles of selection bias and HbA1c assay variation on these preliminary findings before the data can be used to influence health service development. We encourage the development of true population based registers in all health systems and welcome collaborators from other countries.

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The impact of a childhood onset of type 1 diabetes on higher education and labour market outcomes

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Background and aims: Childhood onset of Type 1 diabetes mellitus (DM) may place great strain on the affected individuals, their families and society. The time consuming treatment requires lifestyle changes and the complications of the disease may increase absenteeism and reduced work capacity. This study investigates the effect of a childhood onset of Type 1 diabetes on university education and labour market outcomes in early adulthood.
Materials and methods: Individuals, diagnosed <15 years and born in 1972-1978, were selected from the Swedish Childhood Diabetes Register, which was linked to national population registers including the Longitudinal integration database for health insurance and labour market studies (LISA). For each individual (n=2,485), four controls from the general population, matched for year of birth and residency at the time of the diagnosis, were selected by Statistics Sweden (n=9,940). Data concerning annual earnings was available from 1990 to 2010 which allowed the analysis to consider the time period after graduation from upper secondary school (age 19) to the age of 31. Linear, logistic and panel data (random effects) regression was used to conduct the analysis, controlling for demographics and socioeconomic background.
Results: The results indicate that individuals with diabetes were less likely to have a university degree at 31 years of age (OR 0.81, 95% CI 0.70-0.94 for both women and men) and they were also less likely to have attained higher education for 3 years or longer (OR 0.77, 95% CI 0.66-0.90 and 0.78, 95% CI 0.66-0.92 for women and men, respectively). Further, individuals with diabetes were less likely to be employed at age 31, particularly women (OR 0.64, 95% CI 0.52-0.79 and 0.70, 95% CI 0.55-0.90 for women and men, respectively).

Among those employed at age 31, the results also showed negative effects on earnings, although not significant for women (-5% women, p=0.192 and -13% men, p<0.001). The largest negative effect on earnings was found among men without higher education (-16%; p<0.001). The results using panel data analysis showed lower earnings for people in the ages 19-31 years with diabetes active on the labour market (women -9.30%; p<0.001, -5.27%; p=0.023). The negative effect was larger among those without university education, especially for women, whereas no significant difference was seen among men with university education.
Conclusion: The results showed that Type 1 diabetes with childhood onset negatively impacts the level of education and labor market outcomes among young adults. Women with diabetes were generally worse off, with the greatest impact among women with pre-university education only.

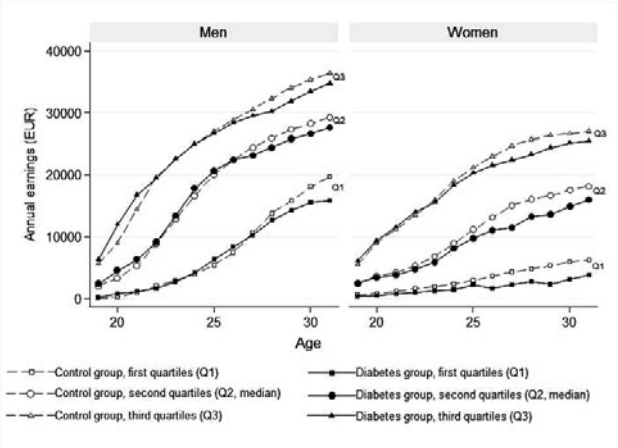

Figure 1: Development of annual earnings for the diabetes group and the control group in the age between 19 to 31 years.

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Early retirement in patients with type 1 diabetes: data from a nationwide multicentre survey in Brazil
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Background and aims: Type 1 diabetes mellitus (T1D) is a chronic disease which carries a great risk of morbidity and mortality, as a result of the microvascular and macrovascular complications that reduce individual's quality of life and life expectancy. In general T1D patients had less probability to be in the workforce, had more work-loss days that could be enhanced by the presence of any diabetes-related complication. Moreover, the rate of unemployment is greater in T1D, of 5.6% in comparison to T2D, of 1.9% and non diabetic individuals, of 2.9% . This data must be interpreted with the knowledge that the majority of T1D were in a labor age and in the most productive years of their lives. Objective: To evaluate the prevalence, causes and predictors of early retirement in patients with T1D in Brazil considering the absence of national data on this topic.
Material and methods: This was a cross-sectional, multicenter study conducted between December 2008 and December 2010 in 28 public clinics in 20 Brazilian cities. Data were obtained from 3,180 patients, aged 22 ± 11.7 years, of whom 56.3% were females, and 57.4% were Caucasians. The mean time since diabetes diagnosis was 10.3 ± 8.1 years. Working status was evaluated in T1D with criteria to be employed or retired, n=2,046 (64.4%), which were subdivided according to self-reported retirement because of diabetes and employed but aged enough to be retired. Patients retired at expected age, n=22 (0.7%), T1D without criteria to be employed or retired (n=1,106, 34.8%) and patients with permanent disability due to other diseases, n=6 (0.2%) [(Down Syndrome, n=4, congenital amaurosis, n=1 and schizophrenia, n=1); were excluded from this study.
Results: The prevalence of retirement because of T1D was 4.2%, without difference between genders. The mean age of retirement in patients retired because of T1D was 35.5 ± 9.3y, resulting in more than 15 y of workforce loss in our society. Retired patients had a significantly higher prevalence of severe hypoglycemia, proliferative and non-proliferative retinopathy, foot disorders, clinical nephropathy, chronic renal disease under conservative and under dialysis treatment and had undergone renal transplantation than employed patients. Cataract, glaucoma, psychological disorders, hypertension and overweight/obesity were also significantly more frequently found in patients that retired because of diabetes in comparison to employed patients. Multivariate logistic regression model performed for retirement (yes/no) as the dependent variable showed an adjusted odds ratio,(95% confidence interval) [OR, 95%CI] of 4.81(2.66-8.69) for the presence of microvascular complications, of 3.5 (1.95-6.59) for the presence of macrovascular complications, of 1.08 (1.05-1.11) for age, of 1.06 (1.02-1.10) for duration of diabetes.
Conclusions: It is imperative in our country, to change the approach given to T1D by the diabetes care team in terms of education and follow-up to a proactive model, in order to avoid or postpone the occurrence of chronic complications and consequently early retirement, which might in many ways worsen an already compromised quality of life. Considering the great workforce loss resulting from early retirement in T1D, this fact must be considered an important issue this group of patients.
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15 year follow-up of quality of life in type 1 diabetes mellitus: no impact of treatment mode (FANTA-1)
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Background and aims: Over the last decades intensified insulin therapy with multiple daily injections (MDI), or continuous subcutaneous insulin infusion (CSII) has become the treatment of choice for patients with type 1 diabetes mellitus (T1DM). The aim of the current study was to evaluate metabolic control and health related quality of life (HRQOL) in a T1DM population treated with different insulin regimes during a 15 year follow-up.
Materials and methods: As part of a prospective cohort study which was initiated in 1995, 283 patients with T1DM were included. To determine long term changes in HRQOL and glycaemic control data from subjects who completed baseline and follow-up measures in 2002 and 2010 were analysed. Linear mixed models were used to calculate estimated values and to test differences between the three moments in time and the three treatment modalities.
Results: At baseline, in subjects who completed follow-up (n=113, 63.7% male) the median age was 37.1 (IQR 29.4, 43.0) years and diabetes duration 13.4 (5.5, 21.2) years. Among these patients significant changes (mean Δ (95% confidence interval (CI)) in BMI (2.4 kg/m^2 (0.98, 3.8)), systolic blood pressure (-6.4 mmHg (-11.4, -1.3)), serum creatinine (-10.1 micromol/L (-14.8, -5.4)) and EuroQol-VAS (-7.3 (-11.4, -3.3)) were observed over time (p<0.05). Regarding mode of therapy, 33 patients switched from MDI to CSII at some point during follow-up. A total of 52 patients remained on MDI and 28 remained on CSII throughout follow-up. Among patients on MDI, HRQOL decreased significant in time: mental component summary (-9.8 (-16.3, -3.2)), physical component summary (-8.6 (-15.3, -1.8)) and EuroQol-VAS (-8.1 (-14.0, -2.3)), p<0.05 for all. The latter also decreased over time among CSII users: -9.6 (-17.5, -1.7, p<0.05). There were no significant differences between patients that remained on either therapy or patients that switched from MDI to CSII).
Conclusion: Although some parameters of HRQOL decreased among patients on MDI and CSII, it remained stable for the whole group of T1DM patients after long-term follow-up, irrespective of changes in BMI and blood pressure. There were no differences with respect to metabolic and HRQOL parameters between the various treatment modalities.

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Explanatory factors of emotional diabetes distress and glycaemic control in type 1 diabetes
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Background and aims: The majority of patients with type 1 diabetes are expected to self-manage their disease. Diabetes is a heavy burden for the pa-

tients and psychological problems are highly prevalent among patients with type 1 diabetes. Little is known about the relationship between social factors, psychological factors and glycaemic control. The objective of this study was to investigate and compare explanatory factors of the variance in diabetes distress and HbA1c respectively - in patients with type 1 diabetes. We investigated the association of low social status, living without a partner, lack of social support and poor self-management behaviours to poor glycaemic control (HbA1c) and to emotional diabetes distress. Furthermore, we investigated the association of HbA1c level to the variance in emotional diabetes distress and the association of emotional, interpersonal, regimen and physician distress to the variance of HbA1c.

Materials and methods: We used a cross-sectional survey of 2419 Danish patients with type 1 diabetes and data from an electronic patient record. Data were analysed using stepwise addition of covariates in multiple linear regression models with HbA1c and emotional diabetes distress as dependent variables. We included patient characteristics: sex, age, diabetes duration, complication status and other chronic illness as covariates in all models.

Results: Results showed that patient characteristics, social status, cohabitation status and social support accounted for 23 % (P<0.0001) of the total variance in emotional diabetes distress. Adding HbA1c and self-management behaviours raised the explained component to 25% (P<0.0001). Furthermore, patient characteristics, social status, cohabitation status and social support accounted for 8 % (P<0.0001) of the variance in HbA1c. Adding emotional, interpersonal, regimen and physician diabetes distress covariates explained 19% (P<0.0001) and adding self-management behaviours raised it to 21% (P<0.0001).

Conclusion: Our study highlights the relationship between social factors, psychological factors and glycaemic control. The study points towards a web of causes influencing psychological distress as well as glycaemic control. The large unexplained component of variance calls for further research into factors explaining the variance in glycaemic control and emotional diabetes distress

OP 08 Incretins: What's new?

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GLP-1 and the DPP-IV inhibitor saxagliptin similarly protect human beta cells from palmitate-induced toxicity: potential role of autophagy regulation

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Background and aims: GLP-1 protects beta cells from lipotoxicity; moreover, the recent discovery of GLP-1 and DPP-IV in human pancreatic islets (HI) has raised interest on the possibility of a direct action of this local GLP-1 system on islet cell pathophysiology. Here we studied the role of GLP-1 and the DPP-IV inhibitor saxagliptin on isolated HI exposed to palmitate toxicity, and explored some of the possible mechanisms involved, including autophagy.

Materials and methods: HI were isolated from the pancreas of 14 multiorgan donors (age: 71±13 yrs, M/F: 8/6, BMI: 26.5±3.0 kg/m2) by enzymatic digestion and density gradient purification. Survival, functional, ultrastructural and molecular studies were performed with HI incubated for 48h at control conditions (Ctrl) or in the presence of 0.5 mM palmitate (lipotoxic condition), with or without the addition of 10 nM GLP-1 or 50 μM saxagliptin, either alone or in combination.

Results: As expected, palmitate caused increased beta cell death (7.0±1.1 vs 1.0±0.2% of Ctrl, p<0.01) and blunted glucose-stimulated insulin secretion (stimulation index: 1.6±0.5 vs 2.2±0.9, p<0.05). This was accompanied by significant reduction of insulin granules, increased ER and mitochondrial volume (all quantified by electron microscopy) and unchanged expression (by qPCR) of glut2 and glucokinase. GLP-1 or saxagliptin alone similarly decreased palmitate-induced beta cell death (-40%), improved insulin secretion (+40%) and preserved, at least in part, organelle ultrastructure. A synergic effect of GLP-1 and saxagliptin was only seen on insulin secretion, that doubled compared to that of palmitate exposed islets. Incubation with palmitate led to the accumulation of autophagosomes in beta cell cytoplasm (0.8±0.1 vs 0.2±0.1 ml% of Ctrl, p<0.01), that associated with increased expression of the autophagic genes Beclin 1, ATG5, ATG7 and LAMP2. Co-incubation with GLP-1 or saxagliptin resulted in normalization of autophagosome volume density and autophagy gene expression.

Conclusion: Since saxagliptin had protective effects on lipotoxicity similar to GLP-1, it is suggested that inhibiting DPP-IV can have direct beneficial actions on human islet cells, which was supported by saxagliptin and GLP-1 synergic potentiation of glucose-stimulated insulin release; changes at the autophagy level may play a role.

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Direct enhancement of insulin secretion by dipeptidyl peptidase 4 inhibitors in pancreatic islets: studies in incretin receptor deficient mice

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Background and aims: Type 2 diabetes (T2D) is characterized by insulin insufficiency and insulin resistance. Successful treatments for T2D have been achieved by improving insulin secretion by targeting the incretin hormones glucose dependant insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP-1). Inhibition of dipeptidyl peptidase 4 (DPP-4), the enzyme that degrades and inactivates GIP and GLP-1, has proven to be a successful strategy for treating T2D. DPP-4 inhibitors effectively lower HbA1C in type 2 diabetic individuals without increasing circulating GLP-1 and GIP to supraphysiological levels. This has led many to question whether the effects of DPP-4 inhibitors are mediated solely through preserving intact intestinally secreted GLP-1, allowing it to exert insulinotropic effects on beta cells. Recent studies have demonstrated that alpha cells express and secrete GLP-1 and GIP and that the islet secreted incretins exert their insulinotropic effect directly on adjacent beta cells. We hypothesize that DPP-4 activity is also present in mouse and human islets and that DPP-4 inhibitors exert some of their effects on insulin secretion by preserving intact GLP-1 and GIP secreted from alpha cells.

Materials and methods: Islets were isolated from female C57BL/6 (WT) mice as well as GIP receptor knockout (GIPR-/-) or double incretin receptor knockout (DIRKO) mice, which are doubly deficient in the GIP and GLP-1 receptors. Islets were incubated in media containing 2.8 or 16.7 mM glucose with or without the addition of the DPP-4 inhibitor NVP-DPP728. Insulin secretion was determined by ELISA. Dipeptidyl peptidase 4 activity was assayed in homogenates of frozen mouse and human islets.
Results: DPP-4 activity was readily detected in mouse and human islet homogenates. The enzymatic activity of DPP-4 in mouse islet homogenates was lower than that in plasma from the same animals (36.0 ± 1.8 vs. 139.8 ± 4.4 pmol/minute/mg protein in islets and plasma, respectively). In frozen human islet homogenates, DPP-4 activity was detectable but lower than that of mouse islets (36.0 ± 1.8 vs. 9.8 ± 1.0 pmol/min/mg protein in mouse and human islets respectively). Glucose stimulated insulin secretion from isolated mouse islets was potentiated by the DPP-4 inhibitor NVP-DPP728. The GLP-1 receptor antagonist Ex9-39 inhibited insulin secretion in both the presence and absence of NVP-DP728. The effect of DPP-4 inhibition on glucose stimulated insulin secretion was further determined in islets from GIPR (-/-) and DIRKO mice. Islets isolated from GIPR (-/-) mice showed robust glucose stimulated insulin secretion and this was significantly potentiated by treatment with NVP-DPP728 (855 ± 49 vs. 1143 ± 95 pmol/min/islet, p=0.03 in untreated vs. NVP-DPP728 treated, respectively). Islets from DIRKO mice showed significantly impaired basal and glucose stimulated insulin secretion and were not responsive to treatment with NVP-DPP728.
Conclusion: Human and mouse islets contain appreciable DPP-4 activity and inhibition of this activity directly potentiates insulin secretion. Islet derived GLP-1 makes a substantial contribution to basal glucose stimulated insulin secretion and the insulin secretion induced by DPP-4 inhibition in vitro. The effect of DPP-4 inhibition on insulin secretion is maintained in islets from GIPR(-/-) mice but not in DIRKO mice lacking both GIP and GLP-1 receptors.
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Pulsatile secretion of glucagon-like peptide-1(GLP-1) from pancreatic alpha cells: evidence for independent mechanism from intestinal GLP-1 secretion in rodents
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Background and aims: It has been reported that pancreatic islet(alpha) cells in rodents have a potential to secrete glucagon-like peptide 1(GLP-1) as well as glucagon, but the secretion profile and its function are not well known. We hypothesize that GLP-1 from alpha cells has the paracrine action to maintain the beta cell function in the islet by the different mechanism from that of the intestinal GLP-1. In this study, the secretion profile of GLP-1 from pancreatic alpha cells was compared with that from the intestine.
Materials and methods: Male F344/JCL rats at the age of 11-13 weeks were fasted for 18 hrs before perfusion study. Rats were divided into 2 groups; simultaneous perfusion of pancreas and intestine (P-I perfusion) and pancreas perfusion without the intestine. Tissues were perfused with Krebs-Ringer-bicarbonate-HEPES with 30 % dextrin, 0.25% BSA, 40 nM vildagliptin and 50 μg/ml aprotinin continuously gassed with 95% O_2 5 %CO_2 mixture at 37 ℃, through the celiac and superior mesenteric artery via a catheter inserted into the abdominal aorta. The perfusate from the portal vein was collected sequentially in 1 min intervals, and then subjected for the measurements of GLP-1, insulin and glucagon. The perifusion study using isolated mouse islets was also performed to directly examine a secretion profile of the pancreatic GLP-1.
Results: In P-I perfusion, 12 mM glucose, 2 mM isobutyl-methylxanthine (IBMX), or 20 nM glucose-dependent insulinotroic peptide (GIP) stimulated significantly both insulin and GLP-1 secretion with the peak of 2 min and 6 min, respectively. In pancreas perfusion, the similar effects of these secretagogues were also observed on insulin secretion, but not on GLP-1 secretion. Instead, the pulsatile secretion of GLP-1 was observed in the pancreas perfusion. Continuously repeated 5-7 cycles with peak and bottom in 60 min perfusion were selected from each experiment. Peak to peak intervals were 8.05±0.09 min (mean±SE, n=39 from total 6 rats). The peak and bottom GLP-1 concentrations were 2.50±0.15 pM and 1.59±0.09 pM, respectively (n=39, p<0.0001 with t-test). Pulsatile GLP-1 secretion was not affected by glucose, IBMX, GIP or IL-6. On the other hand, the secretion of pancreatic GLP-1 as well as the intestinal GLP-1 was suppressed by addition of 20 nM somatostatin (SST). The unique profile of pancreatic GLP-1 secretion was confirmed in the perifusion study using isolated mouse islets.
Conclusion: In the present study, we demonstrated the pulsatile secretion of GLP-1 from pancreatic islet (alpha) cells probably through an independent mechanism of the intestinal GLP-1 secretion. The physiological role of pancreatic GLP-1 remains to be further elucidated.

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Novel glucagon receptor antagonist peptides help alleviate streptozotocin induced diabetes and preserve beta cell function in mice
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Background and aims: Studies on glucagon receptor KO mice have shown that they are resistant to streptozotocin (STZ) induced diabetes and retain normal glycaemic control and glucose tolerance. In this study, the effects of chronic administration of two novel glucagon receptor antagonist (GRA) peptides was assessed in STZ-induced type 1 diabetic mice.
Materials and methods: Male NIH Swiss mice (8 weeks old) were fed a high fat diet (45% fat) for 10 days. Groups of mice (n=8) were treated with either a single intraperitoneal (i.p.) injection of STZ (125 mg/kg body weight) or saline (controls) and maintained on the high fat diet for 6 days with daily i.p. saline injections. Once daily i.p. GRA peptide injections were then commenced for 18 days with either desHis^{1}Pro^{4}Glu^{9}glucagon or its closely related acylated analogue desHis^{1}Pro^{4}Glu^{9}Lys^{12}(glut-PAL)glucagon, or daily saline injections were given to both a STZ-treated control or a healthy control group (no STZ). Body weight, blood glucose and plasma insulin were measured at regular intervals over 18 days. After 18 days treatment, various assessments were performed including an OGTT (2 g glucose/kg bw), insulin sensitivity test (25 U insulin/kg bw), energy expenditure by indirect calorimetry using an Oxymax complete laboratory animal monitoring system, and quantification of terminal plasma glucagon and pancreatic insulin content by RIA.
Results: desHis^{1}Pro^{4}Glu^{9}glucagon and desHis^{1}Pro^{4}Glu^{9}Lys^{12}(glut-PAL) glucagon had no significant effect on STZ-induced weight loss over the course of the study (day 0-18), however, both GRA peptides transiently delayed STZ-induced hyperglycaemia on day 3 (p<0.05). Compared with STZ-treated controls (glucose $AUC_{(0\text{-}60\ min)}$ 750±153, mean±sem), desHis^{1}Pro^{4}Glu^{9}glucagon (AUC 530±189) and desHis^{1}Pro^{4}Glu^{9}Lys^{12}(glut-PAL)glucagon (AUC 513±185) treatment significantly (p<0.05) improved oral glucose tolerance. In an insulin sensitivity test, both desHis^{1}Pro^{4}Glu^{9}glucagon and desHis^{1}Pro^{4}Glu^{9}Lys^{12}(glut-PAL)glucagon significantly (p<0.05) enhanced the glucose lowering effects of insulin by 1.4-fold ($AAC_{(0\text{-}60\ min)}$ 1471±145) and 1.3-fold (AAC 1396±113), respectively when compared to STZ-treated controls (AAC 1079±166). Energy expenditure was also significantly increased (p<0.001) in both GRA treated groups compared with STZ-treated controls. Interestingly, the acylated analogue desHis^{1}Pro^{4}Glu^{9}Lys^{12}(glut-PAL)glucagon partially protected the pancreatic insulin content of STZ-treated mice producing a 40-fold increase (0.85±0.46 μg/g, p<0.001) in pancreatic insulin content compared to STZ-treated mice (0.02±0.02 μg/g, >99% insulin loss versus healthy controls 2.44±0.27 μg/g). STZ treatment resulted in a 1.9-fold (p<0.001) increase in terminal plasma glucagon concentrations compared with healthy controls (50.6±15.0 mg/ml vs 26.7±8.0 mg/ml), but both desHis^{1}Pro^{4}Glu^{9}glucagon and desHis^{1}Pro^{4}Glu^{9}Lys^{12}(glut-PAL)glucagon significantly reduced (p<0.001) the STZ-induced rise in plasma glucagon by 50% (25.5±7.0 mg/ml) and 60% (20.2±2.7 mg/ml), respectively.
Conclusion: These data show that the novel glucagon receptor antagonists desHis^{1}Pro^{4}Glu^{9}glucagon and desHis^{1}Pro^{4}Glu^{9}Lys^{12}(glut-PAL)glucagon can significantly improve glucose tolerance and insulin sensitivity and suppress hyperglucagonaemia in a model of type 1 diabetes. The acylated GRA analogue has pancreatic β-cell protective effects which could prove useful therapeutically.
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Exenatide, a GLP-1 receptor agonist, activates glucose transport in L6 muscle cell by an AMPK-dependent mechanism

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Background and aims: Exenatide (EXE) is a GLP-1 analogue which acts on pancreatic beta cells potentiating glucose-induced insulin secretion in type 2 diabetes treatment. The effects of EXE on the regulation of glucose metabolism in skeletal muscle as well as the molecular mechanisms potentially involved are unknown and a subject of active investigation. We aimed to explore the acute and chronic effects of EXE on L6 skeletal muscle myotubes as well as the existence of a cross-talk between insulin- and EXE-dependent signalling pathways.
Materials and methods: Western blot analysis and 2DG uptake were evaluated in L6 myotubes treated with 10-7 M Exenatide.
Results: Time-course experiments showed that 10-7 M EXE induced glucose uptake up to 48 h, achieving the maximum effect already after 20' (2-deoxyglucose nmol/min/mg prot: basal 1.14±0.09; EXE 20' 2.32±0.18; EXE 2h 2.12±0.27; EXE 4h 1.92±0.14; EXE 24h 2.22±0.15; EXE 48h 2.09±0.37; $p<0.01$) to an extent similar to insulin (2-deoxyglucose nmol/min/mg prot: basal 1.14±0.09; Insulin 100nM 30' 2.84±0.17; $p<0.001$). In order to identify the molecular mechanism through which EXE exerts its effect on glucose uptake, we evaluated the activation state of intracellular signaling pathways. EXE treatment, differently from insulin, was not able to induce IR beta subunit and IRS1 tyrosine phosphorylation, activation of AKT and GSK3beta defined by the phosphorylation at Ser473 and Ser21/9 respectively, as well as ERK1/2 and JNK1/2 phosphorylation. Thus, we hypothesized that an alternative pathway, such as AMPK, could be involved in EXE effect on glucose uptake. EXE acutely increased AMPKalpha and ACC phosphorylation by 2.5-fold compared to control ($p<0.01$). By contrast, insulin neither activated AMPK nor increased EXE effect on AMPK activation when cells were stimulated with both insulin and EXE. To clarify the role of AMPK in the effects of EXE on glucose uptake in L6 myotubes, cells were incubated either with LY294002 (LY), a PI 3-kinase inhibitor, or with tubercidin (Tub), widely used as AMPK inhibitor, prior to treat cells with EXE, insulin or a combination of both. LY inhibited insulin but not EXE-induced glucose uptake (basal 1.23±0.12; Insulin 100nM 30' 2.94±0.15; Insulin 100nM 30' + LY 1.26±0.08; EXE 20' 2.42±0.16; EXE 20' + LY 2.13±0.17 nmol/min/mg prot, $p<0.001$), whilst Tub pre-treatment almost abolished EXE effect on glucose uptake (EXE 20' 2.42±0.16; EXE 20' + Tub 1.45±0.07; $p<0.01$) but decreased insulin-induced glucose uptake only by 20% (Insulin 100nM 30' 2.94±0.15; Insulin 100nM 30' + Tub 2.36±0.18, nmol/min/mg prot, $p<0.05$)
Conclusion: Our results show that acute treatment with Exenatide stimulates glucose uptake by activating AMPK, in L6 myotubes.

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Oxyntomodulin has significant acute glucoregulatory effects comparable to liraglutide in subjects with type 2 diabetes

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Background and aims: Oxyntomodulin (OXM), a gut peptide in the preproglucagon family, appears to have acute glucoregulatory effects in preclinical models, attributed in part to GLP-1 receptor activation, and to induce weight loss (WL) in non-diabetic humans at 4 weeks. However, there are no data with OXM in patients with Type 2 diabetes mellitus (T2DM).
Materials and methods: We tested the hypothesis that OXM has glucoregulatory effects in T2DM, independent of WL, by comparing acute changes in pancreatic beta cell function in response to a single dose of either OXM (continuous intravenous infusion at 3pmol/kg/min, a dose that provides exposures associated with WL) or the GLP-1 analogue, liraglutide (LIRA: 0.6 mg, subcutaneous). In the setting of a randomized, double-blind, placebo-controlled, three-period crossover trial, following an overnight fast, male T2DM (N=12, age: 30.1 ± 8.2 years, BMI: 30.9 ± 1.9 kg/m2, HbA1c:6.9 ± 0.7%, fasting glucose: 5.53 ± 0.42 mmol/L) underwent a graded glucose infusion (GGI), at glucose infusion rates (GIR) of 2, 4, 6 and 10mg/kg/min (40 minutes each), while receiving OXM or LIRA or placebo (PBO).
Results: Data over the duration of the GGI are presented as change from baseline, mean +/- SD, plasma glucose (G) and maximal glycemic excursion (Gmax) in mmol/L, and insulin secretion rates (ISR, derived by C-peptide deconvolution) in ng/min. At matched GIR, OXM and LIRA led to significant augmentation of ISR and blunting of G and Gmax ($p<0.001$ for all comparisons over PBO). Significant changes were observed in G (5.90 ± 1.28 vs.6.80 ± 1.96), Gmax (11.7 ± 3.5 vs 15.1 ± 2.8), ISR (2.78 ± 0.9 vs 1.66 ± 0.7), and slope of ISR/G (0.019 ± 0.01 vs 0.006 ± 0.003) ($p<0.001$ for all comparisons, OXM vs PBO). The effects of OXM and LIRA on blunting of glycemic excursion were comparable [p=NS, OXM vs LIRA, for G (5.90 ± 1.28 vs 6.06 ± 1.20) and Gmax (11.7 ± 3.5 vs 11.6 ± 3.7)].
Conclusion: Our findings demonstrate for the first time that OXM may have significant direct acute glucoregulatory effects in T2DM, independent of weight loss.
Clinical Trial Registration Number: NCT01373450

OP 09 Retinopathy: risk stratification and novel therapies

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Pre-pubertal onset of diabetes may delay development of retinopathy: possible influence of metabolic control and blood pressure

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Background and aims: Diabetic Retinopathy (DR) is infrequent, and hardly ever severe, before puberty. However, it is not known whether this is due to the relatively short duration of diabetes or to a real "protective" effect of the pre-pubertal milieu. We compared the cumulative prevalences of DR in relation to disease duration in patients in whom diabetes onset was before or after puberty.
Materials and methods: Data of 1473 patients with diabetes onset before age 30, on insulin treatment and duration <30 years who underwent screening for DR between 1991 and 2010 were analysed. Age at onset of diabetes was in pre-pubertal age (defined as 0-12 in males and 0-11 in females) in 644 patients (324 females) and later in the remaining 829 (414 females). DR had been assessed by 2-field retinal photography and classified as absent or mild/more severe (at least microaneurysms in one eye). The levels of HbA1c, systolic and diastolic blood pressure at the time of screening were available for 771 patients, 327 pre-pubertal and 444 post-pubertal.
Results: Mean age at first screening was 18.8±8.0 vs 32.4±10.4 in prepubertal and postpubertal onset patients (p<0.001), respectively, and diabetes duration was 11.6±7.8 vs 12.6±8.6 years (NS). The overall prevalence of DR was lower in the pre-pubertal patients, 29.1% (n=186) vs 46.1% (n=378), p=0.001, and remained significantly so between 4 and 14 years of diabetes duration, after which the curves tended to overlap. HbA1c was higher in the pre-pubertal patients (8.3±1,3 vs 8.1±1,4, p=0.03), especially in the first 15 years of disease, whereas systolic (114.9±12.5 vs 120.8±14.4, p<0.001) and diastolic blood pressure (70.5±7.4 vs 72.4±7.7, p=0.0006) were higher in the post-pubertal onset patients throughout.
Conclusion: This case series confirms that DR may appear later and develops more slowly if diabetes onset is before puberty. However, in the long term, its prevalence becomes similar to that of patients with post-pubertal onset. This can be interpreted as a combination of higher blood pressure accelerating DR when diabetes onset is after puberty, with worse overall HbA1c in the pre-pubertal patients, resulting in a "catch up" phenomenon in later years.

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Twenty years on from UKPDS: More or less diabetic retinopathy at diagnosis of type 2 diabetes?

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Background and aims: To compare retinopathy and other characteristics of patients newly diagnosed with Type 2 diabetes mellitus (T2DM) in Gloucestershire between 2005 and 2012 with those recruited to the UK Prospective Diabetes Study (UKPDS) between 1978 and 1990.
Materials and methods: Data were collected from the Gloucestershire Diabetic Eye Screening Service (GDESS) in the UK. Retinopathy status was extracted from the GDESS database and clinical information from primary care records for those newly diagnosed with T2DM between 2005 and 2012 and aged 65 or younger at diagnosis. Clinical characteristics and diabetic retinopathy (DR) grading results were analysed and compared with data from patients with newly diagnosed T2DM originally recruited to UKPDS, all of whom were 65 or younger. GDESS offers nationally-approved DR screening using two-field 45º digital retinal images (macular- and disc-centred), taken and assessed by qualified and quality assured staff. Screening is offered as soon as possible after diabetes diagnosis and annually thereafter. UKPDS four-field 30º film-based imaging (temporal-to-macula, macula, disc and nasal-to-disc), which largely equates to the current two-field screening protocol, was carried out as soon as possible after diagnosis and triennially thereafter. UKPDS images were assessed in a single reading centre by trained and quality assured staff. DR status by eye in GDESS identifies: R0M0, no DR; R1, mild non-proliferative DR (microaneurysms (MA) ± haemorrhages ± exudate ± cotton wool spots (CWS)); R2, intraretinal microvascular abnormalities (IRMA), venous beading or reduplication or multiple deep round or blot haemorrhage; R3, any proliferative feature and M1; presence of two-dimensional surrogate markers for macular-involving DR (only in conjunction with R1, R2 or R3). Grades R2, R3 or M1 are reasons for referral to hospital eye service. UKPDS utilised ETDRS-derived grading severity scales from 10 (no DR); 20 (MA only); 31 (MA and haemorrhage (HMA) ± exudate ± CWS); 41 (IRMA or beading) through to 61+ for proliferative DR. Level 41 and above is here considered a comparable reason for referral although some patients with level 31+ could also have had macular-involving DR.
Results: GDESS data were available for 2,070 men and 1,375 women first screened soon after diagnosis of T2DM. Of these 1,403 (68%) men and 990 (72%) women had no retinopathy. Retinopathy was found in 667 (32%) men of whom 415 (20%) had R1 in only one eye, 202 (10%) had bilateral R1 and 50 (2%) had any referable DR. In women the rates were 385 (28%), 258 (19%), 100 (7%) and 27 (2%) respectively (p=0.0033). In UKPDS, DR proportions at diagnosis amongst 1731 men were 61% no DR, 19% unilateral MA, 5% bilateral MA, 7% HMA and 8% more severe (level 41+) DR. In 1233 women the rates were 66%, 20%, 3.5%, 7% and 4.5%. Overall there was proportionally more DR and referable DR in UKPDS than in GDESS patients (p<0.0001). GDESS patients were of similar age to those in UKPDS but had lower HbA1c (mean difference -1.8%), higher systolic and diastolic blood pressure (+7 and+2 mmHg, respectively) and were heavier (BMI +4 kg/m^2).
Conclusion: Patients newly diagnosed with T2DM in this screening programme have lower rates of DR and of referable DR, are less hyperglycaemic but they have higher blood pressure and are substantially heavier than those recruited to UKPDS. This has implications for screening programs and risk estimation.

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Validation of an individualised screening model for diabetic retinopathy

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Background and aims: The aim of this study was to validate a recently published risk algorithm for determination of individualised screening frequency to monitor diabetic retinopathy.
Materials and methods: An Icelandic study group developed a risk algorithm which recommends individual screening intervals ranging from 6 to 60 months depending on several risk factors (sex, HbA_{1c}, systolic blood pressure, presence of grade 1 to 3 diabetic retinopathy at baseline and diabetes duration). For validation of the risk algorithm clinical data on 2,764 type 2 diabetes patients of the Diabetes Care System (DCS) West-Friesland in the Netherlands were analysed. Two-field fundus photographs were taken every year and graded according to the EURODIAB coding system. Sight threatening retinopathy (STR) was considered EURODIAB grade 3 and 4. Patients with STR at baseline were excluded from the study. Validity of the model was assessed using the ability to predict the number of observed cases of STR during follow-up (calibration) and the ability to distinguish between those who develop STR during follow-up from those who do not (discrimination, area under the curve (AUC)). We compared the number of fundus photographs determined by the model with the actual number of annually taken fundus photographs within usual care of the DCS. Outcomes of omitted fundus photographs according to the model and potentially missed cases of STR were calculated.
Results: Assessment of discrimination of the algorithm in our cohort demonstrated a good fit (AUC 0.77 95% CI 0.71 to 0.82). Calibration showed that the model slightly overestimated the risk of developing STR. Using the model, 72.9% of the patients were appointed a screening interval longer than 12 months ranging from 22 to 60 months. Of those, 11 patients (0.6%) developed STR before the recommended screening interval. 26 patients (3.5%) developed STR before 1 year (usual care) and according to the model, screening in those patients should have been performed 6 months earlier. In the total population 53% of the fundus photographs would be safely omitted during 60 months of follow-up.
Conclusion: This study provides the first validation of the individualised screening algorithm to monitor diabetic retinopathy. We showed that, using the model, the screening interval can be prolonged while efficacy and safety were hardly compromised. Use of the individualised screening model may help to reduce screening costs for diabetic retinopathy.

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Polyphenol-enriched cocoa protects the diabetic retina from glial reaction and oxidative stress through the sirtuin (SIRT-1) pathway

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Background and aims: Sirtuins, Class III histone deacetylases, act in response to various stressors and have currently been listed as having an important molecular function in the regulation of various diseases such as Alzheimer's, chronic obstructive pulmonary disease, and longevity. Silent information regulator 1 (SIRT1) acts in some locus removing the acetyl group from the histone chromatin, but it can also suppress other proteins by deacetylation, including the NFkB-p65 subunit protein. Studies have shown that cocoa is a rich source of polyphenols with antioxidant properties. In this study, we hypothetised that cocoa can protect the diabetic retina by reducing inflammatory citokynes, thus decreasing glial reaction (glial fibrillary acidic protein, GFAP), an early marker of diabetic retinopathy (DR). To test this hypothesis, in vivo and in vitro studies were conducted.
Materials and methods: Diabetes was induced by streptozotocin (SZT) in 12-week-old hypertensive rats (SHR).The animals were randomised to receive polyphenol-enriched cocoa (60% polyphenol, 24mg/kg/day) or water by gavage daily for 16 weeks. Similarly, non-diabetic rats were randomised to be treated with polyphenol-enriched cocoa or water by gavage. In vitro, rat Muller cells (rMCs) were exposed for 24 hours to normal (NG) or high glucose (HG), either combined with cocoa or not combined with cocoa (10μg/ml), with or without SIRT-1 inhibitor EX 527 (10μM), 1 hour pre-treatment. The cells were also treated with H_2O_2 as an oxidative stress inducer.
Results: As expected, the diabetic rats displayed high expression of GFAP and occluding (early markers of DR), as well as elevated nitrotyrosine (NT) levels and decreased SIRT-1 activation ($p<0.05$). The retinal function evaluated by electroretinogram was markedly impaired ($p<0.05$). The treatment with cocoa fully restored all the above-mentioned alterations in the diabetic animals. In vitro, rMCs exposed to HG increased ROS production, GFAP expression and lysine-310-p65-NFkB acetylation ($p<0.05$). By immunofluorescence, SIRT-1 was predominantly present in cytosol, and its protein expression and activity were markedly reduced. The treatment with cocoa prevented these abnormalities and the co-treatment with SIRT-1 inhibitor abolished the effects of cocoa on the rMCs exposed to HG. Similarly, the rMCs exposed to H_2O_2 treated with cocoa prevented the up-regulation of GFAP, p65-NFkB acetylation and decreased the SIRT-1 protein expression and activity ($p<0.05$).
Conclusion: In an animal model of DR, the early markers of DR accompanied by increased NT expression and decreased SIRT-1 activity were prevented by oral administration of polyphenol-enriched cocoa. The presence of cocoa in the rMCs exposed to HG or H_2O_2 prevented p65-NFkB acetylation by increasing SIRT-1 activity, thus reducing GFAP up-regulation. This study reveals, for the first time, the beneficial effect of the oral administration of cocoa on reducing GFAP up-regulation in the pathogenesis of DR through the SIRT-1 activation pathway.
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Pioglitazone increases the risk of diabetic macular oedema in patients with type 2 diabetes

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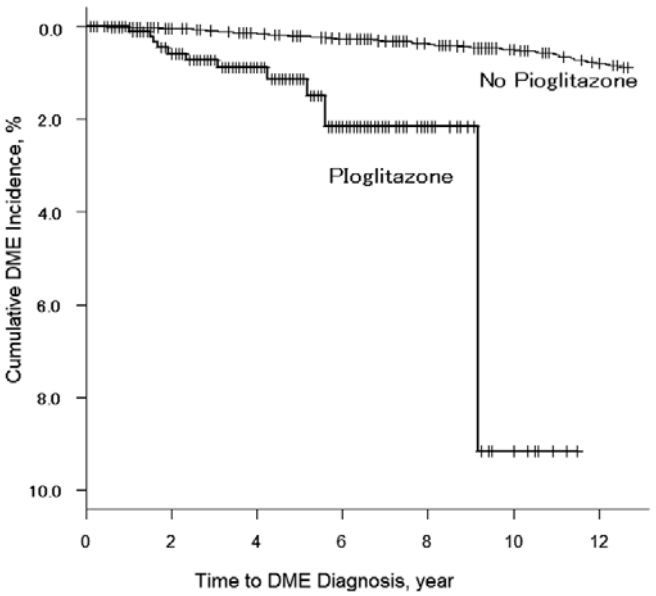
Background and aims: There has been a concern about the potential link of pioglitazone with adverse effects, including an increased incidence of bone fractures, edema, heart failure and bladder cancer. Recent studies have raised a possibility that pioglitazone, which generally causes fluid retention, may develop diabetic macular edema (DME). DME is one of the main causes of visual impairment in patients with diabetic retinopathy. We investigated whether the use of pioglitazone may increase the risk of DME in Japanese patients with type 2 diabetes.
Materials and methods: We retrospectively examined the incidence of DME in patients with type 2 diabetes in relation to pioglitazone administration during 12 years from 2000 to 2011 using the database in our institute. Subjects included a total of 22,115 patients with type 2 diabetes. The incidence of DME was calculated at 2 and 12 years of follow-up. Cox multiple regression analysis was used as the analysis to calculate the hazard ratio (HR). Kaplan-Meier curves were constructed to illustrate graphically the difference in DME incidence according to exposure to pioglitazone.
Results: A total of 99 subjects with type 2 diabetes were found to have DME, and the total prevalence of DME in type 2 diabetes was 0.45 % at 12 years of follow-up. Among 953 patients taking pioglitazone, 11 patients were found to have DME (1.15 %). The unadjusted HR of pioglitazone for development of DME was 7.76 [95%CI: 4.00-15.07] at 12 years of follow-up. After Cox multiple regression analysis (adjusted for age; sex; HbA1c levels; and use of insulin, antiplatelets, or renin-angiotensin system inhibitors), pioglitazone use was associated with an increased risk of DME at 2 years of follow-up (HR 4.71 [95%CI: 1.38-16.10]) and 12 years of follow-up (HR 5.12 [95%CI: 2.61-10.04]). Furthermore, combination therapy with pioglitazone plus insulin was associated with a higher risk for DME (adjusted HR 9.21 [95%CI: 4.06-20.89]) at 12 years of follow-up.
Conclusion: In this study, the association was observed between pioglitazone and DME, suggesting an increased risk of DME by pioglitazone in patients with type 2 diabetes.



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36-month safety and efficacy of ranibizumab in diabetic macular oedema: the RESTORE extension study (final analysis)

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Background and aims: Diabetic macular edema (DME) is the most common cause of vision loss in patients (pts) with diabetes. The randomized, double-masked RESTORE core study demonstrated superior efficacy of ranibizumab monotherapy (RBZ) or combined with laser (RBZ+laser) versus laser monotherapy in patients with visual impairment due to DME. The RESTORE extension open-label study was designed to evaluate the long-term safety and efficacy of RBZ 0.5 mg intravitreal injections.
Materials and methods: 240 out of 303 pts who were treated with RBZ, RBZ+laser, or laser in the core phase and completed the 12-month (M) core study (Day [D] 1-M12) entered the 24M extension study (M12-M36). During the extension phase, all pts were eligible to receive RBZ pro re nata (PRN) according to pre-specified best-corrected visual acuity (BCVA) stability and DME progression-based retreatment criteria. Laser PRN was allowed according to Early Treatment Diabetic Retinopathy Study (ETDRS) guidelines. Here we report overall (12M core phase+ 24M extension phase [D1-M36]) results for the incidence of adverse events (AEs), treatment exposure, mean changes in BCVA, central retinal subfield thickness (CRT), and patient-reported visual functioning (assessed by Visual Functioning Questionnaire [VFQ-25]) outcomes. Last observation carried forward approach was used for BCVA, CRT, and VFQ-25 analysis.

Results: 208 (86.7%) pts completed the extension study. Ocular serious adverse events (SAE) were observed in 2.4%-4.1% of the pts with no reports of endophthalmitis, retinal tear, or retinal detachment over 36 months. Overall, the most frequent nonocular SAEs were coronary artery disease and cerebrovascular accident (1.7%, each). The most frequent ocular AEs were cataract (16.3%) and eye pain (15.4%), while the most frequent nonocular AEs were nasopharyngitis (23.3%) and hypertension (13.3%). The mean number of RBZ injections for prior-RBZ, prior-RBZ+laser, and prior-laser groups was 14.2, 13.5, and 13.9 (D1-M35); 3.9, 3.5, and 4.1 (M12-23); and 2.9, 2.5, and 2.4 (M24-35), respectively. Across all prior-treatment groups, 19% to 25% of pts did not require any RBZ injections from M12-35. The mean BCVA gain and CRT reductions at M12 were maintained over M36 in pts treated with prior-RBZ in both core and extension phase (+8.0 letters, -145.9μm [prior-RBZ], +6.7, -142.1μm [prior-RBZ+Iaser]), while a progressive BCVA improvement (+6.0 letters) and CRT reduction (-142.7μm) was observed from M12-36 in prior-laser treated pts after allowing individualized RBZ treatment during the extension phase. Mean changes in VFQ-25 scores (D1-M36) for prior-RBZ/prior-RBZ+Iaser/prior-laser groups were +12.2/+7.8/+8.4 (near activities), +2.6/+4.1/+3.5 (distance activities), and +5.0/+4.3/+3.9 points (composite score).
Conclusion: No new ocular/nonocular safety findings or increased safety concerns for intravitreal RBZ 0.5 mg PRN with or without laser used over three years in patients with DME were identified. BCVA and CRT improvements in the first year could be maintained with a decreasing frequency of RBZ treatments over time. Individualized RBZ treatment led to an overall improvement or maintenance of BCVA, CRT, and VFQ-25 outcomes.
Clinical Trial Registration Number: NCT00687804

OP 10 In-patient diabetes: rights and wrongs

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Glycaemic variability is not related to postoperative complications in elective cardiac surgery patients on tight glucose control
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Background and aims: Glycemic variability has been recently proposed as an independent factor associated with worsened outcomes in critically ill patients. The aim of our study was to assess the relationship between glycemic variability and postoperative complications in elective cardiac surgery patients with either perioperatively (PERI) or postoperatively (POST) initiated protocols for tight glucose control (TGC).
Materials and methods: 2383 patients (age 18-90 years, 28.1% diabetics) undergoing elective cardiac surgery were randomized into either PERI (1134 subjects, 26.9% diabetics) or POST (1151 subjects, 29.4% diabetics) group according to the time of initiation of intravenous insulin infusion therapy. Target glucose range was set at 4.4-6.1 mmol/l. Glycemic variability was calculated using selected formulas including SD (standard deviation), MAGE-Day (mean amplitude of glycemic excursions during 24 hours), LBGI and HBGI (low and high blood glucose index). Adverse events from any cause were collected during the whole postsurgical hospital stay.
Results: In the whole cohort, perioperatively initiated TGC markedly reduced the number of patients with postoperative complications (23.8 vs. 31.4%, $p<0.001$) in spite of only modest improvement of glucose control (blood glucose 6.6±0.7 vs. 6.7±0.7 mmol/l, $p<0.001$). The positive effect of TGC on postoperative complications was driven by non-diabetic patients (20.3 vs. 31.7%, $p<0.001$; blood glucose 6.6±0.7 vs. 6.5±0.6 mmol/, $p<0.05$) while no significant effect was seen in the diabetic patients subgroup (33.2 vs. 30.5%, n.s.) despite significantly better glucose control in the PERI diabetic group (blood glucose 6.6±0.7 vs. 7.1±0.8 mmol/l, $p<0.001$). In the whole PERI group glycemic variability was only slightly reduced compared to POST group (MAGEDay 2.98 ± 2.09 vs. 3.19 ± 1.36, $p<0.0001$). Similar situation could be seen in the diabetic subgroup (MAGEDay 4.01 ± 3.57 vs. 4.12 ± 1.44, $p<0.01$), while the opposite was true for non-diabetic subjects (MAGE 4.74 ± 4.54 vs. 4.63 ± 1.49, $p<0.05$). The risk of hypoglycemia was low in the whole study cohort (LBGI<2.5) with minimal differences between subgroups. No correlation between the number of postoperative complications and various glycemic variability indices was observed in the study.
Conclusion: Perioperative initiation of intensive insulin therapy during elective cardiac surgery reduces postoperative morbidity only in non-diabetic subjects. Glycemic variability does not seem to play an important role in postoperative outcomes of elective cardiac surgery patients.
Clinical Trial Registration Number: RVO-VFN64165, IGA NT 13299-4 and SVV264503

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Short term continuous subcutaneous insulin infusion (CSII): a boon for type 2 diabetic patients with severe hyperglycaemia undergoing urgent surgical procedures
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Background and aims: To determine the efficacy, safety and cost effectiveness of short term Continuous Subcutaneous Insulin Infusion (CSII) therapy using Insulin Pumps Vs. conventional Continuous Intravenous Insulin Infusion (IVII) among Type-2 Diabetes Mellitus (T2D) patients with severe Hyperglycemia requiring urgent control of Blood glucose pre intervention or surgery.
Materials and methods: This is a pilot study over a period of three months on 50 T2DM patients (26- Males (M), 24-Females (F)) on MSI (Multiple subcutaneous Insulin) and/or Oral hypoglycemic agents who presented with severe hyperglycemia (Random blood glucose > 400mg/dl, fasting blood glucose > 250mg/dl). The patients were in the age group - 34 to 78 years. These patients were planned for various medical and surgical interventions and

were randomized in two treatment groups - 25 patients were put on continuous IVII (14M, 11F) while 25 patients were put on a short term(3-5 days) CSII therapy (12M, 13F) to manage severe Hyperglycemia. The target glucose level was 100-150mg/dl. One hourly capillary blood glucose level was measured in both the groups. Patients in both the groups were matched in terms of blood glucose, HbA1c, BMI, creatinine clearance, h/o diabetic complications, and h/o steroids usage. There was no episode of sepsis in either group.

Results: In the CSII group, 23/25 patients achieved the target BG. While, 25/25 patients in the IVII group achieved the target BG (p=0.26). CSII was clinically safer than IVII. 1/25 patients had an episode of mild hypoglycemia in the CSII group whereas in the IVII group, 1/25 patients had an episode of mild hypoglycemia while 3/25 patients had severe hypoglycemia which required 50% dextrose infusion for management (p=0.051). CSII therapy was more convenient and did not require hospitalization. The cost of the CSII therapy for a period of 3 days was <13.3 % of the total cost of IVII therapy in patients hospitalized for control of hyperglycemia

Conclusion:

- Short term CSII is safer than IVII therapy for treatment of urgent severe hyperglycemia in T2D. There were lesser episodes of hypoglycemia among patients in the CSII group (4%) Vs. IVII therapy (16%).
- CSII is as efficacious as IVII for treatment of severe hyperglycemia in T2D
- Total length of hospital stay was reduced in the CSII group.
- CSII proved to be highly cost effective (<13.3%) as compared to IVII.
- Large scale studies are needed to further evaluate benefits of CSII in this group of patients.

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Comparison of inpatient glycaemic control by continuous glucose monitoring (CGM) and capillary point-of-care (POC) testing in general medicine patients with type 2 diabetes

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Background and aims: Point-of care testing with capillary glucose fingerstick tests is the universally accepted method of glucose monitoring in the hospital. No previous studies have compared the efficacy of CGM in the management of hyperglycemia in general medicine (non-ICU) patients with T2D. Accordingly, this prospective randomized study compared glycemic control by CGM (Sofsensor iPro 2, Medtronic) to bedside POC testing in non-ICU patients treated with basal bolus regimen for ≥ 3 days. Both patients and hospital staff were blinded to the CGM data. POC testing measurements were performed before meals, 2-h after meals, at bedtime and 3 AM. The primary outcomes were differences in daily BG, and number of hypoglycemia (<3.9 mmol/L) and hyperglycemia (>10 mmol/L) events between groups.

Materials and methods: A total of 40 insulin-naïve patients (age: 65.8±8 yr, DM duration 14.7±9 yr, admission BG: 13.9±1 mmol/L, A1C: 9.7±2.4%, ±SD) were treated with glargine and glulisine at a starting total dose of 0.4 U/kg/day if BG was between 140-200 mg/dl and 0.5 U/kg/day if BG was between 200-400 mg/dl, given half as glargine once daily and half as glulisine before meals.

Results: We observed no difference in daily BG after the 1st day of treatment by CGM and POC testing (9.8±2 vs 9.77±2mmol/L, p:0.828). There were 10 patients with hypoglycemia recognized by both methods, but CGM detected higher number of events (55 vs 12, p=0.0001) than POC testing, with 40% occurring between breakfast and dinner and 60% between dinner and 6 A.M. A total of 26.3% of hypoglycemia were asymptomatic and most (86.7%) were only identified by CGM. The proportion of BG >10 mmol/L was 36.8% by CGM and 42.1% by POC, p=0.828.

Conclusion: The use of CGM recognized increased number of hypoglycemic events compared to POC testing. Our study indicates potential benefit of using real-time CGM in the hospital to detect symptomatic and asymptomatic hypoglycemic events in a more timely fashion compared to POC testing.

Supported by: Sanofi, Medtronic

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Randomised clinical trial to assess efficacy of two insulin protocols in hospitalised subjects with type 2 diabetes mellitus receiving medium/high dose of corticosteroids: preliminary results

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Background and aims: To assess efficacy and safety of two different basal-bolus insulin protocols (glargine vs. NPH insulin as basal insulin) in subjects with type 2 diabetes (T2DM) who received medium or high dose of intermediate acting corticosteroid therapy. Primary endpoint: mean blood glucose between 80 and 180 mg/dl. Secondary endpoints: number of mild or severe episodes of hypoglycaemia, glycaemic variability, and length of hospital stay.

Material and methods: Randomised clinical trial that included 51 consecutive subjects aged between 18 and 80 years admitted on the Pneumology ward and who received medium or high dose of intermediate acting corticosteroids between February 2011 and November 2012. Subjects were randomly assigned to receive glargine+glulisine (n=25) or NPH insulin+glulisine (n=26) as insulin therapy during hospitalisation. Sociodemographic, clinical data and days of hospital stay were collected. HbA_{1c} was measured. Information on insulin dose, corticosteroid dose, and capillary blood glucose measurements before and 2hour after meals during the first 6 days after admission was recorded. Continuous glucose monitoring (iPro2 Medtronic) was used for 6 six days in 26 subjects (16 in the glargine group and 10 in the NPH insulin group).

Results: Mean diabetes duration was 8.1±6.7 yrs. Mean HbA_{1c} at admission was 7.4±1.6% and was not statistically significantly different between groups. Diagnosis at admission was: chronic obstructive pulmonary disease exacerbation in 49% of subjects, pneumonia in 27.5%, asthma exacerbation in 13.7% and other causes in 9.9%. Mean length of hospitalisation was 9.0±3.2 days, and although glargine group had a shorter length of stay, it did not reach statistical significance (Glargine 8.2±2.8 days vs. NPH insulin 9.8±3.4 days, p>0.05). Table 1 shows glycaemic control between study groups. Data from continuous glucose monitoring were not significantly different between study groups.

Conclusion: Our preliminary results pointed that an insulin protocol based on the administration of glargine for hospitalised subjects with T2DM treated with medium or high dose of corticosteroids might be safe and have similar efficacy to achieve glycaemic control than a protocol with NPH insulin.

Glycemic control

Variables	Total (n=51)	Glargine (n=25)	NPH insulin (n=26)	p
Sex (men/women) (n)	31/20	19/6	12/14	0.05
Age (yrs)	68.8±7.3	70.2±6.6	67.5±7.8	ns
Daily insulin dose on first day (U/kg/day)	0.5±0.2	0.5±0.3	0.6±0.2	ns
Daily insulin dose on sixth day (U/kg/day)	0.7±0.3	0.7±0.3	0.7±0.4	ns
Mean capillary blood glucose on first day (mg/dl)	224±73	226±82	223±65	ns
Mean capillary blood glucose on sixth day (mg/dl)	189±54	180±58	196±51	ns
Subjects with mean capillary blood glucose between 80-180 mg/dl on first day (%)	37.5	45.8	29.2	ns
Subjects with mean capillary blood glucose between 80-180 mg/dl on sixth day (%)	48.6	57.1	42.9	ns
Episodes of mild hypoglycemia	12	4	8	ns
Episodes of severe hypoglycemia	3	0	3	ns
Mean amplitude of glycemic excursions	114±19	105±58	124±60	ns

Supported by: Sanofi

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The safety and efficacy of a subcutaneous continuous glucose monitoring system compared to point of care measurement in critically ill patients: a randomised controlled trial

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Background and aims: Hyperglycaemia, hypoglycaemia and glucose variability are associated with adverse outcome of critically ill patients. Using a continuous glucose monitoring (CGM) system might reduce these disturbances in glucose regulation. The reliability and accuracy of CGM using subcutaneous measurements has been studied in critically ill patients before. The present study aims to determine whether the clinical use of subcutaneous CGM is safe, effective and feasible in critically ill patients.
Materials and methods: In an open labeled randomized controlled trial patients were assigned to glucose regulation using a subcutaneous CGM system (FreeStyle Navigator®) or frequent point of care measurements (POCM) using Accu-Chek® (Roche) for 5 days or until ICU discharge. Blinded arterial blood glucose measurements were performed on standard times in both groups. Patients with POCM also had subcutaneous CGM but these data were blinded. Data from CGM or POCM were entered in the same computerized glucose regulation protocol which prescribed the insulin dose and the time of next data entering.
Results: 178 Patients were included. Median APACHE IV was 0.32 (IQR 0.55) and 92% were mechanically ventilated. 13% were complicated cardiac surgery patients, the others medical patients. From 15 patients CGM data were lost for technical reasons, therefore 163 were analysed. The median study duration was 70 hrs (IQR 99) in the CGM patients and 60 hrs (IQR 89) in POCM patients (p=0.91). We analyzed 2844 glucose measurements, of which 1358 were paired CGM-POCM. The median time in target range (5-9 mmol/l) was 57.5 hrs (IQR 74.4) in CGM patients and 34.9 hrs (IQR 62.7) in POCM group (p=0.043). The incidence of severe hypoglycaemia (below 2.2 mmol/l) or severe hyperglycaemia (above 25 mmol/l) was similar in both groups (p=0.54 and 0.09) as well as the glucose variability in terms of mean absolute glucose change per hour (MAG). The total number of blood samples per patient was 25 for CGMS and 41 for POCM (p=0.001). Hospital and ICU length of stay and mortality did not show any significant differences.
Conclusion: Glucose monitoring using a subcutaneous device was safe in terms of hypoglycaemia incidence and resulted in significantly more time in target range than the use of a point of care blood measurement. In addition, the number of blood samples was reduced.
Clinical Trial Registration Number: NCT01526044

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Continuous glucose monitoring at the intensive care unit: nursing workload reduction and cost-benefit analysis

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Background and aims: Continuous glucose monitoring (CGM) in ICUs has the potential to improve glycaemic control and thereby enhance patient safety and outcomes. Furthermore, CGM may reduce nursing workload related to glucose control. The primary objective of this study was to assess the influence of real-time CGM on nursing workload for glucose control, compared to intermittent point-of-care glucose measurements (POCM) in patients admitted to the ICU and treated with insulin. We also estimated the net financial benefit or cost of using CGM systems in a critical care setting.
Materials and methods: We used data of a recent randomized controlled trial at the ICU of a teaching hospital in the Netherlands comparing subcutaneous glucose monitoring with real-time CGM (FreeStyle Navigator®) versus conventional POCM (arterial blood sampling by use of the Accu-Chek® glucometer). The main outcome of this substudy was nursing workload defined as the time burden for glucose control per patient per day (24 hours). A time-in-motion design was used to estimate the nursing workload. We additionally performed a cost-benefit analysis in which we assessed the average difference in costs using either real-time GCM or conventional POCM. Cost parameters were nursing personnel costs, costs of the devices and materials used for blood glucose monitoring and laboratory costs. Subsequently, the incremental cost-effectiveness ratio (difference in costs divided by the percentage difference in nursing workload) was calculated.
Results: We analyzed data of 78 patients with CGM versus 77 patients with POCM. The average total time burden for glucose control in the CGM group was 49 [44-58] minutes per 24 hour, whereas in the POCM group the total time burden was 70 [64-81] minutes (p<0.001). The mean reduction in total nursing workload for glucose control was 30% or 21 minutes per patient per 24 hours. Mean total costs per patient per day were slightly less in patients randomized to CGM (EUR 64) compared to patients randomized to POCM (EUR 89, difference EUR 25; 95% CI -43 to 29). The incremental cost-effectiveness ratio was - EUR 83, indicating the superiority of real-time CGM over conventional glucose control.
Conclusion: This study shows that in ICU patients the use of continuous subcutaneous glucose monitoring significantly reduces the nursing workload for glucose control and costs were reduced compared to conventional glucose monitoring with intermittent point of care measurements.

OP 11 Exercise physiology and impact on ageing

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Effect of chronic exercise on skeletal muscle mitochondria dynamics in older adults

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Background and aims: Aging is thought to decrease mitochondrial (Mito) content and function. Acute exercise has been shown to promote Mito remodeling. The extent to which chronic exercise (i.e. training) modifies mitochondrial content and the different transcription factors in skeletal muscle of older adults is not known. The goal of this study was to explore the effects of chronic exercise on Mito volume density (MVd), Mito proteins and transcription factors involved in Mito biogenesis and dynamics. To this aim, we compared skeletal muscle from older sedentary adults to chronically trained athletes matched by age.
Materials and methods: Seven sedentary (S) were compared to 8 master athletes (A) in a cross sectional design (Mean Age 67.3 ± S.E.M. 1.4). Skeletal muscle specimens were obtained by vastus lateralis biopsies in the fasting and resting state (at least 48 hours after the last exercise bout). VO2peak was measured by GXT, lean body mass (LBM, kg) and fat mass (FM, kg) by DXA. Mitochondrial volume density (MVd, %) was measured by transmission electron microscopy. Proteins and mRNA levels were quantified by western blotting and quantitative RT-PCR. Independent T tests were done to compare groups and correlations were analyzed by Spearman rho. To look for patterns of distribution of the transcripts, we performed a principal component analysis (PCA).
Results: S had higher BMI (S=27.55kg/m2 ± 1.34; A=21.83kg/m2 ± 0.39; p=.002) and FM than A (S=28.12kg ± 2.73; A=11.70kg ± 1.00; p=.0001). A had higher VO2peak (S=38.21ml/min/KgLBM ± 1.92; A=48.02 ± 2.41; p=.006) and MVd (S=4.41% ± .48; A=7.71% ± .67; p=.002) than S. A had higher contents of proteins from respiratory complexes (C) I, IV and V (all p≤.01). No group differences were found for CII and III. MFN 1 & 2 protein levels were higher in A (p≤.03), while OPA1 and DRP1 were not different. The following mRNAs were significantly higher in A: CS, Mfn2, ATG5g1, Nampt1 (all p<.05), but not Tfam, Nrf1, Nrf2, Drp1, Mfn1, Sirt1, Ndufa2. VO2peak correlated with CI (p=.02), CIV (p=.0009), CV (p=.03), MFN2 (p=.02). MVd correlated significantly with CIV (p=.03), CV (p=.0002), MFN2 (p=.008). We observed a strong correlation among the protein expression levels of CI, CIV, CV, MFN1&2 on one hand and DRP1, CII and CIII on the other. At the gene expression level, we identified groups of transcripts that were highly correlated. The PCA identified 4 factors that explained 96% of the variance of the mRNA data. These were: [CS, Drp1, Sirt1, Tfam, Mfn2], [Nampt1, Nrf2, Ndufa2, ATG5g1], [Mfn1] and [Nrf1].
Conclusion: In this study, chronically active older subjects had a higher content of mitochondria, electron transport chain components and mitochondrial fusion proteins as compared to their sedentary counterparts. These results highlight the effect that lifestyle has at the molecular level on mitochondria. Indeed, aging per se might not explain the mitochondrial dysfunction observed in older humans. On the contrary, our data suggests that a sedentary lifestyle decreases specific mitochondrial proteins. Furthermore, we describe patterns of gene expression that may be involved in the maintenance of mitochondrial content with age and exercise. Some but not all of the genes explored were increased at the mRNA level in the athletes. This could be explained by the lack of an acute effect as this study was built solely to observe chronic effects.
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The effect of exercise training on skeletal muscle mitochondrial content in older adults correlates with exercise efficiency and fat oxidation

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Background and aims: Skeletal muscle mitochondrial content has been implicated in age-related metabolic dysfunction. Cross-sectional studies have shown that trained older adults have more mitochondrial content than untrained. It is known that substrate usage during exercise also changes with chronic training to rely more on fat than carbohydrates. The extent to which mitochondrial content impacts fat oxidation during exercise is not known. The purpose of this study was to examine the changes in skeletal mitochondrial content, exercise efficiency and exercise substrate use in older sedentary adults undergoing an exercise intervention.
Materials and methods: In this pre/post intervention design, 13 sedentary older (age 66.15±0.82, range 63-71) men (N=7) and women (N=6) underwent four months of an endurance exercise intervention. Volunteers trained 3 supervised sessions per week at 60-75% of their maximal heart rate. Mitochondrial volume density (MVD) was measured in vastus lateralis biopsy specimens by using transmission electron microscopy and classic stereological measures. Gross efficiency (GE, %) and proportion of energy expended from fat (EEF, %) were determined during one-hour of submaximal (55% of VO2peak) cycle ergometry exercise in tightly controlled conditions (fasting and 72 hours from last exercise bout). VO2peak was determined by a GXT. Intervention effects were measured by Paired T Test and correlations by Spearman rho. Results are presented in mean ± SEM.
Results: Mean weight, BMI, and percent fat decreased by 2.3 ± 0.9, 2.3 ± 0.9 and 8.5 ± 2.6 % respectively (all p<0.05). VO2peak, GE and MVD all increased by 16.3 ± 4.5, 5.9 ± 1.7 and 58.2 ± 18.3 % (all p<0.05). EEF increased by 36.3 ± 9.7% (p=0.002). MVD at baseline correlated with GE (r=0.73, p=0.016) but not with EEF or carbohydrate oxidation. GE correlated with VO2peak (r=0.78, p=.002) but not BMI or weight. EEF did not correlate with VO2peak. The change in EEF with intervention was related to the change in MVD (r=0.70 p=0.022) but not to the change in VO2peak or GE.
Conclusion: This data confirms that older adults are able to respond to a moderate intensity exercise training protocol in terms of improved fitness, exercise efficiency and mitochondrial content. Interestingly, improvements in mitochondrial content predicted a greater reliance on fat as an energy source during submaximal prolonged exercise.
Clinical Trial Registration Number: NCT01224886
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Electrodiab study: impact of bi-quadricipital muscular electrostimulation on insulin sensitivity in type 2 diabetic subjects

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Background and aims: Physical activity improves insulin sensitivity and promotes type 2 diabetes (T2D) control. However, physical activity guidelines are rarely applied due to lack of motivation or to concomitant diseases (diabetic foot, coronary artery disease···). Muscular electro-stimulation (MES) enhances both muscles strength and volume. Metabolic effect of MES on insulin sensitivity is explored in this study, as an alternative to physical activity.
Materials and methods: Prospective multicentric 4 weeks pilot study. Inclusion criterias : T2D ; 18-75 years ; BMI > 25 ; 6-9% HbA1c ; treatment with oral hypoglycemic agents (OHA) and/or GLP1 analogs > 3 months. Usual physical activity was controlled with a body monitoring system and dietary intake was controlled with dietary self reports during the study. Insulin sensitivity was evaluated with 3 euglycemic hyperinsulinemic clamps (EHC) (80mUI/m2/min) for each subject : baseline (T0), one hour after a unique MES session (T1), and after one week of ambulatory daily MES training (T2). Each MES session was a 25 minutes bi-quadricipital simultaneous stimulation with a 4 channel electrostimulator. Patients were asked to increase stimulation to maximal tolerated intensity. Indirect calorimetry energy expenditure (EE) was also evaluated at rest and during the first MES session.
Results: 15 patients T2D were included (age 60.1±9.0 years ; diabetes duration 12.3±7.0 years; BMI 32.9±5.2 ; HbA1c 7.3±0.8%). For overall population (responders and non-responders), insulin sensitivity (M-Value) increased of 11.3±40.8% (ns) and 28.3±38.4% (p<0.05) at T1 and T2 vs T0, respectively. For the 8/15 responders, M-Value increased of 36.8±37.7% (ns) and 58.3±24.9% (p<0.05) at T1 and T2 vs T0, respectively. During the first MES session, for a mean stimulation intensity of 25.8±5.6/100, energy expenditure (EE) (indirect calorimetric measure) increased of 93.6±176.8 Kcal/h (ie +5.2±9.4% from EE at rest) (ns). During the daily MES training week, patients increased stimulation intensity with a week mean of 37.1±12.9/100. Concerning tolerance, 8/15 subjects felt moderate muscle pain (n=7) and/or

skin pain (n=4) during or after MES sessions. 14/15 patients would perform regular MES sessions on the long term, 3 times/week for 10/14 patients.
Conclusion: A one week training with daily biquadricipital 25 minutes MES sessions significantly improves insulin sensitivity of ~ 30% in orally treated type 2 diabetic patients. This substantial improvement of insulin sensitivity occurs despite a weak EE increase during MES (+5,2±9,4%, p=0,055). This discrepancy suggests a specific metabolic effect of MES. Furthermore, this weak EE increase allows to consider the extent of this physical treatment in T2D patients with cardiovascular deconditioning, a usual sedentary population. All together, these data encourages further longer term research on the effect of MES on glycemic control.
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Increased IGF-I bioavailability ameliorates the decline in insulin sensitivity that occurs with ageing and type 2 diabetes
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Background and aims: Cross sectional and epidemiological studies have shown a correlation between low circulating levels of total IGF-I and the risk of developing type 2 diabetes and disease prognosis. These findings have been reinforced by data from clinical and experimental animal models which provide evidence linking reduced levels of free IGF-I and diabetes. The corollary of these data suggest that increased free IGF-I might have beneficial effects on insulin sensitivity and glucose homeostasis. The aim of this study was to investigate the effects of aging and consumption of a diabetogenic diet on insulin sensitivity and glucose homeostasis in an animal model of increased free IGF-I.
Materials and methods: These studies utilise mice deficient in IGFBP-1, a specific high affinity binding protein that sequesters IGF-I, as a model of increased free IGF-I . In this longitudinal study, we have assessed glucose and insulin tolerance, insulin secretion and endocrine profiles in IGFBP-1 knockout (BP1KO) mice that have been fed either a diabetogenic (high fat) or a normal diet.
Results: Although glucose tolerance was similar in both groups at 8 weeks of age, BP1KO mice were significantly more insulin sensitive (AUC KO v WT 376.5 ± 20.05 v 483.94 ± 21.6 $p < 0.05$). Of interest, we noted that BP1KO mice were heavier than age matched controls (KO v WT 26.4 ± 0.4 g v 25.8 ± 0.25 $p < 0.05$) and analysis of body composition showed this was mainly due to a difference in body fat (KO v WT 3.7 ± 0.42 g v 2.3 ± 0.32 $p < 0.05$). Glucose and insulin tolerance tests were repeated after 16 weeks of feeding a normal chow or high fat diet (45% kcal from fat). There was evidence of impaired glucose tolerance (AUCKO v WT 615 ± 63 v 803 ± 40.6 $p < 0.01$) and insulin resistance (AUCKO v WT 360 ± 12.4 v 517.5 ± 15.9 $p < 0.01$) in both groups on both diets but the impairment was significantly ameliorated in BP-1KO mice, particularly in the high fat fed cohort. BP1KO mice also secreted significantly less insulin than controls in response to a glucose challenge at both timepoints. Weight gain was similar in both groups of chow fed mice but high fat feeding resulted in greater weight gain in wild type mice than BP1KO mice (weight gain KO v WT 12.7 ± 0.3g v 19.5 ± 0.44g $p < 0.01$). Analysis of body composition revealed that wild type mice lost lean muscle mass and gained fat when maintained on either diet whereas BP1KO mice gained lean muscle mass and acquired less fat mass than controls regardless of diet. Endocrine profiling (table 1) indicated differences in leptin resistin and TNFα all of which were lower in BP1KO mice than controls at all timepoints. Free IGF-I is approximately 3 fold higher in BP1KO mice than controls although levels of total IGF-I are similar.
Conclusion: These data support the hypothesis that increased levels of free IGF-I are beneficial in ameliorating the deterioration in glucose homeostasis and insulin sensitivity that occurs with aging and type 2 diabetes and suggests that factors which enhance free IGF-I may have therapeutic applications in treating insulin resistance in diabetes and other states.

		KO NC	WT NC	KO HF	WT HF
Total IGF-I (ng/ml)	B	241.3 ± 14.6	206.4 ± 17.1		
Free IGF-I (ng/ml)	B	2.578 ± 0.34 †	0.940 ± 0.17		
Insulin	B	0.782 ± 0.35	0.277 ± 0.07	-	-
(ng/ml)	E	0.288 ± 0.09	0.353 ± 0.08	0.654 ± 0.21	0.765 ± 0.28
GH	B	2.933 ± 0.50	6.002 ± 4.01	-	-
(ng/ml)	E	6.729 ± 2.11	3.735 ± 1.20	6.055 ± 2.54	5.166 ± 2.12
Leptin	B	0.186 ± 0.08	0.630 ± 0.299	-	-
(ng/ml)	E	0.340 ± 0.14 † γ	1.490 ± 0.53 δ	6.316 ± 2.55	8.173 ± 2.46
Resistin	B	2.254 ± 0.22	2.445 ± 0.17	-	-
(ng/ml)	E	1.777 ± 0.09 γγ	2.309 ± 0.32 γγ	3.491 ± 0.44	4.065 ± 0.34
IL-6	B	6.45 ± 0.81	8.31 ± 2.15	-	-
(pg/ml)	E	29.17 ± 10.67	18.94 ± 6.25	21.64 ± 11.20	56.71 ± 32.26
MCP-1	B	10.02 ± 1.59	6.63 ± 2.37	-	-
(pg/ml)	E	8.57 ± 5.48	3.62 ± 1.19	2.49 ± 0.36	14.12 ± 2.43
TNFα	B	15.78 ± 3.86	17.59 ± 3.99	-	-
(pg/ml)	E	32.21 ± 5.63	18.13 ± 3.25	39.63 ± 5.59	43.46 ± 6.45 γγ
FFA	B	1.94 ± 0.25	1.89 ± 0.18	-	-
(μmol/ml)	E	1.70 ± 0.35	1.64 ± 0.20	1.64 ± 0.21	2.30 ± 0.40

Table 1. Plasma/Serum parameters in mice by genotype, diet and timepoint

† significantly different by genotype † P<0.05
γ significantly different by diet γ P <0.05 , γγ P<0.01
Analyzed using unpaired Student's *t* test at each timepoint
KO, IGFBP-/-; WT, wildtype
HF, high fat diet; NC, normal chow diet
B, baseline; E, endpoint

Supported by: UCD Life Sciences Fund

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Effect of exercise training and resveratrol on HFD-induced changes in skeletal muscle PDHa activity: impact of PGC-1 alpha
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Background and aim: Obesity is associated with increased risk of dysregulated glucose metabolism and insulin resistance which potentially can lead to the development of type 2 diabetes. High fat diet (HFD) is known to impair glucose metabolism in skeletal muscle. One important protein in glucose metabolism is the pyruvate dehydrogenase (PDH) complex, which converts pyruvate into acetyl CoA in a reaction which represents the only entry of carbohydrates into mitochondria for oxidation. It is well known that exercise can improve whole body glucose homeostasis and increase oxidative capacity in skeletal muscle. Resveratrol (RSV) is a polyphenol located in the skin of dark grapes and fruits. RSV has been shown to mimic some the beneficial effects of exercise in rodents activating some of the same molecular pathways inside the cell as exercise. Peroxisome proliferator-activated receptor co-activator (PGC)-1alpha is a transcriptional co-activator involved in mitochondrial biogenesis and suggested to play a role in substrate regulation. The aim of the present study was to investigate the effect of exercise training and RSV on HFD induced changes in the activity of PDH in the active form (PDHa) in skeletal muscle.
Materials and methods: Muscle specific PGC-1alpha knockout (KO) and littermate wild type (WT) mice were put on either high fat diet or chow diet for 16 weeks. The mice on HFD were divided into 4 groups, HFD, HFD+RSV (4g/kg food), HFD+exercise training and HFD+exercise training+RSV (4g/kg food). The respiratory exchange ratio (RER) was measured during 24 hours in metabolic chambers. Mice were euthanized by cervical dislocation and Quadriceps muscles were removed. The PDHa activity was measured in homogenates from the Quadriceps.
Results: The PDHa activity in resting quadriceps muscle was in all HFD groups lower ($p<0.05$) in PGC-1alpha KO than in WT mice. The PDHa activity tended to be lower ($0.05<p<0.1$) in WT and was lower ($p<0.05$) in PGC-1alpha KO in the HFD group than in the chow group. Exercise training increased ($p<0.05$) the PDHa activity in WT, but this effect was blunted in PGC-1alpha KO mice. There were no effects of RSV or exercise training+RSV. The RER was in both genotypes lower ($p<0.05$) in all the HFD groups than in chow, and the only difference within the HFD groups was a higher ($p<0.05$) RER in HFD+exercise training than HFD in PGC-1alpha KO mice.
Conclusion: The main finding of the present study is that exercise training prevented a HFD-induced decrease in PDHa activity in mouse skeletal muscle and that muscle PGC-1alpha is required for this effect. In addition, RSV had no effect on PDHa activity and the combination of RSV+exercise even seemed to block the effect of exercise alone. Therefore it seems that exercise

training alone and not RSV can normalize the PDHa activity in mice on a HFD, properly due to increased PDH protein content.
Supported by: The Danish Medical Research Council

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Exercise - but not resveratrol - increases performance and oxidative proteins in elderly human skeletal muscle
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Background and aims: Aging is associated with reduced physical performance, decreased muscle mass and strength as well as reduced oxidative capacity in skeletal muscle. Altogether this may affect the overall health status and quality of life for the individual. Exercise training has been shown to increase muscle mass, mitochondrial biogenesis and oxidative capacity in skeletal muscle and thus has the potential of postponing many of the observed age-associated metabolic complications. Recently, the naturally occurring antioxidant resveratrol (RSV) has been shown to exert "exercise like" effects on mitochondrial biogenesis and oxidative capacity in skeletal muscle of high fat diet challenged rodents. However, little is known about the effect of RSV on metabolism in healthy aging. The aim of the present study was to investigate whether RSV supplementation (suppl.) alone or in combination with exercise training has beneficial effects on whole body performance as well as metabolic parameters in human skeletal muscle of elderly subjects.
Material and methods: 43 physical inactive healthy aged men (age: 64.9 0.5 years; BMI: 24.9 0.6 kg $2h^{-1}$; VO_{2max} 2605 70.2 mlO_2 min^{-1}) were randomized into 4 groups with 8 weeks of daily suppl. of 250 mg RSV or placebo with or without concomitant high-intensity exercise training. Before and after the intervention all subjects performed an incremental cycle ergometer VO_2 max test and time to exhaustion during one-leg knee-extensor exercise. Vastus lateralis muscle biopsies were obtained before and after the intervention period.
Results: Whereas RSV suppl. alone did not affect VO2 max, both exercise groups increased VO2 max, but the placebo group increased more than the RSV group (19 % and 13 % increase respectively). Time to exhaustion increased similarly in the two exercise groups (22% and 58 % increase respectively). The content of oxidative proteins cytchrome c and cytochrome c oxidase I in skeletal muscle increased equally ~1.5 fold in both exercise groups, whereas no effect of RSV suppl. alone was observed. Sirtuin 1 protein content, a potential mediator of both exercise and RSV-induced adaptations in skeletal muscle did not change with any of the interventions.
Conclusion: Exercise training increased overall physical performance and the capacity for oxidative phosphorylation in human skeletal muscle of elderly subjects, but RSV alone or in combination with exercise training had no effect in the present settings. The present findings support a potent effect of exercise training rather than RSV on metabolism in healthy aging.
Supported by: The Ministry of Culture Committee on Sports Research, Denmark

OP 12 FGF21 cures it all?

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High glucose represses fibroblast growth factor 21 (FGF21) action through peroxisome proliferator-activated receptor gamma in mouse pancreatic islets
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Background and aims: Fibroblast growth factor 21 (FGF21) is a distinctive member of the FGF family whose actions require the cofactor β-klotho. Restricted expression of β-klotho in liver, pancreas and adipose tissue provides the mechanistic basis for tissue-specific actions of FGF21 and thus implicates the important roles of β-klotho in these tissues. Circulating FGF21 levels are elevated in diabetic subjects and correlate with abnormal glucose metabolism, while pharmacologically administered FGF21 can ameliorate hyperglycemia. The pancreatic islet is a target of FGF21, yet the actions of FGF21 in islet under normal and diabetic conditions are not fully understood. This study aimed to investigate the role of high glucose in islet FGF21 action by using animal models of diabetic db/db mice and FGF21 knockout (KO) mice.
Materials and methods: Pancreatic islets were isolated from db/db diabetic mice and m+/db normal mice, as well as FGF21 KO mice and their wild-type littermates. FGF21 and insulin levels were assessed by a specific mouse enzyme-linked immunosorbent assay (ELISA) kit. mRNA expression was evaluated by real-time RT-PCR; protein expression and post-receptor signaling pathways were analyzed by Western blotting. Islet morphology was evaluated by immunofluorescence.
Results: Serum levels of FGF21 were increased in diabetic db/db mice at 12 wks (m+/db 12.76 ± 4.53 pg/ml; db/db 39.00 ± 6.08 pg/ml; $^{**}P < 0.01$). The expression of β-klotho at both mRNA and protein levels, but not all FGF receptors, was reduced (72% and 53% respectively; $^{***}P < 0.001$) in islets isolated from adult db/db mice in an age-dependent manner. FGF21-induced phosphorylation of FGF receptor substrate 2 (FRS2) was impaired in islets from overt diabetic mice. In corroboration, *ex vivo* studies revealed that treatment with elevated glucose concentration (28 mM) down-regulated β-klotho expression (55%; $^{**}P < 0.01$), compared to those treated with normal glucose (5.6 mM). Weakened FGF21-induced FRS2 phosphorylation was also observed after high glucose treatments. Reduced β-klotho expression and FGF21 signaling in islets from adult diabetic mice or treated with high glucose were reversed by rosiglitazone (20 μM), an anti-diabetic peroxisome proliferator-activated receptor gamma (PPARγ) ligand. Both diabetic mice islets and high glucose-treated islets showed decreased PPARγ expression and activity, which were prevented by rosiglitazone. Meanwhile, islets of FGF21 KO mice showed distorted architecture and impaired glucose-stimulated insulin secretion.
Conclusion: Hyperglycemia in type 2 diabetes mellitus (T2DM) may lead to FGF21 resistance in pancreatic islets, probably through the reduction of PPARγ expression and activity which represents a novel mechanism for glucose-mediated islet dysfunction.
Supported by: General Research Fund and Collaborative Research Fund

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Glucagon like peptide 1 (GLP1) in combination with fibroblast growth factor 21 (FGF21) enhances browning of white adipose tissue
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Background and aims: GLP1 causes body weight loss via suppression of appetite, while FGF21 decreases body weight via a rise in energy expenditure. We have recently reported that GLP1 and FGF21 when administered in combination generate synergistic body weight loss in diet-induced obese (DIO) mice greater than that observed with either agent alone. In the present study, we aimed to investigate the molecular mechanism underlying this synergistic weight loss.
Materials and methods: DIO C57/Bl6 male mice were treated via subcutaneous osmotic pumps with either vehicle (Phosphate Buffered Saline, 12 μl/mouse/day), GLP1 (10 nmol/kg/day), FGF21 (10 nmol/kg/day) or GLP1 (10 nmol/kg/day) + FGF21 (10 nmol/kg/day) combination (COMBO) for 14 days. Body weight and food intake were monitored daily, In addition basal metabolism was also measured from Day 6 to Day 8 via indirect calorimetry.

Following sacrifice, several tissues (brown adipose tissue [BAT], white adipose tissue [WAT] and liver) were collected for analysis of mRNA expression of candidate genes by QPCR.
Results: Treatment with GLP1 or FGF21 alone led to 7% or 12% body weight loss, respectively, as compared to vehicle. However, COMBO treatment resulted in significantly greater body weight loss (27% decrease from vehicle). Even though GLP1 alone treatment had no effect on the energy expenditure, GLP1 in combination with FGF21 significantly potentiated the FGF21-induced energy expenditure increase during light photoperiod. As summarized in the table, COMBO treatment up-regulated PGC-1α and UCP-1 mRNA expression in epididymal white adipose tissue indicative of "browning" of WAT. Moreover GLP1+FGF21 combination increased leptin receptor expression in metabolically active peripheral tissues.
Conclusion: Combination of GLP1 and FGF21 produces a profound decrease in body weight via enhanced transformation of adipose tissue from a WAT to BAT-like state and the marked increase in peripheral mRNA expression suggests that leptin signaling and leptin sensitivity may be enhanced.

Table. mRNA expression of candidate genes in white adipose tissue and liver following 14 day treatment of DIO mice
(* indicates significant from control; + significant from GLP1 or FGF21).

Treatment	Tissues (fold increase from control)									
	White Adipose Tissue						Liver			
	UCP-1	PGC-1α	FGF21	β-Klotho	Leptin	Leptin R	PGC-1α	FGF21	β-Klotho	Leptin R
Control	1	1	1	1	1	1	1	1	1	1
GLP1	2.6	1.3	0.6*	1.2	0.6*	1	1.4	0.8	1.2	1.4
FGF21	3.2	1.2	0.4*	1	0.5*	1.2	1	1	1	4*
COMBO	15.5*+	2.8*+	0.4*	2*+	0.2*+	1.2	2.2*+	0.6	1.4*	15*+

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FGF21 knockdown exacerbates hepatic insulin resistance via STAT3/ SOCS3 pathway
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Background and aims: Insulin resistance is a metabolic disorder often associated with type 2 diabetes. Recent reports have demonstrated that FGF-21 plays an important role in the progression of insulin resistance. Nonetheless, the biochemical and molecular mechanisms by which changes in FGF-21 activation result in changes in the rates of hepatic gluconeogenesis and glycogenolysis remain to be elucidated. This study aimed to explore the effects of FGF-21 knockdown on HGP, especially the relative contribution of gluconeogenesis and glycogenolysis to HGP, and STAT3/ SOCS3 signal pathways.
Materials and methods: We have developed an adenovirus-mediated RNA interference technique in which short hairpin RNAs (shRNAs) were used to inhibit FGF-21 expression in the liver. Insulin sensitivity was examined by euglycaemic-hyperinsulinaemic clamping. Glucose rates of appearance (GRa) were determined with 3-[3H] glucose. Whole body GRa and glucose uptake (GRd) were calculated using the non-steady-state equation. mRNA and protein expressions were measured by qRT-PCR and Western blot, respectively.
Results: Liver-specific knockdown of FGF-21 in HFD-fed ApoE-/- mice produced a 39% increase in glycogenolysis and a 75% increase in gluconeogenesis and were accompanied by increases in the hepatic expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. FGF-21 knockdown further suppressed phosphorylation of STAT3 in HFD-fed mice. And SOCS3 mRNA and protein levels in FGF-21 knockdown plus HFD-fed mice were decreased to 10% and 50% compared to that in HFD-fed mice alone.
Conclusion: We present evidence suggesting that FGF-21 knockdown exacerbated hepatic insulin resistance in the liver is a consequence of increased gluconeogenesis and glycogenolysis in concert with activation of G6Pase and PEPCK via the STAT3/ SOCS3 pathway.
Supported by: National Natural Science Foundation of China

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Alpha lipoic acid induces hepatic FGF21 expression via up-regulation of Nur77 and CREBH
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Background and aims: Fibroblast growth factor 21 (FGF21) is a potential candidate for the treatment of metabolic syndrome due to its beneficial effects on glucose and lipid metabolism. FGF21 stimulates hepatic fatty acid oxidation,ketogenesis and gluconeogenesis in the response to long-term fasting. The mechanisms underlying the transcriptional regulation of FGF21 are not fully elucidated. Nur77 is a transcription factor that belongs to a nuclear hormone receptor superfamily and implicated in hepatic gluconeogenesis during fasting. Alpha lipoic acid (ALA), naturally occurring thiol antioxidants, decreases lipogenesis and increases insulin sensitivity in liver. Previously we demonstrated that ALA induces Nur77 induction in vascular cells. The aim of this study is to determine whether ALA induces Nurr77 and increases FGF21 expression in liver.
Materials and methods: The alterations of FGF21, Nur77, cyclic AMP response element binding protein H (CREBH) and fasting-related metabolic gene expression were compared in mice under both fed and fasted conditions. The effects of ALA and adenovirus-mediated Nur77 and CREBH on hepatic FGF21 production in vitro and in vivo were investigated. We also examined whether downregulation of endogenous Nur77 by small interfering (si) RNA restores the ALA effect on hepatic FGF21 production.
Results: We found that fasting and ALA treatment induced Nur77 and CREBH production. ALA treatment and adenovirus-mediated overexpression of Nur77 and CREBH increased hepatic FGF21 expression and serum FGF21 levels. Moreover, the inhibition of endogenous Nur77 expression by siRNAs markedly abolished ALA-induced CREBH and FGF21 expression. Nur77 and CREBH synergistically increased FGF21 expression in liver cells.
Conclusion: This study suggests Nur77 as novel positive regulator of hepatic FGF21 expression during fasting state and ALA has potential metabolic benefits by upregulation of FGF21.

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Brown adipose tissue, a sink of glucose disposal? Effects of FGF21 on glucose metabolism in brown adipose cells
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Background and aims: Brown adipose tissue (BAT) is a site of adaptive thermogenesis in mammals. BAT is more active and abundant in adult humans than expected, and its activity is negatively correlated with obesity and type II diabetes. BAT is a sink of lipid utilization but evidence of active uptake of radiolabeled glucose, as detected in PET-scan procedures, has led to the hypothesis that BAT could be an important site of glucose utilization, and BAT activation could be useful to prevent hyperglycemia. FGF21 is a novel hormonal factor with beneficial metabolic effects on glycemia, and BAT is a potential site of FGF21 action. Here we determined the effects of FGF21 on glucose metabolism in brown adipocytes, in an attempt to evaluate the capacity of FGF21 to induce the potential of BAT as an active site glucose disposal.
Materials and methods: We evaluated brown adipocytes (mouse primary cultures) as targets of FGF21 on glucose metabolism, by determining the effects of FGF21 (5nM, or a range of 5nM to 50 nM) on: a) expression of the components of the FGF21 response machinery (FGF receptor-1 and beta-Klotho cor-receptor): b) potential intracellular responses to FGF21 (ERK1/2, p38MAP kinase, protein kinase A), by immunoblot detection of the phosphorylated forms, c)3H-2-deoxyglucose uptake in absence or presence of insulin, d) 14C-glucose oxidation to 14C-CO2; d) expression of glucose transporters GLUT1 and GLUT4 and other marker genes of oxidative (cytochrome oxidase activity, spectrophotometric) and thermogenic (UCP1 expression) activity; e) overall respiratory activity (Biosensor; f) lipolysis (glycerol in the medium, Sigma). Transcript levels were measured by standard qRT-PCR (TaqMan, Applied Biosystems) and specific protein levels by immunoblot. Statistical analysis used ANOVA.
Results: Differentiation of brown adipocytes led to a strong induction of beta-Klotho expression. FGF21 caused a strong and rapid (15 min) activation of ERK1/2 and p38 MAP kinase but did not alter protein kinase-A activity.

Exposure to FGF21(5nM) caused a induction in glucose uptake both in the presence (2.2-fold, P=0.03) and absence (2.6-fold, P=0.02) of insulin; and a 3.8-fold induction (P=0.01) of glucose oxidation in brown adipocytes. Induction of overall cellular respiration was 1.4-fold (P=0.03). In parallel, FGF21 caused a dose-response induction of GLUT4 and GLUT1 expression (overall, a range of 2-3-fold maximal induction in specific transcript and protein levels for each transporter) This was paralleled by a significant induction of cytochome oxidase activity (2.3-fold, P=0.02) and expression of the thermogenic marker UCP1 (2.9-fold, P=0.008). FGF21 caused a significant induction of lipolysis (1.8-fold, P=0.03).
Conclusion: Brown adipocytes are sensitive targets of FGF21. FGF21 induces glucose uptake and overall oxidation, and a specific strong induction of oxidation of glucose. This happened in association with a coordinate activation of the overall machinery for mitochondrial uncoupled oxidation plus a specific induction in the expression of glucose transporters. This effect appears to be independent from responsiveness to insulin. Thus, the induction of brown adipocyte amounts and/or activity appears as a foreseeable strategy to enhance glucose disposal. FGF21 is a promising tool to promote this process with potentially beneficial effects in conditions of hyperglycemia
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FGF21 resistance relevant to insulin resistance in skeletal muscle of patients with type 2 diabetes
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Background and aims: Insulin resistance and hyperinsulinemia are the hallmark features of type 2 diabetes (T2DM) and obesity, but the detailed pathogenesis underlying the initiation of insulin resistance is still poorly understood. Recently, fibroblast growth factor 21 (FGF21), a novel member of the fibroblast growth factor family, was identified as a potent metabolic regulator with specific effects on glucose and lipid metabolism. We investigated the mechanisms involved in the induction of FGF21 resistance in human skeletal muscle of T2DM patients.
Materials and methods: We recruited 16 T2DM subjects and 12 subjects with normal glucose tolerance for this study. Whole-body insulin-mediated glucose uptake was determined using a euglycemic hyperinsulinemic clamp test. A percutaneous biopsy sample of the vastus lateralis muscle was obtained from 15 to 20 cm above the knee using a Bergstrom needle. We used western blotting to determine levels of FGF21 and active FGF receptor and FRS2α in skeletal muscles from T2DM subjects and normal glucose tolerant subjects. Differentiated human skeletal muscle myoblasts (HSMMs) were pretreated with palmitate at three different concentrations for 24 hours, and then were stimulated for 10 minutes with recombinant FGF21 (100 ng/ml). We measured levels of FGF21, FGF Receptor, FRS2, and ERK1/2 using antibodies in HSMMs treated with palmitate.
Results: Levels of FGF21 were significantly increased in skeletal muscles from T2DM subjects but levels of active FGF receptor and FRS2α were significantly decreased in skeletal muscles from T2DM subjects. Similarly, FGF21 increased levels of active FGF receptor, FRS2α and ERK1/2 in HSMMs but there were significantly decreased levels of FGF21-stimulated FGF receptor, FRS2α and ERK1/2 in HSMMs treated with palmitate.
Conclusion: In conclusion, FGF21 resistance apparently was involved in insulin resistance in skeletal muscle in type 2 diabetes.
Supported by: Ministry of Health and Welfare, Republic of Korea

OP 13 Clinical interventions and cardiovascular outcomes

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Baseline characteristics of participants enrolled in the cardiovascular outcome study of linagliptin versus glimepiride in early type 2 diabetes (CAROLINA)
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Background and aims: Current evidence on the cardiovascular (CV) safety of diabetes drugs remains inconclusive, in particular for sulphonylureas (SUs). There is therefore a need for properly designed, long-term, active controlled studies in type 2 diabetes (T2D) to provide solid evidence on the potential benefit or lack thereof of different glucose-lowering therapies on CV events and mortality.
Materials and methods: In CAROLINA potential differing effects on CV outcomes of linagliptin (5 mg/day) therapy versus glimepiride (up to 4 mg/day) will be determined in participants with early T2D at high CV risk or with CV complications. The study is a double-blind, double dummy, event driven (targeting 631 events [time to first event of a composite of non-fatal myocardial infarction, non-fatal stroke, CV death or hospitalisation for unstable angina pectoris]) trial powered for CV non-inferiority and superiority.
Results: Between Dec 2010 and Dec 2012, 581 clinical sites globally randomised 6103 patients. At baseline, data on clinical history, symptoms and medications along with centralised evaluations of ECGs and lab specimens were obtained. Most participants come from Europe (45.3%) or North-America (19.5%), 40.2% are women and 34.2% have previous CV complications. Diabetes duration was ≤5 years in 41% and mean HbA1c was 7.2% (HbA1c <7.5%: 73%). 67% were on 1 and 23% on 2 glucose-lowering agents, with the vast majority taking metformin (TABLE). The population was overweight/obese, with BMI ≥30 kg/m^2 in 46% of participants.
Conclusion: Baseline characteristics indicate that an appropriate target population has been recruited to address the study question of potential differing effects of linagliptin and glimepiride on CV outcomes and thus will help inform clinical decision-making when selecting second line therapy in T2D.

Table. Baseline characteristics of the study population in CAROLINA

Age (years), mean ± SD	64±10
Race or ethnicity, n (%)	
White	4442 (73.3%)
Asian	1061 (17.5%)
Hispanic or Latino	1064 (17.6%)
Black/African American	333 (5.5%)
American Indians/Alaska Natives	214 (3.5%)
HbA1c (%), mean ± SD	7.2±0.6
BMI (kg/m²), mean ± SD	30.1±5.3
Systolic/Diastolic blood pressure (mmHg), mean ± SD	138 ± 17/80 ± 10
Resting pulse rate (beats per minute), mean ± SD	72 ± 10
eGFR (MDRD; ml/min/1.73m²)	77±20
Qualifying CV severity risk category for trial entry, n (%)*	
Previous CV complications	2071 (34.2 %)
Microvascular complications (proliferative retinopathy, albuminuria, eGFR 30-59 mL/min/1.73 m²)	496 (8.2%)
Advanced age (≥70 years)	1187 (19.6%)
Multiple CV risk factors (hypertension, current smoking, dyslipidaemia, T2D duration ≥10 years)	2296 (37.9%)
Oral glucose lowering treatment at baseline, n (%)	
No therapy	554 (9.1%)
Monotherapy	4066 (67.1%)
% of patients on monotherapy receiving metformin	*89.1%*
Dual therapy	1400 (23.1%)
% of patients on dual therapy receiving metformin + SU <5 years	*87.4%*
Other therapies, n (%)	
ASA	2965 (48.9%)
Statins	3684 (60.8%)
Any antihypertensive therapy	5197 (85.8%)

Based on 6058 randomised and treated patients (database cutoff: 16 Feb 2013). *: 0.1% missing categorisation.

Clinical Trial Registration Number: NCT01243424
Supported by: Boehringer-Ingelheim

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Liraglutide effect and action in diabetes: evaluation of cardiovascular outcome and results (LEADER) trial design and methods

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Background and aims: Diabetes is independently associated with a two-fold excess risk for a broad range of cardiovascular adverse events including cardiovascular death, stroke, and non-fatal myocardial infarction. Liraglutide is a human glucagon-like peptide (GLP-1) receptor analog approved for use in patients with type 2 diabetes (T2DM). Its cardiovascular safety is being formally evaluated in the ongoing LEADER trial.
Materials and Methods: LEADER is Phase 3B, multi-center, international, double-blind, placebo-controlled clinical trial with long-term follow-up. Patients with T2DM at high risk for cardiovascular disease (CVD) who are treated with either oral antidiabetic (OAD) agents or selected insulin regimens alone or combined with OADs were eligible for inclusion. Patients with and without prior CVD were randomized to liraglutide once daily (maximum dosage of 1.8 mg) or placebo. The primary endpoint is the time from randomization to the first occurrence of cardiovascular death, non-fatal myocardial infarction, or non-fatal stroke. Estimated minimum enrollment of 8,754 randomized subjects was based on accrual of no less than 611 primary events with minimum follow-up of 42 months after last subject randomized. Consistent with United States Food & Drug Administration guidelines for demonstrating cardiovascular safety in anti-diabetes drugs, noninferiority of liraglutide will be established if the upper 2-sided 95% confidence limit for the relative risk of the primary endpoint is less than 1.3.
Results: LEADER enrolled patients from 2010-2012. Baseline demographics of the enrolled population (N=9,340) are shown in the Table. Of the 9,340 patients, 7,633 (81.7%) had prior CVD and 1,707 (18.3%) did not. There were 1,854 (19.9%) patients with estimated glomerular filtration rate (eGFR) 30-59 mL/min/1.73m²) and 177 (1.9%) with eGFR < 30 mL/min/1.73m².
Conclusions: LEADER is an ongoing phase 3B time- and event-driven randomized double blind clinical trial evaluating the cardiovascular safety of liraglutide in patients with T2DM at heightened risk for cardiovascular complications. Over 80% of the population has a history of prior CVD. It is anticipated that LEADER will provide conclusive results on the cardiovascular safety of liraglutide, with results expected in 2016.

Data are mean +/- standard deviation or percent

	Previous CVD N=7,592	No Previous CVD N=1,748
Age, y	63.9 ± 7.6	65.8 ± 5.2
Gender (male)	66.5	54.6
White	78.7	72.3
Weight, kg	92.3 ± 20.9	89.6 ± 21.4
Hypertension	90.7	87.0
Coronary artery disease	69.7	1.0
Diabetes duration, y	12.8 ± 8.1	12.3 ± 7.5
Hemoglobin A1c, %	8.7 ± 1.5	8.8 ± 1.6
Insulin use	42.9	36.9
LDL cholesterol, mg/dL	88.0 ± 35.5	96.5 ± 34.6
HDL cholesterol, mg/dL	44.9 ± 12.1	48.0 ± 12.7

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk

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Effects of metformin versus placebo in combination with insulin analogues in patients with type 2 diabetes mellitus: the CIMT randomised clinical trial

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Background and aims: It is unknown whether metformin versus placebo in combination with insulin analogues offer more benefits than harms in patients with T2D. In the Copenhagen Insulin Metformin Therapy (CIMT) trial we assessed the effects of 18-month metformin versus placebo in combination with one of three insulin analogue regimens in T2D patients on mean carotid intima-media thickness and adverse events.
Materials and methods: A centrally randomised, 2 x 3 factorial, treat-to-target (HbA1c ≤ 7.0%) clinical trial conducted at eight clinics assessing blindly metformin (1g twice daily) versus placebo. Inclusion criteria: T2D, HbA1c > 7.5%, BMI 25 to 40 kg/m2, oral antidiabetic agents for one year and/or insulin for at least 3 months. Insulin regimens were insulin detemir, insulin aspart biphasic, or a combination of aspart and detemir. Primary outcome: change in mean carotid intima-media thickness of the common carotid arteries. Secondary outcomes: serious adverse events, hypoglycaemia, weight, HbA1c, and dose of insulin (treatment variable). After multiple imputations for the primary outcome, intention-to-treat analyses were performed adjusted for stratification variables plus entry mean carotid intima-media thickness with two-sided significance tests (alpha 0.01) using general linear univariate model (continuous data) or Chi-square test as appropriate.
Results: Only 413 of 950 planned patients were randomised 1:1 to metformin vs. placebo. The two groups did not differ regarding insulin regimens. The 18-month ratio between the mean carotid intima-media thickness did not differ significantly (1.013, 95% CI 0.995 to 1.030, P=0.16; metformin mean 0.784 (95% CI 0.771 to 0.798) mm vs. placebo 0.774 (95% CI 0.761 to 0.787) mm). The number of patients with serious adverse events was 29.6% vs. 24.8% (P=0.27), with severe hypoglycaemia 5.8% vs. 5.3% (P=0.83), and with non-severe hypoglycaemia 76.2% vs. 75.2% (P=0.82). The 18-month insulin dose did not differ significantly between the two groups (mean difference -9.24 IE, 95% CI -24.6 to 6.16 IE; P=0.24). The 18-month HbA1c (mean ratio 0.96, 95% CI 0.93 to 0.98, P=0.0015; mean 7.65 vs. 8.00%) and weight (mean ratio 0.98, 95% CI 0.96 to 0.99, P=0.002; mean 97.5 vs. 100.0 kg) and the AUC of insulin doses during the 18 months (mean difference -7425 IE, 95% CI 2115 to 12735 IU, P=0.006) were significantly less in the metformin group.
Conclusion: We did not observe significant effects of metformin versus placebo on mean carotid intima-media thickness, serious adverse events, severe hypoglycaemia, non-severe hypoglycaemia, or 18-month insulin dose. Metformin significantly decreased the 18-month HbA1c and weight as well as the total insulin dose during the trial. However, the trial only reached 43% of the planned sample size, and our results underscore the need for large randomised clinical trials with adequate methodologies to determine the impact

of long-term combination of metformin with insulin analogues versus insulin analogues alone in T2D.
Clinical Trial Registration Number: NCT00657943
Supported by: An unrestricted grant from Novo Nordisk

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LV remodelling in patients with type 2 diabetes: determinants of the beneficial effect of olmesartan
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Background and aims: Cardiac structural adaptation develops early in hypertension and diabetes and is aggravated if both conditions coexist. Blockade of the angiotensin receptors may attenuate these cardiac processes and we analyzed clinical determinants (including therapy with olmesartan) of preventing or delaying left ventricular (LV) remodeling in a large-scale prospective study.
Materials and methods: In the ROADMAP study, a double blind, placebo controlled, multicenter, phase IIIb study, comparing olmesartan 40mg (OM) vs. placebo (Pb; additional anti-hypertensive medication allowed, except RAAS inhibitors) on microalbuminuria prevention in diabetic patients, also 12-lead ECGs have been conducted at baseline and in yearly intervals over the mean study duration of 3.2 years. Patients were eligible to enter the prespecified ECG-LVH analysis if they had an evaluable ECG at baseline and one after at least 2 years. All ECGs were centrally and independently evaluated by two investigators in a blinded fashion. The primary ECG parameter of LV remodeling and hypertrophy was Cornell voltage QRS duration product, and the Cornell voltage index and Sokolow-Lyon index were secondary parameters.
Results: Of the 14.074 ECG recorded in the ROADMAP study, 9.418 have been found evaluable and 1.513 patients were identified in whom interpretable ECGs were available at baseline and at last assessment (N=736 Pb and N=777 OM patients). Dividing the study population into 4 quartiles defined by the Cornell voltage QRS duration product at baseline, the prevalence of patients in the highest quartile (i.e. >200 mV*msec) increased from 24% to 26,5% in the placebo but decreased from 25.5% to 22.3% in the olmesartan group (odds ratio 0.598 [95% CI: 0.440-0.813], p=0.0011). When adjusted for potential differences in clinical baseline parameters (incl. blood pressure), the odds ratio did not change substantially. Analysis of determinants for LV remodeling in these diabetic patients revealed that the prevalence of the Cornell voltage QRS duration product was significantly reduced in diabetic patients in the OM group with a BP either < or > 130/80 mmHg at last assessment (Risk reduction [RR] 39.2%, and 45.6 %, p=0.0081 and 0.0406, respectively). In addition, it was also significantly reduced in the OM group independently of the age (> or ≤ 58 years; RR 37.9% and 42.3%, p=0.0347 and 0.0121, respectively) or being male or female (RR 46.5% and 36.3%, p=0.0448 and 0.0136, respectively). A significant reduction for OM was also seen in patients with a duration of diabetes > 5 years (RR 37.7%, p=0.0039), with anti-diabetic medication at baseline (RR 41.5%, p=0.0008), in obese patients (BMI >28 kg/m^2; RR 44.2%, p=0.0011), in patients with an HbA1c >7,0% (RR 46.1%, p=0.0023) at baseline, as well as in patients with an increase in HbA1c during the study period (RR 52.6%, p=0.0010).
Conclusion: Olmesartan significantly decreased ECG left ventricular remodeling in type 2 diabetes independent of differences in blood pressure, age and gender. This effect was most striking in patients with longer duration of diabetes, intake of anti-diabetic medication, obesity and a high HbA1c at baseline or its increase during the study. Thus, in addition to clinical parameters, olmesartan delayed ECG signs of cardiac structural adaptation in type 2 diabetes.
Clinical Trial Registration Number: NCT00185159
Supported by: Daiichi Sankyo

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Predictors of congestive heart failure after treatment with an endothelin receptor antagonist: a post-hoc analysis of the ASCEND trial
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Background and aims: The Avosentan on time to doubling of Serum Creatinine, ENd stage renal disease or Death (ASCEND) trial tested the potential renoprotective effect of the endothelin receptor antagonist (ERA) avosentan versus placebo in patients with diabetic nephropathy. Yet, the trial was prematurely terminated due to high incidences congestive heart failure (CHF) in the avosentan arms likely due to fluid retention. The aim of this study was to identify risk markers associated with congestive heart failure (CHF) after treatment with the endothelin receptor antagonists (ERA) avosentan.
Materials and methods: In a post-hoc analysis of ASCEND (N=1392) we assessed whether baseline CHF risk was modified by avosentan treatment. Furthermore, post-randomisation changes between baseline and the first available measurement of the fluid retention surrogates body weight (BW) and haemoglobin (Hb) were examined for their relationship with CHF development.
Results: Relative to placebo, avosentan increased CHF risk (HR 2.72; 1.66 to 4.47; p<0.001). The avosentan induced CHF risk increased with lower baseline cholesterol levels (p=0.03) and concomitant statin use (p=0.060), while it decreased with lower eGFR (p=0.040). Patients allocated to avosentan had a median BW increase of 0.6 kg (IQR 0.0 to 2.0 kg) and a median Hb decrease of 14 g/L (IQR -21 to -7 g/L) at the first post-randomisation measurement (p<0.001 vs. placebo). Avosentan induced BW increase was a predictor of CHF (p=0.038), while Hb decrease was not (p=0.603). The increase in BW was particularly pronounced in patients with a cardiovascular disease history and in those using statins.
Conclusion: In avosentan treated patients with diabetic nephropathy, BW increase, but not Hb decrease, was an early risk marker for CHF development, indicating that close BW monitoring could be used to identify high risk subjects in future trials with ERAs. Monitoring of statin use is recommended as avosentan-exposure may increase in combination with these agents.
Clinical Trial Registration Number: NCT00120328
Supported by: Speedel Pharma

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Effects of bariatric surgery on ectopic fat depositions and cardiovascular function in obese type 2 diabetes patients
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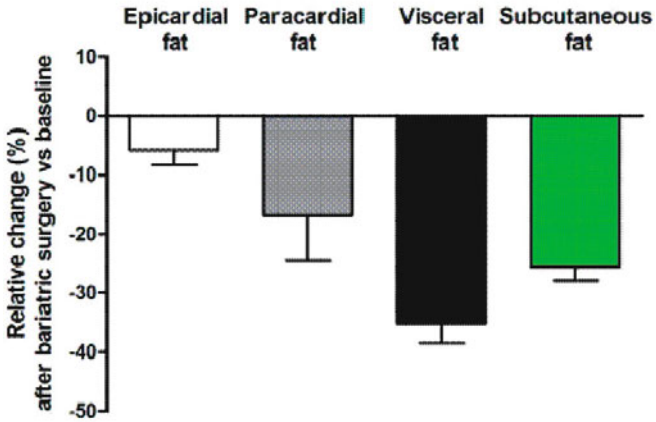
Background and aims: Cardiovascular disease (CVD) is the main cause of death in type 2 diabetes (T2DM) patients. Lipotoxicity, caused by ectopic fat depositions in and around the heart, is thought to play a role in the pathogenesis of CVD. Diet-induced weight loss results in a decrease in cardiac ectopic fat stores, however whether this is the same for weight loss after bariatric surgery is less clear. Therefore, we assessed myocardial TG content, pericardial fat and cardiac function in obese, insulin-dependent T2DM patients before and 16 weeks after a Roux-en-Y gastric bypass (RYGB) surgery.
Materials and methods: Nine obese patients (40% male, age 54 ± 9 years (mean ± SEM)) with insulin-dependent T2DM, without a history of cardiovascular disease, scheduled to undergo RYGB surgery were enrolled in this study. Cardiovascular function and ectopic fat accumulation were assessed with magnetic resonance (MR) imaging and myocardial TG content with MR spectroscopy before and 16 weeks after the RYGB surgery.
Results: BMI decreased from 41.6 ± 1.5 kg/m2 at baseline to 34.2 ± 1.0 kg/m2 (p<0.001) 16 weeks after RYGB surgery. Glycemic control improved as well, as shown by a decrease in HbA1c from 8.0 ± 0.4 to 6.8 ± 0.5 % (p<0.05). Total daily insulin dose decreased (-115 ± 19 units/day) and 2 patients discontinued insulin therapy completely. A significant decrease of abdominal and pericardial fat depots was seen, with a wide variation in the relative changes. There was a high relative decrease in visceral (-35.2 ± 3.4%) as compared

to subcutaneous (-25.7 ± 2.3%) fat volume and a higher relative decrease in paracardial (-16.9 ± 7.7%) as compared to epicardial (-5.8 ± 2.6) fat volume (Figure 1). We did not observe an effect of the RYGB surgery on cardiac function, pulse wave velocity or myocardial TG content.
Conclusion: This study shows that surgical-induced weight loss results in tissue-specific changes in body fat distribution.

Figure1. Relative changes in fat depots 16 weeks after RYGB.



Supported by: CTMM

OP 14 Technologies to transform diabetes

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The pharmacologic profile of rapid-acting insulin administered by jet-injection in patients with diabetes
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Background and aims: We recently showed in a euglycemic clamp study among healthy subjects faster absorption and action of rapid-acting insulin when injected by a jet injector rather than by a conventional pen. To determine whether these benefits exist in patients with diabetes in a more real-life situation, we compared the pharmacologic profile of rapid-acting insulin administration by jet injection to that by conventional insulin pen prior to a standardized meal in patients with type 1 or type 2 diabetes.
Materials and methods: In a randomized, double-blind, double-dummy cross-over study, 12 patients with type 1 and 12 patients with type 2 diabetes (mean±SD age: 49.9±16.0 years, BMI: 26.4±2.1 kg/m^2, HbA1c: 7.5±0.8%) received insulin aspart either by jet injection or by conventional pen followed by a standardized high-glycemic index meal (108g carbohydrates). Blood was sampled for six hours for determination of plasma glucose and insulin levels to calculate pharmacologic parameters.
Results: Insulin administration by jet injection resulted in shorter time until peak plasma insulin levels (51.3±6.4 versus 91.9±10.2 min, P=0.003) and reduced hyperglycemic burden during the first hour (154.3±20.8 versus 196.3±18.4 mmol·min·l^{-1}, P=0.041) when compared to conventional administration. Jet injection did not significantly reduce the hyperglycemic burden during the hours thereafter. There were no differences between the jet injector and conventional pen with respect to number of patients requiring exogenous glucose to prevent hypoglycemia (17 versus 18, P=0.75) or the amount of glucose administered (21.0±5.5 g versus 23.7±5.7 g, P=0.61). Both injection methods elicited a similar experience of pain (VAS, 1.96±1.9 versus 1.40 ±1.6, P=0.14). The jet injector did not perform significantly different in patients with type 1 or type 2 diabetes, although there was a suggestion that the jet injector had a stronger effect in patients with type 2 diabetes.
Conclusion: In patients with type 1 or type 2 diabetes, insulin administration by jet injection resulted in considerably more rapid insulin absorption compared to administration by conventional pen. This translated into a significant decrease in early postprandial hyperglycemia. Beyond one hour, the benefit of jet injection on postprandial glycemic burden was no longer statistically significant. The improved postprandial glucose control may specifically benefit patients with difficulty limiting postprandial glucose excursions.2
Clinical Trial Registration Number: NCT01438632
Supported by: European Pharma Group

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Clinical evaluation of the Imperial College bio-inspired artificial pancreas overnight and after breakfast in adults with type 1 diabetes
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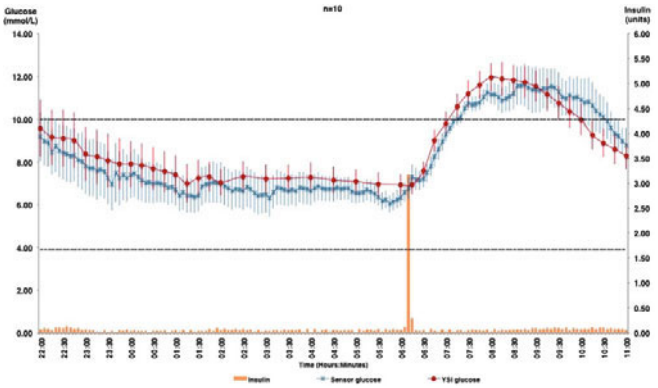
Background and aims: The aim of this study was to assess the safety and efficacy of the Imperial College Bio-inspired Artificial Pancreas in adults with type 1 diabetes mellitus (T1DM) during overnight and post-prandial conditions. The bio-inspired closed-loop control algorithm, developed by our group, is based on a mathematical model of beta-cell physiology and is implemented on a microchip within a handheld device.
Materials and methods: This was a non-randomised open label study. Each subject underwent 13-hours of closed-loop control starting at 22:00. The system comprises a Medtronic Enlite sensor, the Imperial College bio-inspired controller and a Roche Accu-Chek Combo insulin pump. A standard breakfast containing 40 grams of carbohydrates was given at 06:00 the next day with the meal announced to the controller.
Results: 10 adult subjects with T1DM (60% male, mean (SD) age 45 (11) years, duration of diabetes 19 (11) years, duration of pump therapy 3.1 (3.2)

years, HbA1c 7.6 (0.6) %, body mass index 25 (3) kg/m2) participated in the study. The percentage time (median (IQR)) in target (3.9-10.0 mmol/l) was 67.6 % (60.8-69.0) and 38.4 % (33.1 - 51.0) in euglycaemia (3.9-7.8 mmol/l). 0.0 % (0.0 - 0.0) of time was spent in hypoglycaemia (<3.9 mmol/l) or severe hyperglycaemia (<15 mmol/l). The graph below shows the mean glucose (dotted line) and sensor glucose (starred line) with standard error bars and mean insulin (bars) delivered over the 13-hours closed-loop study. No algorithm decisions were rejected by the study team and no rescue carbohydrate was given.

Conclusion: The Imperial College Bio-inspired Artificial Pancreas is safe and achieves good glycaemic control in subjects with T1DM overnight and after a meal challenge, demonstrating the safety and efficacy of a physiologically based glucose controller in a closed-loop insulin delivery system. Reassuringly, no time was spent in the hypoglycaemic or severe hyperglycaemic range.



Clinical Trial Registration Number: NCT01534013
Supported by: Wellcome Trust

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Using CGM data to test the hypothesis that higher levels of fear of hypoglycaemia are related to poorer glycaemic control

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Background and aims: Fear of hypoglycemia (FoHypo) is recognized as a major potential barrier to optimal diabetes control; however, few studies have found relationships between FoHypo and measures of glycemic control such as HbA1c. Most of these studies have used the Hypoglycemia Fear Survey-II (HFS-II) to measure FoHypo. We have recently published findings showing that the Behavior Subscale of the HFS-II contains two factors, one describing behaviors to avoid hypoglycemia that involve maintaining higher BG levels (Maintain High BG Subscale), and one describing other types of behaviors aimed at avoiding hypoglycemia (Avoidance Subscale). The primary purpose of this study was to test the hypothesis that higher patient scores on the Maintain High BG subscale would be related to glucose profile variables indicating poorer glycemic control (e.g. higher average BG levels). Glucose profile variables were computed based on data from continuous glucose monitoring (CGM) devices used for a one-month period. No previous studies have used CGM data to investigate the impact of FoHypo on diabetes control. In addition, this study tested the hypothesis that a new measure of fear of hyperglycemia (FoHyper) would be related to glucose profile variables indicative of problems with hypoglycemia.
Materials and methods: A total of 60 patients (58% female) participated in the study. Mean/SD for age was 40.4 ± 13.6 years and for diabetes duration was 23.9 ± 10.8 years. All participants used continuous subcutaneous insulin infusion (CSII) therapy and, for the purposes of this study, used CGM devices for a one-month period. The mean number of glucose readings obtained from participants during this period was 7,586 ± 972. Prior to beginning CGM data collection, participants completed psychosocial questionnaires including the HFS-II and the newly-constructed FoHyper survey.
Results: As predicted, higher scores on the Maintain High BG Subscale correlated significantly with higher average BG levels (r = .29, p = .043) and higher BG variability (r = .31, p = .028). Higher scores on the Avoidance Subscale correlated with a higher number of low (<3.9mmol/L) BG readings (r = .39, p = .005) and with higher hypoglycemic risk (r = .38, p = .007). Higher scores on the FoHyper Behavior Subscale were associated with more frequent low BG levels (r = .39, p = .005), lower average BG levels (r = -.49, p < .001), and higher hypoglycemic risk (r = .31, p = .03).
Conclusion: This is the first study to investigate the relationship between FoHypo and FoHyper on glucose profiles using CGM data. These preliminary findings support the hypothesis that patient anxiety about both hypo- and hyperglycemia may influence diabetes management behaviors and control.
Supported by: NIH/NIDDK RO1 DK 085623 and R21 DK 080896

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In type 1 diabetic patients insulin pump treatment is associated with reduced arterial stiffness

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Background and aims: Insulin pump treatment is often associated with reduced glucose variability and in small improvements in average glycemic control. This might reduce development of vascular complications. We investigated the relationship between arterial stiffness, evaluated by pulse wave velocity (PWV), and treatment with insulin pump in patients with type 1 diabetes, and examined if this association was dependent of glucose control.
Materials and methods: Cross-sectional study, from 2009-2011, including 639 Caucasian patients with type 1 diabetes. PWV measurements (SphygmoCor, AtCorMedical, Australia) were available in 58 patients with insulin pump (35 with albuminuria ≥ 30mg/d) and 542 (274 with albuminuria ≥30mg/d) treated with multiple daily insulin injections ((MDI) ≥2 injections). ANCOVA compared groups and adjusted linear regression analyses examined the association between PWV and treatment group as well as HbA_{1c}-level.
Results: Insulin pump *vs.* MDI treated patients were 52% *vs.* 57% men, 51±11 *vs.* 54±12 years of age, 34±13 *vs.* 32±16 years diabetes duration, mean arterial pressure was 93±11 *vs.* 93±11 mmHg, and HbA_{1c} 62±10 *vs.* 64±13mmol/mol (p>0.08 for all). PWV was lower in patients treated with insulin pump (9.3±2.8 *vs.* 10.4±3.4m/s; p=0.016). This difference remained significant (p=0.001) after adjustment for gender, diabetes duration, eGFR, urine albumin excretion rate, HbA_{1c}, total-cholesterol, smoking, mean arterial pressure, heart rate and BMI. In patients with albuminuria, PWV was also higher in the MDI *vs.* insulin pump treated groups (11.5±3.4 *vs.* 9.5±2.4 m/s; adjusted p<0.001). In adjusted regression analysis, treatment with insulin pump was significantly (p = 0.026) associated with lower PWV, while HbA_{1c}- level was not associated with PWV neither in patients treated with or without pump ($p \leq 0.925$).
Conclusion: Insulin pump treatment was independently associated with reduced arterial stiffness, while HbA_{1c} was not. Although glucose variability was not assessed, our results suggest that glucose variability and not HbA_{1c}-level affects arterial stiffness. This needs confirmation in randomised prospective studies.
Clinical Trial Registration Number: NCT01171248

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Interim 12 month results from a post market clinical trial of DJBL treatment outcomes in subjects with type 2 diabetes and/or obesity

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Background and aims: The Duodenal-Jejunal Bypass Liner (DJBL) is an endoscopically placed and removed device that creates a bypass of the proximal small intestine mimicking the Roux-en-Y and Duodenal-Jejunal Bypass without the risks associated with surgery. The DJBL is indicated for the treatment of type 2 diabetes and/or obesity and remains implanted for up to 12 months. The aim of this study was to observe the efficacy and safety of DJBL in the post market setting in the United Kingdom.
Materials and methods: 45 subjects implanted with DJBL were included in the study. Interim twelve month data are available for 32 subjects and data collection is ongoing for the remainder. Baseline mean BMI was 39.6 kg/m2 (range: 30.1-50.4 kg/m2), weight 245.5lb (range: 167.4-381.4lb). Subjects were reported to have had type 2 diabetes for a mean duration of 4.6 years (range: 1-10 years), with a baseline HbA_{1c} of 8.5% (7.2-10.5%). Adverse events were monitored continuously throughout the implant period. Data were collected at baseline and 3, 6 and 12 months post implant/removal as well as 3 and 6 months post-removal. Twelve month post-implant weight, blood pressure,

HbA_{1c}, fasting plasma glucose, insulin, total cholesterol, LDL, and TG are presented here.
Results: At twelve months post-implantation the mean BMI (35.4, -4.4kg/m2,), weight (219.1, -26.4lb), HbA_{1c} (7.5, -1.0%), fasting plasma glucose (7.8, -1.5 mmol/L), insulin (14.0, -3.8 mU/L), SBP (129.4, -8.3mmHg), DBP (76.6, -2.7mmHg), total cholesterol (3.9, -0.47mmol/L), LDL (2.15, -0.10 mmol/L) and TG (1.64, -0.24 mmol/L) were reduced from baseline/implant. The most common reported device related adverse events were reported as mild to moderate and gastrointestinal in nature. Three (6.7%) subjects had their device removed for device related reasons (abdominal pain, anchor migration, melena). Ten (22.2%) subjects had their device removed for non device related reasons.
Conclusion: Use of the DJBL is safe and leads to improved glycaemic control, body weight, and associated biomarkers at 12 months compared to baseline in subjects with type 2 diabetes and/or obesity in the post-market setting.
Clinical Trial Registration Number: NCT01114438
Supported by: GI Dynamics, Inc.

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Clinical effectiveness and cost-effectiveness of using positive airways pressure to manage sleep apnoea in patients with type 2 diabetes in the UK
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Background and aims: To assess the clinical effectiveness and cost-effectiveness of using positive airways pressure (PAP) to manage sleep apnoea in patients with type 2 diabetes from the perspective of the UK's National Health Service (NHS).
Materials and methods: Using a case-control design, a model was constructed depicting the treatment paths and associated resource use attributable to managing a cohort of sleep apnoea patients with type 2 diabetes. The model was based on the case records of 150 PAP-treated patients and 150 matched non-PAP treated patients from the THIN database (a nationally representative database of patients registered with general practitioners in the UK). Differences between groups were tested for significance using either a Mann-Whitney U-test or Chi-Square test. Regression analyses were also performed to assess the relationship between baseline parameters and outcomes. The model estimated the total NHS cost and outcomes of patient management in each group over 5 years and the cost-effectiveness of PAP compared to no PAP treatment.
Results: Patients' mean age was 53.7 years, 82% were male and >80% of patients in both groups were obese at 5 years. The time between diagnosis of sleep apnoea and starting PAP treatment was a mean 19.6±31.2 months. Compliance with PAP ranged from 93% in year 1 to 89% in year 5. PAP-treated patients had significantly lower HbA1c levels (66.0 versus 108.4 mmol/mol (8.2% versus 12.1%); $p<0.03$), indicative of better controlled diabetes. Logistic regression showed that females were more likely than males to have lower HbA1c levels at 5 years (Odds ratio: 13.03; $p<0.01$) and patients with angina were more likely to have uncontrolled diabetes (Odds ratio: 0.033; $p<0.02$). Diabetic retinopathy was an independent predictor of patients experiencing anxiety disorder (Odds ratio: 6.79; $p<0.03$). Time from diagnosis to starting PAP was an independent predictor of increasing the relative risk of having a stroke (Odds ratio: 10.46 for each 10 months; $p<0.04$). Multiple regression showed that HbA1c values increased by 2.7 mmol/mol (2.4%) for each 10 years of age ($p<0.05$) and decreased by 24.6 mmol/mol (4.4%) among PAP-treated patients ($p<0.05$). Use of PAP significantly increased patients' health status by 0.89 quality-adjusted life years (QALYs) (from 2.11 to 3.00 QALYs; $p<0.0001$) per patient over 5 years. PAP treatment also resulted in an increased NHS cost of £5,555 (from £11,973 to £17,528) per patient. Hence, the cost per QALY gained with PAP is £6,242. PAP remained a cost-effective intervention for plausible changes in the model's inputs.
Conclusion: Initiating treatment with PAP in sleep apnoea patients with type 2 diabetes leads to significantly better controlled diabetes and affords a cost-effective use of NHS resources.
Supported by: RM

OP 15 Neuropathy: peripheral and central mechanisms and outcomes

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Diabetes induces neuronal loss in the rostroventromedial medulla of streptozotocin-diabetic rats: the preventive effects of antioxidant treatment
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Background and aims: Diabetic neuropathy (DN) is one of the most frequent complications of diabetes, presenting repercussions at the somatossensory nervous system. Diabetes is associated with structural and functional changes at brain areas involved in ascending transmission of nociceptive input, as the spinal cord and thalamus. This is likely to concur to the exacerbated pain in patients with diabetic neuropathy. The effects of diabetes on brain areas involved in descending pain modulation are almost unknown. We previously reported a significant increase in oxidative stress damage in the rostroventromedial medulla (RVM), a key brainstem area involved in serotoninergic descending pain modulation, in the streptozotocin (STZ)-diabetic rat. Such damage may impair serotonin-mediated pain inhibition and explain the low analgesic efficacy of serotonin-selective reuptake inhibtitors (SSRIs) in diabetic neuropathic pain. In the present study we evaluated the effects of a treatment with epigalocathechin gallate (EGCG), a potent antioxidant present in green tea, in oxidative stress damage, neuronal density and number of serotoninergic neurons at the RVM of STZ- diabetic rats.
Material and methods: Diabetes was induced in male Wistar rats by intraperitoneal injection of STZ (60 mg/kg). Control animals (CTR group) received the vehicle solution. During the 10 weeks post-injection, a group of STZ rats received EGCG (2g/l) in drinking water while the other experimental groups received only water (untreated-STZ and CTR). Mechanical hyperalgesia and tactile allodynia were evaluated using the paw pressure test and the plantar aesthesiometer, respectively, before treatment onset and after its completion. Oxidative stress damage was quantified by densitometry in RVM sections immunoreacted against 8-OHdG, a marker of acid nucleic oxidative damage, and neuronal density was determined by counting the number of cells immunoreactive to Neu-N (a marker of neurons). Serotoninergic neurons were identified by immunodetection of tryptophan hydroxylase (TpH-IR; enzyme involved in serotonin synthesis).
Results: All STZ rats developed hyperglycemia, which was not affected by EGCG treatment. EGCG ameliorated the mechanical hyperalgesia and tactile allodynia detected in untreated-STZ rats. The untreated-STZ rats presented increased oxidative stress damage and decreased neuronal density at the RVM which was accompanied by a reduction in the number of serotoninergic neuron. EGCG treatment prevented those changes.
Conclusion: Diabetes induces increased oxidative stress and loss of neurons in the RVM. The decrease neuronal density detected in the RVM of STZ rats may, in part, be due to the reduced numbers of serotoninergic neurons. EGCG elicited neuroprotective effects during diabetes by preventing the oxidative stress and loss of RVM neurons, namely of serotoninergic neurons involved in descending modulation of nociceptive transmission, which may explain the analgesic effect here reported. EGCG could be a promising agent in preventing diabetes-induced neurodegeneration and pain.
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Altered baseline brain activity in type 2 diabetes: a resting-state fMRI study
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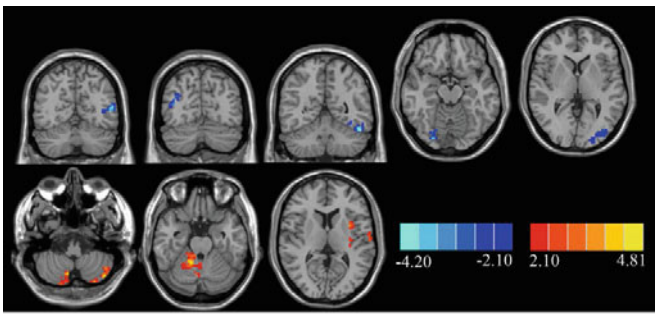
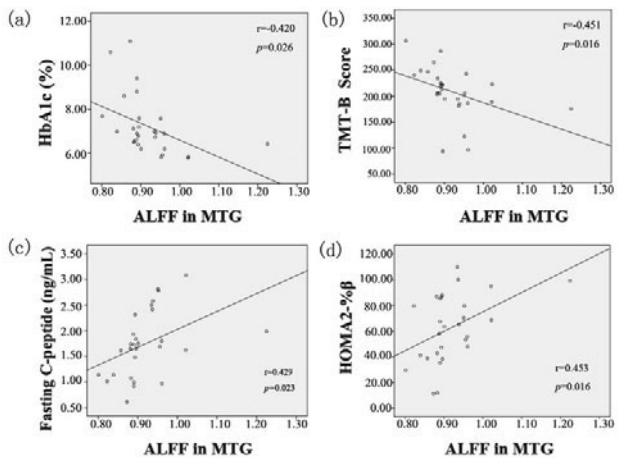
Background and aims: Type 2 diabetes mellitus (T2DM) has been associated with cognitive impairment, such as mild cognitive impairment (MCI) and Alzheimer's disease (AD). Our study aims to investigate whether there exists altered baseline brain activity in T2DM patients using resting-state functional magnetic resonance imaging (rs-fMRI).

Materials and methods: T2DM patients (n=28) were compared with non-diabetic age-, sex-, and education-matched control subjects (n=29) using rs-fMRI. We computed amplitude of low-frequency fluctuations (ALFF) of fMRI signal to measure the spontaneous neuronal activity, and detect the relationship between rs-fMRI information and clinical data.
Results: Compared with healthy controls, T2DM patients had significantly decreased ALFF values in bilateral middle temporal gyrus (MTG), left fusiform gyrus, left middle occipital gyrus, right inferior occipital gyrus; and increased ALFF values in both cortical and subcortical regions, including the bilateral cerebellum posterior lobe, right cerebellum culmen, and insula lobe. Moreover, we found an inverse correlation between the ALFF values in the middle temporal gyrus and both the HbA1c (r= -0.420, p= 0.026) and the score of Trail Making Test-B (TMT-B) (r= -0.451, p= 0.016) in the patient group. On the other hand, C-peptide level and HOMA2-%ß has positive correlation(r= 0.429, p= 0.023; r=0.453, p=0.016, respectively) with the ALFF value in the middle temporal gyrus.
Conclusion: The present study provides evidence that T2DM patients have altered ALFF in many brain regions, which links with poor neurocognitive performances, the severity of consistent hyperglycemic state and the impaired ß-cell function. Abnormal ALFF values in MTG may be regarded as a potential marker to identify cognitive decline associated with T2DM. Those findings may contribute to further understanding of neurological pathophysiology underlying T2DM, and present some enlightenment to clinical diagnosis in the future.





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Reduced cutaneous Langerhans cell number, endothelial area, and nerve fibre density in recently diagnosed type 2 diabetic subjects
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Background and aims: Microvascular abnormalities and immune-mediated processes have been implicated in the pathogenesis of diabetic polyneuropathy. Langerhans cells (LCs) are the sole dendritic cells located in the epidermis and are thus the first antigen-presenting cells exposed to pathogens at the skin surface. LCs play a pivotal role in the regulation of cutaneous immunity, exert tolerogenic properties, and are surrounded by sensory nerve endings. The aim of the present study was to determine whether cutaneous immune-mediated and neurovascular alterations are present in recently diagnosed type 2 diabetes.
Materials and methods: We assessed skin biopsies and peripheral nerve function in 97 participants of the German Diabetes Study (GDS) with recently diagnosed type 2 diabetes (age: 55.5±11.3 (SD) years; male: 70.1%; BMI: 32.0±6.0 kg/m^2; diabetes duration: 1.5±1.2 years; HbA1c: 6.5±1.0%) and 83 healthy controls (age: 54.0±18.1 years; male: 65.1%; BMI: 24.7±3.6 kg/m^2). Subepidermal endothelial area and the number of epidermal LCs were determined in subgroups of 59 patients with type 2 diabetes and 54 controls. Intraepidermal nerve fibre density (IENFD) was assessed by immunohistochemistry using PGP 9.5 antibody in 3-mm punch biopsies from the distal leg. CD31 immunohistochemistry was used to determine subepidermal endothelial area. Epidermal LC density was quantified using langerin (CD207) staining. Peripheral nerve function was assessed by motor and sensory nerve conduction velocity (MNCV, SNCV), sensory nerve action potential (SNAP) amplitudes, vibration perception thresholds (VPT), and thermal detection thresholds (TDT), neuropathy symptom score (NSS), and neuropathy disability score (NDS). Insulin sensitivity (M-value) was determined by a hyperinsulinaemic euglycaemic clamp. All comparisons were adjusted for sex, age, and BMI.
Results: When compared to the control group, type 2 diabetic patients showed reduced IENFD (10.0±3.1 vs 8.0±3.0 fibers/mm; P=0.001), peroneal MNCV (47.6±4.4 vs 43.2±5.8 m/s; P<0.0001), sural SNCV (46.9±4.4 vs 42.6±6.2 m/s; P<0.0001), and cold TDT on the foot (28.8±2.1 vs 26.2±6.5 °C; P=0.002) as well as elevated malleolar VPT (1.22±1.11 vs 2.94±4.20 µm; p=0.01). Subepidermal endothelial area was smaller in the diabetic group compared to the control subjects (3.48±2.24 vs 4.17±2.40%; P<0.0001). Moreover, a reduction of epidermal Langerhans cell number by approximately 30% was noted in the diabetic subjects compared to the control group (405±157 vs 582±194 cells/mm^2; P=0.002). In the diabetic group, multiple linear regression analysis adjusting for age, sex, BMI, and HbA1c revealed a positive association between LC number and M-value (ß=0.401; p=0.004. No relationship was noted between IENFD, epidermal LC number, and subepidermal endothelial area.
Conclusion: Apart from intraepidermal nerve fibre loss, recently diagnosed type 2 diabetic subjects show reduced subepidermal endothelial area and a marked reduction of tolerogenic Langerhans cells. The loss of Langerhans cells could promote an immunogenic imbalance toward inflammation and thereby provide a link to insulin resistance. Prospective studies are needed to determine whether these early neuro-immuno-vascular alterations contribute to the development of diabetic neuropathy.

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p66Shc mediates bone marrow denervation and impairs stem cell mobilisation in diabetes
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Background and aims: Mobilization of bone marrow (BM) stem and proangiogenic cells is impaired in diabetes, contributing to chronic complications. Sympathetic innervation of the BM is critical for stem cell mobilization. Previous data indicate that induction of p66Shc expression mediates hypeglycemic oxidative damage, but there is no data on diabetic autonomic neuropathy (DAN). Herein, we tested whether p66Shc, a life-span regulating gene linked to oxidative stress, is involved in the diabetic stem cell mobilopathy.
Materials and methods: p66Shc gene expression was quantified by real time PCR in PBMC of 20 non diabetic patients and 20 diabetic patients (10 of whom had DAN), as well as in BM cells of control and streptozotocin (STZ)-induced diabetic mice. BM innervation was assessed by immunofluorescence staining for tyrosine hydroxilase (Tyr-OH). Stem cell mobilization was assessed by quantifying Lin-cKit+Sca1+ (LKS) cells and CD34+Flk1+ endothelial progenitor cells (EPC) before and after 4 days of G-CSF treatment in wild type and p66Shc-/- STZ mice
Results: First, we found that expression of p66Shc is increased in PBMC of diabetic patients, which is worsened by the presence of DAN. In addition, BM cells from diabetic mice showed increased p66Shc expression compared to controls. Diabetic mice showed reduced Tyr-OH immunoreactive nerve fibers and impaired stem cells and EPC mobilization. The functional role of p66Shc was tested using *p66Shc*$^{-/-}$ mice which, once made diabetic by STZ injection, showed protection from BM sympathectomy, as evidenced by the preserved Tyr-OH immunostaining of sympathetic nerve fibers. In addition, induction of diabetes in *p66Shc*$^{-/-}$ mice did not impair LKS and EPC mobiliza-

tion after G-CSF treatment, despite they were as hyperglycaemic as wild type STZ mice.
Conclusion: Deletion of p66Shc, by preserving BM SNS innervation, restores stem cell mobilization in diabetes. This can exert a protective effect against the development of complications.
Supported by: The Italian Ministry of Health, EFSD Clinical Research Grants

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Factors associated with reversibility of erectile dysfunction in men with type 1 diabetes: longitudinal findings from DCCT/EDIC
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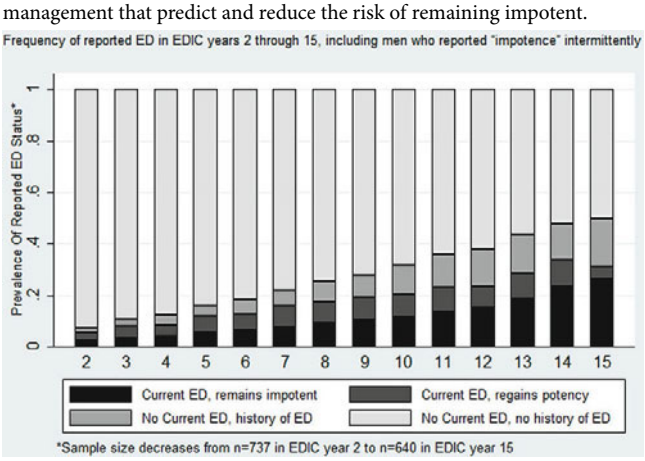
Background and aims: Diabetes imparts a 3-fold higher risk of erectile dysfunction (ED), which is associated with neuropathy, glycaemic control, microvascular complications, and duration of diabetes. The goal of this study is to describe the course of diabetic ED over time and determine factors that are related to a return of potency versus irreversible progression of ED.
Materials and methods: The Diabetes Control and Complications Trial (DCCT) randomised 761 men with T1DM to intensive or conventional glycaemic therapy from 1983-1989. The cohort (n=720) was then followed in the Epidemiology of Diabetes Interventions and Complications (EDIC) study. Within EDIC, ED was defined as a "Yes" response to a yearly single yes/no question querying the presence of "impotence". Subjects were classified as regaining potency if they answered "No" (absence of impotence) at any point during the five years following the index ED report. Pharmacologic therapy for ED was reported by a small minority of men and was not considered in this analysis. HbA_{1c} was measured at baseline, quarterly during DCCT, and annually in EDIC. Multivariable logistic regression estimated the odds of regaining potency by number of consecutive years of prior reported ED after age adjustment.
Results: Mean age of subjects at the most recent assessment was 50.4 years. The incidence of ED increased from 5.5% (n=42) at EDIC year 1 (1994) to 33.6% (n=219) by EDIC year 16 (2009). The majority of men reported ED at least once during the 16 years of follow up (53.5%) indicating that ED is not a fixed condition in all cases (see Figure). In subjects with at least 5 consecutive years of follow up within the time span of EDIC, the likelihood of regaining potency within 5 years decreased with every additional year of prior reported ED: for subjects with a single report of ED, (n=198), 67.7% regained potency within 5 years; with 2 consecutive years of ED (n=138) 50.7% regained potency; with 3 consecutive years of ED (n=96), 37.5% regained potency; with 4 consecutive years of ED (n=70), 32.9%; Those with 5 or more consecutive years of ED (n=44) had less than 20% subsequent chance of regaining potency. A1c and age were significantly associated with regaining potency only in subjects with four or less consecutive years of ED.
Conclusion: ED is a dynamic state in men with T1DM that may be modifiable when first experienced. Progressively longer periods of ED were associated with a lower likelihood of return to potency, approaching a point of no return when HbA1c no longer is associated with potency. Future work in this population needs to explore biomarkers, behaviour modification and clinical management that predict and reduce the risk of remaining impotent.



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QTc prolongation is associated with three-year mortality after above ankle amputation in type 2 diabetic patients
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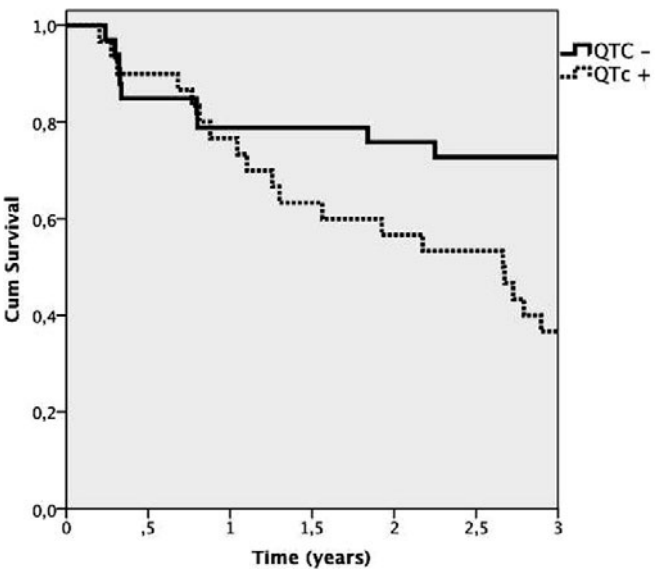
[1]Clinical Sciences, Lund University, [2]Internal Medicine, Ängelholms Hospital, Sweden.

Background and aims: Diabetic patients have not only an increased lower extremity amputation (LEA) risk as compared to non-diabetic individuals, but also an enhanced mortality risk after above ankle amputation. There are still no clinically useful markers to identify patients with the highest mortality risk after a LEA. Heart rate corrected QT interval (QTc) interval prolongation is a potential indicator of increased cardiovascular risk in the general population, but also, as recently shown, in patients with chronic diabetic foot ulcers. The aim of this study was to evaluate whether QTc-time is a significant factor for long-term survival after above ankle amputation in patients with type 2 diabetes.
Materials and methods: All patients with type 2 diabetes during three consecutive years, who underwent an above ankle amputation at any our two Diabetic Foot Clinics were included in this study and followed for three years. Patients older than 80 years were excluded. Preoperative ECGs were analysed to calculate QTc time and patients were grouped according to QTc time ≤ and >440 ms. Mortality data was obtained from the National Death Registry. Patients who died within 14 days after amputation were excluded from the final analysis. Data are given as percentages or median and range. Appropriate non-parametric statistics were used to compare differences between groups. Cox proportional hazard regression models were applied to study the independent associations. A two-tailed p-value <0.05 was considered statistical significant.
Results: 68 patients with a median age of 72 (57-80) years were included. Two patients with pacemaker were excluded, as QTc time could not be evaluated. QTc prolongation (QTc+ group) was present in 31 patients (47%) and not present in 35 (QTc- group). There were no differences in smoking habits, diabetes medications, dyslipidemia, hypertension, dialysis, previous vascular surgical interventions or ABI between groups. A history of myocardial infarction was more commonly present in the QTc+ group; 50 vs. 30%, p=0.04. Creatinine levels in patients not on dialysis were similar between groups, 84 (43-264) vs. 81 (38-276) μmol/L. Three-year mortality was 51.5%. Three patients (1 in QTc+ vs. 2 in QTc-) died within 14 days of amputation. Mortality was significantly higher in patients with QTc-prolongation, 67% vs. 27%, p=0.002. In a cox-proportional hazard analysis including presence of myocardial infarction, renal function, age, HbA1c and QTc-prolongation only age (decades) (HR 2.1 (1.01-4.2), p=0.049) and QTc prolongation (HR 3.4 (1.4-8.4), p=0.007) were significantly associated with 3-year mortality. A history of myocardial was not a significant risk factor in this analysis, HR 1.2 (0.5-2.7), p=0.70.
Conclusion: This study indicates that QTc prolongation is associated with increased mortality in type 2 diabetes patients after above ankle amputation.



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OP 16 Clinical nephropathy: focus on novel biomarkers and improving outcomes

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Genome-wide association studies of renal diabetes complications

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Background and aims: Diabetic kidney disease (DKD) is a leading cause of end-stage renal disease (ESRD) and the risk of developing DKD is partly determined by genetic factors. In the SUMMIT consortium we performed meta-analysis of genome-wide association studies in European T2D patients to identify genetic determinants of DKD. Because the genetic variants regulating early stages of disease may differ from those that determine progression to severe disease we also analyzed microalbuminuria (MiAU), reflecting early pathologic changes and endothelial dysfunction, and more severe DKD, i.e. macroalbuminuria (MaAU) and ESRD, separately.
Material and methods: DKD was defined as MiAU, MaAU or ESRD, whereas controls had normoalbuminuria and diabetes duration > 10 years. The study included four cohorts of T2D patients: GoDARTS (n=2526), the Scania Diabetes Registry (SDR, n=1313), Steno (n=322) and MNI (n=203). We analyzed ~2.3 million single nucleotide polymorphisms (SNPs), imputed based on the CEU HapMap2 reference panel, using logistic regression, adjusting for sex, age at onset and duration of diabetes, for four phenotypes: DKD, MiAU, MaAU+ESRD and ESRD alone. Meta-analyses were performed using a fixed effects model. We also performed joint analyses with four T1D studies analyzed using similar methods and phenotype definitions: FinnDiane (n=3435), Eurodiab (n=812), SDR (n=513) and Cambridge (n=400).
Results: In the T2D analyses the most strongly associated SNPs were rs2424306 (OR=0.72, $p=1.4x10^{-7}$) in the C20orf26 gene for DKD (2326 cases, 2038 controls), rs11157816 (OR=1.65, $p=2.7x10^{-7}$) close to *TMX1* for both ESRD (335 cases ,1698 controls) and MaAU+ESRD (OR=1.37, $p=7.8x10^{-7}$; 1063 cases, 1848 controls), and rs162693 (OR=1.95, $p=3.2x10^{-6}$) close to GRM7 for MiAU (1198 cases, 1681 controls). In the joint T1D+T2D meta-analysis of MiAU (2008 cases, 4246 controls), rs5749503 in SYN3/TIMP3 reached genome-wide significance (OR=0.78, $p=4.8x10^{-8}$). The association effect size was similar in T1D (OR=0.77, $p=6.5x10^{-5}$) and T2D (OR=0.80, $p=1.8x10^{-4}$). Nominally significant associations were observed also in the analysis of ESRD (OR=0.86, p=0.006) and DKD (OR=0.91, p=0.002). Interestingly, this SNP was also associated with risk of diabetic retinopathy in a combined T1D/T2D meta-analysis in the SDR sample (OR=0.64, $p=5.7x10^{-5}$, 827 cases and 283 controls).
Conclusion: We have identified a new candidate locus for DKD situated in the *SYN3* (synapsin III) gene, 50kb upstream of TIMP3 (TIMP metallopeptidase inhibitor 3). Based on available literature *TIMP3* is a likely effector gene. TIMP3 has been shown to play a role in animal models of DKD and mutations in TIMP3 cause fundus dystrophy in humans. *SYN3* is a modulator of neurotransmitter release in synapses. Our results support a role of TIMP3 in human DKD and possibly in diabetic retinopathy.
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Predictors of rapid decline in renal function in type 2 diabetes

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Background and aims: Trials of therapies for preventing end stage renal disease in type 2 diabetes (T2D) are typically conducted in those with estimated Glomerular Filtration Rate (eGFR) in the range 30-60 ml/min/m^2. Improving the power of such trials or shortening their duration by over-sampling those most at risk of progression is an important goal. Here we aimed to identify biomarkers of renal function decline in T2D beyond baseline eGFR.
Methods: From a large cohort of T2D patients from Scotland (Go-DARTS), we identified people with a baseline eGFR in the range 30-60 ml/min/m^2 at baseline. Among these we defined cases as those who had lost ≥40% of their baseline eGFR over a follow up of ≤42 months (median = 22months) and controls as those who at a follow up of ≥42 months had a decline in eGFR of <5%. In these samples (154 cases 155 controls) we measured 24 serum biomarkers - 13 by standard ELISAs or automated immunoassays and 11 by MS. We chose candidate biomarkers based on prior reports of association with renal function in diabetic or non-diabetic cohorts or based on pathways reported as involved in diabetic kidney disease. Data analysis was by logistic regression using 50-fold cross validation with forward selection and by 50-fold cross validated backward selection with LASSO penalty. Clinical factors included were age, sex, diabetes duration, eGFR, creatinine, BMI, HbA1c, BP, smoking, insulin use and antihypertensive drugs use.
Results: 6 biomarkers - N-terminal pro-B type Natriuretic Protein (NT-proBNP), Cystatin C, Asymmetric Dimethylarginine (ADMA), Symmetric Dimethylarginine (SDMA), N-Acetyl-B-D-Glucosaminidase (NAG), and Vascular Cell Adhesion Molecule 1 (VCAM1) were associated with rapid decline in renal function independently of clinical factors (table) while osteopontin may also contribute to prediction. 9 other markers made lesser contributions to improving prediction. The only clinical factors that contributed to the risk models were male sex, systolic BP and baseline eGFR. The increment in the area under the ROC curve was 0.19 (AUC for model of clinical factors and all selected biomarkers = 0.82, AUC for clinical factors only = 0.63).
Conclusion: In this cross validated study we confirm the previously reported association of Cystatin C, ADMA, SDMA and VCAM1 with subsequent decline in renal function. We report novel findings that NT-proBNP predicts renal function decline independently of eGFR with which it is associated and serum NAG, a marker of lysosomal enzyme release, may also be of use. The selected biomarkers have potential to improve prediction of decline in renal function and further validation of these biomarkers is warranted.

Logistic regression model adjusted for all clinical covariates and all selected biomarkers

Normalised Covariate	Odds Ratio per standard deviation of marker (95% Confidence Interval)	Wald p-value
Cystatin C	4.24 (2.13, 8.95)	<0.001
N-Terminal Prohormone B-type Natriuretic Peptide	2.61 (1.61, 4.52)	<0.001
Asymmetric Dimethylarginine	0.37 (0.23, 0.57)	<0.001
Vascular Cell Adhesion Molecule 1	2.20 (1.37, 3.69)	0.002
N-Acetyl-B-D-Glucosaminidase	1.88 (1.23, 2.94)	0.004
Symmetric Dimethylarginine	2.27 (1.14, 4.65)	0.022
Osteopontin	1.41 (0.92, 2.26)	0.127

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Urinary proteomics predict onset of microalbminuria in normoalbuminuric type 2 diabetic patients in the DIRECT 2 study

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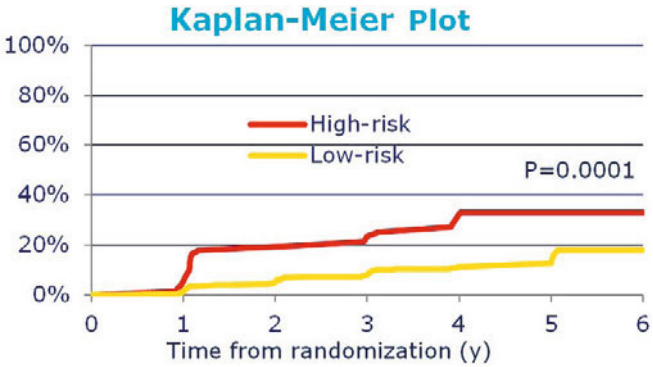
Background and aims: Early prevention of diabetic nephropathy is not successful as early interventions have shown diverging results. Urinary proteomics has shown promise as an early indicator of future development of diabetic nephropathy and could guide need for treatment. In this study we investigate that ability in a large type 2 diabetic cohort with normoalbuminuria.
Materials and methods: In a post-hoc study of the DIRECT-Protect 2 study, a randomized, controlled clinical trial of candesartan for slowing the progression of retinopathy, we studied patients with type 2 diabetes and normoalbuminuria (n=792), followed for a mean of 4.7 years. We determined a previously defined CKD risk score based on proteomic measurement of 273 urinary peptides (CE-MS), the risk-score was selected from previous cross sectional case-control studies. A Cox regression model for progression of albuminuria was built. The primary endpoint was development of persistent microalbuminuria (MA) (3 out of 4 samples).
Results: Persistent MA developed in 92 patients (11,6%). At baseline the CKD risk score was able to predict development of MA during follow-up, independent of treatment (candesartan/placebo), age, gender, baseline systolic BP, baseline UAER, baseline eGFR, baseline HbA_{1c} and diabetes duration (HR 2.0 (95% CI 1.17-3.45), p=0.012). In the placebo treated group the HR was 2.1 (1.1 to 4.2) compared to 1.6 (0.8 to 3.1) in the candesartan group.
Conclusions: In this cohort of patients with type 2 diabetes and normoalbuminuria from a large intervention study, the CKD classifier was an independent predictor of MA. This may provide a better opportunity to select normoalbuminuric patients for early prevention of diabetic nephropathy as treatment with candesartan seems to mitigate this risk.



Supported by: EU's FP7

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Soluble RAGE predicts progression of late-stage diabetic nephropathy in type 1 diabetes

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Background and aims: Soluble receptor for advanced glycation end-products (sRAGE) may ameliorate the deleterious effects that activation of its membrane bound counterpart RAGE exerts by binding to the same circulating ligands. High sRAGE concentrations in non-diabetic subjects are considered beneficial; in contrast, in diabetic nephropathy (DN) high concentrations are associated with disease. A causal relationship between sRAGE and DN has, nevertheless, not been established. To assess whether baseline sRAGE concentrations predict the development or progression of DN and also whether a temporal change in sRAGE predicts the development and progression of DN, we studied sRAGE in Finnish type 1 diabetes patients.
Patients and methods: At baseline 3107 T1D patients were studied and followed up for 6.3 years (range 1-14years). In a subset of 1143 patients, sRAGE was also measured at follow-up. Patients were grouped based on their AER in at least two out of three timed urine collections into patients with normal AER (n=2063), microalbuminuria (n=418), macroalbuminuria (n=457), and end-stage renal disease (ESRD; n=169). During follow-up 349 patients progressed (11 %) in their kidney status. sRAGE was measured from serum using a commercial kit. Repeated measures ANOVA was applied to evaluate significance of change between the time points. Nonparametric tests and Cox regression analyses were used accordingly. SPSS 15.0 was used for the analyses.
Results: Mean baseline sRAGE concentrations were higher in macroalbuminuric (1582 ± 892 pg/ml; p=5.47 x 10^{-24}) as well as in ESRD patients (1780 ± 1548 pg/ml; p= 3.08 x 10^{-5}) when compared with those with normal AER (1176 ± 435 pg/ml). Mean baseline sRAGE concentrations and quartiles did not correlate with the progression from normal AER to microalbuminuria or micro- to macroalbuminuria. On the other hand, baseline sRAGE predicted the progression from macroalbuminuria to ESRD independently of eGFR. However, although the highest baseline sRAGE quartile was associated with the progression from macroalbuminuria to ESRD (HR 4.45, 95% CI 2.15-9.20), this was no more significant after entering eGFR into the model. Baseline and follow-up sRAGE concentrations were significantly different F(1, 1142) = 12.36, (p=0.00045) and correlated with HDL, HbA_{1c}, creatinine, eGFR, AER, urate, and BMI. The sRAGE change (ΔsRAGE) between the time points was -73 ± 543 pg/ml (-5519 - 3888 pg/ml). ΔsRAGE was not significant between the groups and did not predict progression of kidney status. However, ΔsRAGE did correlate with change in eGFR, AER, HDL, BMI, and WHR.
Conclusion: Baseline sRAGE concentrations predicted progression from macroalbuminuria to ESRD independently of eGFR. A temporal change in sRAGE did not predict development or progression of DN. However, ΔsRAGE correlated with the change of several risk factors associated with DN. These data suggest that sRAGE is a predictor of progression of late-stage DN.

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Long-term renal outcome in the Steno 2 study

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Background and aims: To describe the progression in loss of renal function in patients with type 2 diabetes and microalbuminuria, originally enrolled in the Steno-2 study.
Materials and methods: 160 patients were randomized to conventional or intensified, multifactorial lifestyle and pharmacological intervention for an average period of 7.8 years. After this time point all patients received intensified intervention in an open follow-up trial. Renal function was assessed directly (51Cr-EDTA clearance) and indirectly (eGFR, MDRD-equation) after an average of 2, 3.8, 7.8, 13.3, 16 and 19 years of follow up. The primary end point for the current analysis was a composite of dialysis treatment (Danish Renal Registry) and death by any cause, secondary end point was 50% reduction in GFR or eGFR, or doubling of s-creatinine. A tertiary end point was progression to macroalbuminuria.
Results: A total of 87 patients (33 intervention, 54 control) reached the primary end point (p = 0.001). Four patients in the intervention group compared to eight in the control group required dialysis treatment and the patients in the control group underwent dialysis (median)6.6 years earlier than patients in the intervention group (p = 0.093). 63 patients (39%) (33 patients in the intervention group and 30 in the control group) reached the secondary end point (p = 0.31). Patients in the control group reaching the primary end point and/or renal function less than 60ml/min/1.73m2 had an increased risk of mortality compared to patients not progressing (p = 0.006). This was not found in the intervention group. The risk of progression to macroalbuminuria was reduced by 66% in the intervention group, p = 0.004.
Conclusion: Intensified multifactorial treatment decreased mortality and the need for dialysis. Although there was significantly decreased macroalbu-

minuria there was no difference in the renal function in survivors after 19 years of follow up. This is probably due to competing risk from cardiovascular death in the control group. Patients in the control group with deteriorating renal function had a significant increase in mortality.

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Proteinuria reduction with probucol and telmisartan in patients with type 2 diabetic nephropathy

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Background and aims: Diabetic nephropathy is the leading cause of end-stage renal disease. Telmisartan is known to not only reduce proteinuria of diabetic nephropathy, but also slow its progression. Probucol is an antioxidant and has cholesterol-lowering effects. However, combination therapy with telmisartan and probucol in the treatment of diabetic nephropathy has not been proved with sufficient clinical evidence. This study examined the clinical efficacy of telmisartan and probucol in proteinuria reduction of type 2 diabetic nephropathy.
Materials and methods: A total of 160 type 2 diabetic nephropathy patients with proteinuria (0.5 g/24h< urinary protein <3.0 g/24h) were enrolled in a randomized, double-blind, placebo-controlled, multicenter study. The patients were randomly divided into two groups. One group (telmisartan group, n=80) was administered with telmisartan (80 mg/day) and probucol placebo (1000 mg/day for 24 weeks, and then reduced to 500 mg/day for next 24 weeks). The other group (probucol group, n=80) was administered with telmisartan (80mg/day) and probucol (1000 mg/day for 24 weeks, and then reduced to 500 mg/day for next 24 weeks). All patients were further categorized into two subgroups based on urinary protein level. One subgroup has 83 patients who had urinary protein <1.0g/24h at baseline, and the other has 77 patients with urinary protein level ≥1.0g/24h at baseline. All patients were followed throughout the 48-week period, and the percentage change of urinary protein from baseline to 48 weeks was assessed.
Results: The baseline characteristics of the two groups were similar, as well as the BP and HbA1c profile over the study period. There was a significant reduction (27.02%) in urinary protein level in the probucol group from baseline to 48 weeks. However, urinary protein level was increased in the telmisartan group, with an average increase of 1.45% (P < 0.001 for the comparison with probucol group). A further analysis between the subgroups showed, that for patient subgroup with lower urinary protein (<1.0g/24h at baseline), the reduction in urinary protein level from baseline is significantly greater in probucol group than in telmisartan group (36.54% vs. 8.55%, P=0.013). For the patient subgroup with higher urinary protein (≥ 1.0g/24h at baseline), there was a significantly reduction of 15.38% in urinary protein level in probucol group from baseline. However, urinary protein was increased in telmisartan group, with an average increase of 10.96% (P=0.011 for the comparison with probucol group).
Conclusion: The combination therapy of probucol and telmisartan lowers urinary protein more effectively than telmisartan alone. This indicates administering probucol and telmisartan in combination have additive or synergistic effect to reduce proteinuria and prevent diabetic nephropathy, and maybe a new treatment for type 2 diabetic nephropathy.
Clinical Trial Registration Number: ChiCTR-TRC-10001177
Supported by: Science and Technology Project of Beijing, China (Grant No.D09050704310904)

OP 17 New players in the regulation of insulin secretion

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GPR55 deletion is coupled to impaired glucose tolerance and increased islet cell apoptosis

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Background and aims: G-protein coupled receptor 55 (GPR55) has been classified as a novel cannabinoid receptor due to its sensitivity to distinct cannabinoid ligands. In addition, the non-cannabinoid lipid L-alpha-lysophosphatidylinositol (LPI) has also been identified as an endogenous ligand of GPR55. We have previously reported GPR55 expression by mouse and human islets, and demonstrated that GPR55 activation with LPI and O-1602, an exogenous agonist, stimulates insulin secretion *in vitro*. This study investigated the phenotype of GPR55 knockout (KO) mice under normal conditions and diet-induced obesity.
Materials and methods: C57BL/6 (WT) and GPR55 KO mice were randomly assigned to two groups at 4 weeks of age and fed *ad lib* either standard chow (SC, fat: protein: carbohydrate: 14%:28%:58%) or a high fat diet (HFD, 55%:16%:29%) for 19 weeks. Daily energy consumption was calculated by multiplying daily food intake by the physiological fuel values of SC and the HFD. Glucose and insulin tolerance tests were performed following a single i.p. administration of glucose (2g/kg body weight) or insulin (0.75U/kg body weight), respectively. Tail vein blood glucose concentrations were determined using a glucose meter. Blood samples were collected before and after i.p. administration of glucose and serum insulin concentrations were determined using an ultra-sensitive ELISA. Caspase-3/7 activities of isolated islets were quantified using a luminescent assay.
Results: GPR55 KO and WT mice fed on SC did not show significant differences in weight gain over 19 weeks (KO week 1: 21.4±0.3g, week 19: 32.7±1.1g; WT week 1: 22.0±0.3g, week 19: 31.3±0.4g, n=6-7, P>0.2). However, GPR55 KO mice had a significantly higher daily energy intake on a HFD than did WT mice (17.6±0.5 kcal vs. 13.3±0.4 kcal; P<0.001), and KO mice showed a significantly greater weight gain on a HFD than WT mice over the same period (KO: 21.1±0.5g, 53.0±1.9g; WT: 20.8±0.5g, 35.9±1.3g; n=6-7, P<0.001). A small impairment in glucose handling following GPR55 deletion was observed in mice fed SC (AUC: WT vs. KO: 1,504±81 vs. 1,688±67), and this was more pronounced when mice were fed a HFD (2,477±231 vs. 2,969±218). The glucose intolerance observed in GPR55 KO mice fed a HFD was accompanied by blunted insulin secretion following i.p. injection of glucose (WT vs. KO: 136±1 vs. 98±17 pmol/ L serum insulin). Furthermore, GPR55 KO mice fed a HFD showed significantly lower insulin sensitivity than WT mice fed a HFD (HOMA-IR; WT vs. KO: 9.1±0.0 vs. 29.4±0.2; P<0.001). Finally, islets isolated from GPR55 KO mice exhibited significantly higher levels of cytokine-induced apoptosis both from mice fed SC (caspase3/ 7 activities; luminescence; WT vs. KO: 35,071±536 vs. 42,364±2,222; P<0.05) and a HFD (36,539±1,768 vs. 53,816±2,194; P<0.001).
Conclusion: GPR55 KO mice are hyperphagic and are more susceptible to diet-induced obesity. They also show impaired glucose tolerance and insulin resistance, which is most apparent under the increased metabolic demand caused by diet-induced obesity. The impaired glucose tolerance is at least partly due to reduced insulin secretion, increased β-cell apoptosis and the subsequent failure to compensate for increased insulin requirement. These data are consistent with GPR55 playing an important role in glucose homeostasis, and suggest its potential as a target for Type 2 diabetes therapies.
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Modulation of beta cell function and glucose homeostasis by the bile acid farnesoid X receptor (FXR)

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Background and aims: Type 2 diabetes mellitus coincides with alterations in the size and composition of the bile acid (BA) pool. We have recently shown that the BA taurochenodeoxycholic acid increases insulin release via FXR-dependent inhibition of K_{ATP} channels. We now extended our study and tested the influence of different conjugated and unconjugated BAs on stimulus-secretion coupling. In addition, we investigated the significance of FXR in a mouse model of diet-induced glucose intolerance.
Materials and methods: Islets or islet cells from wild type (WT) and FXR knockout (FXR-KO) mice were used to determine $[Ca^{2+}]_c$ by fura-2 fluorescence and insulin release by a radioimmunoassay. High fat diet (HFD) was applied to WT and FXR-KO mice for 12 weeks (45% kcal fat vs. 10% kcal fat vs. groups with 0.5% chenodeoxycholate (CDC)). Glucose and insulin tolerance were measured by intraperitoneal injection of glucose (ipGTT 2g/kg BW) and insulin (ipITT 1U/kg BW), respectively. Morphometric analysis was carried out on slices of paraffin-embedded pancreatic tissue by immunostaining of insulin and glucagon.
Results: The BAs taurochenodeoxycholate (TCDC), CDC, and glycochenodeoxycholate (GCDC) (500 nM) increased glucose-induced insulin secretion (TCDC: 1.2±0.3 ng/(islet*h), CDC: 1.0±0.2 ng/(islet*h), and GCDC: 1.1±0.2 ng/(islet*h) vs. 15 mM glucose: 0.7±0.1 ng/(islet*h) n=5, p≤0.05). The effects of the 3 BAs were absent in FXR-KO islets (n=4). In agreement with a stimulatory effect on insulin release, the BAs augmented $[Ca^{2+}]_c$ in WT but not in FXR-KO islet cells (n=3). By contrast ursodeoxycholate (UDC, 500 nM) that has no affinity to FXR showed an inhibiting effect on glucose-stimulated insulin secretion in both genotypes (n=4). FXR-KO protected against HFD-induced impairment of fasting glucose concentration and glucose tolerance. In the FXR-KO group values did not differ from WT animals on normal chow (ipGTT AUC (mM*min)): control WT 1131±48, HFD FXR-KO 1111±34 vs. HFD WT 1245±40 n=9 p≤0.05). Improved glycemic control of FXR-KO mice fed with HFD was not due to differences in body weight or insulin resistance. Adding CDC to the chow reduced weight gain of WT mice under HFD and protected against HFD-induced glucose intolerance (ipGTT AUC (mM*min)): control WT 1131±48, HFD+CDC WT 1160±34 vs. HFD WT 1245±40, n=8 p≤0.05). Insulin resistance was not altered. HFD led to an increase of the beta-cell area in WT and FXR-KO mice (HFD WT 113099±7193 (a.u.), HFD FXR-KO 116826±9715 (a.u.), vs. control WT 83220±6942 (a.u.), n=98 p≤0.001) and augmented the number of beta-cells/islet (HFD WT 114±8, HFD FXR-KO 125±11, vs. control WT 77±7, n=98 p≤0.001) The beta-cell size did not differ, indicating hyperplasia under HFD. Interestingly, CDC supplementation protected against this hyperplasia, because number and area of beta-cells were reduced.
Conclusion: BAs that activate FXR increase insulin secretion of lean mice in vitro. Surprisingly, under HFD conditions FXR-KO mice profit from receptor deficiency. Feeding WT animals with a BA that activates FXR in addition to HFD leads to improved glucose tolerance. Obviously, the nutritional status critically determines the effects of FXR on glucose tolerance. Further studies with lean and obese mice are needed to evaluate the different signalling pathways contributing to nutrition-dependent effects of FXR activation.
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Intestinal gluconeogenesis controls pancreatic beta cell function

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Background and aims: Pancreatic β-cells producing insulin play a key role in the control of glucose homeostasis. A defect in insulin secretion is a crucial factor in the development of diabetes. The pancreatic function is controlled by the sympathetic/parasympathetic nervous system. The appearance of glucose produced by the intestine in the portal vein (via intestinal gluconeogenesis, IGNG) initiates a nervous signal ("portal glucose signal") resulting in beneficial effects in the control of food intake and glucose homeostasis. We considered the role of IGNG in the regulation of the pancreatic function. We used mice knockout for the gene of the catalytic subunit of glucose-6 phosphatase (G6PC, the key enzyme of GNG) specifically in the intestine by an inducible Cre-Lox approach.
Materials and methods: We first performed in vivo insulin release test (3g/kg intraperitoneal (IP) glucose injection). Then, we studied the in vitro insulin secretion on isolated pancreatic islets from mice: 1/under basal and glucose-stimulated conditions; 2/after portal glucose infusion; 3/after treatment with a specific inhibitor of the α2 adrenergic receptors, yohimbine.
Results: In control mice, a 1.7- fold increase in insulin secretion was observed at 3 min after IP glucose injection and the insulin level re-increased at 12 min, indicating a second-phase insulin response. In I-G6pc-/- mice, the acute first-phase insulin secretory response to glucose was blunted. However, the insulin levels slightly rose after the glucose challenge, suggesting some retention of the second-phase insulin secretion in I-G6pc-/- . Then, we measured insulin secretion in response to glucose stimulation in islets isolated from I-G6pc-/- and control mice. Pancreatic islets from I-G6pc-/- mice showed an increase in insulin secretion under non-stimulated conditions (5.5mM glucose) (0.33 ± 0.02 vs 0.14 ± 0.03 % of insulin content). Besides this defect in insulin secretion in the basal state, pancreatic islets isolated from I-G6pc-/- mice exhibit an alteration in glucose stimulated insulin secretion (GSIS). While insulin secretion from control islets was increased 3-fold at 16.7mM glucose compared to 5.5mM glucose, I-G6pc-/- islets didn't show any response to glucose. Interestingly, mimicking IGNG by infusing portal glucose corrected insulin secretion defects in I-G6pc-/- mice. Indeed, the infusion of portal glucose decreased insulin secretion in the basal state and restored normal GSIS in I-G6pc-/- mice. On the other hand, the plasma concentration of epinephrine was increased in I-g6pc-/- mice (15 ± 2 vs 2 ± 1 ng / ml). A yohimbine treatment for 15 days decreased insulin secretion in the basal state and improved GSIS in I-G6pc-/- mice.
Conclusion: The absence of the intestinal IGNG is sufficient to alter the β-cells function. The activation of the sympathetic nervous system could explain this defect. These data strongly suggest the existence of an intestine-brain-pancreas nervous circuit, controlling the secretion of insulin and initiated by the intestinal IGNG.

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Functional role of ERK1 on pancreatic beta cell physiology

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Background and aims: Insulin secretion from pancreatic β-cells is triggered by glucose and is amplified by hormones/neurotransmitters through the activation of second messengers and protein kinases. The Extracellular signal Related Kinase 1 & 2 (ERK1/2) are known to be involved in the regulation of the β-cell mass, and it has also been proposed that they could play a role in glucose-induced insulin secretion. Since the independent role of each kinase has never been investigated in β-cells, our aim was to investigate, for the first time, the specific role of ERK1 using ERK1 knockout mice ($ERK1^{-/-}$).
Materials and methods: Free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) changes and insulin secretion were recorded in perifused islets isolated from $ERK1^{+/+}$ and $ERK1^{-/-}$ mice. The translocation of ERK1-YFP and the endogenous ERK1/2 activities (using EKAR) were monitored beneath the plasma membrane in living mouse β-cells using total internal fluorescence (TIRF) microscopy. The islet number and areas were determined by morphometric analysis of pancreatic sections stained with eosin/hematoxylin. The activation and regulation of ERK1/2 and of MSK1 were evaluated by western blots.
Results: $ERK1^{-/-}$ mice showed similar body weight, blood glucose and plasma insulin concentration than $ERK1^{+/+}$ mice, but displayed a mild glucose intolerance (p<0.01). In β-cells, ERK1-YFP is actively recruited beneath the plasma membrane by glucose (1.01±0.008 vs 1.25±0.038 a.u., p<0.001), a phenomenon that is abrogated by U0126 (a blocker of the upstream kinases MEK1/2), at a concentration (1μM) that neither affected cellular metabolism (NADPH fluorescence) nor $[Ca^{2+}]_c$ changes. Additionally, the natively expressed ERK1/2 were also recruited beneath the plasma membrane under glucose stimulation as shown by the phosphorylation of EKAR (a FRET-based biosensor of ERK1/2 activity) (p<0.05). Nevertheless, an increase in glucose concentration (from 3 to 15 mM) induced similar changes in $[Ca^{2+}]_c$ and insulin secretion in $ERK1^{-/-}$ and $ERK1^{+/+}$ islets. Therefore, glucose-induced a recruitment of the activated ERK1 to the plasma membrane, that is not involved in the regulation of insulin secretion. The activation of the Glucagon Like Peptide-1 (GLP-1) receptor increases insulin secretion in response to glucose, but also leads to the activation of ERK1/2. GLP-1 induced a similar two fold increase of insulin secretion in the presence of 15 mM glu-

cose in ERK1$^{-/-}$ and ERK1$^{+/+}$ islets, indicating that the kinase is not involved in the insulinotropic effects of GLP-1. To determine if ERK1 plays a role in the regulation of the β-cell mass, morphometric analysis of pancreatic sections were performed. ERK1$^{-/-}$ mice displayed a small proportion of very large islets (~1%; p<0.05), while the islet number was similar compared to ERK1$^{+/+}$ mice. Interestingly, the absence of ERK1 was not compensated by ERK2 (ie no increase in both ERK2 protein expression and/or activation by glucose), and more importantly we report for the first time that the activation of MSK1, one known key target of ERK1/2, is entirely dependent on ERK1 activity.
Conclusion: ERK1 is unlikely to play a role in the regulation of insulin secretion induced by glucose or GLP-1, even if the activated kinase is recruited beneath the plasma membrane under glucose stimulation. By contrast ERK1 seems to regulate the islet mass. Interestingly, we report that the activation of MSK1 is entirely dependent on ERK1 activity.
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The orphan receptors GPRC5B and GPRC5C are highly expressed in mouse and human islets of Langerhans and regulate insulin secretion and islet cell survival
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Background and aims: GPRC5B and GPRC5C are orphan G protein-coupled receptors whose role in beta-cells is unknown. We have examined their expression in human and mouse islets and their impact on mouse beta-cell function.
Materials and methods: Mouse islet Gprc5b and Gprc5c were down-regulated using Lentiviral particles encoding gene-specific shRNAs. Insulin secretion and cell viability were determined by radioimmunoassay and MTS assay respectively.
Results: GPRC5B and GPRC5C mRNAs are abundantly expressed in human and mouse islets (expression rel. GLP1R: GPRC5B: 95.2+-28.4%; GPRC5C: 85.6+-11%). In islets from newborn mice, Gprc5b expression was reduced (-45.8+-18.9%, p<0.05) whereas Gprc5c expression was strongly upregulated (+150+-64.1%, p<0.05) compared to adult mouse islets. Downregulation of Gprc5b, increased basal and glucose-stimulated insulin secretion (1 mM G: +125+-25%; 20 mM G: +46.7+-16%, p<0.05), and attenuated the detrimental effects on islet cell survival following exposure to pro-apoptotic cytokines (+40±3.4% survival, p<0.01). In contrast, down-regulation of Gprc5c had no effect on basal insulin secretion and inhibited glucose stimulated insulin secretion (20 mM G: -46.6+-16%, p<0.05), whereas islet cell survival following exposure to cytokines was drastically reduced (-66.1+-3.9% survival, p<0.01). Finally, mRNA (+55± 2.5%, p<0.001) and protein (+56±3.1%; p<0.01) expression of GPRC5B was upregulated in islets from human diabetic donors, whereas GPRC5C expression was unaffected.
Conclusion: These data demonstrate that the orphan receptors GPCR5B and GPRC5C are expressed in mouse and human islets and that they mediate opposing effects on beta-cell function that make them potential targets for the development of novel therapies for the treatment and/or prevention of type 2 diabetes.

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Membrane raft integrity constrains basal insulin secretion, but is impaired in type 2 diabetes
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Background and aim: Patients with type 2 diabetes typically exhibit elevated basal insulin secretion under non-stimulatory conditions together with impaired stimulated insulin release, e.g. in response to glucose. Glucose-stimulated insulin release is tightly controlled by the actions of voltage- gated Ca^{2+} channels (Ca_v channels) and exocytotic SNARE proteins. The SNARE complexes are targeted to cholesterol-glycosphingolipid rich membrane raft regions in the plasma membrane. These specialised regions also anchor different types of ion channels such as the voltage-gated L-type $Ca_v1.2$ channel. The accumulation of these regulators of insulin exocytosis throws the limelight onto the role of membrane rafts in insulin secretion. By contrast, little is known about the cellular mechanisms controlling basal insulin secretion. The present study aims at addressing the importance of membrane rafts for basal and regulated insulin release.
Materials and methods: Models: Human donor islets, rat clonal INS1 832/13 cells, and rat primary islets were used as models of human insulin secretion. The human donor islets were obtained from the Nordic Network of Clincial Islet Transplantation in collaboration with the Lund University Diabetes Center Human Tissue Lab. Agents: Membrane rafts fractions were disrupted using cholesterol oxidase (COs) or Sphingomyelinase (SMase). Membrane raft localisation: Cells were stained for membrane rafts with ATTO 647N-Sphingomyelin or BODIPY FL C_5-ganglioside G_{M1}. Confocal imaging was employed to study raft localisation and for measurements of intracellular $[Ca^{2+}]$. Insulin secretion was measured by RIA. Electrophysiology: The patch clamp technique was used to measure whole-cell currents, depolarization-evoked exocytosis and single-channel activity.
Results: Membrane rafts were easily visualised in pancreatic clonal beta cells, rat islets as well as human islets. Treatment with cholesterol oxidase (COs) effectively reduced the raft fractions by 47% in human islets (p<0.01, n=3). Furthermore, high-glucose incubations for 72 hrs in 21.1 mM glucose resulted in a dramatic washout of rafts fractions in clonal beta-cells. When compared with cells cultured in normal glucose (9.7 mM glucose), fluorescence intensity fell dramatically by 91% (p<0.05, n=3). In line with this finding, membrane raft fluorescence intensity in islets from T2D donors was only 40% of that observed in islets from healthy donors (p<0.001, n=3). Interestingly, in the T2D islets, COs failed to affect membrane raft content that only decreased by 13% (n.s., n=3). COs failed to significantly impact stimulated secretion, but markedly increased basal release in all model system tested without signs of compromised cell viability. In human islets, the increase amounted to 213% (p<0.001, n=3). The latter effect coincided with enhanced intracellular Ca^{2+} signalling at both basal and stimulated conditions, which was due to increased voltage-gated Ca^{2+} influx (-19±2.2 pC vs -28±2.9 pC at 0 mV; p<0.05; n=22 and 21), in turn caused by increased single-Ca^{2+} channel open probability, as well as increased single-channel conductance.
Conclusion: a) Membrane raft integrity is a prerequisite for controlled insulin secretion. b) Membrane raft disruption leads to hyperactivation of voltage-gated Ca^{2+} channels under basal conditions. c) Impaired membrane raft organisation in islets may contribute to elevated basal insulin release in T2D.
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OP 18 Central control of metabolism

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Central apelin generates a hypothalamic ROS production that controls hepatic glucose metabolism during the establishment of type 2 diabetes

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Background and aims: Hypothalamus is a key area involved in the control of glycaemia which receives information from various origins (hormones, neurotransmitters, metabolites) and in turn, modifies peripheral glucose utilisation and production by targeting the liver via the autonomic nervous system (ANS). Among all factors, we have demonstrated that apelin is an adipokine able to control glucose metabolism by acting directly on peripheral tissues, but also by acting as a neurotransmitter through hypothalamic neurons. We have recently published that intracerebroventricular (icv) injection of high dose of apelin, similar to that observed in the hypothalamus of obese/diabetic mice, triggers T2D characteristics in normal mice such as fasted hyperglycaemia. These data suggest the existence of a hypothalamic apelin to liver axis. Nowadays, nothing is known concerning the molecular hypothalamic actors implicated in the hypothalamic effect of apelin on hepatic function. One potential target of central apelin could be the Reactive Oxygen Species (ROS) since hypothalamic ROS are involved in central regulation of glucose homeostasis and are impaired during T2D. Moreover, data from literature show that apelin generates ROS release in cultured neurons. In this study, we demonstrate that high levels of central apelin generate ROS hypothalamic production leading to over-activation of ANS and hepatic glycogenolysis and gluconeogenesis. Thus, the brain apelin-to liver axis participates in establishment of a T2D by generating fasted hyperglycaemia.
Materials and methods: We measured fasted glycemia, biochemical assay of activity of liver enzymes, hepatic glycogen content, plasma catecholamines, real-time amperometric ROS detection (especially hydrogen peroxide H_2O_2) in the hypothalamus on 1) C57bl6 mice fed with normal, and treated with acute icv apelin, and 2) mice that lentivectors expressed-apelin in the ventromedial nuclei of the hypothalamus (VMH).
Results: Real-time amperometric measurement reveals that apelin stimulates hypothalamic ROS release (approximately 100nM in 20 minutes $p<0.001$). Moreover, we show that high levels of apelin in the hypothalamus generate fasted hyperglycaemia (raise of 20%, $p<0.05$), in accordance to an increase of hepatic glycogenolysis and neoglucogenesis activities. This results in a decrease of hepatic glycogen stocks (0.70µg/mg for the apelin group vs 2 for control group; $p<0.05$) and an increase of activity of hepatic enzymes. Plasma norepinephrine is significantly increased in icv apelin treated mice (19.6ng/ml for the apelin group versus 7.5 for control group; $p<0.005$) suggesting an over-activation of sympathetic nervous system. All these deleterious effect of apelin are reduced by icv antioxidant pre-treatment. Finally, we confirm the specific hypothalamic effect of apelin in lentivectors expressed-apelin model.
Conclusion: We demonstrate that high levels of apelin in the hypothalamus (and more particularly in the VMH) generate a hypothalamic ROS signaling pathway which targets liver via the ANS. This original signalling pathway leads to an increase of hepatic glucose production resulting in fasted hyperglycaemia, a characteristic of T2D. Thus, central apelin could be considered as a new target for the treatment of metabolic disorders.

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Lipoprotein lipase inhibition into hypothalamus increases glucose tolerance and insulin sensitivity in mice

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Background and aims: It has been shown that non-esterified fatty acids could activate or inhibit hypothalamic neurons, and that this lipid sensing could modulate food intake and hepatic glucose production. After a meal, plasma NEFA concentration is physiologically decreased whereas plasma triglycerides increase, thus we hypothesize that a local TG hydrolysis into hypothalamus may provide NEFA and modulate lipid sensing. Here we inhibited hypothalamic Lipoprotein Lipase (LPL) and measured glucose homeostasis.
Materials and methods: LPL lox/lox mice were bilaterally injected into ventromedian nucleus of hypothalamus (VMN) with an adenovirus coding for Cre recombinase. Food intake, weight and fat/lean body mass were monitored; energy expenditure, locomotor activity and respiratory quotient were assessed one month after injection. We performed OGTT and insulin tolerance tests.
Results: Homozygous Lpllox/lox injected with Cre adenovirus (Hyp-Lpl-/-) displayed a 30% decrease in LPL activity ($p<0.05$ vs. WT mice) whereas heterozygous mice had no phenotype. Hyp-Lpl-/- mice gained less weight during the 12 weeks of the study (28.3±0.6g for controls vs. 26.4±0.8g for Hyp-Lpl-/-, $p<0.05$), whereas their food intake and energy expenditure were unchanged. In addition, Hyp-Lpl-/- mice had better glucose tolerance (AUC of glycaemia decreased by 45% vs. controls, $p<0.05$) and better insulin sensitivity (AUC of glycaemia increased by 60% vs. controls, $p<0.05$). Using WT-mice food-restricted to match Hyp-Lpl-/- mice weight gain over time, we demonstrated that the low weight was not entirely responsible for the improvement of glucose tolerance and that specific mechanisms controlling glucose homeostasis were involved.
Conclusion: We evidenced a role for hypothalamic LPL in the control of glucose homeostasis: a decreased LPL activity inside VMN lead to a moderate underweight and improved insulin sensitivity and glucose tolerance.
Supported by: French ANR Lipobrain

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Lipoprotein lipase deletion into hippocampus leads to glucose intolerance and impaired insulin secretion in mice

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Background and aims: Hippocampus is involved in the control of food intake, body weight and energy expenditure. In addition, it is the major site of expression and activity and of Lipoprotein Lipase (LPL) in brain, the main enzyme responsible for the hydrolysis of triglycerides into free fatty acids. We hypothesize that hippocampal LPL may play a role in the control of energy balance by modulating the brain lipid sensing.
Materials and methods: Lpl lox/lox mice were crossed with mice expressing Cre recombinase, under the control of a partial CamK2α promoter that exhibits a major expression in CA1 layer of hippocampus. The absence of recombination in other peripheral organs like pancreas was checked. Food intake, body weight and composition, energy expenditure were characterized for the descendants. Glucose homeostasis was explored by OGTT and by insulin tolerance test (ITT); glucose-stimulated insulin secretion was measured both in vivo and in vitro.
Results: Heterozygous mice for Camk2α-Lpl gene deletion presented a significant decrease in hippocampal LPL activity (-30%, $p<0.05$) and developed a transient overweight without over-eating, associated with an increase in fat mass and a decrease in energy expenditure, compared to control mice. These mice were also found to be glucose intolerant (28% increase in glycaemia AUC vs. controls, $p<0.05$), due to a defect in in vivo insulin secretion. In vitro, islets were normally responsive to glucose. In addition, Hipp-Lpl +/- mice were more sensitive to exogenous insulin (at t=15 min post-injection, glycaemia: 138 mg/dl in Hipp-Lpl+/- vs. 188 in controls, $p<0.05$). Homozygous mice did not present any phenotype or decrease in hippocampal LPL activity (due to a putative compensatory LPL expression).
Conclusion: Heterozygous LPL gene deletion behind CamK2α promoter leads to a glucose intolerance associated with a defect in in vivo insulin secretion, probably due to changes in sympatho-vagal balance. These results support a role of hippocampal LPL activity in nervous regulation of glucose homeostasis.
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Revealing a functionally-relevant map from the brain to pancreatic islets

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Background and aims: For more than 150 years it has been known that certain brain injuries can lead to diabetes, and that the central nervous system

(CNS) is able to exert a degree of control over insulin and glucagon secretion. However, the precise CNS area(s) that link to the endocrine pancreas are essentially unknown. Here, we reveal this direct neuronal circuit in mice with and show that it is relevant in the regulation of pancreatic endocrine secretions.
Materials and methods: A pseudorabies virus strain (PRV-BaBlu) expressing the LacZ reporter gene (PRV-BaBlu) was used as a specific neuronal retrograde transynaptic tracer to track the neuronal link from the endocrine pancreas to the CNS. Essentially, mouse pancreata were infected with PRV-BaBlu and traced over a 96h period for appearance in the CNS. Immunofluorescence (IF) analysis was used to examine β-gal expression from PRV-BaBlu, and co-localisation with glucose-sensing glucokinase (GK) in the CNS neurons. Stereotaxic brain injections of adenoviral-driven Hexokinase I (HKI) specifically to CNS regions enriched by β-gal/GK+ neurons was conducted in mice to lower glucose-sensing from the mM toward the μM range. Intraperitoneal glucose and insulin tolerance tests (IPGTT/IPITT, respectively) were carried out to examine whether altering CNS glucose-sensing affected pancreatic islet endocrine cell secretory functions.
Results: The PRV-BaBlu neuronal tracing revealed that a major neuronal circuit originating in the Arcuate (ARC), Ventromedial (VMN), Dorsomedial (DMN), and Lateral (LHA) regions of the hypothalamus, on to the Periventricular (PeVN) and paraventricular (PVN) hypothalamus around the 3rd ventricle of the brain to the periaqueductal gray (PAG) relay center, then to the dorsal motor (DMX) and solitary (NTS) nuclei in the brain stem, and ending on the endocrine pancreas. IF analysis indicated enriched GK co-localisation with PRV-BaBlu in the ARC, VMN and LHA. HK1 overexpression specifically in the ARC resulted in no effect on insulin sensitivity but significant glucose-intolerance ($p \leq 0.05$), relative to control mice. No effect was observed with HK1 overexpression specifically in the VMN. In contrast, HK1 overexpression in the LHA indicated improved glucose tolerance ($p \leq 0.05$). These contrasting effects in the ARC versus the LHA were associated with distinct changes in the balance between insulin and glucagon levels during the glucose/insulin tolerance tests.
Conclusion: Our results reveal a topographical map between the CNS and pancreatic islets that has functional relevance. Moreover, the data indicate that glucose sensing in different hypothalamic regions can have quite distinct consequences to glucose homeostasis instigated by a CNS-augmented control of insulin and glucagon secretion in vivo.
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Hepatic glycogen loading increases the counter-regulatory response to hypoglycaemia by a liver-brain neural connection
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Background and aims: The risk of iatrogenic hypoglycemia is a persistent barrier to the safe, effective treatment of people with type 1 diabetes. In response to insulin-induced hypoglycaemia, increased secretion of glucagon and epinephrine stimulates hepatic glucose production and inhibits muscle glucose utilization, thereby restoring glucose homeostasis. However, the secretion of both glucagon and epinephrine in response to insulin-induced hypoglycaemia is impaired in patients with type 1 diabetes, making augmentation of their release of therapeutic value.
Materials and methods: During the first 4h of each study, 18h fasted dogs received somatostatin and basal amounts of intraportal insulin and glucagon. Arterial plasma glucose was doubled by glucose infusion into a peripheral vein and either saline (SAL; n=6) or fructose (7.15 μmol/kg/min; n=11) was infused into the hepatic portal vein, with the latter significantly stimulating hepatic glucose uptake and glycogen deposition. This glycogen loading period was followed by a 2h hyperglycaemic/normoinsulinemic period during which the intraportal infusion of SAL or fructose was discontinued. During the final 2h period, hypoglycaemia was induced in all animals using a 16x basal intraportal infusion of insulin. Animals from FRU were divided into two groups; one group that had intact liver innervation (FRU; n=6) and one that had severed hepatic nerves (FRU-DEN; n=5).
Results: Fructose infusion led to a large increase in hepatic glycogen in both FRU groups (286±28, 446±33 and 418±44 μmol/g liver in SAL, FRU and FRU-DEN, respectively; $p<0.05$). During the final hour of the 2h hyperinsulinemic/ hypoglycaemic period, arterial plasma glucose levels were similar among the three groups (2.6±0.1, 2.6±0.1 and 2.7±0.1 mmol/l in SAL, FRU and FRU-DEN, respectively) as were the hepatic sinusoidal insulin levels (2538±114, 2340±186 and 2472±180 pmol/l, respectively). Hypoglycaemia caused greater net hepatic glucose output during this period in FRU compared to SAL (25.3±1.1 vs 8.4±1.1 μmol/kg/min, respectively; $p<0.01$) as a result of a greater increase in plasma glucagon (109±25 and 65±7 ng/l, respectively; $p=0.06$) and epinephrine (1820±365 and 902±185 ng/l, respectively; $p<0.05$). Hepatic denervation blocked each of these responses such that, despite the presence of elevated hepatic glycogen, net hepatic glucose output (12±3 μmol/kg/min), plasma glucagon (62±17 ng/l) and epinephrine (892±337 ng/l) levels were similar in FRU-DEN and SAL.
Conclusion: Our data indicate that an increase in hepatic glycogen is associated with augmented secretion of both glucagon and epinephrine in response to insulin-induced hypoglycaemia. This increase is blocked by hepatic denervation indicating that it resulted from altered afferent neural input from the liver to the brain. Thus, hepatic glycogen content can be a determinant of the counter-regulatory response to hypoglycaemia.
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Hypothalamic nesfatin-1 knockdown increases gluconeogenesis by mTOR/STAT3 pathway
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Background and aims: Nesfatin-1, an 82 amino acid neuropeptide, has been recently characterized as a potent metabolic regulator. However, the metabolic mechanisms directly associated with the action of nesfatin-1 have not been well delineated. To determine the role of hypothalamic nesfatin-1/NUCB2 in glucose homeostasis and metabolic signal pathway, we have determined whether attenuation of central nesfatin-1/NUCB2 signal leads to the change in peripheral glucose kinetics using a loss-of-function animal model by knocking down hypothalamic nesfatin-1/NUCB2 in rats. More importantly, we determined the molecular mechanisms by which the central nesfatin-1/NUCB2 knockdown leads to the alteration of the insulin signaling cascade.
Materials and methods: We established a loss-of-function model of hypothalamic nesfatin-1/NUCB2 signaling in rats using an adenoviru-mediated RNAi. Using this model, we assessed the effects of central nesfatin-1/NUCB2 knockdown on glucose metabolism and changes in transcription factors and signaling pathway during euglycemic-hyperinsulinemic clamping. Glucose rates of appearance (GRa) were determined with 3-[3H] glucose. Whole body GRa and glucose uptake (GRd) were calculated using the non-steady-state equation. mRNA and protein expressions were measured by qRT-PCR and Western blot, respectively.
Results: We showed that inhibition of central nesfatin-1/NUCB2 activity markedly increased feed intake in normal chow diet (NCD)-fed rats and increased hepatic glucose fluxes and decreased glucose uptake in peripheral tissue in both NCD- and high fat diet (HFD)-fed rats. The change of hepatic glucose fluxes in the hypothalamic nesfatin-1 deficiency rats was accompanied by increasing in the hepatic expression of G-6-Pase and PEPCK and decreasing in InsR, IRS-1, and AKT phosphorylation. Furthermore, knockdown of hypothalamic nesfatin-1 led to decreased phosphorylation of STAT3 and mTOR and subsequent SOCS3 expression.
Conclusion: We demonstrate here that a rapid loss of function in hypothalamic nesfatin-1/NUCB2 signaling can be induced in vivo using an adenoviru-mediated RNAi. The targeting of this vector to hypothalamus should provide a formidable tool for dissecting the contribution of this pathway to glucose metabolism and insulin signal transduction. Using this experimental tool, we unveil the critical role of hypothalamic nesfatin-1 knockdown in the regulation of liver glucose fluxes and insulin signal pathway as well as the involvement of mTOR-STAT3-SOCS3 pathway. Our data delineate that modulation of the hypothalamic nesfatin-1/NUCB2 signaling may represent a promising strategy for the treatment of obesity and the underlying metabolic syndrome by regulating mTOR-STAT3-SOCS3 signal pathway.
Supported by: National Natural Science Foundation of China

OP 19 State of the art of inhibiting DPP-4

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Dipeptidyl peptidase-4 inhibitor sitagliptin preserves islet beta cell function in patients with new-onset latent autoimmune diabetes in adults

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Background and aims: Latent autoimmune diabetes in adults (LADA) is thought to result from chronic autoimmune destruction of pancreatic β cells. The ideal agents for LADA should aim to promote β cell proliferation and modulate immune system. The incretin drugs dipeptidyl peptidase-4 (DPP-4) inhibitor can stimulate β-cell proliferation and increase insulin content in vitro and has been shown to improve β cell function in type 2 diabetic patients. Besides, it also plays a fundamental role in immune regulation, especially in T-cell activation. Therefore, we hypothesized that LADA patients might benefit from DPP-4 inhibitor treatment and this study aimed to investigate the effect of sitagliptin on β cell function in LADA patients.
Research design and methods: Thirty newly diagnosed LADA patients with remained β cell function (fasting C-peptide more than 0.2nmol/L) were enrolled and randomized 1:1 to receive insulin plus sitagliptin (100mg/day) (insulin+SIT group, n=15) or insulin alone (insulin group, n=15) for 12 months. Plasma glucose, HbA_{1c}, fasting C-peptide (FCP) and 2h postprandial C-peptide (2hCP) during a mixed-meal tolerance test (MMTT) were determined at the baseline, 3th month, 6th month, 9th month and 12th month during the treatment. Glutamic decarboxylase antibody (GAD-Ab) and C-peptide were measured by radioimmune assays. Islet β cell function was evaluated by FCP, 2hCP and ΔCP (ΔCP=2hCP-FCP).
Results: There were no significant changes in the level of FCP, 2hCP and ΔCP in insulin+SIT group during the 12 months' observation compared with the baseline (all $P>0.05$). Whereas in the insulin alone group, not only FCP level decreased from 407.2 pmol/L at baseline to 323.3 pmol/L at the 3th month ($P<0.05$), and continued to decline to 256.8 pmol/L at the 12th month ($P<0.05$), but also 2hCP level decreased gradually from 1496.pmol/L at the baseline to 830.2 pmol/L at the 12th month (3th, 6th, 9th, 12th vs baseline, all $P<0.05$) and ΔCP level decreased significantly from the baseline of 1090.2 pmol/ L to the 12th month of 580.9 pmol/ L (3th, 9th and 12th vs baseline, all $P<0.05$). Furthermore, 2hCP and ΔCP levels were both significantly higher in insulin+SIT group than those in the insulin alone group at the 12th month (both $P<0.05$).
Conclusion: Our study demonstrates, we believe for the first time, that the sitagliptin therapy protects β-cell function in LADA patients, which provides important therapeutic implications for DPP-4 inhibitors in autoimmune diabetes.

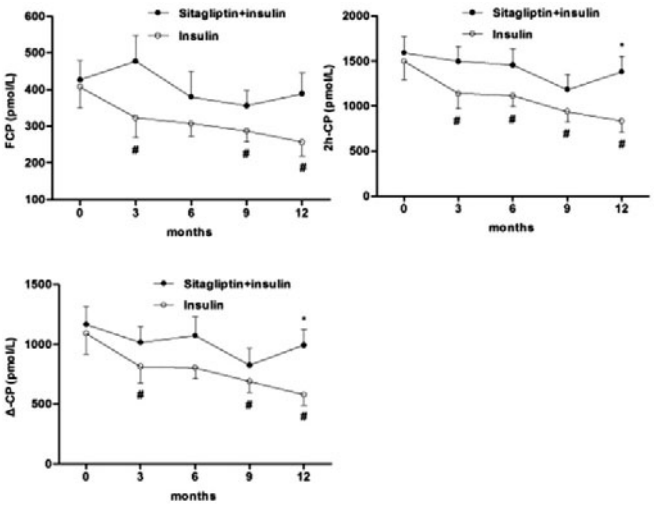

FIG.2. FCP, 2hCP and ΔCP levels changes in LADA patients treated with insulin plus sitagliptin or not during the 12months' period.

Black circles=sitagliptin+insulin group; white circles= insulin group. Data are means± SE of 15 patients in each group.
Vs. the baseline, *P<0.05; vs. the control group, #P<0.05

Clinical Trial Registration Number: NCT01159847
Supported by: EFSD/CDS/Lilly

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Improved glucagon dynamics during hypoglycaemia and food-rechallenge by DPP-4 inhibition by vildagliptin in insulin-treated patients with type 2 diabetes

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Background and aims: Glucagon counter-regulation is critical for defending hypoglycemia and lowering of glucagon is a key component for improved post-meal glycemia in type 2 diabetes (T2D). Glucagon sensitivity to glucose is increased in hyperglycemia by GLP-1 and increased in hypoglycemia by GIP. We previously showed that the DPP-4 inhibitor, vildagliptin (VILDA), reduces glucose levels without increasing hypoglycemia in patients on insulin therapy with either T2D or type 1 diabetes (T1D), reduces glucagon at hyperglycemia in T2D and T1D, and sustains glucagon counter-regulation during hypoglycemia in drug-naïve and insulin-treated T2D patients. We have now explored the effects of VILDA on glucagon dynamics during meal ingestion, during insulin-induced hypoglycemia and during subsequent food re-challenge after hypoglycemia in patients with insulin-treated T2D to examine whether VILDA improves the glucagon dynamics also when used in combination with insulin.
Materials and methods: The study was a single-center, double-blind, randomized, placebo (PBO)-controlled crossover study involving 29 patients with T2D (16M, 13F, mean age 58 yrs, diabetes duration 15 yrs, duration of insulin therapy 7 yrs, HbA1c 60 mmol/mol (7.7%), BMI 30.7 kg/m^2) treated with long acting (mean 33U/day) and, in 26 patients, additionally short-acting (mean 28U/day) insulin. Patients received VILDA (50 mg BID) or PBO as add-on to insulin for four weeks in random order with a four week washout in-between. On day 28 of the respective treatment, patients were served a standard meal (500 kcal) followed by a hyperinsulinemic hypoglycemic clamp (2.5 mmol/l for 30 min) and a subsequent food re-challenge.
Results: Glucose levels were lower during the meal with VILDA than with PBO (120 min AUC: 1.1±0.1 vs. 1.4±0.1 mol/l x min, P<0.001) whereas glucose levels were similar between the groups during the clamp (2.6±0.1 mmol/l in both groups, NS). During the meal, glucagon levels were lower with VILDA than with PBO (120 min AUC: 7.1±0.5 *vs.* 7.7±0.6 ng/ml x min, P<0.02). The glucagon counter-regulation to the insulin-induced hypoglycemia was sustained by VILDA (23.1±4.0 vs.24.2±4.2 pg/ml, NS). Also the hypoglycemia counter-regulatory responses in adrenaline, noradrenaline and pancreatic polypeptide were not different between the groups. During the food re-challenge after hypoglycemia, glucagon levels were, again, significantly lower after VILDA than after PBO (4.8±0.4 vs. 5.3±0.4 pg/ml x

min, P<0.05); glucose levels did not differ between the groups (0.12±0.01 vs. 0.14±0.01 mol/l x min, NS). After the four week treatment period, HbA1c was reduced with VILDA -4.7±0.6 mmol/mol (-0.5±0.1%) and with PBO by -1.1±0.6 mmol/mol (-0.1±0.1%(; in-between group difference -3.6±1.0 mmol/mol (-0.4±0.1%; P<0.001).
Conclusion: In insulin-treated patients with T2D, add-on with the DPP-4 inhibitor VILDA reduces glucagon during meal but preserves glucagon counter-regulation during hypoglycemia. VILDA also sustainably lowered the glucagon response after the food re-challenge following hypoglycemia. The results thus show that VILDA improves glucagon dynamics during hypoglycemia and food re-challenge in insulin-treated patients with T2D.
Clinical Trial Registration Number: NCT01219400
Supported by: VR, Faculty of Medicine, Region Skåne, Novartis

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Gemigliptin added to ongoing metformin therapy provides sustained glycaemic control over 52 weeks and was well tolerated in patients with type 2 diabetes

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Background and aims: Many people with type 2 diabetes cannot maintain target blood glucose levels as monotherapy, because of the complex nature of the disease and the progressive deterioration in pancreatic β-cell function. This was a 28-week extension to a study of the efficacy and safety of gemigliptin compared with sitagliptin added to ongoing metformin therapy in type 2 diabetes patients with inadequate controlled with metformin alone. This study was designed to evaluate the long-term efficacy and safety of gemigliptin and to assess the efficacy and safety of switching from sitagliptin to gemigliptin in type 2 diabetes patients receiving background treatment with metformin. All patients received once daily gemigliptin 50 mg during extension period.
Materials and methods: We conducted a 24-week, randomized, double-blind, active-controlled study of gemigliptin with an open-label extension to 52 weeks. A total of 604 patients diagnosed with type 2 diabetes mellitus were screened and 425 eligible patients were then enrolled. About 90% of the patients who completed 24 weeks of treatment in each group consented to continue the study to receive further treatment with gemigliptin 50 mg qd for 28 weeks. Among 335 patients who consented to participate in the extension study, 315 patients (94%) completed 52 weeks of treatment. The primary endpoint for extension period was the mean change in HbA1c from baseline to week 52. Safety and tolerability based on adverse events (AEs) also assessed.
Results: All treatment groups showed clinically and statistically (*P*<.0001) significant HbA1c reduction after 52 weeks of dosing regardless of previous treatment given during initial 24 weeks. The 95% confidence interval (CI) for HbA1c reduction at week 52 was (-1.28%, -0.85%) in patients continued to receive once daily gemigliptin 50 mg. Proportion of patients achieving HbA1c <7% at week 24 in gemigliptin 25 mg bid (53.5%) and gemigliptin 50 mg qd (54.6%) was comparable to the results with once daily sitagliptin 100 mg (45.5%). It slightly increased in each group at the end of week 52 reaching 60.3% (25 mg bid) and 70.9% (50 mg qd) in gemigliptin groups and 52.3% in sitagliptin group. Gemigliptin was generally well tolerated with a low incidence of hypoglycemia and an absence of body weight gain over 52 weeks.
Conclusion: In conclusion, this study demonstrated the long term efficacy and safety of once daily administration of gemigliptin 50 mg as an add-on therapy to metformin in type 2 diabetes mellitus patients.
Clinical Trial Registration Number: NCT01602003
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Cardiovascular (CV) safety of linagliptin in patients with type 2 diabetes: a pooled comprehensive analysis of prospectively adjudicated CV events in phase 3 studies

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Background and aims: Incidence of CV events is increased in T2D, but the potential for CV risk modulation with glucose lowering therapies is debated.
Materials and methods: We compared the incidence of CV events and CV mortality in patients with T2D treated with linagliptin (lina), a once daily DPP-4 inhibitor, with non-lina comparators (placebo or active; comp) in 19 double-blind RCTs (duration ≥ 12 weeks). CV events were prospectively adjudicated by a blinded independent expert committee. The primary endpoint was a composite of CV death, non-fatal stroke, non-fatal myocardial infarction, and hospitalization for unstable angina pectoris. Other secondary and tertiary CV endpoints were also assessed.
Results: Of 9459 patients, 5847 received lina (5 mg: 5687, 10 mg: 160) and 3612 comp (placebo: 2675, glimepiride: 775, voglibose: 162). The cumulative exposure (person years) was 4421.3 for lina and 3254.7 for comp. Changes (mean±SEM) in HbA1c and weight, for linagliptin and comp, respectively were -0.68±0.01%/-0.27±0.02% and -0.1±0.1/+0.4±0.1 kg whereas there were similar changes in systolic/diastolic blood pressure and lipids. In total, 60 primary events were reported in the lina group and 62 in the comp group (36 in the placebo and 26 in the active comp group). Incidence rates of the primary endpoint (/1000 years at risk) were lower for lina (13.4) than for the comp group (18.9) in line with the hazard ratio (0.78) (TABLE).
Conclusion: This updated pooled analysis of adjudicated CV events in a large Phase 3 program continues to support that lina is not associated with an increased risk for CV events. Potential CV benefits with lina will be tested prospectively in more than 14000 patients in the active-comparator (glimepiride) **CAROLINA trial (NCT01243424) and the placebo-controlled CARMELINA trial.**

	Linagliptin (n=5847)	Comparator (n=3612)	
Characteristics of study cohort and exposure according to study arms			
Mean age (years)	58±11	59±10	
Female gender (%)	45.6	43.5	
Mean baseline HbA1c (%)	8.1±0.9	8.1±0.9	
T2D duration > 5 years (%)	54.9	56.8	
Baseline BMI (kg/m²)	29.0±5.2	29.5±5.2	
Mean (maximum) exposure (days)	276 (776)	329 (804)	
Impact on primary, secondary and tertiary CV endpoints according to study arms			
	Incidence rate/1000 pt-yr		**Hazard ratio (Cox proportional model) (95% CI)**
Primary CV endpoint	13.4	18.9	0.78 (0.55, 1.12)
Secondary CV endpoints			
CV death, stroke, or MI	9.3	14.0	0.74 (0.49, 1.13)
All adjudicated CV events	21.5	29.1	0.82 (0.61, 1.09)
Tertiary CV endpoints			
CV death	2.4	2.4	1.04 (0.42, 2.60)
Non-fatal MI	5.1	6.1	0.86 (0.47, 1.56)
Non-fatal stroke	2.0	5.8	0.34 (0.15, 0.75)*
Transient ischemic attack	0.2	2.4	0.09 (0.01, 0.75)*
Hospitalization for UAP	4.9	4.8	1.08 (0.56, 2.06)

*Significant lower Hazard ratio (upper 95% CI <1.0). Abbreviations: MI – myocardial infarction, UAP – unstable angina pectoris.

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Durability of the efficacy and safety of alogliptin compared to glipizide over 2 years when used in combination with metformin

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Background and aims: This three-arm, multicenter, randomized, double-blind, active-controlled study was conducted to evaluate the durability of the efficacy and safety of alogliptin (ALO) compared to glipizide in combination with metformin (MET) in patients with type 2 diabetes inadequately controlled on stable-dose MET.
Materials and methods: The duration of the study was 104 weeks. The three treatment arms were: ALO 12.5 mg QD + MET (ALO 12.5) (n=880), ALO 25 mg QD + MET (ALO 25) (n=885), and glipizide 5 mg titrated up to a

maximum of 20 mg + MET (GLIP) (n=874). The primary endpoint was least square mean change from baseline in HbA1c at 104 weeks.
Results: The majority of patients were white (62.3%) and 50.3% were women. The mean age was 55.4 years; body mass index, 31 kg/m^2; diabetes duration, 5.5 years; and baseline HbA1c, 7.6%. Reductions in HbA1c at Week 104 were 0.68%, -0.72%, and -0.59% for ALO 12.5, ALO 25, and GLIP, respectively. Significantly more patients achieved an HbA1c ≤7% at Week 104 with ALO 25 (48.5%) vs GLIP (42.8%) (P=0.004); the proportion for ALO 12.5 was 45.6% (not significant vs GLIP). Changes in fasting plasma glucose at Week 104 were -0.9 mg/dL for ALO 12.5, -3.2 mg/dL for ALO 25, and 5.4 mg/dL for GLIP; both ALO reductions were significant compared with the GLIP increase (P<0.001). Mean weight changes at 104 weeks were -0.68, 0.89, and 0.95 kg for ALO 12.5, ALO 25, and GLIP, respectively; both ALO decreases were significantly different from the GLIP increase (P<0.001). More GLIP patients (23.2%) experienced one or more hypoglycemic events compared to ALO 12.5 (2.5%) and ALO 25 (1.4%) patients; severe hypoglycemia occurred in six GLIP patients, one ALO 12.5 patient, and no ALO 25 patients. Numbers of patients experiencing at least one adverse event (AE), a serious AE, or an AE leading to treatment discontinuation were similar across the three groups. The most frequently reported AEs overall and within each treatment group were upper respiratory tract infection, nasopharyngitis, diarrhea, hypertension, headache, and back pain. Pancreatitis occurred in one ALO 25 patient and three GLIP patients. Eleven deaths occurred: three in the ALO 12.5 group, three in the ALO 25 group, and five in the GLIP group.
Conclusion: The efficacy of alogliptin was sustained through 104 weeks. The safety profile was similar among the treatment arms, although considerably lower incidences of hypoglycemia were observed in the ALO dose groups.
Clinical Trial Registration Number: NCT00856284

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Comparison of trough dipeptidyl peptidase-4 inhibition in patients with type 2 diabetes treated with saxagliptin, sitagliptin, and vildagliptin
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Background and aims: Saxagliptin (Saxa), sitagliptin (Sita), and vildagliptin (Vilda) are dipeptidyl peptidase-4 (DPP-4) inhibitors currently approved as oral antihyperglycemic agents (AHAs) for use in treating type 2 diabetes. These agents have substantially different pharmacokinetic properties and DPP-4 binding characteristics that may have important implications for their pharmacodynamic profiles. Our present understanding is limited, however, because (1) in some prior studies, assay conditions were not optimized for comparison between agents, and (2) no prior study has directly compared DPP-4 inhibition in patients treated with these agents. Here, using an assay that minimized ex vivo plasma dilution (a potential source of error), we have assessed trough DPP-4 inhibition in a single cohort of patients treated with these three agents.
Materials and methods: This was a randomized, placebo-controlled, open-label, five-period crossover study. Eligible patients were 18-65 years of age and had A1C ≥6.5% and ≤10.0% when treatment-naïve or off prior AHA therapy for ≥6-12 weeks. The treatments were 5 mg Saxa q.d., 100 mg Sita q.d., 50 mg Vilda q.d., 50 mg Vilda b.i.d., and placebo q.d (PBO). Treatments lasted 5 days and were separated by ≥10 days. Plasma was prepared from blood samples collected predose on Days 1-5 and 12, 24, 36, 48, and 96 h following the morning dose on Day 5. Glycyl-propyl-amino-methylcoumarin hydrobromide solution was combined with plasma in a 1:9 ratio and DPP-4 activity was measured fluorometrically. Percent DPP-4 inhibition (%DPP-4i) was calculated for each treatment relative to the predose DPP-4 activity in each period. The primary endpoint was trough %DPP-4i measured 24 h after the morning dose on Day 5. It was analyzed using a linear mixed-effects model with fixed-effects terms for treatment and period. Pharmacokinetic data were evaluated using WinNonlin version 5.0.1.
Results: In the randomized cohort (N=22), mean (range) baseline A1C was 7.4% (6.4-9.0). Least-squares (LS) mean (95% CI) trough %DPP-4i was 73.5% (66.6%, 79.0%), 91.7% (91.4%, 92.1%), 28.9% (17.9%, 38.4%), 90.6% (88.9%. 92.1%), and 3.5% (−0.7%, 7.5%) after Saxa, Sita, Vilda-q.d., Vilda-b.i.d., and PBO treatments, respectively. In comparisons with Sita, LS-mean differences were 18.2% vs Saxa (p<0.001; positive values favor Sita), 62.9% vs Vilda-q.d. (p<0.001), 1.1% vs Vilda-b.i.d. (p=0.128), and 87.8% vs PBO (p<0.001). Given that mean %DPP-4i was ~90% at 12 h postdose for each active treatment, these between-group comparisons primarily reflect differences in duration of action. Mean apparent $t_{½}$ was 9.5 h for Saxa (14.7 h for its active metabolite 5OH-saxagliptin), 12.0 h for Sita, and 2.4-3.4 h for Vilda. Ten adverse events were reported overall, none serious. All were transient and characterized as mild or moderate in intensity. Three patients were discontinued because of hyperglycemia, one because it was determined after randomization that she did not meet eligibility criteria, and one patient withdrew consent.
Conclusion: In this study, treatment with Sita provided significantly greater DPP-4 inhibition than either Saxa or Vilda once daily and DPP-4 inhibition similar to that provided by Vilda twice daily.
Clinical Trial Registration Number: NCT01582308
Supported by: Merck Sharp & Dohme Corp.

OP 20 Inflammation and insulin resistance: switching on and off

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Alteration of CD28 T cell costimulatory pathway protects against obesity-induced inflammation and metabolic disorders

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Background and aims: Lymphocytes have been shown to be critical in adipose tissue (AT) inflammation and contribute to the metabolic consequences of obesity. The CD28-B7 costimulatory molecules are essential for T-cell activation and regulatory T cell homeostasis. Nevertheless their role in obesity has never been assessed. In this present study, we assess the role of T cell activation and T cell homeostasis in diet-induced obesity models by modulating CD28-mediated T cell costimulation using targeted mutations of CD28 gene or CD28/B7 pathway pharmacological blockade.
Materials and methods: CD28 wild-type (WT), knock-out (KO), knock-in (KI) with a loss of the cytoplasmic tail mice were fed a high-fat diet (HFD, 65% kcal from lipids) for 16 weeks. An additional group of WT mice was injected with CTLA4-Ig (Abatacept), an inhibitor of CD28-B7 interactions and fed HFD for 8 weeks. Metabolic and inflammatory parameters were evaluated by glucose tolerance test, FACS analysis, RNA quantification, immunohistochemistry and ELISA.
Results: CD3+, CD3+CD4+, CD3+CD8+ T cell population were reduced and regulatory T cells (CD3+CD4+CD25+FoxP3+) were almost absent in epididymal AT from CD28KO and KI compared to WT mice. In addition while T-cell activation in spleen and AT was prevented in CD28KO, it was partially retained in CD28KI mice. Despite an established obesity, CD28KO and KI mice displayed improved insulin sensitivity. Furthermore, CD28 deficiencies protect from fatty liver accumulation. In some instances the protective effect was stronger in CD28KI than in CD28KO mice with improved systemic glucose tolerance, higher levels of lipogenesis and insulin signaling mRNA in AT. In agreement, CD28KI mice exhibited higher levels of anti-inflammatory M2-macrophages while the global AT-macrophages infiltration did not differ between the groups. Short CTLA4-Ig treatment of WT mice decreased CD4+ and Tregs in AT but CD8 content and metabolic parameters were not altered.
Conclusion: These findings demonstrate that CD28 deficiencies prevent insulin resistance and liver steatosis induced by high fat diet without affecting obesity. Cell activation is not required to recruit macrophage in adipose tissue but it is for macrophage polarization, emphasizing the cooperation between activated lymphocytes and macrophages within AT.
Supported by: SFD

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Intra-islet inflammation in type 2 diabetes: evidence that the E3 ligase TRAF2 is a key protective factor in beta cells

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Background and aims: Activation of the JNK and NF-κB signaling pathways are associated with beta cell failure and apoptosis. TNFα-receptor associated factor 2 (TRAF2), an E3 ubiquitin ligase, mediates activation of both these pathways. We predicted that genetic deletion of TRAF2 in beta cells would improve beta cell function and prevent diabetes; an idea previously untested in islets.
Materials and methods: We generated beta cell specific TRAF2 knockout mice (bTRAF2), examined TNFα signaling and subjected mice to a diet-induced obesity model.
Results: bTRAF2 islets appeared normal, showed robust insulin labeling, and bTRAF2 mice displayed normal glucose tolerance at 8 weeks of age. Analyzing TNFα induced signaling ex vivo, bTRAF2 islets displayed delayed degradation of IκBα compared to wild type (WT). Phosphorylation of JNK was delayed, and also profoundly prolonged. Deleting TRAF2 in Min6 beta cells using siRNA emulated delayed and prolonged JNK activation. Further, we found increased processing of p100 to p52 in unstimulated bTRAF2 islets indicating cell intrinsic activation of the non-canonical NF-κB pathway. bTRAF2 islets were hyper-responsive to TNFα stimulation compared to WT showing 3-7 fold (**P<0.005) increased gene expression for CXCL10, ICAM1 and TNFα, genes regulated by the non-canonical pathway. Also the basal levels for these genes were increased ~2.5 fold (*P<0.05) compared to WT islets. In vivo, bTRAF2 mice subjected to an i.p. GTT showed a trend towards worsened glucose tolerance at 16 weeks of age (mean AUC±SEM: WT: 969.7±41.71, bTRAF2: 1181±80.13; n≥12; *P<0.05). When fed a high-fat diet for 8 weeks, glucose intolerance in bTRAF2 versus WT mice was greatly exacerbated (mean AUC±SEM: WT: 1420±37.8, bTRAF2: 1903±108.7; n≥12; ***P<0.001). This data was further corroborated by a trend towards lower fasting blood insulin levels and a defect in first phase insulin secretion in bTRAF2 mice determined by i.v. GTT (mean AUC±SEM: WT: 2.47±0.16, bTRAF2: 1.96±0.15; n≥10; *P<0.05). Intriguingly, TRAF2 mRNA levels were reduced in WT islets isolated from high-fat diet fed mice versus islets from chow fed mice.
Conclusion: Thus, mice lacking TRAF2 in islets exhibit severe defects in glucose control and insulin secretion under a high fat feeding regimen and bTRAF2 islets exhibit a hyper-inflammatory profile. At the molecular level TRAF2 controls the cellular tempo of JNK and NF-κB activation and reigns in the non-canonical NF-κB pathway. Loss of this control circuit exacerbates diabetes suggesting that TRAF2 functions as a key protective factor in beta cells.
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Delayed intervention with pyridoxamine improves metabolic and vascular function in high-fat diet-induced obese mice

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Background and aims: The development of obesity is associated with insulin resistance and is accompanied by a cluster of risk factors for cardiovascular disease, including hypertension, dyslipidaemia, and inflammation. We and others previously demonstrated increased levels of inflammation, oxidative stress and glycation in obese visceral adipose tissue (VAT). Pyridoxamine (PM), one of the three natural forms of vitamin B6, has been identified as an anti-inflammatory, anti-oxidant and anti-glycating agent. The objective of the present study was to examine the effect of a delayed PM intervention on metabolic and vascular function in high-fat diet-induced obese mice.
Materials and methods: After a run-in period on low-fat diet (Research Diets D12450H), 12 week old male C57BL/6J mice were divided into two groups. The low-fat diet group (LFD, n=15) continued on the same diet, whereas the other group switched to a high-fat diet (D12451, HFD,n=30). After 6 weeks half of the mice fed the HFD group received PM (2 g/L) in their drinking water (HFD+PM, n=15). All mice were sacrificed 18 weeks later. Glucose tolerance (GT) and insulin sensitivity (IS) were measured with glucose or insulin IP injections. Metabolic variables were measured by ELISA and colorimetric assays. Adipose inflammation was addressed with qPCR and immunohistochemistry. Vascular reactivity of aortic ring segments was measured *ex vivo* with wire myography.
Results: Bodyweight (28.2±0.4 gram), plasma glucose (10.4±0.3 mM) and plasma cholesterol (2.4±0.1 mM) were significantly (p<0.001) increased after 6 weeks of HFD compared with the LFD group (25.3±0.3, 8.0±0.4, and 1.8±0.1 respectively). Supplementation of PM, during the subsequent 18 weeks of HFD, attenuated the bodyweight gain (32.2±1 versus 37.5±2 gram), hyperglycaemia (8.1±2 versus 9.1±1 mM) and hypercholesterolemia (3.0±0.4 versus 4.2±0.3 mM) (for all, p<0.05). Furthermore also plasma insulin (1.1±0.2 ng/mL) and leptin levels (12.7±3.1 ng/mL) were significantly (p<0.05) lower in the PM supplemented group compared to the HFD untreated group (2.4±0.4 and 54.4±18 ng/mL, respectively). The decrease in GT and IS (AUC 2263±142 and 750±72 units, p<0.05) observed in the HFD group compared with the LFD group (1566±74 and 541±24) was improved by PM supplementation (1800±108 and 672±38, p<0.05). Intra-arterial diastolic (72±2 mmHg) and systolic blood pressure (100±1 mmHg) were mildly increased in the HFD group compared with the LFD group (68±2 and 93±4 respectively), and tended to be decreased in the HFD+PM group (70±1 and 97±1 respectively). Furthermore, the VAT had a pro-inflammatory profile as indicated by the significant up-regulation of MCP-1, TNFα, leptin, CD11c and MHC-II mRNA levels, and down-regulation of adiponectin compared to the LFD group (for all, p<0.05). PM supplementation significantly (p<0.05) prevented these changes in mRNA levels. Finally, myograph experiments showed an improvement in NO-independent aortic relaxation in the HFD+PM group compared with the HFD group (p<0.05).

Conclusion: Our study shows that a delayed intervention with PM is associated with improvement of several aspects of obesity including insulin resistance, adipose inflammation, and of aortic dysfunction in high-fat diet-induced obese mice.
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IL-37 protects against obesity-induced inflammation and insulin resistance
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Background and aims: Cytokines of the IL-1 family are important modulators of obesity-induced adipose tissue inflammation with the subsequent development of systemic insulin resistance. Here, we report that IL-37, the anti-inflammatory member of the IL-1 family, plays an important role in obesity-induced inflammation and insulin resistance, in both mice and human.
Materials and methods: A high-fat-diet intervention study was performed in human IL-37-transgenic mice (hIL-37tg-mice). Furthermore, *in vitro* IL-37 silencing experiments were performed in HEPG2 cells. Lastly, adipose tissue biopsies were taken from healthy lean and obese persons.
Results: IL-37 transgenic mice (IL-37tg) did not develop an obese phenotype in response to a high-fat-diet (HFD). Unlike WT mice, IL-37tg mice exhibited reduced numbers of adipose tissue macrophages and preserved glucose tolerance and insulin sensitivity when subjected to 16 weeks of HFD. A short-term HFD intervention also revealed that the IL-37-mediated improvement in glucose tolerance is independent of bodyweight differences. Moreover, IL-37tg mice manifested a beneficial metabolic profile with higher circulating levels of the anti-inflammatory adipokine adiponectin. Additionally, *in vitro* data show that IL-37 protects insulin signalling. Importantly, in human subjects with varying levels of obesity, steady-state IL-37 adipose tissue mRNA levels were positively correlated with insulin sensitivity, with lower adipose tissue levels of leptin and with a lower inflammatory status of the adipose tissue.
Conclusion: These findings reveal IL-37 as an important anti-inflammatory modulator during obesity-induced inflammation and insulin resistance in both mice and humans and suggest IL-37 as a potential target to tackle obesity-induced insulin resistance and type 2 diabetes.
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Fas (CD95) expression in myeloid cells promotes obesity-induced muscle insulin resistance
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Background and aims: Low grade inflammation in adipose tissue and liver has been implicated in obesity-associated insulin resistance and type 2 diabetes. Yet, the contribution of inflammatory cells to the pathogenesis of skeletal muscle insulin resistance remains elusive. The aim of this study was to investigate the contribution of Fas expression in myeloid cells to metabolic dysregulation in obesity.
Materials and methods: Glucose metabolism was determined in standard chow, high fat diet (HFD)-fed or LPS injected control and myeloid-cell specific Fas depleted mice on a C57Bl6/J or ob/ob background. In vitro, co-culture experiments with conditioned media from Fas-depleted RAW cells and L6 muscle cells, 3T3-L1 adipocytes or HepG2 liver cells were performed. In addition, monocytic Fas expression was analyzed in a large cohort of obese human individuals and correlated with insulin sensitivity and other metabolic parameters.
Results: Diet-induced obesity and LPS treatment increased Fas protein levels in myeloid cells. Consequently, myeloid specific Fas depletion (FasΔmye) prevented the development of glucose intolerance and skeletal muscle insulin resistance in high fat diet-fed (IS-GDR 34.3±3.2 mg/kg*min in control vs. 63.8±7.4 mg/kg*min in FasΔmye mice, $p<0.05$) in lipopolysaccharide (LPS)-treated C57Bl6/J mice (AUC ipGTT 1856±51 mmol*min in control vs. 1636±24 mmol*min in FasΔmye mice, $p<0.01$) as well as in ob/ob mice (IS-GDR -6.1±3.4 mg/kg*min in control vs. 3.0±1.3 mg/kg*min in FasΔmye mice, $p<0.05$). Mechanistically, Fas mediated LPS-induced TNFα production in myeloid cells promoting insulin resistance in skeletal muscle but not in adipose tissue and liver. Such muscle specific effect of myeloid-cell expressed Fas is confirmed in vitro. Conditioned medium from LPS-treated and Fas-depleted RAW cells (a mouse myeloid cell line) affected insulin sensitivity only in L6 muscle cells (insulin stimulated glucose uptake: 79.0±1.8% LPS-untreated (control) vs. 98.4±2.6% LPS-untreated (Fas-depleted), $p<0.01$) but not 3T3-L1 adipocytes or HepG2 liver cells. Importantly, in a large cohort of obese human individuals, monocytic Fas expression correlates with skeletal muscle insulin sensitivity ($r2=0.31$, $p<0.0001$) as well as serum LPS ($r2=0.2$, $p<0.0001$) and TNFα levels ($r2=0.06$, $p<0.0001$).
Conclusion: Our results reveal an important role for myeloid cell expressed Fas in mediating obesity-induced insulin resistance in skeletal muscle.
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Angiopoietin like protein 2 (Angptl2) induced adipose tissue macrophage activation causes impaired glucose tolerance
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Background and aims: Obesity causes chronic low-grade inflammation which may contribute to systemic metabolic disorders including insulin resistance, type 2 diabetes, atherosclerosis and ischemic heart disease. Angiopoietin-like protein 2 (Angptl2) is a novel pro-inflammatory cytokine mainly secreted from adipose tissues as one adipokine. Serum concentration of Angptl2 increases in patients with obesity, type 2 diabetes, and cardiovascular disease. Elevated serum Angptl2 levels positively associated with the development of type 2 diabetes in a general Japanese population. Deletion of Angptl2 gene reduced inflammation of adipose tissues and ameliorated systemic insulin resistance in mice, although, the underlying mechanism is still unknown. This study aimed to explore the molecular mechanisms of Angptl2 on insulin sensitivity and identify macrophage activation as the key factor for the effects of Angptl2 in diabetic animal model.
Materials and methods: Adenovirus mediated lacZ (Ad-LacZ) or human Angptl2 (Ad-hAngptl2) was delivered via tail vain in 12 weeks old diabetic db/db mice. Oral glucose tolerance test was performed at 14 weeks old. We measured serum lipid, insulin and adipokine levels. We also examined gene expression of pro-inflammatory cytokines, lipogenesis, and glyconeogenesis related genes. Adipose tissue macrophages were isolated from epididymal fat pad and characterized by FACS analysis. We also prepared recombinant human Angptl2 (rhAngptl2) protein and treated on human endothelial cells and THP-1 monocytes /macropages, examined pro-inflammatory gene expression by quantitative PCR and TNF-α production by ELISA.
Results: Body weight, epididymal WAT and liver weight, total cholesterol, and liver cholesterol between Ad-LacZ and Ad-hAngptl2 did not differ. Fasting glucose level, liver triglyceride level, and liver glyconeogenesis related gene expression levels were greater in Ad-hAngptl2 treated mice. Also, Ad-hAngptl2 treated mice significantly impaired glucose tolerance compared to Ad-LacZ treated mice. FACS analysis on adipose tissue macrophages revealed that Ad-hAngptl2 treated mice significantly increased adipose tissue macrophage ($F4/80^+CD11b^+$) cell. Furthermore, M1 macrophage ($F4/80^+CD11b^+CD11c^+$) cells fraction significantly increased. We also investigated that rhAngptl2 protein treatment induced pro-inflammatory gene expression in human endothelial cells and THP-1 monocytes /macropages. Furthermore, rhAngptl2 protein treatment significantly increased humanTNF-α production in the conditioned media of PMA-treated THP-1 macrophages.
Conclusions: Ad-hAngptl2 treatment in db/db mice impaired glucose tolerance and increased not only adipose tissue macrophages but also pro-inflammatory M1 macrophages in adipose tissues. Angptl2 may play an important role in the development of adipose tissue inflammation and glucose intolerance by pro-inflammatory effect in peripheral tissues.

OP 21 Health economic aspects of diabetes: happy, healthy and prosperous

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Impact of targeting intensive versus conventional glycaemic control on quality of life in patients with type 2 diabetes: a systematic review

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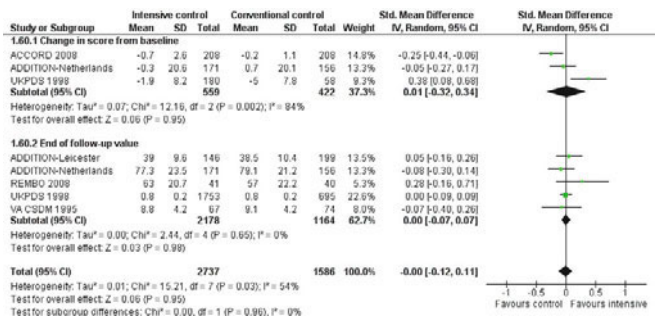
Background and aims: Targeting intensive glycaemic control in patients with type 2 diabetes (T2D) demands extra efforts for the patients and increases the risk of hypoglycaemia. Possible benefits remain controversial. The present systematic review assesses the effects of targeting intensive versus conventional glycaemic control on physical and mental quality of life in patients with T2D.
Materials and methods: Randomised clinical trials (RCTs) were identified through searches of The Cochrane Library, MEDLINE, EMBASE, Science Citation Index Expanded, LILACS, and CINAHL. We included RCTs with a prespecified difference in glycaemic targets between intervention groups in adult patients with T2D. Two authors independently extracted data and additional information was obtained from authors of the included trials. We meta-analysed quality of life in two components: physical and mental quality of life. Meta-analyses were conducted applying the random-effects model. Due to the use of different scales in the included trials, meta-analyses were performed with standardised mean difference (SMD) and 95% confidence intervals (CI).
Results: Twenty-five RCTs including a total of 33467 patients, whereof ten trials reported health-related quality of life or well-being. The included trials were heterogeneous in design, inclusion criteria, and scales used to assess quality of life. Four of the trials reported the quality of life data in a way so they could not be applied in a meta-analysis. Six of the trials reported six different quality of life scales and evaluated quality of life in a total of 3996 patients. Three of the trials reported the 36-Item Short Form Health Survey scale or a modified version. The physical component of quality of life did not show any statistical significance between the interventions when meta-analysed with change from baseline (SMD 0.01, 95% CI -0.32 to 0.34) or with the end of follow-up values (SMD 0.00, 95% CI -0.07 to 0.07; Fig 1). The mental component of quality of life showed no statistical significance between targeting intensive versus conventional glycaemic control when meta-analysed as change from baseline (SMD 0.12, 95% CI -0.10 to 0.35) or at the end of follow-up (SMD -0.09, 95% CI -0.19 to 0.01).
Conclusion: Targeting intensive glycaemic control does not seem to influence physical or mental quality of life. Uniform reporting of quality of life in patients with T2D is recommended in future RCTs.



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Long term consequences on annual earnings after childhood onset of type 1 diabetes

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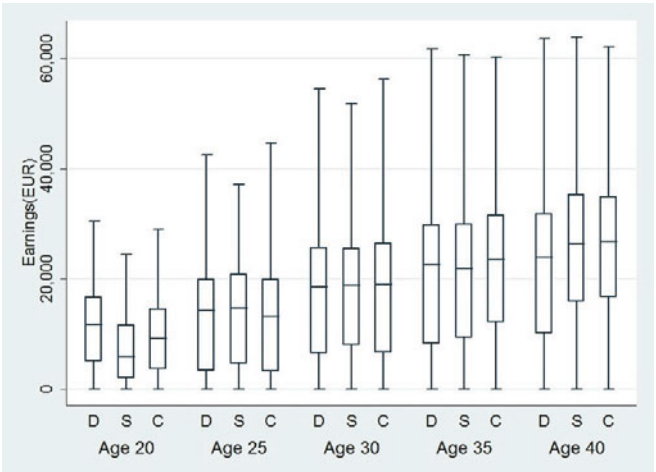
Background and aims: Results from the Swedish Childhood Diabetes Register Study group have shown that onset of type 1 diabetes during childhood (ages 0-14) was associated with lower grades from compulsory and upper secondary education. Also, a detrimental effect on annual earnings has been found for people with onset of type 1 diabetes in young adulthood (age 15-34). Socioeconomic consequences of disease may vary or even accumulate over time. This study investigates long term consequences on earnings of childhood onset (ages 6-15) using two control groups 1) siblings; and 2) population controls matched for year of birth and municipality of residence.
Materials and methods: Covering the period 1990-2010, annual education and earnings data from national population registers was added to individuals from the Swedish Childhood Diabetes Register. We retrieved data on 1,381 individuals, born in 1962-1971 and diagnosed with type 1 diabetes in ages 6-15, together with data on 1,205 siblings to the individuals with diabetes, and 5,554 general population controls matched by year of birth and residence at the time of diagnosis. The development of annual earnings was analysed using Mann-Whitney tests at 5-year intervals from young adulthood into middle age (20-40 years) and using fixed-effects panel data methods. People with type 1 diabetes were compared to both siblings (with same socioeconomic status but different year of birth) and general population controls (with same year of birth but any socioeconomic status). All analyses were based on year 2007 standardised earnings calculated using the consumer price index from Statistics Sweden.
Results: The development of median earnings in ages 20-40 showed a lag in earnings for the diabetes group compared to siblings and population controls (Figure 1). Comparing median earnings at certain ages, the diabetes group had higher earnings than the population controls at age 20 ($p<0.001$) but lower from age 35 and onwards ($p<0.001$). Compared to siblings, the diabetes group had higher earnings at age 20 ($p<0.001$) but lower at age 40 ($p<0.001$). At age 40, the median annual earnings in the diabetes group was EUR 2,402 and EUR 2,860 lower than for siblings and population controls. Controlling for diabetes duration, education, demographics, and fixed effects, crude regression results confirmed an increasingly negative impact on earnings for people with diabetes compared to both siblings and population controls.
Conclusion: The significant effect of type 1 diabetes on earnings was in line with previous associations found between diabetes and earnings. The seemingly accumulative effect was seen irrespective of choice of comparator group and appeared to increase by age and duration of diabetes. Figure 1: Median annual earnings at 20, 25, 30, 35, and 40 years of age for people with type 1 diabetes onset in ages 6-15 years (D) compared to siblings (S) and population age-matched controls (C).



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Health-related quality of life in adults with and without type 2 diabetes: results of a pooled analysis of German population-based cohort studies

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Background and aims: Population-based data concerning health related quality of life (HRQL) in type 2 diabetes (T2DM) are essential for health economic decision models to estimate disease burden. Results presented here analyse longitudinal individual-level data from three population-based cohort studies in Germany (KORA, SHIP, CARLA). By comparing persons with prevalent T2DM to persons with normal glucose metabolism as well as incident T2DM cases, we evaluated change in HRQL over a period of up to 14 years.
Materials and methods: This study is part of the DIAB-CORE (Economic and Health Care Research in Type 2 Diabetes) research project, drawing on a data pool of population-based studies in Germany. T2DM is defined based on self-report of physician-diagnosed diabetes or self-reported intake of oral anti-diabetic agents. HRQL was assessed by the German version of the SF12/36 (version 1). Data were pooled and SF-12 composite scores for physical and mental health (PCS, MCS) were calculated. Linear regression modeling was used to describe differences in absolute change in PCS/MCS scores per observation year between persons with prevalent T2DM, incident T2DM cases and non-diabetics, adjusted for age, sex and study region.
Results: A total of 3,739 persons aged 45-74 years had completed the SF-12 at baseline and at follow-up. Of those, 6.9% reported T2DM at baseline and 14.1 % at follow-up. Mean follow-up time was 7.3 years. The decline in HRQL was more pronounced in older age. Furthermore, the results showed a more pronounced decrease of 0.35 points per year (95% CI: 0.54; 0.16) on the PCS scale for the prevalent T2DM cases and of 0.18 points (95% CI: 0.37; 0.01) for incident cases compared to persons without T2DM. On the MCS scale, the prevalent T2DM cases showed an additional loss of 0.45 points per year (95% CI: 0.67; 0.23) against persons without T2DM and of 0.39 points (95% CI: 0.69; 0.10) against incident T2DM cases.
Conclusion: Our results provide evidence of the impact of T2DM on HRQL over 7 years on average in a large population-based sample. Using longitudinal data, we can show that the gap in HRQL between persons with and without T2DM which has been reported previously in cross-sectional analysis becomes even more apparent. Population-based surveys and longitudinal cohorts provide important data to estimate the incremental burden of diabetes.

Impact of T2DM on change in HRQL

SF-12 composite scores	total n=3739	Least Square Means* Change per year	Standard Error	95% CI lower	95% CI upper
PCS					
Diabetes	258	-0.57	0.09	-0.75	-0.39
Incident Diabetes	269	-0.40	0.09	-0.58	-0.22
No Diabetes	3212	-0.22	0.03	-0.27	-0.17
MCS					
Diabetes	258	-0.50	0.11	-0.71	-0.29
Incident Diabetes	269	-0.11	0.11	-0.31	0.10
No Diabetes	3212	-0.05	0.03	-0.11	0.01

* adjusted for age, sex and study region

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Diabetes care from the patient's perspective: combining risk factors with patient reported outcomes measures and capabilities in the national diabetes registry in Sweden

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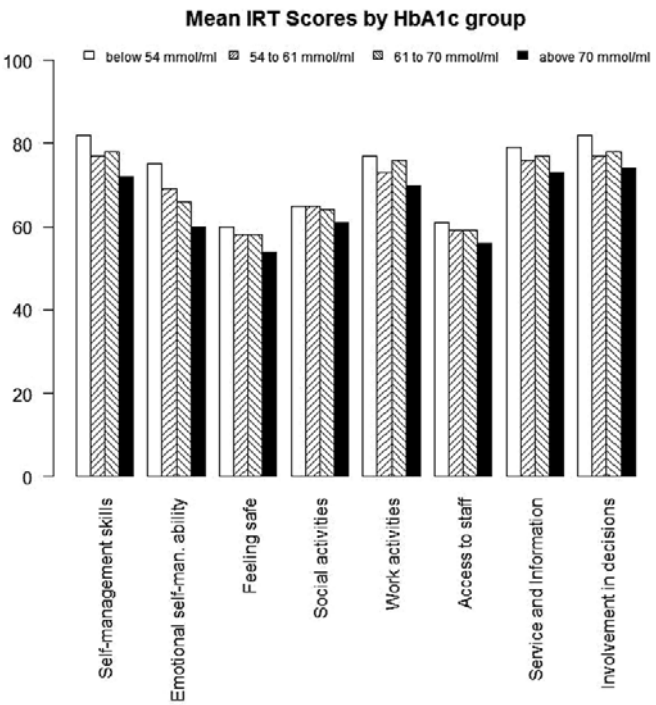
Background and aims: Diabetes demands patients to be engaged in their disease and its treatment. However, the patient perspective is often missing when Diabetes care is evaluated. To better evaluate Diabetes care, we have taken on the patient perspective inspired by the Capability Approach. A person's capability is the set of things possible for the person to achieve. Functioning is what is actually achieved. HbA1c, BP and LDL are risk factors for diabetes related comorbidities, that we let represent the perspective of healthcare. We believe it is important to develop Diabetes care according to both perspectives. Our aim was to measure patient capabilities and functionings, and judgment of quality of provided care, and use these together with risk factors to evaluate the patient's health. Furthermore, to identify any differences between the perspectives of the patient, and healthcare.
Material and methods: We used patient reported outcomes measures (PROM) collected by a questionnaire developed by NDR, and Item Response Theory (IRT) to estimate values (IRT Scores) on capabilities, functionings and judgments. Risk factors were extracted and deterministically linked with the IRT Scores on the individual patient level. The analysis included 1 124 type 1, and 1 792 type 2 patients. Another 1 656 type 1 and 1 431 type 2 patients were used for validation.
Results: We obtained measures of capabilities and functionings: Self-management skills, Emotional self-management ability, Feeling safe, Ability to carry out Social and Work activities, and judgments: Access to staff, Service and information, and Involvement in decisions. For each of those, an IRT scale was successfully developed, validated, and used for estimating IRT Scores. The questionnaire was able to detect patients with poor health-related quality of life (HRQOL) according to EQ-5D. They reported significantly lower IRT scores than the examined population overall. In Type 1 and 2 Diabetes, increased HbA1c was associated with lower IRT scores in all dimensions. No such patterns were seen for BP or LDL. Neither patients with poor BP control, nor with poor LDL control, reported significantly lower IRT Scores in any dimensions. Patients with poor HbA1c control reported significantly lower Self-management skills. The risk factors could not be used to detect poor HRQOL according to EQ-5D.



Conclusion: We found that the patient perspective, i.e. capabilities, functionings, and judgments, differs from the perspective of healthcare, i.e. risk factors. Knowledge of both types of data is required in order to optimize the patient's HRQOL and to avoid diabetes related comorbidities.
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Factors associated with self-rated health quality in patients with diabetes mellitus

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Background and aims: Self-rated health (SRH) is a powerful predictor for a number of important health outcomes, such as mortality, morbidity or utilisation of healthcare services. The tool has good psychometric properties, monitors broad spectrum of factors, reflects overall feeling of health in people, and could be easily obtained. Health-related quality-of-life and self-rated health may also provide additional information on patient risk independent of clinical and biochemical risk factors for invalidity and mortality. The aim of this study was to investigate differences in determinants of self-rated health between patients with type 1 and 2 diabetes mellitus (T1DM/T2DM).
Materials and methods: The study involved of 188 patients; 43 patients with T1DM (mean age 45,2 years, mean duration of diabetes 17,1 years) and 145 patients with T2DM (mean age 59,2 years, mean duration of diabetes 11,7 years). The analysis was based on self-rated health questionnaire Short Form 36 Health Subject Questionnaire, (SF-36), self-esteem was measured using the Rosenberg's Self-Esteem Scale (RSES) and overall life satisfaction was measured by the Cantrill's ladder of life. The study was approved by the Ethical Committee of the University.
Results: In the group of patients with T1DM we found a significant positive association between self-rated health and age ($r = 0,62$, $p < 0,05$). Negative associations were found between SRH and two clinical variables (duration of diabetes, $r = -0,45$, $p < 0.05$ and the number of microvascular complications $r = -0,54$, $p < 0,05$). Low SRH was also associated with the presence of hypertension and coronary heart disease. The self-rated health was significantly positively associated with psychological variables (satisfaction with treatment; $r = 0,57$, $p < 0,05$ and Cantrill´s ladder; $r = 0,74$, $p < 0,05$), too. We did not find any association between SRH and the frequency of hypoglycaemic episodes. In the group of T2DM patients we found weaker but significant negative associations between self-rated health and the duration of diabetes ($r = -0,22$, $p < 0,05$), BMI ($r = -0,20$, $p < 0,05$) and the number of complications ($r = -0,40$, $p < 0,05$). Low SRH was significantly associated with the presence of hypertension ($r = 0,23$, $p < 0,05$). Patients on insulin revealed lower SRH as compared with those on diet or oral drugs. The associations with Rosenberg Self-Esteem Scale and Cantrill´s ladder were similar as in T1DM ($r = 0,45$, $p < 0,05$ and $r = 0,44$, $p < 0,05$ respectively) Regular control of body weight was also associated with better SHR ($r = 0,17$, $p < 0,05$).
Conclusion: The study revealed the importance of subjective attitude of patients to their disease and a close interrelationship between clinical symptoms of illness and its psychological aspects. One should also bear in mind that T1DM and T2DM are different diseases from the point of view of their pathogenesis and early manifestation. However later in the course of disease the influence of complications on their quality of life is similar. From the aspect of everyday diabetes care the subjective dimension of this chronic disease is as important as the clinical symptoms and the results of laboratory investigations

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Psychometric properties of the hypoglycaemia perspectives questionnaire (HPQ) in type 2 diabetes mellitus

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Background and aims: The Hypoglycemia Perspectives Questionnaire (HPQ) was developed with clinician and patient input to assess symptoms, behaviors, and impact of hypoglycemia on diabetic patients.
Materials and methods: The HPQ was administered to adult patients with type 2 diabetes mellitus (T2DM) on antidiabetic treatment as part of a cross-sectional, epidemiological study evaluating hypoglycemia and health-related quality of life (HRQoL) in Cyprus. Demographic and clinical data were collected. Patients also completed the Audit of Diabetes Dependent Quality of Life (ADDQoL-19), treatment satisfaction questionnaire, and EuroQol-5 Dimensions (EQ-5D). The original HPQ consisted of 45 items rating current status or behavior related to hypoglycemia on an 11-point numeric rating scale (NRS) and 7 additional descriptive hypoglycemia event frequency items. Analyses included examination of HPQ item performance, item reduction, and factor structure. Measurement properties (reliability, construct validity, known-groups validity) of the final HPQ were evaluated.
Results: 500 T2DM patients completed the HPQ with a mean age of 61±10 years; 32.6% women. Based on item evaluation, the original HPQ item pool was reduced to 22 items. Exploratory and confirmatory factor analysis identified 21 items contributing to 3 hypoglycemia domains (Symptoms [8 items], Compensatory Behaviors [7 items], Worry [6 items]) and a single-item of global symptom awareness. HPQ domains had high internal consistency reliability (Cronbach's alpha=0.78-0.92). Construct validity was demonstrated by significant correlations between HPQ scores with HRQoL, treatment satisfaction, and health status. HPQ also demonstrated ability to discriminate between known groups. Compensatory behaviors and symptom awareness were higher for patients with a recent low blood sugar event ($p<0.001$) and high symptom awareness corresponded to less concern about experiencing symptoms of low blood sugar and worry ($p<0.05$).
Conclusion: These results provide preliminary evidence that HPQ is reliable and valid for assessing the experience and impact of hypoglycemia on T2DM patients.
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OP 22 Drilling down on lipid mechanisms in diabetes

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Intensified LDL-c target of statin therapy and risk of incident diabetes: meta-analysis of randomised statin trials

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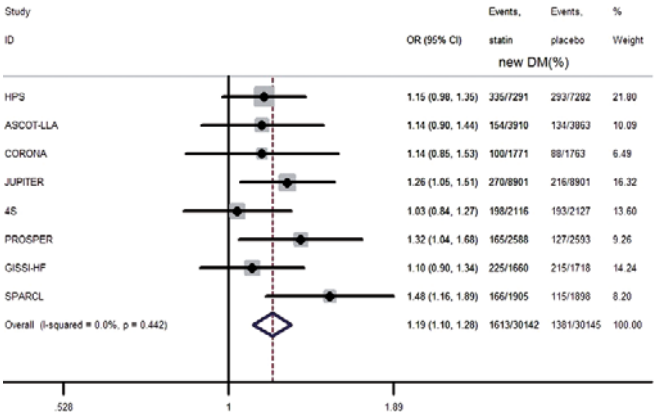
Background and aims: Intensified low-density lipoprotein cholesterol (LDL-c) goal may provide greater cardiovascular benefits. However, findings suggest that statin therapy increase the development of new-onset diabetes. We aimed to identify whether relationship exists between intensified LDL-c target goal of statin use and development of diabetes by doing a meta-analysis of large randomized controlled trials.
Materials and methods: We searched Pubmed, Embase and the Cochrane Central Register of Controlled Trials, no time limited, for randomized controlled endpoint trials of statins. We included only trials which recruited at least 1000 participants with scheduled treatment duration of at least 2 years. We excluded trials don't meet intensified target goal level of LDL-c <2.59mmol/L (100 mg/dl), or relative LDL-c reduction at least 30% of baseline in statin group. We used the I^2 statistic to measure heterogeneity between trials and calculated an overall OR with a fixed-effects model meta-analysis to identify potential effects of intensified LDL-c target of statin therapy on incident diabetes.
Results: Among 60287 participants, 2994 patients (1613 assigned to statins and 1381 assigned to placebo) developed diabetes during a mean of 4 years. Intensified LDL-c target of statin therapy was associated with an 18% increased risk for diabetes (odds ratio [OR] 1.19; 95% CI 1.10-1.28), with little heterogeneity (I^2=0.0%) between trials. There was one additional case of diabetes per 130 patients taking statin therapy for 4 years. Fail-safe number was 53(P=0.05), funnel plot was basically symmetrical and all scattered inside 95%CI (p=0.557 in egger test) in this meta-analysis.
Conclusion: Intensified compared to general LDL-c target of statin therapy increases risk of incident diabetes. Although intensified LDL-c target practice in patients with cardiovascular risk or existing cardiovascular disease should not change, blood glucose monitoring is suggested to watch out for incident diabetes.



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Effect of pitavastatin on the incidence of diabetes in Japanese individuals with impaired glucose tolerance

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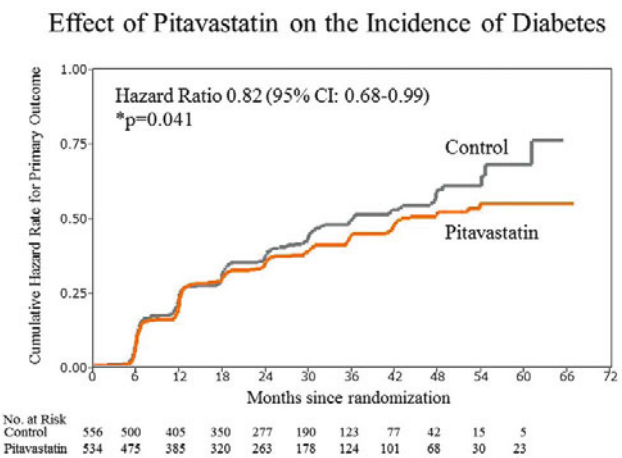
Background and aims: Although statin therapy is known to reduce cardiovascular risk, trial data and meta-analyses suggest that statins may also increase the risk of development of diabetes. However, in those trials, data were analyzed retrospectively, and the diagnostic criteria of diabetes differed. Accordingly, we performed the Japan Prevention Trial of Diabetes by Pitavastatin in Patients with Impaired Glucose Tolerance (J-PREDICT) study, to evaluate the effect of pitavastatin on the incidence of diabetes using a prospective study design.
Materials and methods: In a multicenter, open-label, randomized controlled trial, 1,269 individuals with impaired glucose tolerance (IGT) were randomized to either the pitavastatin group (lifestyle modification and pitavastatin [1-2 mg/day]) or the control group (lifestyle modification only). Every six months, a 75-gram oral glucose tolerance test was performed with other standard laboratory tests. The primary outcome was the incidence of diabetes mellitus, as determined by a 2-h plasma glucose ≥200 mg/dl or a fasting plasma glucose ≥126 mg/dl measured at least once.
Results: The diabetes incidence rates for the pitavastatin and control groups were 163 and 186 cases per 1,000 person-years, respectively; the hazard ratio for progression from IGT to diabetes in the pitavastatin group was 0.82 (95% CI: 0.68-0.99; P = 0.041). Analyses of patients stratified by hypertension status suggested heterogeneity in the treatment effect, with a benefit for patients without hypertension and no benefit or hazard in patients with hypertension (P = 0.01 for interaction). Even in any subgroups, pitavastatin did not accelerate the incidence, unlike the effects of statins in previous reports.
Conclusion: Pitavastatin in combination with lifestyle modification was associated with a lower incidence of diabetes than was lifestyle modification alone in Japanese patients with IGT. Statins are now used with the understanding that a slightly increased risk of diabetes is outweighed by cardiovascular benefits of the drugs. However, based on our results, it may be necessary to reconsider whether all statins really increase the risk of developing diabetes.



*P value was calculated using a log-rank test that was stratified according to the 5 assignment factors (sex, age, Body mass index, 2-h plasma glucose, and presence of hypertension).

Clinical Trial Registration Number: Gov.Number, NCT00301392
Supported by: The Waksman Foundation of Japan Inc./ Kowa Pharmaceutical Co. Ltd.

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Serum lipidomic profiling identifies biomarkers associated with progression to type 2 diabetes in the METSIM study

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Background and aims: Traditional serum lipid markers are of limited clinical utility as predictors of progression to type 2 diabetes (T2D). Better tools are needed for early detection of T2D in order to prevent the disease and its co-morbidities. Here we applied global lipidomics in a prospective study setting, with the objective of identifying novel predictive markers of progression to T2D

Materials and methods: Age-matched cases (N=110; mean age ± SE = 59.21 ± 0.55) and controls (N=220; mean age ± SE = 59.92 ± 0.36) were selected from the METSIM (Metabolic Syndrome in Men) observational study. All subjects were male and non-diabetic by both OGTT and HbA1c criteria at baseline. Case subjects were diagnosed with T2D within the 5 year follow-up period and displayed this glycemic profile at baseline: 45% IFG, 7% IGT, and 38% combined IFG/IGT. Control subjects displayed normal fasting glucose, normal glucose tolerance, and HbA1c <6.5% both at baseline and at the 5-year follow-up. Lipidomic analyses of plasma samples at baseline and follow-up were performed using ultra performance liquid chromatography coupled with time-of-flight mass spectrometry

Results: Cases and controls had very distinct lipidomic profiles at baseline as well as at follow-up. Univariate association of each lipid with T2D as assessed from logistic regression analysis after adjusting for age, BMI, blood pressure, HDL-C and LDL-C revealed that 196 out of 337 molecular lipids were significantly different between cases and controls at baseline (FDR q<0.05). Interestingly, the distinct pre-diabetic lipidomic profile remained similar, although more pronounced, after the diagnosis of progression to T2D. Progression to T2D was associated with increased triglycerides, particularly those with lower double bond and carbon content, and specific diacyl phospholipids, as well as with decreased lysophosphatidylcholine (with the most significant being LPC (18:2)). In order to develop predictive model of progression to T2D based on baseline lipidomics as well as other available clinical data, we applied stepwise multivariate logistic regression. The lipid-based model predicted T2D with AUC = 0.86, 95% CI = (0.80, 0.92), which was better than FINnish Diabetes RIsk SCore (FINDRISC) alone (AUC = 0.81, 95% CI = (0.73, 0.86)). Combining the lipid-based model with the FINDRISC significantly improved the predictive model as compared to FINDRISC alone (AUC = 0.88, 95% CI = (0.82, 0.94)).

Conclusion: We have identified molecular lipids which are associated with progression to T2D. Elevation of triglycerides with lower double bond and carbon content replicates earlier findings and highlights the potential role of fatty liver in pre-diabetes. A candidate biomarker panel has been developed which predicts T2D.

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Pleiotropic effects on lipid levels and obesity identified in multi-trait meta-analysis of genome-wide association studies (GWAS) of type 2 diabetes related traits

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Serum lipid levels, fat storage and obesity are related to T2D risk through shared biochemical pathways and can be influenced by common genetic factors. Analysis of the genetic effects on multiple phenotypes simultaneously allows dissection of multi-trait associations. Within the ENGAGE consortium, we assessed multi-trait genetic effects on four blood lipids (high-/low-density lipoprotein and total cholesterol, triglycerides [HDL/LDL/TC/TG]) and body-mass index (BMI). The 1000 Genomes reference panel (06/2011) was used for imputation in up to 41,752 individuals from 18 European GWAS. Each study carried out multi-trait analysis by fitting a multiple logistic regression on SNP genotypes allowing for joint effects of four lipid traits and BMI and combining evidence across study-specific likelihoods. Single-trait meta-analyses, conditional on remaining traits, were used to verify the independence of trait-specific genetic effects. Joint analysis enabled identification of 26 signals with genome-wide significant (P_{LRT}<5.0x10^{-8}) multi-trait effects. At 9 loci associations were driven by the individual trait effects: a) *TRIB1* on BMI, b) *GCKR*, *FADS1*, *MLXIPL* on TG; c) *CEPT* on BMI/HDL, d) *LPL, APOA1* on BMI/TG; e) *LIPC* on HDL/TG, f) *APOE* on HDL/LDL/TG. At *TRIB1*, *CEPT*, *LPL*, *APOA1* where association with obesity was identified for the first time, higher BMI was related to higher HDL and lower TG indicating complex relationships between obesity and regulation of lipid levels. Effects on lipids at *CELSR2, ABCA1*, *HNF4A*, *MADD*, *PPP1R3B* and *RANB10* were determined by the association with HDL. At the remaining 11 loci, multiple traits contributed to the signal. We detected a substantial proportion of T2D-related metabolic trait loci with complex patterns of genetic effects, some of which may not follow epidemiological correlations.

Supported by: ENGAGE

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Large-scale genome-wide association meta-analysis of the 1000 genomes project imputed data identifies novel susceptibility loci for glycaemic and obesity traits

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Background and aims: Genome-wide association studies (GWAS) have detected numerous associations with quantitative glycemic and obesity-related traits: only ~10-12% and 3.2-4.4% of phenotypic variance, respectively, been explained mainly by common variants (minor allele frequency, MAF >5%). To extend detection of novel association signals, especially to variants of lower frequency, we performed fixed-effects meta-analysis of GWAS data

following imputation using the 1000 Genomes Project reference panel (June 2011 release).
Materials and methods: These analyses included up to 12.7 million SNPs and were performed in up to 18 European GWAS for the following traits: fasting glucose (FG, n=46,694), fasting insulin (FI, n=24,245), body-mass-index (BMI, n=87,048) and waist-to-hip ratio adjusted for BMI (WHR, n=54,572). Both sex-combined and sex-differentiated analyses were conducted for each trait. To find independent signals at a given locus, we used approximate conditional analyses methods applied to summary-level association data.
Results: We identified novel loci reaching genome-wide significance for FG (1 locus near RMST, $p=2.0x10^{-10}$, MAF=0.10) and BMI (5 loci near GALNT10, DTX2P1, GRID1, EPYC and AKAP6; $p<4.5x10^{-8}$, MAF=0.07~0.49). We additionally detected a novel, low-frequency, female-specific FG signal near EMID2 (rs6947345, MAF=0.02, female β=0.16, $p=3.8x10^{-8}$, male β=-0.02, p=0.50, sex-differentiated $p=2.1x10^{-7}$, sex-heterogeneity $p=3.7x10^{-5}$). Through approximate conditional analysis within known GWAS loci, we identified a novel low-frequency FG-associated variant independent of the lead SNP at G6PC2 (rs150171632, MAF=0.012, β=0.18, $p=3.0x10^{-19}$, conditional $p=1.7x10^{-21}$). At the known GWAS signal for WHR near RSPO3, we detected a novel independent lead signal with stronger association and larger effect size than the previously reported signal (rs72959041, MAF=0.08, β=0.11, $p=1.7x10^{-13}$, conditional $p=9.8x10^{-11}$ vs. previous lead rs9491696, MAF=0.49, β=0.04, $p=2.1x10^{-11}$, conditional $p=1.2x10^{-8}$).
Conclusion: Our results highlight the potential advantages of imputation using the high-density reference panels for the identification of novel associated signals for both common and low frequency variants for quantitative metabolic traits.
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DNA methylation changes influence type 2 diabetes
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Background and aims: Type 2 diabetes (T2D) is a global epidemic, becoming increasingly prevalent due to a rising incidence of obesity caused primarily by poor diet and lack of physical activity. The incidence of T2D is particularly high in Mexican American individuals and although disease development is well known to be genetically regulated, implicated loci explain only a small portion of the genetic liability. Epigenetic regulation, such as DNA methylation, is a novel mechanism that may lead to gene dysfunction and disease development. However, to date, no studies have investigated genome-wide DNA methylation changes associated with diabetes in a large population.
Materials and methods: Using Illumina HumanMethylation450 BeadChips, we performed genome-wide DNA methylation profiling of >450,000 CpG sites in peripheral blood cells from 950 Mexican American individuals from ~40 large pedigrees. For each individual, we have an array of phenotypic data relating to T2D (disease status, medication usage, fasting glucose, fasting insulin), obesity, dyslipidemia and cardiovascular disease. DNA methylation data was normalized using inbuilt controls on the arrays, BMIQ normalization to reduce technical variance and inverse normalization to correct for residual deviation from normality. Using SOLAR, we tested for heritability of each CpG site, and for association with T2D status and fasting glucose (in non-diabetic samples only). Age, age2, sex, age x sex interactions, BeadChip number and BeadChip position were incorporated as covariates. We used the Bonferroni method to correct for multiple comparisons.
Results: Of the 471,142 CpG sites that were assessed for heritability, we identified 111,600 statistically significant associations (24%; p<1.06x10-7). Of the 482,421 CpG sites that were assessed for association with T2D, we identified 12 statistically significant associations (p<1.04x10-7). The top association was for a CpG site in the *TXNIP* gene (beta=-0.51, p=5.57x10-18). Txnip is known to modulate glucose metabolism and insulin sensitivity, likely playing a role in T2D and possibly contributing to the therapeutic action of metformin. We also identified a significant association between diabetes and *ABCG1* (beta=0.39, p=5.45x10-12), which is involved in reverse cholesterol transport. Mouse knockout studies implicate ABCG1 in insulin secretion and glucose tolerance. Significant associations between DNA methylation and T2D status were also seen in other genes previously implicated in diabetes, including *CPT1A* (beta=-0.35, p=5.92x10-10) and *DHCR24* (beta=-0.33, p=7.66x10-9), as well as in potentially novel genes. Most of these genes were also at least nominally significantly associated with fasting glucose in the non-diabetic portion of our dataset (n=745). In a subset of the population (n=250) we have DNA methylation profiles and phenotypic data available at two time points, approximately 5 years apart. We are currently analyzing this data to determine whether DNA methylation changes over time influence fasting glucose levels and the evolution of diabetes.
Conclusion: Our study has identified a number of genes that show DNA methylation changes associated with T2D in a Mexican American population. We are currently conducting pyrosequencing studies to validate these findings. We show here that DNA methylation profiling is a powerful means to identify genes involved in T2D, which may ultimately aid in the development of targeted therapeutics for diabetic treatment.
Supported by: DK087749, HL045522, MH059490

OP 23 T cells and autoantigens

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MicroRNA profiling of impaired CD4+ T regulatory cells residing in the pancreatic draining lymphnodes of patients with type 1 diabetes

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Background and aims: Pancreatic draining lymphnodes (PLN) and peripheral blood (PB) of patients with type 1 diabetes (T1D) undergoing pancreas transplantation and of non-diabetic individuals were collected. We previously showed that PLN of patients with T1D have CD4+CD25bright T cells epigenetically imprinted to be regulatory (Treg cells) but that, for still unknown reasons, do not function as such in vitro. Importantly, this functional defect is present only in Treg cells residing in the PLN of T1D patients and not in those circulating in their PB. MicroRNAs (miRNAs) are an abundant class of small non-coding RNAs that regulate gene expression by affecting the degradation and translation of target mRNAs. When poorly regulated, miRNAs are critically involved in a range of human diseases. We hypothesize that there might be post-transcriptional regulations in Treg cells and these regulations may affect Treg cells when residing in the PLN but not when circulating in the periphery of subjects with T1D.
Materials and methods: CD4+-CD25++-CD127-Treg cells were sorted from PLN and PB of 4 patients with T1D and from PLN and PB of 3 and 8 non-diabetic donors, respectively. From each sample, 200 sorted cells were analyzed and miRNA expression profiles were performed by RT-PCR using Taqman miRNAs array card A comprising 380 unique miRNAs. Data analysis was performed using 2-ddCt method. Graphical analysis and bioinformatic miRNAs target prediction were performed using Spotfire 5.0 software and Targetscan 6.2, respectively.
Results: Specific miRNAs differentially expressed between Treg cells residing in the PLN of T1D patients and those in their PB or in PLN of non-diabetic donors were identified. Interestingly, we found 4 microRNAs with a particular expression pattern. Namely, miR-125a-5p, miR-642, and miR-155 were increased in Treg cells isolated from PLN as compared to those isolated from PB of T1D patients (p=0.017, p=0.0005, p=0.0007- paired t-test); this differential expression was not identified in Treg cells isolated from non-diabetic donors uncovering a disease-specific miRNA expression pattern. In addition, miR-146a was increased in Treg cells isolated from PLN as compared to those isolated from PB, both in T1D patients and in non-diabetic donors suggesting a tissue (i.e., PLN) specific miRNA expression pattern irrespective of the disease status. Interestingly, FOXP3 was identified as a predicted target of miR-125a-5p by Targetscan 6.2
Conclusion: Disease- and tissue-specific miRNA signatures of Treg cells isolated from PLN and PB of patients with T1D and of non-diabetic donors were for the first time delineated. These patterns may explain functional defects of Treg cells in patients with T1D in controlling autoreactive immune responses. Specifically, miR-125a-5p by targeting FOXP3, may lead to reduced FOXP3 expression and induce dysfunction in Treg cells residing in the PLN. Studies are ongoing to confirm this hypothesis also in a bigger cohort of donors collected from the nPOD network and to assess the miRNAs' role in the functional control of Treg cells.
Supported by: JDRFi nPOD Grant

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A novel approach for immunotargeting of islet reactive CD8 T cells to prevent diabetes

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Background and aims: CD8 T cells play an important role in the pathogenesis of Type 1 diabetes in both humans and the Non Obese Diabetic (NOD) mouse. We have developed a new approach for selectively targeting the CD8 T cells that recognize peptides of insulin and IGRP. This utilizes beta2-microglobulin linked to a selected MHC class I binding peptide and the intracellular activation domain of CD3-zeta. When a T cell expresses this construct, combining with endogenous MHC class I, it redirects the T cell against CD8 T cells recognizing the specific MHC class I/peptide complex. We have previously shown, by a transgenic approach, that targeting insulin reactive cells inhibits CD8 T cell attack on islet beta cells and thereby prevents the onset of T1D. Our current strategy uses mRNA transfection to express an islet autoantigenic peptide construct that reprograms polyclonal CD8 T cells to target and kill islet autoantigen-specific CD8 T cells.
Materials and methods: mRNA constructs were generated, consisting of peptide-beta2microglobulin-CD3zeta. A construct encoding EGFP was used as a control. Peptides used included insulin B chain15-23 and IGRP206-214. We tested the functional expression of the constructs using a hybridoma B3Z to stimulate an insulin B15-23 reactive T cell hybridoma containing NFAT-lacZ. Specific antigenic stimulation of the insulin-reactive hybridoma was detected by a colorimetric change. We also electroporated the peptide constructs to redirect polyclonal CD8 T cells from NOD mice which were tested for their ability to kill the insulin-reactive T cell hybridoma as well as G9 insulin reactive CD8 T cells or 8.3 IGRP reactive CD8 T cells in vitro. To test for longevity of expression of mRNA constructs in vivo, CD8 T cells transfected with the EGFP mRNA construct were i.v. injected into young NOD mice and cells expressing EGFP were tested for at various times after injection in the spleen and pancreatic lymph nodes (PLN). Finally, we electroporated polyclonal CD8 T cells from NOD mice with insulin and IGRP constructs and i.v. injected these redirected CD8 T cells into young NOD mice to test for inhibition of diabetes.
Results: The hybridoma B3Z when transfected with the insulin peptide construct and K^d stimulated the insulin-reactive hybridoma cells, inducing a color change indicating that expression of the construct induced recognition by the insulin-reactive hybridoma. We then showed that redirected polyclonal CD8 T cells were able to kill the insulin-reactive hybridoma (35% specific lysis at 10:1 E:T ratio), G9 TCR transgenic CD8 T cells that recognize the insulin B15-23 peptide and 8.3 TCR transgenic CD8 T cells that recognize the IGRP peptide (both 10% specific lysis at 10:1 E:T ratio), in vitro. We next demonstrated that redirected polyclonal T cells, expressing the EGFP construct, i.v. injected into young NOD mice were detected in the spleen and PLN at least 7 days after injection (2.4% of CD8 T cells). Finally, mRNA transfected cells were i.v. injected into young NOD female mice to assess whether this strategy can be used to prevent autoimmune diabetes in NOD mice and this experiment is currently ongoing.
Conclusion: Our experiments show that, in principle, mRNA transfection can be used to induce expression of constructs that redirect CD8 T cells to kill islet autoantigen reactive CD8 T cells. This may be a novel means of selectively targeting islet reactive cytotoxic T cells to prevent diabetes which we are currently testing in vivo.
Supported by: EFSD/JDRF/Novo Nordisk

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A novel immune regulatory peptide, PEPITEM, controls T cell trafficking during inflammation, a tonic inhibitory pathway that is lost in type 1 diabetes mellitus

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Background and aims: T-cells are recruited from the blood into extra-vascular tissues during an inflammatory response. In chronic inflammatory diseases such as T1D, an inappropriate accumulation of T-cells in the pancreatic islets results in the destruction of insulin producing beta cells. We aimed to explore the role of adiponectin, an adipose derived cytokine with recognised anti-inflammatory properties, on T cell migration in T1D. Here we describe that adiponectin stimulates the expression of a unique immune-regulatory peptide that imposes a tonic inhibition of T-cell trafficking during inflammation. PEPtide Inhibitor of Trans-Endothelial Migration (PEPITEM) introduces a new paradigm into the pathways that regulate the inflammatory response. We present evidence that this new pathway is compromised in patients with T1D. We propose that loss of this regulatory pathway makes the immune system 'leaky', facilitating the inappropriate access of T-cells into the pancreatic islet, thus leading to islet inflammation.
Materials and methods: In vitro, videomicroscopy was used to assess the migration of lymphocytes isolated from healthy donors or patients with T1D across TNF-α/IFN-γ activated endothelial cells (EC). In vivo, lymphocyte recruitment was assessed in a model of zymosan driven peritoneal inflammation. PEPITEM was identified using mass spectrometry and adiponectin receptors (AR1, AR2) expression was measured by flow cytometry.

Results: Using an in vitro migration assay, we observed that the migration of human lymphocytes was dose-dependently blocked by adiponectin (EC50=37nM). The effect of adiponectin was lost when B cells were absent, but could be regained by the addition of supernatants from adiponectin stimulated B-cells. Therefore adiponectin achieved its effects on T-cell migration through the induction of a B-cell secreted mediator. We used mass spectrometry to isolate and identify the B-cell derived agent, subsequently named PEPITEM. PEPITEM corresponds in the human genome to a proteolytic excision product of the 14.3.3ζδ protein. Synthetic PEPITEM could effectively inhibit T-cell migration (EC50=18pM). Interestingly, the B-cell derived product did not act directly on T-cells; rather, it stimulated EC to release the lipid mediator sphingosine-1-phosphate, which in turn inhibited the migration of T-cells. In zymosan-induced peritonitis, T-cell recruitment was significantly increased in a mouse strain lacking B cells when compared to wild-type animals. This excess of T-cell recruitment was ameliorated by treatment with PEPITEM. Lymphocytes isolated from patients with T1D expressed lower levels of both adiponectin receptors (Frequency AR1: HC=20.2±2.3, T1D=12.6±1.1 and AR2: HC=21.1±2.5, T1D=11.2±0.8) and were released from the inhibitory effects of adiponectin. However, this regulatory pathway could be re-established by the addition of exogenous PEPITEM.
Conclusion: We propose that low adiponectin receptor expression by lymphocytes in T1D facilitates the inappropriate accumulation of islet reactive T cells in the pancreas. We also propose that targeting the PEPITEM pathway has therapeutic potential for T1D.
Supported by: Diabetes UK

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Identification of two distinct patterns of immune cell infiltration in the insulitic lesions in human type 1 diabetes
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Background and aims: Type 1 diabetes is believed to result from the selective destruction of pancreatic β-cells by an immune-mediated process associated with islet insulitis. However, the mechanisms that promote and sustain islet inflammation in humans are poorly understood and the composition of the insulitic infiltrate has rarely been examined in detail. In one such study, pancreas specimens recovered from a cohort of recent-onset type 1 diabetes patients collected within the UK were investigated to establish the profile of immune cell infiltration into the islets during the active phase of β-cell destruction. This suggested that cytotoxic CD8+ T-cells form the major cellular component of the infiltrate but also revealed the ingress of significant numbers of CD20+ B-cells. In order to determine whether this pattern is seen more widely in patients with type 1 diabetes, we have now compared the immune cell composition of the insulitis in the UK cohort with that in patients from among the JDRF nPOD collection.
Materials and methods: A total of 30 T1D cases (4 nPOD; 26 UK) with proven insulitis were studied. 4um sections of formalin fixed paraffin embedded pancreas were analysed for insulin content and immune cell numbers by immunocytochemical methods. Sections were stained to reveal cells expressing CD45, CD4, CD8, CD20 or CD68 and their numbers analysed in each islet section.
Results: Initial examination of 3 nPOD cases immediately revealed a major difference from the profile seen previously within the UK samples. In particular, while CD8+ cells still formed the major component, the insulitic lesions contained markedly reduced numbers of CD20+ cells. One important variable between the 2 cohorts was the period since disease diagnosis, with the nPOD cases typically having a longer disease duration (up to 11 years). Therefore, we re-examined the larger UK cohort to determine whether this altered CD20+ cell profile could also be found in patients with shorter disease duration. This confirmed that two distinct patterns of insulitis are distinguishable in both cohorts - one in which elevated numbers of CD20+ cells are present ("CD20 hi"; 11.5±1.9 cells per islet) and another with markedly reduced CD20+ cells ("CD20 lo"; 0.8±0.1 cells per islet ($p<0.001$)). These patterns were distinct and representative of all insulitic islets within any given case. They were independent of disease duration, although "CD20 hi" cases were diagnosed at a younger age (7.8±1.5 vs 13.9±1.4 years; $p<0.01$) and contained a reduced proportion of residual insulin-positive islets (21.6±7% vs 38±6%). The total number of infiltrating immune cells was also reduced in the insulitic lesions of "CD20 lo" cases during the active phase of β-cell destruction. Nevertheless, CD8+ cells were always predominant and the major difference was in the ratio of CD8+ to CD20+ cells. This ratio was increased from a mean of only 1.5 in "CD20 hi" cases to 7.1 in "CD20 lo" cases.
Conclusion: We have identified 2 distinct patterns of insulitis in human type 1 diabetes. These differ in both the absolute numbers of immune cells present and, more particularly, in the relative proportions of CD8+ to CD20+ cells. The data imply that the presence of elevated numbers of CD20+ cells within the lesions may be indicative of a more aggressively destructive phenotype.
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***Mafa* knockout NOD mice are protected from type 1 diabetes despite accelerated infiltration of lymphocytes into islets**
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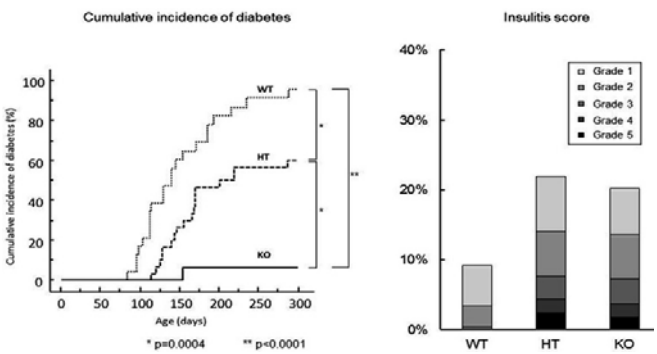
Background and aims: Type 1 diabetes is an organ-specific autoimmune disease against pancreatic islets, characterized by lymphocyte infiltration into islets, leading to destruction of insulin-producing beta cells. *Mafa* is an insulin transactivator that is specifically expressed in pancreatic beta cells. Recently, we reported that "intra-thymic" expression of *Mafa,* as well as *Ins2*, was reduced in NOD (non-obese diabetic) mice in comparison with that in control mice, and identified unique variants in the promoter region of $Mafa^{nod}$, which cause reduced promoter activity of *Mafa*. Systemic disruption of *Mafa* resulted in reduction of *Ins2* and *Mafa* expression in the thymus, indicating that *Mafa* is a key regulator of insulin expression in the thymus. To clarify the role of *Mafa* in the development of insulitis and type 1 diabetes *in vivo*, we generated *Mafa* knockout NOD mice and studied the phenotypic changes and islet pathological features in comparison with those in *Mafa* knockout control mice.
Materials and methods: All mice were housed under specific pathogen-free conditions. $Mafa^{-/-}$ ICR mice were backcrossed for five generations onto the NOD/Shi background. Microsatellite markers in the vicinity of *Idd* loci were genotyped by PCR method in each generation for selective breeding. Pancreata were removed from the three groups of pre-diabetic NOD females ($Mafa^{-/-}$: KO, $Mafa^{-/+}$: HT and $Mafa^{+/+}$: WT) at 8 weeks of age. Four non-consecutive tissue sections were prepared for hematoxylin-eosin staining. More than twenty islets in each pancreas were examined by light microscopy, and the degree of insulitis was scored (grade 0: insulitis-free, grade 1: peri-insulitis, grade 2: 0~25%, grade 3: 25~50%, grade 4: 50~75%, grade 5: 75~100%). The cumulative incidence of diabetes was compared using the Kaplan-Meier method with 95% confidence intervals; p values less than 0.05 by the log-rank test were considered statistically significant. Populations of lymphocytes infiltrated around islets were analyzed by immunohistochemical staining.
Results: All microsatellite markers were confirmed to be replaced with the NOD genotype at N5 generation. Thecumulative incidence of spontaneous development of diabetes in N5 females was significantly lower in KO mice (5.6%) than in HT mice (60.0%, $p=0.0004$) and WT mice (95.6%, $p<0.0001$), and was significantly lower in HT mice than in WT mice (60.0% vs. 95.6%, $p=0.0004$, Figure, left panel). However, insulitis scores of KO mice (proportion of grade 1 to 5: 20.3%) and HT mice (21.9%) were higher than that of WT mice (9.2%) at 8 weeks of age (Figure, right panel).
Conclusion: Disruption of *Mafa* caused accelerated infiltration of lymphocytes into pancreatic islets, but suppressed spontaneous development of diabetes in the NOD mouse, suggesting the involvement of a regulatory mechanism in benign insulitis.



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Relationships between major epitopes on the IA-2 autoantigen in type 1 diabetes

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Background and aims: Studies on the natural history of Type 1 diabetes indicate that spreading of autoimmunity within and between autoantigens represents a critical element of disease progression. Intra- and inter-molecular spreading is clearly demonstrated in IA-2 autoimmunity, where antibodies appearing early in disease frequently bind linear epitopes in the juxtamembrane (JM) region, subsequently spreading to epitopes in the tyrosine phosphatase (PTP) domain and to IA-2beta. By the time of disease onset, antibodies to the PTP domain predominate. In animal models of diabetes, B-cells play a critical role in epitope spreading. We hypothesise that B-cells reactive to the JM domain may facilitate spreading of autoimmunity by promoting uptake and presentation of peptides in the PTP domain, potentially as a consequence of close structural alignment of these determinants. The aim of this study was to investigate structural and immunological relationships of JM and PTP domain epitopes by competitive binding studies with IA-2 antibodies and investigating associations of T- and B-cell responses to these regions.
Materials and methods: Sera were obtained from Type 1 diabetic patients within 6 months of diagnosis. Human monoclonal IA-2 antibodies were generated by transformation of B-cells from diabetic patients and mouse monoclonal antibodies by immunization with IA-2 cytotoplasmic domain. Reactivity of antibodies to radiolabelled constructs representing the cytoplasmic (605-979) or JM (605-693) domains of IA-2 were determined by radioligand binding assay after capture of immune complexes with protein A or protein G Sepharose. Linear epitopes in the JM domain were localised by blocking antibody reactivity with synthetic 20-mer peptides. Overlap of antibody epitopes was analysed by competition studies with Fab fragments of monoclonal antibodies. T-cell reactivity to synthetic IA-2 peptides was determined in peripheral blood lymphocytes from diabetic patients by cytokine ELISPOT.
Results: Three distinct patterns of binding of antibodies to the JM domain in sera of diabetes patients could be categorized according to ability of synthetic peptides to block reactivity to the JM construct: JM1: blocked by 601-620 and 611-631 peptides, JM2: blocked by 611-631 and 621-640 peptide and JM3: blocked only by 611-630 peptide. Mouse monoclonal antibodies to JM epitopes were identified that could also be categorized as JM1, JM2 or JM3 epitope-reactive according to peptide blocking. Fab fragments of these mouse antibodies inhibited binding of JM-reactive autoantibodies in patients' sera. The mouse JM antibodies also inhibited binding of human monoclonal autoantibodies to a defined epitope in the 831-860 region of the IA-2 PTP domain. Furthermore, T-cell reactivity in Type 1 diabetic patients to peptide 841-860 of IA-2 was strongly associated with presence of antibodies to JM domain epitopes ($p<0.001$).
Conclusion: The epitope specificities of JM antibodies generated after immunization of mice with IA-2 protein mimic those of the human IA-2 autoantibodies developing early in the Type 1 diabetes. Blocking studies with Fab fragments of the mouse antibodies indicate close structural relationships of JM and PTP domain epitopes. The association of antibodies to the IA-2 JM domain with T-cell responses to PTP domain determinants supports a role for B-cells in promoting intramolecular spreading of the IA-2 autoimmune response.
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OP 24 Basic nephropathy: novel mechanisms and potential therapies

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The mechanism of action of the splice variant VEGF165b in decreasing proteinuria in mouse models of type 1 diabetic nephropathy

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Background and aims: Several studies have shown diabetes to be associated with increased levels of vascular endothelial growth factor (VEGF, main isoform VEGF165), both in the plasma and urine. Studies in rodents have also shown that inhibition of VEGF in diabetic nephropathy models reduces the levels of proteinuria. VEGF165 increases permeability to water in the glomerulus, however its splice isoform, VEGF165b, displays the opposite actions. We investigated the effect of VEGF165b on proteinuria in mice with type I diabetes as well as its mechanism of action, both in vitro and in vivo.
Materials and methods: Transgenic C57Bl/6 mice (TG) over-expressing VEGF165b under a nephrin promoter were generated. Both TG and wild-type (WT) littermate controls were rendered diabetic using STZ injections (100mg/kg for 3 consecutive days) and the subsequent urinary albumin:creatinine ratio (uACR) was measured in each group. Triple transgenic mice with podocyte-specific over-expression of both human VEGF165b (constitutively) and mouse VEGF164 (inducible) were rendered diabetic using the low-dose AMDCC STZ protocol (50 mg/kg for 5 consecutve days). 4 weeks after the onset of hyperglycaemia, VEGF164 overexpression was induced for 7 days by adding doxycycline in drinking water. Diabetes was also induced in DBA2J mice using the AMDCC low-dose STZ protocol. After 2 consecutive weeks of hyperglycaemia (>20mmol/L), baseline albumin-creatinine ratios (uACR) were measured, and mice received saline, 1µg VEGF165b or 5µg VEGF165b injections i.p. twice weekly for 6 weeks. VEGFR-2 expression and phosphorylation was assessed by immunofluorescence staining on the diabetic DBA/2J kidney sections with/without VEGF165b treatment. In vitro studies involved treating glomerular endothelial cells (GEnCs) with 30mM glucose for 7 days and assessing phosphorylation of VEGFR-2 in the presence or absence of VEGF165b (2.5nM).
Results: Control STZ-injected mice had a significantly increased uACR compared to sham-treated controls and this was partially rescued in heterozygous neph-VEGF165b STZ mice ($n=5$-9, $p<0.05$). Over-expression of VEGF164 for 7 days led to an increased uACR in STZ-induced diabetic mice and this was partially rescued if the mice also constitutively over-expressed VEGF165b ($p<0.05$). In diabetic DBA/2J mice uACR increased progressively in the sham group while in the treated groups remained constant at pre-treatment levels (1µg VEGF165b) or decreased (5µg VEGF165b) (two-way ANOVA for treatment $p< 0.01$). Glomeruli from diabetic DBA/2J mice showed both an increase in expression and phosphorylation of VEGFR-2 when treated with VEGF165b compared with diabetic controls ($n=3$, $p<0.05$). GEnCs treated with 30mM D-glucose have increased phosphorylation of VEGFR-2, compared with 30mM Mannitol controls, which is further increased by the addition of VEGF165b.
Conclusion: The over-expression of VEGF165b in the podocytes of diabetic mice partially rescues high levels of albuminuria. This is also the case when podocytes are over-expressing VEGF164, suggesting that the balance between the two isoforms determines the phenotype. VEGF165b is shown to be signalling, at least in part, through VEGFR-2 leading to an increase in phosphorylation of the receptor GEnC in vivo and this can be also reproduced in vitro in GEnCs in culture exposed to high glucose concentrations. Surprisingly, VEGF165b administration in vivo also increases expression of VEGFR2 in GEnC.
Supported by: MRC

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Clusterin/apolipoprotein J attenuates angiotensin II-induced renal fibrosis

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Background and aims: The blockade of angiotensin II (Ang II) is a main therapeutic target of diabetic nephropathy. Ang II acts through two receptors, angiotensin type 1 receptor (AT1) and angiotensin type 2 receptor (AT2). Most of the known actions of Ang II, including vasoconstriction and fibrosis, are due to AT1 activation, which is upregulated via sequential activation of the transcription factors NF-kB, ELK-1 and AP-1. In addition, AT2 decreases AT1 expression and function via nitro oxide (NO)/cyclic guanosine monophosphate (cGMP). Clusterin/apolipoprotein J is a conserved secreted glycoprotein expressed in a wide array of tissues and being implicated in several physiological processes. Increased expression of clusterin has been established in several renal diseases and nephrotoxicity models suggesting that clusterin may have a protective role against nephropathogenesis. Here, we examined clusterin prevents Ang II-induced renal fibrosis via downregulation of AT1 but upregulation of AT2.
Materials and methods: Clu-/- and wild type mice were infused with Ang II using osmotic mini pump. The effects of clusterin on renal fibrosis and AT receptor expression in vivo were assessed by Ang II-induced renal fibrosis model. NRK-52E cells, a stable cell line derived from rat proximal tubules were cultured. The effects of adenovirus mediated overexpression of clusterin on Ang II-induced fibrorsis and AT receptor expression in NRK-52Es were measured by Western blot and qRT-PCR analysis. The effect of clusterin on NF-kB-DNA binding activity was examined by co-immunoprecipitation assay. The inhibitory effect of the AT2 receptor on AT1 receptor expression was measured by the nitric oxide synthase inhibitor L-NAME or cGMP inhibitor 1H-[1,2,4] oxadiazolo-[4,3-a] quinoxalin-1-one.
Results: In kidneys of Clu-/- mice, renal fibrosis and expression of AT1 were increased compared with those in kidneys of wild-type mice. In addition, loss of clusterin accelerated Ang II-stimulated renal fibrosis and AT1 expression. But, AT2 expression was markedly decreased in kidneys of Clu -/- mice. Adenovirus mediated overexpression of clusterin in NRK-52E cells inhibited Ang II-stimulated PAI-1, collagen 1 type, fibronectin and AT1 expressions, but increased AT2 expression in a dose dependent manner. Moreover, infusion of Ang II using osmotic minipump markedly increased PAI-1, type I collagen, fibronectin and AT1 expressions but decreased AT2 expression. These changes were prevented by clusterin over-expression by adenovirus. Clusterin inhibited Ang II-induced NF-kB translocalization to nucleus. Furthermore, inhibition of NO production or blockade of cGMP abolished the inhibitory effect of clusterin on AT1 expression.
Conclusion: This study shows that clusterin attenuates renal fibrosis by inhibiting the AT1 but activating AT2 expression. Therefore, the present study raises the possibility that clusterin can be a target for the prevention of Ang II-induced renal fibrosis.

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Pivotal involvement of CX3CL1-CX3CR1 system in the pathogenesis of diabetic nephropathy in streptozotocin-treated mice

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Background and aims: Several lines of accumulating evidence implied that CX3CL1 and CX3CR1 seem to be involved in the progression of diabetic nephropathy. Actually, we found that urinary CX3CL1 levels were significantly higher in diabetic patients with albuminuria, compared with those with non-albuminuria and non-diabetic ones. Thus, in the present study, we established a model of diabetic nephropathy in streptozotocin (STZ)-induced diabetic mice, and examined pathogenic roles of CX3CL1-CX3CR1 system in diabetic nephropathy using CX3CR1-deficient mice.
Materials and methods: Male CX3CR1$^{+/+}$ (WT) and CX3CR1$^{-/-}$ (KO) mice aged 8-12 weeks were employed. For the induction of diabetic nephropathy, mice were intraperitoneally injected STZ (200 mg/kg ip). At the indicated time intervals after STZ injection, urine and blood samples were collected to measure the urinary albumin excretion and to evaluate blood glucose and blood urea nitrogen levels, respectively. Systolic blood pressure was also measured using tail-cuff method. After the sacrifice of mice at 3 and 6 month after STZ challenge, kidney samples were harvested, histopathological and immunohistochemical analyses were performed to evaluate fibrotic changes and macrophage recruitment. Moreover, intrarenal gene expression of cytokines, collagen and adhesion molecules was examined by real time RT-PCR.
Results: In WT, the intrarenal gene expression of CX3CL1 and CX3CR1 were apparently enhanced at 3 and 6 months after STZ challenge. Immunhistochemically, CX3CR1 protein was mainly expressed on intrarenal macrophages, and CX3CL1 protein could be detected on macrophages and renal tubular cells. These observations implied that CX3CL1-CX3CR1 axis might be involved in the pathogenesis of STZ-induced diabetic nephropathy. Blood glucose levels were Increased, to a similar extent, in KODM mice, compared with WTDM mice. Moreover, there was no significant difference in systolic blood pressure and blood urea nitrogen levels between WTDM and KODM mice. Urine albumin excretion, a hallmark of diabetic nephropathy, was elevated in both WTDM and KODM mice at 3 and 6 months after STZ challenge, whereas it was significantly lower in KODM mice than in WTDM ones. Histopathologically, the PAS-positive area in the glomeruli and interstitial fibrotic changes were less evident in KODM mice than in WTDM ones at 6 months after STZ administration. Actually, the gene expression of type IV collagen and TGF-β was significantly attenuated in KODM mice. Additionally, macrophage accumulation in the glomeruli was attenuated in KODM mice, compared with WTDM mice. These observations indicated that the absence of CX3CR1 could alleviate STZ-induced diabetic nephropathy, with a concomitant of attenuated macrophage recruitment. Moreover, intrarenal gene expression of ICAM-1, VCAM-1, iNOS and MMP-2 was less enhanced in KODM mice, compared with WTDM mice, implying that CX3CL1-CX3CR1 axis could regulate these molecules.
Conclusion: CX3CL1-CX3CR1 system plays detrimental roles in STZ-induced diabetic nephropathy, and might be a good therapeutic target for diabetic nephropathy.

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PDK1 protects podocytes from apoptosis

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Background and aims: Apoptosis is one of the mechanisms of podocyte loss in diabetic nephropathy. We hypothesized that 3-phosphoinositide-dependent kinase-1 (PDK1), an activator of protein kinase B (PKB)/Akt, would be a prosurvival factor for podocytes by increasing the phosphorylation level of Akt.
Matherials and methods: To study the role of PDK1 in podocyte apoptosis, PDK1 was knocked down in cultured human podocytes using lentiviral small hairpin RNAs (shRNAs). The apoptotic rate of podocytes was measured by Annexin V staining and flow cytometry. The involvement of phosphatidylinositol 3-kinase/Akt (P13-K/Akt) and p38 mitogen-activated protein kinase (p38MAPK) signaling cascades and the expression levels of proapoptotic BAX and antiapoptotic BCL-2 after knockdown of PDK1 were analyzed by quantative Western blotting. Expression level of PDK1 was also studied in podocytes treated with serum obtained from patients with type 1 diabetes and macroalbuminuria and compared to cells treated with serum obtained from microalbuminuric patients. Further, the expression level of PDK1 in glomeruli of lean and obese Zucker rats and in human patients with diabetes was studied.
Results: PDK1 is expressed in rat glomeruli and tubules and in cultured human podocytes. Knockdown of PDK1 increased apoptosis in cultured human podocytes, and inhibited the PI3-K/Akt and activated the p38MAPK pathways. BCL-2 level was decreased and BAX increased after PDK1 knockdown. Expression of PDK1 was lower after treatment with serum from macroalbuminuric patients compared to serum from normoalbuminuric patients. Furthermore, PDK1 was found to be downregulated in glomeruli of obese Zucker rats compared to lean littermates and in podocytes of human patients with diabetes.

Conclusion: Our data show that PDK1 may protect podocytes against apoptosis. Downregulation of PDK1 in Zucker rat glomeruli prior to proteinuria suggests that PDK1 could have a protective role in the development of podocyte injury.
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Linagliptin and the angiotensin II receptor blocker telmisartan show comparable efficacy but different renoprotective pathways in rats with 5/6 nephrectomy

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Background and aims: DPP-4 inhibitors were shown to delay disease progression in experimental diabetic nephropathy and may also have renoprotective properties and diabetes-independent effects. In this study, we compared the effect of linagliptin with an angiotensin II receptor antagonist in preventing renal disease progression in non-diabetic rats with 5/6 nephrectomy. Furthermore, we analysed kidney tissue- and plasma-biomarkers at study end in order to determine the potential differences in the underlying mechanisms of action.
Materials and methods: Male Wistar rats were allocated to 4 groups: sham operated; 5/6 nephrectomy (5/6 Nx) plus placebo treatment (placebo); 5/6 Nx plus linagliptin (0.083 mg/kg/d in chow); 5/6 Nx plus telmisartan (5 mg/kg/d in drinking water). Study duration was 130 days. Histological analysis for interstitial fibrosis, glomerulosclerosis, and nephrin was performed. Plasma was investigated for TIMP-1, calbindin, osteopontin, and β2 microglobulin (B2M), as well as circulating macrophage-derived chemokine (MDC) and plasma macrophage colony-stimulating factor-1 (M-CSF-1).
Results: Interstitial fibrosis increased by 69% in placebo vs sham rats ($p<0.05$), and decreased by 48% with linagliptin ($p<0.05$) and 24% with telmisartan (p=ns) vs placebo rats. Glomerular size increased by 28% in placebo vs sham rats ($p<0.01$), and decreased by 18% ($p<0.001$) with linagliptin but not significantly with telmisartan vs placebo rats. The glomerulosclerosis index was significantly increased in placebo vs sham rats ($p<0.05$). There was a trend towards decreased glomerulosclerosis with linagliptin and telmisartan. The urinary albumin/creatinine ratio increased 14-fold in placebo vs sham rats ($p<0.001$), and decreased by 66% with linagliptin ($p<0.05$) and 92% with telmisartan ($p<0.01$) vs placebo rats. Blood pressure was lowered by telmisartan (31 mmHg; $p<0.05$) and unaffected by linagliptin. Expression of the tissue split membrane forming protein nephrin increased 3.2-fold ($p<0.01$) in placebo vs sham rats. Linagliptin and telmisartan treatment abolished this effect. Plasma TIMP-1, calbindin, osteopontin, and B2M were all significantly increased (all $p<0.05$) in the placebo vs sham rats. Linagliptin decreased plasma levels of TIMP-1, calbindin, osteopontin, and B2M vs placebo rats (all $p<0.05$), whereas telmisartan significantly decreased osteopontin ($p<0.05$). M-CSF-1 concentration increased by 45% ($p<0.05$) in placebo vs sham rats. Linagliptin had no effect on the M-CSF-1 concentrations, whereas telmisartan abolished this effect. MDC concentrations were significantly reduced by 51% in linagliptin-treated rats only ($p<0.05$). Telmisartan had no effect on MDC plasma concentrations. Plasma concentrations of the T-cell-specific protein RANTES increased by 2.5-fold ($p<0.05$) with telmisartan. Linagliptin abolished this effect.
Conclusion: Linagliptin is as effective as telmisartan in preventing renal disease progression in rats with 5/6 Nx. Furthermore, analysis of biomarkers revealed that the underlying molecular mechanisms of renoprotection appear to be substantially different.
Supported by: Boehringer Ingleheim

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Luseogliflozin (TS-071), a novel SGLT2 inhibitor, protects against diabetic nephropathy in diabetic db/db mice

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Background and aims: SGLT2 is responsible for reabsorption of ~90% of filtered glucose in the S1 segments of proximal tubule. Now, SGLT2 represents a novel target for normalizing glycemia. To address in vivo the issue of the efficacy and tolerance of a novel SGLT2 inhibitor Luseogliflozin (LUSEO), we investigated whether LUSEO can prevent renal insufficiency and glomerulosclerosis in diabetic mice, and determined potential mechanisms of renoprotecitve effects.
Materials and methods: Initially, eight weeks old male diabetic *db/db* mice or nondiabetic *db/m* mice were treated with 15 mg/kg LUSEO for eight weeks. Body weight, fasting blood glucose, HbA1c, blood pressure, and urinary albumin were measured. Renal histology, expressions of SGLT2, hypoxia-probe pimonidazole were evaluated at the end of the study. Second, we examined the expression of SGLT2 or inflammatory molecules in human renal proximal tubular epithelial cells (HRPTEC) by RT-qPCR.
Results: Diabetic *db/db* mice showed higher body weight and fasting blood glucose levels, HbA1c, compared with control *db/m* mice. LUSEO had an impact on urinary glucose excretion (UGE) even in *db/m* mice (0.97±0.67 *vs.* 367.31±45.92 mg/day, *db/m*, *db/m*+LUSEO), without hypoglycemia and weight loss. LUSEO had significant effects on plasma glucose levels in *db/db* mice (HbA1c; 7.32±0.85 *vs.* 13.50±0.44 %, *db/db*+LUSEO, *db/db*, respectively, $p<0.001$), which apparently lead to rescue pancreatic islet loss. Then, consistent with lowerd filtrated glucose load, UGE in *db/db* treated with LUSEO was decreased (1,127.06±537.82 *vs.* 2,041.7±713.03 mg/day, *db/db*+LUSEO, *db/db*, respectively, $p<0.05$). Moreover, LUSEO showed renoprotective effects, which significantly attenuated urinary albumin excretion rates, glomerular mesangial matrix expansion, glomerular and interstitial fibronectin accumulation, and Armanni-Ebstein lesions in *db/db* mice to almost the level observed in *db/m* mice. Consequently, LUSEO preserved the expression of SGLT2 in brush border membrane of the proximal tubules of *db/db* mice against tubular injury. Of interest, LUSEO augmented pimonidazole staining of the S3 segment of the proximal tubules cells which expressed SGLT1, in outer stripe of the kidney of *db/db* mice, suggesting hypoxia caused by the overload of glucose and sodium via blocking SGLT2 in the S1 segment. Subsequently, we evaluated the expression of its target molecule SGLT2 using HRPTEC. HRPTEC constitutively expresses SGLT2 mRNA. High glucose (25.5 mM) did not affect the expression of SGLT2 mRNA, and insulin (100 nM) slightly increased SGLT2 mRNA expression by ~120% of control. Whereas, profibrotic TGF-ß1 markedly decreased SGLT2 mRNA expression (48.3±10.4 %, $p<0.01$). Intriguingly, Hepatocyte Nuclear Factor (HNF)-1alpha, a modulator of SGLT2 expression, was drastically decreased by TGF-ß1 treatment, thereby providing a mechanism by which TGF-ß1 represses SGLT2 mRNA expression through decreased HNF-1alpha. In addition, LUSEO alone failed to affect the expressions of SGLT2 and the inflammatory molecules, however, significantly inhibited TGF-ß1-induced fibronectin expression.
Conclusion: These results suggest that chronic treatment with the SGLT2 inhibitor LUSEO can protect against the progression of diabetic nephropathy, which is probably sufficient to overcome the adverse effects of TGF-ß1.
Supported by: Taisho Pharmaceutical Co., Ltd.

OP 25 Novel therapeutic agents and insights

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Dissociation between metformin plasma exposure and its glucose-lowering effect: a novel gut-mediated mechanism of action

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Background and aims: We tested the hypothesis that metformin (Met) reduces plasma glucose primarily by stimulating release of glucoregulatory hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) from enteroendocrine L-cells via a mechanism that is independent of Met plasma concentration. We designed a delayed release (DR) Met tablet that targets the lower bowel and exhibits reduced bioavailability relative to currently available metformin formulations.
Materials and methods: The pharmacokinetics of 1000 or 500 mg Met DR twice daily (BID) was compared to commercially available immediate release (IR) Met (1000 mg BID) and extended release (XR) Met (2000 mg QD) in healthy subjects. Subsequently, a randomised, double-blind, crossover study compared 500 and 1000 mg Met DR BID and 1000 mg Met IR BID for 5 days in patients with type 2 diabetes withdrawn from diabetes therapy 2 weeks prior to randomisation.
Results: In healthy subjects, a single day of treatment with 1000 or 500 mg Met DR BID was associated with plasma Met concentrations that were 52% and 68% lower than 1000 mg Met IR BID and 48% and 65% lower than 2000 mg Met XR QD (N=19; all $p<0.0001$). In patients with type 2 diabetes, Met exposure also was reduced by 45% and 57% with 1000 and 500 mg Met DR compared to 1000 mg Met IR (N=19, both $p<0.0001$). The reduction in fasting plasma glucose from baseline (197 to 200 mg/dl) was similar for all treatments (LSmean±SE: -22±7 mg/dl, 1000 mg Met IR; -20±5 mg/dl, 1000 mg Met DR; 16±4 mg/dl, 500 mg Met DR; all $p<0.01$ vs. baseline). The 10h glucose AUC was similarly reduced in all treatments by 8 to 14% (all $p<0.0001$ vs. baseline). Insulin AUC was unchanged from baseline. All treatments increased fasting and postprandial PYY and GLP-1 (all $p<0.05$ vs. baseline). The GLP-1 AUC increased by 87, 62 and 69% and the PYY AUC increased by 55, 38, and 46% for 1000 mg Met IR, 1000 mg Met DR and 500 mg Met DR (all $p<0.0001$ vs. baseline). Nausea (9%) and vomiting (9%) occurred with Met IR but not Met DR. Diarrhea (10 to 15%) occurred in all treatments.
Conclusion: Despite marked reductions in exposure, a Met DR formulation that targets the lower bowel was as effective as Met IR with better tolerability. These results support the hypothesis that metformin's glucose-lowering effects are predominantly due to actions on enteroendocrine L-cells in the lower bowel and that plasma Met exposure is not required for efficacy.
Clinical Trial Registration Number: NCT01677299

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Efficacy of bile acid sequestrant treatment for glycaemic control in type 2 diabetes - systematic review with meta-analysis of randomised controlled trials

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Background and aims: Studies suggest that bile acid sequestrants (BASs) can lower blood glucose in addition to their lipid-lowering effects. Three oral BASs are approved for hypercholesterolaemia: colestipol, colestyramin and colesevelam. In 2008, the American Food and Drug Administration approved colesevelam for treatment of hyperglycaemia in type 2 diabetes. We evaluated the glucose-lowering effect of BASs in a systematic review with meta-analyses of randomised clinical trials (RCTs).
Materials and methods: The protocol was registered in the Prospero database and the results are reported in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA). Literature searches included manual searches and electronic searches in The Cochrane Library, MEDLINE and EMBASE. Included RCTs assessed patients with type 2 diabetes randomised to BAS, placebo or an active comparator. The primary endpoint was change in HbA1c. Secondary endpoints included changes in fasting plasma glucose, and adverse events.
Results: Ten RCTs (10 on colesevelam and 7 on colestimide) with a duration of 2 to 52 weeks, including 2,118 patients were included in the analysis. Six RCTs in which 399 patients were randomised to BAS treatment versus 391 in the control group reported end of treatment HbA1c. Random effects meta-analysis showed that BASs reduced HbA1c compared with controls (weighted mean difference (WMD) (95% confidence interval (CI)): -0.59% (-0.70 to -0.48%). Six RCTs on colesevelam reported end of treatment fasting plasma glucose and random effects meta-analysis revealed a greater reduction in patients randomised to BAS treatment (n=769 vs. n=759 patients; WMD (95% CI): -0.52% (-0.68 to -0.37)). No evidence of small study effects was identified, but we found evidence of bias related to the trial design. Treatment with BASs increased the risk of drug-related adverse events (relative risk (95% CI): 1.89 (1.32 to 2.67)), constipation being the most common. No increased risk of serious adverse events was reported.
Conclusion: This review found that BAS treatment improves glycaemic control in patients with type 2 diabetes.
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A new anti-inflammatory and immune-modulatory peptide therapeutic improves glycaemic control and beta cell function in the db/db and STZ diabetes models

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Background and aims: Loss of glycaemic control and beta cell function contribute to the pathologies of type I and II diabetes. Using the alpha-1-antitrypsin protein as template, we identified a small anti-inflammatory and immune-modulatory peptide, SP16. To test if SP16 treatment would reduce inflammation and thereby improve glycemic control and beta cell function, we administered the peptide to db/db mice and mice with STZ-induced diabetes.
Materials and methods: Peptides: The SP16 peptide (17 aa) was synthesised by CPC Scientific and the INGAP peptide (15 aa) was provided by Exsulin. Animal studies: Five week old, pre-diabetic db/db animals were assigned to groups of 10 and IP injected with 12 μg SP16, 25 mg/kg Rosiglitazone or saline twice a week for 36 days. C57BL/6j animals were injected daily with 50mg/kg STZ for 5 days to induce beta cell loss and development of diabetes. Groups of 5 animals received 12 μg SP16, 0.5 mg INGAP, 12 μg SP16 plus 0.5 mg INGAP, or saline by daily injection for 41 days. For both studies, non-fasted blood glucose levels were determined twice a week, and HbA_{1c} and c-peptide levels measured at the end of the study. Mechanism of action: An engineered TLR-2 indicator cell line (HEK-Blue mTLR2, Invivogen) was incubated with SP16 or Pam3SK for 24 hours, in the presence or absence of a blocking antibody. A scrambled peptide was included as negative control. Assays were done in triplicate. In vitro safety testing: For initial safety assessment of the SP16 peptide, non-GLP hERG testing and receptor panning studies were executed.
Results: Animal studies: In db/db animals, SP16 and Rosiglitazone treated animals showed improved glycaemic control (lowered non-fasted blood glucose and HbA_{1c} levels, $p<0.05$), improved beta cell function (increased c-peptide levels and decreased islet hyperplasia, $p<0.05$), improved glucose tolerance in an oral glucose tolerance test, reduced inflammation (decreased levels of c-reactive protein, $P<0.05$) and increased serum TGFβ levels ($p<0.05$). In the STZ study, the combination of SP16 and INGAP resulted in significantly lowered non-fasted blood glucose levels ($p<0.05$) and all animals of this group were diabetes free at the end of the study. Mechanism of action: Cell-based assays showed that SP16 induces TLR2 signaling, in a concentration dependent manner, and the signaling could be blocked by addition of a TLR2 blocking antibody. We hypothesise SP16 induces expansion of Fox3P+ Tregs via TLR2, to favorably shift the Teff/Treg balance. In vitro safety testing: The *in vitro* safety studies showed that SP16 had no impact on hERG activity at up to 25 uM and did not impact the activity of the 111 different human receptors and enzymes of the GenSep explorer panel. The SP16 peptide did not elicit any immune response in the animal studies. In summary, SP16 is expected to have a favorable safety profile, without immuno- and cardiotoxicity.
Conclusion: SP16 is a novel, alpha-1-antitrypsin derived, anti-inflammatory and immune-modulatory peptide. SP16 treatment improves glycaemic control and beta cell function in db/db mice and in the STZ model of diabetes. Our cell-based assay data support the hypothesis that SP16 works by TLR2 mediated expansion of protective Tregs. Together, our data, combined with the fact that SP16 is derived from a drug that has been used for treatment of

AAT-deficient patients for over two decades, predict that SP16 is a safe drug with significant therapeutic potential.
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Effect of a dual agonist of glucagon-like peptide-1/glucose-dependent insulinotropic peptide (MAR701) on insulin secretion rate and gastric emptying in healthy volunteers

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Background and aims: MAR701 is a novel long-acting peptide analogue with activity on both glucagon-like peptide-1 and glucose-dependent insulinotropic peptide receptors. The effects of MAR701 on insulin secretion rate (ISR) and gastric emptying (GE) were assessed in this phase I, two part, placebo- and active-control, sequential, single blind study in healthy volunteers.
Materials and methods: Part 1. After a >10 h overnight fast subjects (Cohort 1; n=6, all male, mean age 35.0 years, mean BMI 26.9 kg/m^2) received 2 s.c. injections of placebo (-120 and 0 min) in the abdomen on Day 1 followed by 2 s.c. injections of exenatide 5 μg (10 μg total at -120 and 0 min) in the abdomen on Day 2. Part 2: Subjects (n=18, 15 male, mean age 35.9 years, mean BMI 26.9 kg/m^2) received placebo on Day 1 followed by MAR701 8 mg on Day 2 (Cohort 2, n=6), placebo on Day 1 followed by MAR701 4 mg on Day 2 (Cohort 3, n=6), and placebo on Day 1 followed by MAR701 16 mg on Day 2 (Cohort 4, n=6). ISR was assessed using a 2.5 hr graded glucose infusion (GGI) at 2, 4, 6, 8, and 12 mg/kg/min (30 min per step of 20% dextrose, IV) with sampling for glucose, insulin and C-peptide. GE was assessed by measuring absorption of 1000 mg oral acetaminophen elixir over 240 min.
Results: A dose-dependent increase in ISR was observed in response to exenatide at each GGI rate compared with placebo (p<0.001). MAR701 at all doses significantly increased ISR in a dose-dependent manner during each GGI step (p<0.0001). In contrast to exenatide, treatment with MAR701 did not significantly delay GE at any dose. Treatment with MAR701 was generally safe and well tolerated. The most common adverse events associated with treatment with MAR701 were hypoglycaemia (occurring after discontinuation of the GGI) and mild nausea that was not dose-dependent.
Conclusion: MAR701 significantly increased ISR at doses that had either no or minimal effects on GE.

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Pharmacogenetic study of selected gene variants related to the pharmacodynamics of metformin with response to metformin treatment in type 2 diabetes

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Background and aims: The glucose lowering effect of metformin is mediated by activating AMP-activated protein kinase (AMPK) and possibly also by another mechanism. Previous pharmacogenetic studies observed associations of several gene variants related to pharmacodynamic effect of metformin with the response to metformin treatment. Among those genes, PRKAA1 encodes the A-subunit of AMPK. Proteins encoded by STK11 and probably also by ATM genes activate AMPK. The inhibition of gluconeogenesis downstream of AMPK is also mediated by inhibition of phosphoenolpyruvate carboxykinase encoded by PCK1 gene. The aim of the present study was to examine possible associations of the variants of the mentioned genes with the therapeutic response to metformin.
Materials and methods: 148 drug-naive patients with newly diagnosed type 2 diabetes (72 men/76 women) were included in the present study. Mean age (± SEM) was 57.5±0.9 years, mean baseline HbA1c was 7.64±0.09% (60.0±0.9 mmol/mol), and the mean daily metformin dose was 1400±40 mg. PRKAA1 rs249429 A>G, ATM rs11212617 A>C, STK11 rs8111699 C>G, and PCK1 rs4810083 C>T were genotyped using real-time PCR with subsequent melting curve analysis. HbA1c reduction after 6-month metformin treatment (ΔHbA1c) was the primary endpoint of the study. Genetic models were statistically adjusted for age, sex, BMI, metformin dose, baseline HbA1c and creatinine level (p_adj).
Results: In the entire study group, 6-month treatment with metformin led to a reduction in HbA1c by 0.66±0.08%. All examined genotypes followed the Hardyho-Weinberg equilibrium. PCK1 rs4810083 C>T variant was significantly associated with ΔHbA1c [CC (n=38): 0.98±0.16%; CT (n=76): 0.62±0.12%; TT (n=34): 0.39±0.13%; p=0,038]. In the dominant genetic model, the CC homozygotes had significantly greater reduction in HbA1c in comparison with T-allele carriers (CC: 0.98±0.16% vs. CT+TT: 0.55±0.09%; p=0.021; p_adj=0.029). Beside the PCK1 genotype, significant predictors of the HbA1c reduction were also the baseline HbA1c (p<0.001) and the daily metformin dose (p=0.010). Further examined ATM, STK11, and PRKAA1 variants were not significantly associated with the response to the metformin treatment.
Conclusion: To the best of our knowledge, we observed for the first time an association between rs4810083 variant in PCK1 and the therapeutic response to metformin in patients with type 2 diabetes. 25% of patients who were CC homozygotes had greater decrease in HbA1c by 0.4% (4.7 mmol/mol) in comparison with the rest of the study group. This study needs further replication in a different population.
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The novel glucagon-neutralising Spiegelmer NOX-G15 ameliorates hyperglycaemia in murine models of type 1 and type 2 diabetes

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Background and aims: Excessive secretion of glucagon significantly contributes to hyperglycemia in type 1 and type 2 diabetes mellitus (T1DM and T2DM). Accordingly, immunoneutralization of glucagon or genetic deletion of the glucagon receptor improved glucose homeostasis in animal models of diabetes. Despite this strong evidence, agents that selectively interfere with endogenous glucagon have not been implemented in clinical practice yet. We aimed to discover a mirror-image mixed aptamer (Spiegelmer) that binds and inhibits glucagon, and hypothesized that glucagon neutralization with this agent would reduce hyperglycemia in murine models of T1DM and T2DM.
Materials and methods: Using an in vitro selection process we have generated NOX-G15, a plasma-stable mirror-image (L)oligonucleotide (a so-called Spiegelmer), that directly binds and inhibits glucagon. The Spiegelmer was optimized in terms of pharmacokinetics by site-directed PEGylation. Affinity to glucagon and related peptides was determined using surface plasmon resonance (Biacore). Inhibition of glucagon action was demonstrated in cell-based cAMP formation assays using glucagon receptor-expressing CHO cells. A pilot pharmacokinetics study was performed in healthy mice with a single intraperitoneal injection of 10 mg/kg. Intraperitoneal glucose tolerance tests were performed in streptozotocin (STZ)-treated mice (T1DM) and high-fat diet plus STZ-treated mice (T2DM) after a single intraperitoneal injection of 10 mg/kg or 1 mg/kg. The glucagon receptor antagonist Des-His1-Glu9-glucagon and vehicle were used as controls (n=8-9).
Results: The Spiegelmer candidate NOX-G15 binds glucagon with an affinity of 6.3 nM. NOXG15 shows no cross-reactivity with related peptides, such as glucagon-like peptide1 (GLP-1), GLP-2, gastric-inhibitory peptide, and prepro-vasoactive intestinal peptide (prepro-VIP). *In vitro*, NOX-G15 inhibits glucagon-stimulated cAMP-production in CHO-cells overexpressing the human glucagon receptor with an IC_{50} of 3.4 nM. NOX-G15 has a terminal plasma half-life of 7 h. A single intraperitoneal injection of NOX-G15 (10 mg/kg or 1 mg/kg) ameliorated glucose excursions in intraperitoneal glucose tolerance tests (ipGTT) in mice with streptozotocin-induced (T1DM) diabetes and in a non-genetic mouse model of T2DM as shown by significantly reduced AUC (p<0.01 by One-way ANOVA and Tukey post-test).
Conclusion: The data suggest the novel glucagon-neutralizing Spiegelmer NOX-G15 as a therapeutic candidate with the potential to acutely attenuate hyperglycemia in T1DM and T2DM.

OP 26 Incretins: mechanisms of cardiovascular protection

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Inhibition of DPP-4 activity: potential role of non-GLP-1-mediated cardioprotection in the post MI diabetes setting

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Background and aims: In addition to degrading glucagon-like peptide-1 (GLP-1), di-peptidyl peptidase 4 (DPP-4) also inactivates several chemokines. Among these is stromal derived factor-1 (SDF-1), a pro-angiogenic and cardiomyocyte protective protein that is currently in human trials for ischaemic heart disease. Accordingly, we hypothesized that DPP-4 inhibition may confer benefit following myocardial infarction (MI) in the diabetic setting as a consequence of enhanced SDF-1 availability rather than potentiating GLP-1. To test this hypothesis, we compared DPP-4 inhibition with GLP-1 agonism. In order to demonstrate SDF-1 specificity, we then antagonised SDF-1's cognate receptor, CXCR4, with AMD3100.
Materials and methods: To avoid the confounding effects of glucose-lowering of GLP-1, studies were conducted in Fischer F344 rats with streptozotocin (STZ)-diabetes. Rats were randomized to receive the DPP-4 inhibitor, saxaglitpin (10 mg/kg/d), liraglutide (1mg/kg/sc/d), saxagliptin and AMD3100 (1mg/kg/sc), or vehicle. Two weeks later, animals underwent experimental MI, induced by ligation of the left anterior descending coronary artery. Cardiac function, assessed by conductance catheterisation and echocardiography, were examined 4 weeks post-MI.
Results: Glycemic control was similar in all groups, as was MI size, systolic BP, and heart size (P=NS). DPP-4 activity was increased in diabetic animals when compared with control, and reduced by saxagliptin treatment ($p<0.05$). Four weeks post MI, mortality was reduced in saxagliptin-treated animals when compared with those receiving vehicle ($p<0.05$). When compared with untreated post-MI rats, those receiving saxagliptin had improved fractional shortening ($p<0.05$) and better function in both early and late phases of diastole, as measured by Tau and the slope of the end diastolic pressure-volume relationship, respectively ($p<0.05$ for both). By contrast, cardiac function in liraglutide-treated animals closely resembled that of vehicle-treated animals. Antagonism of CXCR4 in saxagliptin-treated animals prevented the improvement in systolic and diastolic function and was associated with increased mortality (all $p<0.05$) when compared with saxagliptin-treated animals.
Conclusion: Saxagliptin-mediated DPP-4 inhibition but not liraglutide-mediated GLP-1 agonism improved ventricular systolic and diastolic function post myocardial infarction in a model of type 1 diabetes. These findings suggest that non-GLP-1 effects such as SDF-1 potentiation may underlie the cardioprotective effects of DPP-4 inhibition.
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Insights into the mechanism of action of linagliptin in reducing ischaemic brain damage following stroke in diabetic and non-diabetic mice

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Background and aims: Type 2 diabetes (T2D) is a strong risk factor for stroke. Linagliptin is a dipeptidyl peptidase (DPP)-4 inhibitor in clinical use for the treatment of T2D. We recently demonstrated that linagliptin had superior neuroprotective efficacy to glimepiride in a stroke animal model in both normal and diabetic mice. The aim of this investigation was to determine the effect of linagliptin and glimepiride treatment on adult neurogenesis and neuroinflammation. Additional experiments were conducted to determine if linagliptin's neuroprotective effect was mediated via increased glucagon-like peptide (GLP)-1 levels in the brain.
Materials and methods: C57BL/6J male mice were fed a high-fat diet for 32 weeks to induce T2D. Mice were treated for 7 weeks with either linagliptin (10 mg/kg) or glimepiride (2 mg/kg). Stroke was induced at Week 4 of treatment by transient middle cerebral artery occlusion (MCAO). Blood DPP-4 activity, GLP-1 and glucose levels were assessed throughout the experiments. Ischaemic brain damage was measured by determining stroke volume and by stereological quantification of surviving neurons in striatum/cortex. Stroke-induced adult neural stem cell proliferation and neuroblast formation were assessed by quantifying Ki67 and doublecortin-positive cells, respectively. Striatal neurogenesis and gliogenesis were assessed by quantifying BrdU/NeuN- and BrdU/S100-positive cells, respectively. Neuroinflammation was assessed by quantifying Iba-1-positive microglial cells in striatum and cortex using stereological methods. GLP-1 levels in the brain were quantified after perfusion by ELISA in naïve mice treated with vehicle or linagliptin.
Results: Anti-stroke efficacy of linagliptin was shown in both diabetic and normal mice, whereas glimepiride was efficacious against stroke in normal mice only. These results suggest a linagliptin-mediated neuroprotective effect that is glucose-independent and may involve increased GLP-1 levels both in the blood and brain. Additional studies demonstrated that linagliptin treatment increased levels of active GLP-1 in the brain. Furthermore, adult neural stem cell proliferation increased in linagliptin-treated diabetic mice compared with glimepiride and vehicle controls. Interestingly, no differences between the treatment groups were observed in normal mice. Neuroblast formation, neurogenesis, and gliogenesis were unaffected between the groups. Thus, because anti-stroke efficacy by linagliptin was achieved in both normal and diabetic mice, no correlation was found between neural stem cell activation and linagliptin-mediated anti-stroke efficacy. Finally, no regulation of brain inflammation was observed with either linagliptin or glimepiride.
Conclusion: The mechanism of action at the basis of linagliptin's anti-stroke efficacy requires further study. However, if translated to humans, our findings may provide a basis for the use of DPP-4 inhibitors for the prevention and treatment of stroke in both diabetic and non-diabetic patients. Moreover, we hypothesise that diabetic patients at high risk for stroke may derive further benefit because of the increased proliferation of stem cells.
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The suppressive effect of dipeptidyl peptidase-4 inhibitor on atherosclerosis is mainly attributable to the action of incretin in both non-diabetic and diabetic mice

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Background and aims: Several recent reports have revealed that dipeptidyl peptidase (DPP)-4 inhibitors have a suppressive effect on atherosclerosis in hypercholesterolemic mice. It remains to be seen, however, whether this suppressive effect stems from increased levels of the active incretins, namely, glucagon-like peptide-1(GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).
Methods: Male apolipoprotein E-null ($Apoe^{-/-}$) mice and db/db mice were fed an atherogenic diet for 4 weeks. Diabetes was induced in a subset of the $Apoe^{-/-}$ mice by multiple injections of streptozotocin. Mice were administered the DPP-4 inhibitor vildagliptin in drinking water over the same period. While receiving vildagliptin, the mice were subcutaneously infused with either saline, the GLP-1 receptor blocker exendin-9 (Ex-9), the GIP receptor blocker (Pro3)GIP, or both Ex-9 and (Pro3)GIP, via osmotic minipumps. Aortic atherosclerosis and oxidized low-density lipoprotein-induced foam cell formation in exudate peritoneal macrophages were determined. The gene expressions of GLP-1 and GIP receptors were measured by RT-PCR using 10-11 primers covering all exons. The direct effect of DPP-4 on foam cell formation was also determined.
Results: Vildagliptin treatment increased GLP-1 and GIP levels without affecting the food intake, body weight, blood pressure, or plasma lipid profile, though it reduced HbA1c only in diabetic mice. Diabetic $Apoe^{-/-}$ mice exhibited further-progressed atherosclerotic lesions and foam cell formation compared with nondiabetic counterparts. Nondiabetic and diabetic $Apoe^{-/-}$ mice showed a comparable response to vildagliptin, namely, remarkable suppression of atherosclerotic lesions with macrophage accumulation and foam cell formation in peritoneal macrophages. Ex-9 or Pro3 partially attenuated the vildagliptin-induced suppression of atherosclerosis. The two receptor blockers in combination abolished the anti-atherosclerotic effect of vildagliptin in nondiabetic mice but incompletely attenuated it in diabetic mice. Vildagliptin

treatment also suppressed foam cell formation in peritoneal macrophages in nondiabetic Apoe$^{-/-}$ and diabetic (Apoe$^{-/-}$ and db/db) mice, and this suppressive effect was abolished by infusions with Ex-9 plus Pro3. Gene expressions of GLP-1 and GIP receptors were detectable in peritoneal macrophages and J774 cells. Although these receptor genes were far less abundant than those in the vasculature or the pancreas, incretins elicited cyclic AMP generation in J774 cells, Incubation of DPP-4 or vildagliptin had no effect on macrophage foam cell formation.
Conclusion: Vildagliptin exhibits a substantial anti-atherosclerotic effect in both nondiabetic and diabetic mice, mainly via the action of the two incretins. However, the partial attenuation of atherosclerotic lesions by the dual incretin receptor antagonists in diabetic mice implies that vildagliptin partly confers an anti-atherogenic effect beyond that from the incretins.

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The DPP-IV inhibitor saxagliptin inhibits palmitate-induced apoptosis of human endothelial cells and cardiac progenitor cells
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Background and aims: Exposure of endothelial cells and cardiomyocytes to the fatty acid palmitate results in cell dysfunction and apoptosis. DPP-IV inhibitors may exert protective actions on the cardiovascular system. The aim of this study was to investigate the protective effects of the DPP-IV inhibitor saxagliptin on palmitate-induced apoptosis of human umbilical vein endothelial cells (HUVEC) and human cardiac progenitor cells (hCPC).
Materials and methods: Human cardiac progenitor cells isolated from right auricle biopsies and HUVEC were exposed to 0.25 mM palmitate up to 24 h, with or without pretreatment with the DPP-IV inhibitor saxagliptin (0.05 mM). Gene expression was evaluated by quantitative RT-PCR. Expression and phosphorylation levels of the proteins under investigation were evaluated by immunoblotting techniques. Cell apoptosis was detected by measurements of caspase-3 cleavage and cytosolic release of oligosomes.
Results: Expression of DPP-IV in HUVEC and hCPC was confirmed at the mRNA level by quantitative RT-PCR, and at the protein level by immunoblotting and immunofluorescence. Exposure of HUVEC to 0.25 mM palmitate for up to 24 h resulted in reduced Akt phosphorylation, increased expression of the adhesion molecule ICAM-1 ($p<0.05$) and of the pro-apoptotic mediator Bax ($p<0.05$), and was associated with a 5- to 7-fold increase in cellular apoptosis ($p<0.05$). Continuous long-term exposure to palmitate also increased apoptosis of hCPC by 5-fold ($p<0.05$). Pretreatment with the DPP-IV inhibitor saxagliptin (0.05 mM) for short times (5 to 15 min) resulted in a dose-dependent increase in the phosphorylation of Akt, Erk-1/2 and eNOS in HUVEC, and of Akt in hCPC ($p<0.05$). Importantly, both in HUVEC and hCPC exposed to palmitate, saxagliptin restored Akt phosphorylation and almost completely abrogated palmitate-induced apoptosis ($p<0.05$); however, this response required preincubation of the cells for at least 2 h, whereas shorter times were ineffective. Pretreatment with the GLP-1 receptor agonist exendin-4 (20 nM for 2 h) resulted in inhibition of palmitate-induced apoptosis in HUVEC, and this effect was abrogated in the presence of the GLP-1 receptor antagonist exendin 9-39. However, pretreatment of the cells with exendin 9-39 did not alter the ability of saxagliptin to counteract palmitate-induced apoptosis.
Conclusion: Saxagliptin prevents palmitate-mediated apoptosis of HUVEC and hCPC, thereby augmenting the potential for vessels and heart regeneration under conditions of lipotoxicity. Even though saxagliptin can activate Akt signaling within minutes, inhibition of cellular DPP-IV for at least 2 h is required to observe the anti-apoptotic effect of the drug, which appears to occur independently of the GLP-1 receptor. Altogether, these findings suggest that saxagliptin may promote cell survival via increased bioavailability of non-GLP-1 peptides.
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Exendin-4 counteracts palmitate-induced apoptosis by inhibiting de novo production of ceramides in human cardiac progenitor cells
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Background and aims: Ceramides have been suggested to promote lipotoxicity-induced apoptosis in various cell types. Increased apoptosis of cardiomyocytes and cardiac progenitor cells in response to metabolic and oxidative stressors has been proposed as a mechanism of myocardial damage and dysfunction. Glucagon-like peptide-1 (GLP-1) and GLP-1 receptor (GLP-1R) agonists have been shown to exert prosurvival effects on cardiac cells. The aim of this study was to investigate the mechanisms of fatty acid-mediated apoptosis and the protective effects of the GLP-1R agonist exendin-4 in human cardiac progenitor cells (hCPCs).
Materials and methods: Human cardiac progenitor cells were isolated from right auricle biopsies and exposed to 0.1 to 0.5 mM palmitate up to 24 h, with or without pretreatment with 20 nM exendin-4 for 16 h. Expression and activation levels of the proteins under investigation were evaluated by immunoblotting techniques. Cell apoptosis was detected by TUNEL assay and by the evaluation of caspase-3 activation, caspase-3 cleavage and cytosolic release of oligosomes. Intracellular ceramide content was evaluated by fluorescent microscopy. Fumonisin-B1 was used to inhibit ceramide synthase (CerS5), a key enzyme in ceramide generation. Silencing of CerS5 was obtained by specific siRNA sequences. Exendin 9-39, a GLP-1R antagonist, was used as a competitive inhibitor of exendin-4.
Results: Palmitate led to apoptosis of hCPCs in a dose- and time-dependent manner ($p<0.05$). In hCPCs, palmitate induced significant increases in both intracellular ceramide content and mRNA and protein levels of CerS5. Conversely, palmitate did not modify gene and protein expression of serine palmitoyltransferase and dihydroceramide desaturase 1, other enzymes involved in de novo ceramide synthesis. Co-incubation of hCPCs with fumonisin-B1 prevented ceramide accumulation and partially inhibited palmitate-induced apoptosis. Moreover, knockdown of CerS5 with specific siRNAs partially protected hCPCs from the apoptotic effects induced by palmitate. When cells were pretreated with the GLP-1R agonist exendin-4, the palmitate-induced increase in ceramide levels was prevented ($p<0.05$), and cell apoptosis was coordinately reduced ($p<0.05$). Furthermore, exendin-4 prevented the increase in CerS5 expression, both at the gene and protein levels, which occurred in response to palmitate. The protective effects of exendin-4 were abolished when cells were co-incubated with the GLP-1R antagonist exendin 9-39.
Conclusion: Palmitate induces CerS5, a key enzyme of de novo ceramide synthesis, and this results in increased cellular content of ceramide and apoptosis of hCPCs. The GLP-1R agonist exendin-4 prevents palmitate-mediated increase in CerS5 expression, thus counteracting palmitate-induced ceramide accumulation and cell apoptosis. Amelioration of lipotoxicity via ceramide modulation may contribute to the cardioprotective effects of GLP-1R agonists in individuals with type 2 diabetes.
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Incretin-based treatments suppress human macrophage foam cell formation and VSMCs migration and proliferation contributing to atherosclerosis prevention
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Background and aims: We previously reported the suppressive effects of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) on macrophage-driven atherosclerosis in apolipoprotein E-deficient (Apoe$^{-/-}$) mice. Our present study investigated the suppressive effects of these incretins as well as GLP-1 analogs on human macrophage foam cell formation and human vascular smooth muscle cells (VSMCs) migration and proliferation in relation to atherosclerotic lesion prevention in Apoe$^{-/-}$ mice.
Materials and methods: Receptors for GLP-1 and GIP in human VSMSc, monocytes and macrophages were measured with real time PCR. GLP-1 (10

nmol/L), GIP (1 mol/L) or exendin-4 (100 nmol/L) was added to the cultured medium of human monocytes and adherent monocytes were incubated for 7days.Cellular lipids were extracted and the radioactivity of the cholesterol [3H]oleate and cholesterol esterification assay was measured by thin-layer chromatography.CD36 in macrophages was measured by Western blot. VSMCs were seeded and cultured in smooth muscle cell basal medium(SmBM) and the cells were exposed to 5'-bromo-2'-deoxyuridine(BrdU). VSMCs were incubated for 48~72 hours with platelet-derived growth factor-BB(PDGF-BB)and the indicated concentrations of GLP-1 or GIP. BrdU-positive cells were visualized by immunostainning and determined under the microscope. Cell migration was measured using modified Boyden chambers. VSMCs were plated and incubated for 20 hours with PDGF-BB and the indicated concentrations of GLP-1 or GIP. Migration was quantified by counting the number of stained cells. Apoe-/- mice at 17-week-old were infused of GLP-1(2.2 nmol/ kg/ day), GIP(25nmol/kg/day) or liraglutide(107nmol/kg/day) for 4 weeks and were measured their aortic atherosclerotic lesions.

Results: Receptors for GLP-1 and GIP were expressed in human VSMCs, monocytes and macrophages.GLP-1 (10 nmol/L), GIP (1 nmol/L) or exendin-4 (100 nmol/L) significantly suppressed oxidized low-densitylipoprotein(oxLDL)-induced foam cell formation mainly via CD36 down-regulation in human monocyte-derived macrophages. GLP-1(1nmol/L),GIP(1nmol/L),exendin-4 (100 nmol/L) and liraglutide (100 nmol/L) significantly suppressed the proliferation of VSMCs, via the individual receptors. GLP-1(1nmol/L), GIP(1nmol/L) suppressed the migration of VSMCs. Above experiments were performed with the treatment of dipeptidyl peptidase-4 inhibitors to prevent the degradation of GLP-1 and GIP. Infusion of GLP-1 or GIP into Apoe$^{-/-}$mice significantly suppressed the occupied areas of migrated monocytes/macrophages and proliferative phenotype of VSMCs within aortic atherosclerotic lesions. Further, infusion of liraglutide into Apoe$^{-/-}$mice significantly suppressed aortic atherosclerotic lesions associated with the suppression of oxLDL-induced foam cell formation and CD36 expression in macrophages.

Conclusion: Incretin treatments suppress human macrophages foam cell formation and VSMCs migration and proliferation, leading to the prevention of atherosclerotic lesion development.

OP 27 Pregnancy: GDM diagnosis and foetal outcomes

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Gestational diabetes mellitus (GDM) using the new IADSPG criteria: relative importance of different abnormal glucose values and evaluation of risk factors

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Background and aims: The adoption of the new IADPSG diagnostic GDM criteria based on the HAPO study has significantly increased its prevalence. An interesting finding of this study was the observed differences in the relative diagnostic importance of the fasting (FPG) or one of the two post glucose values, which may give the opportunity for a simplification of the testing procedure.The aim of this study was to apply retrospectively the IADPSG GDM criteria on a large sample of pregnant women and to evaluate (a) the contribution of different abnormal glucose OGTT values on GDM diagnosis; (b) the significance of risk factors on GDM development

Materials and methods: Over a period of 15 years, at a tertiary hospital, 9829 pregnant women without known DM underwent a 100g or 75g OGTT in the third trimester of pregnancy. For GDM diagnosis the IADPSG criteria were applied. Age, pre-pregnancy BMI, family history (FH) of DM, parity, education status and smoking were recorded. For statistical analysis stepwise logistic regression was applied and odds ratios (OR-95%CI) calculated.

Results: 5300/9829 (53.9%) women presented with GDM, using IADPSG criteria vs 33% with the previous ADA-2000 criteria. The percentage of GDM diagnosed by each glucose measure in the OGTT were as follows: FPG≥threshold without regard to 1-h and 2-h value=56%, 1-h PG≥threshold without regard to 2-h value and FPG<threshold=34% and only 2-h PG≥threshold=10%. Further, independent risk factors for GDM prevalence, using a stepwise logistic regression model, were: BMI>25 kg/m2 OR=1.99 (1.82-2.17), Age>30y OR=1.98 (1.81-2.16), Maternal FH OR=1.73 (1.48-2.02), Paternal FH OR=1.34 (1.16- 1.56). When all the four risk factors were present in a woman the possibility to develop GDM was calculated to 83.3%. Parity, education status and smoking were not found to be risk factors for GDM.

Conclusion: The adoption of the new IADPSG criteria for GDM diagnosis, as expected, increased significantly the prevalence of GDM. As 90% of GDM women in our center were diagnosed based on the abnormal values of fasting and 1-h samples, it seems reasonable to consider omitting the 2-h sample for patient convenience and cost effectiveness. Further, this large study evaluated the importance of risk factors for GDM prevalence independently and showed that BMI>25 kg/m2 and age>30y are of equal value, while the family history of DM on the maternal side is more important risk factor than on the paternal side

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Maternal insulin resistance affects foetal brain activity

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Background and aims: Fetal programming plays an increasingly important role in the attempt to explain the pathogenesis of metabolic syndrome and type 2 diabetes mellitus. Recent studies underline the importance of the insulin resistance of the central nervous system in this process. The aim of the present study was to investigate whether maternal metabolic changes during OGTT (oral glucose tolerance test) have an impact on fetal brain activity in humans.

Materials and methods: 14 healthy pregnant women (mean gestational age 31 ± 3 weeks) underwent an oral glucose tolerance test (75g). Glucose and insulin levels were measured after 0, 60 and 120 minutes, and insulin resist-

ance (HOMA IR) was calculated. Each blood extraction was preceded by fetal brain activity recordings on a fetal magnetoencephalographic device (fMEG). Response latencies of auditory evoked fetal brain responses were extracted.
Results: During OGTT, insulin increased roughly 15 fold and glucose 2 fold 60 minutes after glucose ingestion as expected. A MANOVA was performed to test the effect of time during OGTT on response latencies There was a marginally significant main effect of time on response latency ($F(2)=2.89$, $p=0.073$) resulting in a delay of response to the auditory stimulus during OGTT from baseline to 60 minutes after glucose administration (0.043 post hoc test). Furthermore, there was a positive correlation between insulin resistance of the mother and auditory evoked response at 60 minutes after glucose ingestion ($p=0.018$, $r2=0.34$). The auditory evoked response showed also a positive correlation of the mothers insulin level 60 minutes after glucose ingestion and the corresponding auditory evoked response of the fetus ($p=0.022$, $r2=0.31$).
Conclusion: Higher maternal insulin resistance leads to slower fetal brain responses in postprandial conditions suggesting that the genesis of central insulin resistance may occur already during fetal development.

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Maternal glucose variability and neonatal metabolic outcome in women with pregnancy complicated with type 1 diabetes
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Background and aims: Diabetic pregnancy is associated with impaired perinatal outcome also in women who improved their metabolic control throughout gestation. Therefore, novel markers of metabolic control are tested for their usefulness in diabetic pregnancy. In our study, we investigated whether there is any association between maternal parameters of glucose variability (GV) and metabolic outcome in newborns born to women with type 1 diabetes mellitus (T1DM).
Materials and methods: A prospective observational study including N=85 women with T1DM, in early singleton pregnancy. In all participants, 18-point daily glycaemic profile was taken once in each trimester and GV was calculated for each trimester. Upon delivery, following parameters were taken in newborn: birth weight, neonatal glycaemia (measured within six hours after delivery), umbilical cord insulin, glucose and IGFBP levels (collected immediately after delivery). Then, we sought for associations between GV (mean, SD, mean amplitude of glycaemic excursion-MAGE, JINDEX, low blood glucose index-LBGI and high blood glucose index-HBGI) and data collected from newborns.
Results: Characteristics of the study group: gestational age at booking: 13.5±7.5 weeks, HbA_{1c} at booking: 7.7±1.1%, prepregnancy body weight: 63.8±12.7 kg, prepregnancy BMI: 23.7±4.3 kg/m2, microvascular complications: N= 42 (49.4%). We did not find any association between neonatal characteristics and GV from Ist and IInd trimester. We found significant correlations (for all, $p<0.05$) between cord insulin and IIIrd trimester mean, SD, JINDEX and HBGI (Spearman's coefficient: 0.30, 0.26, 0.32, 0.32, respectively), between cord insulin/ glycaemia ratio and IIIrd trimester mean, SD, JINDEX and HBGI (Spearman's coefficient: 0.33, 0.27, 0.33, 0.29, respectively), between neonatal glycaemia and IIIrd trimester mean and JINDEX (Spearman's coefficient: 0.26, 0.25, respectively) and neonatal glucose/ insulin ratio and IIIrd trimester mean, SD, JINDEX and HBGI (Spearman's coefficient: 0.39, 0.32, 0.39, 0.36, respectively). Neonatal hypoglycaemia was associated with significantly higher mean and JINDEX (for all, $p<0.05$). Birth weight above 4000g was associated with significantly lower LBGI ($p<0.05$).
Conclusion: Maternal GV is associated with increased markers of poor metabolic neonatal outcome in women with T1DM, confirming the role of proper and stable glucose levels during diabetic pregnancy.
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Parameters of maternal glucose variability as predictors of birth weight and neonatal metabolic status in pregnancy complicated with gestational diabetes mellitus
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Background and aims: Gestational diabetes mellitus (GDM) is a perinatal complication of increasing prevalence. As neonatal risk is elevated also in women who managed to improve their metabolic control throughout gestation, different maternal characteristics are studied to find out whether they can add to prediction and prevention of neonatal complications in this population.
Materials and methods: A prospective observational study including N=115 women with GDM, in singleton, viable pregnancy, referred to our Department for further treatment. In all participants,18-point daily glycemic profiles were taken in the IIIrd trimester and parameters of glucose variability (GV) were calculated using EasyGV version 8.7 software. Upon delivery, following parameters were taken in newborn: birth weight, neonatal glycemia (measured within six hours after delivery), umbilical cord c-peptide, insulin, glucose and IGFBP levels (collected immediately after delivery). Then, we investigated whether GV (mean, SD, mean amplitude of glycemic excursion-MAGE, M-value, JINDEX, low blood glucose index-LBGI and high blood glucose index-HBGI) can serve as predictors for neonatal metabolic status.
Results: Characteristics of the study group: gestational age at booking: 28.5±7.5 weeks, HbA1c at booking: 6.5±1.1%, prepregnancy body weight: 69.5±16.0 kg, prepregnancy BMI: 26.5±6.0 kg/m2, insulin therapy: N=38 (33.0%), prepregnancy obesity: N=36 (31.3%). IIIrd trimester LBGI and M-value were significant predictors for birth weight above 4000g (for LBGI: AUC 0.74, $p<0.001$, sensitivity 80.0%, specificity 64.9% for a cut-off value 3.18, for M-value: AUC 0.74, $p<0.001$, sensitivity 70.0%, specificity 76.3 for a cut-off value 3.44). None of studied GV parameters were predictive of neonatal hypoglycemia (glucose level below 40 mg/dL/ 2.2 mmol/L), low cord glycemia (measured as below 25th percentile for a study group), high c-peptide cord level (measured as above 75th percentile for a study group) or high/ low IGFBP cord level (measured as above 75th/ below 25th percentile for a study group).
Conclusion: 1/ short term maternal glucose variability measured in the last trimester has an impact on fetal growth in women with GDM.

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Decreased cord blood serum dipeptidyl-peptidase 4 (DPP4) enzymatic activity in gestational diabetes mellitus
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Background and aims: Tissue-specific DPP4 dysregulation in adults with diabetes mellitus including that diabetes reduced DPP4 activity in the bone marrow stem cell niche was described. Alterations of the DPP4-incretin axis have not been studied in fetal life in gestational diabetes mellitus (GDM). We have assessed the DPP4 activity and the GLP-1 hormone levels in cord blood samples taken at delivery of healthy and GDM pregnancies.
Materials and methods: One hundred-twenty five pregnant women were enrolled to the study after signing the informed consent: 54 women with GDM and 71 age-matched pregnant females with normal glucose tolerance (age: Mean±SD: GDM: 32.6±5, Controls: 31.6±5.1 years). Obviously there were significant ($p<0.05$, independent T-test) differences between the GDM and control groups in the OGTT values assessed on the 24th-28th gestational week (plasma glucose 0': 4.47±0.54 vs 4.75±0.62 mmol/L; 60': 6.72±1.6 vs 9.52±0.6 mmol/L; 120': 5.54±1.14 vs 8.04±1.46 mmol/L in the control and GDM group, respectively). Women with GDM gained more weight during pregnancy than controls (13.8±4.8 vs 10.3±6.3 kg, $p=0.0005$, independent T-test), but neither in the pregestational BMI nor in the neonatal birthweight were differences detected between the two groups. There were 2 twin pregnancies in the GDM group. DPP4 enzymatic activity was measured in human cord blood serum at 37°C in continuous monitoring microplate based kinetic assay on Varioskan Flash (Thermo Scientific) reader at 405nm for 30 min using Gly-Pro-PNA (Bachem) as substrate. Results were expressed in unit per liter (U/L). Cord plasma samples were taken in tubes containing EDTA, pro-

tease inhibitor cocktail (Sigma-Aldrich) and sitagliptin to measure biologically active forms of plasma GLP-1 [GLP-1 (7-36 amide) and GLP-1 (7-37)]. Fluorescence ELISA assay (GLP-1 active kit, Millipore) was used under conditions as suggested by the manufacturer on a Varioskan Flash ELISA reader. For statistical analysis Shapiro-Wilks, Mann-Whitney-U (MW-U) and independent T-tests were used.
Results: DPP4 activity in human cord serum samples from GDM pregnancies (n=56) was significantly lower than that of controls (n=71) (Mean±SD: 29.31±10.37 U/L vs 35.84±12.8 U/L, MW-U p=0.0017). There was no difference in the serum cord blood DPP4 activity within the GDM group when the insulin treated (12 pts) and non-insulin treated (32 pts) groups were compared (27.71±7.08 U/L vs 30.39±11.5 U/L, p=0.64). The level of biologically active GLP-1 hormone was significantly higher in the cord plasma of neonates born from GDM pregnancies (Mean±SD: 4.99±2.95 pM) than that of controls (3.07±1.39 pM, p=0.022, MW-U). Due to the smaller set of samples in GLP-1 measurements (GDM n=11, control n=21) applied additional cord plasma samples should be assessed and be analyzed for potential correlations with other data.
Conclusion: To our knowledge this is the first study that assessed the DPP4 activity and GLP-1 hormone levels in cord samples of neonates born from GDM and healthy pregnancies. The decreased serum DPP4 activity in neonates from GDM pregnancies might be the result of an adaptive fetal response or part of an early dysregulation in entero-insular axis in fetal life. The consequences of decreased DPP4 activity might be beyond the alterations of incretin hormone levels.
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Earlier management with continuous monitoring blood glucose decrease gestational weight gain in gestational diabetes mellitus: a randomised clinical trial
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Background and aims: To examine the effectiveness of continuous glucose monitoring on maternal glycemic control, gestational weight gain, infant birth weight in women with gestational diabetes mellitus.
Materials and methods: A total of 106 pregnant women at 22-28 gestational weeks diagnosed with gestational diabetes mellitus (GDM) were randomly allocated to have continuous glucose monitoring system (CGMS, n =51) or self-monitoring blood glucose (SMBG, n = 55), based on similar dietary, aerobic exercise and antenatal care. In 24 out of 51 used CGMS during second trimester, the rest (n =27) used during third trimester. The primary outcome was maternal glycemic control, total weight gain and drug-treated proportion. Birth weight, Apgar score and customized birth weight centiles were defined as secondary outcome. Statistical analyses were done on an intention to treat basis.
Results: Women randomized to continuous glucose monitoring had lower gestational weight gain compared with women randomized to self-monitoring blood glucose (difference between groups, p =0.040). The data also found that wearing CGMS during second trimester lead to lower proportion maternal weight gain than those during third trimester, p =0.018).In patients monitored with insulin-treated was introduced (16/51) whereas only 7/55 in the self-monitoring group were drug-treated (difference between groups, p = 0.032). There were no statistically significant differences between the groups regarding maternal age, family history of diabetes, hypertension in pregnancy, pre-pregnancy BMI, HbA1c, fasting plasma insulin, fasting C-peptide, postprandial 2h C-peptide , total cholesterol, HDL cholesterol, triglycerides and gestational weeks at delivery, Apgar score, infant birth weight or neonatal hypoglycemia.
Conclusion: Continuous glucose monitoring system, especially used during second trimester is associated with lower gestational weight gain, a higher proportion of insulin-treated patients were found using CGMS.
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OP 28 Replacement of beta cell mass and function

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Conditional expression of a constitutively active EGF-R in beta cells protects against diabetes
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Background and aims: EGF-R signalling is required for normal pancreatic beta-cell development and proliferation. The beta-cell specific dominant negative EGF-R mice (E1-DN) become diabetic two weeks after birth with a 70-85 % reduced beta-cell mass. In the present study we investigated the effects of a constitutively active EGF-R on beta cell replication, mass and survival.
Materials and methods: We generated transgenic mice with tetracycline-inducible expression of a constitutively active EGF-R in beta cells (INS-CA-EGFR). This was done by crossbreeding INS-rtTA mice with TetOP-hEGFR-L858R mice. The double transgenic mice were given doxycycline-containing (DOX) diet to activate the transgene. Mice of different ages (E12.5-3 months) were given DOX for different time periods (1-9 months) and beta-cell proliferation and mass were analysed. Furthermore, the mice were challenged either with a single or multiple low doses of streptozotocin (MLDS) followed by blood glucose and insulin monitoring. Isolated islets from INS-CA-EGFR and INS-rtTA DOX-treated mice were exposed to cytokines (IL-1β, IFNγ, Tnf-α), and cell death was evaluated by live-dead assays. The expression profiles of selected genes were studied by quantitative RT-PCR.
Results: DOX administration and thus CA-EGFR expression from E12.5 to P30 increased beta cell volume as analysed by optical projection tomography (insulin positive volume 1,6% vs 1,1%; $p < 0.05$; $n = 6$). When DOX was given to adult mice during one or three months, there was no significant increase in beta cell proliferation or beta cell mass. Expression of the transgene improved survival and glycemia of the INS-CA-EGFR mice after a single injection of STZ: at two weeks post-injection 90% of the CA-EGFR mice were alive (mean blood glucose 21,4 mM), whereas only 60% of the STZ-injected control mice survived (mean blood glucose 33,2 mM, $p < 0.01$). Furthermore CA-EGFR improved glycemia and reduced lymphocyte infiltration after MLDS treatment (cumulative percentage of diabetes 100% in the control group vs 43% in INS-CA-EGFR mice, $p < 0.01$). In addition CA-EGFR protected isolated mouse islets from cytokine induced beta cell death (after 48h cytokine stimulation 9% dead cells vs 19% in control islets, $p < 0.05$; $n = 6$), which was likely mediated through repression of the proapoptotic protein Bim (1.7 vs. 1.0 fold induction in control vs. INS-CA-EGFR islets, $p<0.05$).
Conclusion: Expression of constitutively active EGFR in the developing pancreas, but not in adult pancreas, increases beta cell mass. Interestingly, EGF-receptor mediated signalling protects beta cells against streptozotocin- and cytokine-induced cell death.

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PTP1B a key player in the improvement of survival of transplanted islet grafts
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Background and aims: Pancreatic islet transplantation is recognized as a hopeful and potentially curative treatment for type 1 diabetes, however clinically it remains limited by a post-transplantation massive islet loss and/or graft failure. Protein tyrosine phosphatases contribute for the regulation of critical phospho-tyrosine levels in the signal transduction pathways essential for intracellular signalling, cell growth, apoptosis or gene transcription. Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of insulin, leptin and growth-factor signalling. Recent studies have shown that PTP1B also negatively regulates angiogenic signalling. In view of these data, we hypothesised that PTP1B inhibition might be involved in graft survival and revascularisation and therefore constitute a potential therapeutic target for islet transplantation protocols.

Materials and methods: Islets isolated from wild-type and PTP1B-knockout mice were transplanted (150-200 islets/animal) into the anterior chamber of the eye of BALB/c diabetic mice (single injection of streptozotocin - 160mg/Kg, 8 days before transplantation). The animals were divided into 3 groups (n=9 animals per group): T - transplanted with wild-type isolated islets; T - KO - transplanted with PTP1B-KO isolated islets; CTRL - non-transplanted. In vivo studies were performed during 25 days of treatment to evaluate weight, glycaemic levels, and after treatment accessing cell viability and islet graft revascularisation. Post-mortem morphometric analysis and functional studies were conducted on graft-containing eyes.
Results: The T group exhibited a slight decrease in glycemia as compared to the CTRL group (T:418±48mg/dL, CTRL:528±20mg/dL, p<0.05). Remarkably, the T-KO group showed a more robust decrease in glycaemia (211±26 mg/dL) both when compared to the T group (49% decrease, p<0.01) or to the CTRL group (60% decrease, p<0.01). In vivo propidium iodide injection revealed a 73% decrease in cell death in the T-KO relative to the T group (0.25% and 0.95%, respectively; p<0.001). Concerning islet revascularization (functional vasculature), accessed by in vivo dextran injection, a 58% increase in vascular density (0.0297±0.0035% versus 0.0187±0.0053%; p<0.05) as well as a 2-fold increase in vascularisation area (23.180±1.9% versus 10.027±2.6%; p<0.001) was found for the T-KO when compared to the T group. In accordance with these findings, morphometric analyses of the graft-containing eyes revealed a 3-fold increase in the percentage of endothelial cells on islets from the T - KO group when compared with the T group (0.121±0.0096% and 0.0351±0.005%; p<0.001). No differences were found regarding proliferation rates measured by Ki-67 immunostaining.
Conclusion: Our results support the hypothesis that PTP1B plays a negative role on the survival and revascularisation of islet grafts, and may be a potential target for therapeutic treatment after islet transplantation. Future work will focus on further unravelling the molecular mechanism involved in these findings.
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Acetyl-CoA carboxylase 1 is a critical regulator of beta cell mass
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Background and aims: An adequate, functional beta cell mass is essential to maintain glucose homeostasis. Beta cells sense glucose and trigger insulin secretion using oxidative mitochondrial pathways. A distinctive feature of beta cells is the relatively high proportion of glucose-derived pyruvate entering mitochondrial anaplerotic pathways (non-oxidative), driving citrate export and cytosolic acetyl-CoA production, supporting the concept of an alternative glucose-sensing pathway. Acetyl-CoA Carboxylase-1 (ACC1) is the cytosolic, citrate-activated enzyme, coupling glucose metabolism to lipid biosynthesis by catalysing malonyl-CoA production from acetyl-CoA. We sought to test the role of this ACC1-coupled pathway in vivo.
Materials and methods: Beta cell ACC1 activity was ablated in mice using Cre/loxP mediated gene deletion.
Results: Constitutive ACC1-ablation in beta cells using Ins2-cre (bACC1KO) caused impaired in vivo glucose-stimulated insulin secretion (GSIS) and glucose intolerance (mean±SEM blood glucose excursion after 2g kg-1 i.p. bolus: cre-control 2023±76, bAcc1KO 2470±64, mmol l-1 120min-1; P<0.001 by unpaired 2-tailed t-test; n=17/29), with no effect on insulin action nor adiposity. Surprisingly, in vitro GSIS from sized-matched isolated islets was normal. Histological analysis revealed a marked reduction in beta cell mass in bACC1KO pancreata (cre-control 2.203±0.169, bACC1KO 1.042±0.107, mg; P<0.01; n=3), due to a reduction in both beta cell size and proliferation. Finally, we utilised a tamoxifen-inducible system to delete ACC1 in the beta cells of adult mice: this caused glucose intolerance four weeks post-ACC1 ablation, indicating that ACC1 plays an active role in maintaining a functional beta cell mass.
Conclusion: Our data reveal a critical role for ACC1, and potentially glucose-driven lipid synthesis, in regulating beta cell mass.
Supported by: Diabetes Australia Research Trust

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The LRH-1 agonist BL001 improves human islet survival and blunts development of hyperglycaemia in the RIP-B7.1 mouse model of experimental autoimmune diabetes
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Background and aims: We have previously shown that the orphan nuclear receptor LRH-1 is expressed in human and mouse islets and that its over expression protects β-cells against stress-induced apoptosis. Herein we evaluated whether a small LRH-1 agonist codenamed BL001 could improve human islet survival and function as well as to prevent development of hyperglycaemia in the RIP-B7.1 mouse model of experimental autoimmune diabetes (EAD).
Materials and methods: Mouse and human islets were pre-treated with 10 μM BL001 for 24 hours and subsequently exposed to cytokines for an additional 24 hours prior to assessing apoptosis. Insulin secretion in response to 16.5 mM glucose was measured on islets derived from healthy and Type 2 diabetic (T2D) human donors that were treated or not with BL001. For in vivo studies, RIP-B7.1 mice were pretreated with BL001 (10 mg/Kg body weight) once daily for 5 days prior to preproinsulin cDNA vaccination to induce the autoimmune response. Animals were then injected daily for up to 8 weeks with BL001 and blood glucose levels were measured weekly. Pancreas and spleen were extracted at various time points to performed histological studies and splenocyte proliferation assays.
Results: Cytokine-treated human and mouse islets exhibited a 60% increase in apoptosis as compared to control islets. In contrast, islets pretreated with BL001 were refractory to cytokine-induced apoptosis. Of note, control islets treated exclusively with BL001 exhibited a 40% reduction in cell death suggesting an improved viability subsequent to isolation. We next evaluated whether BL001 could ameliorate impaired insulin secretion of T2D islets as compared to healthy islets. The stimulatory index (SI, glucose-stimulated/basal insulin secretion) of T2D islets was 1.5 as compared to 2 for healthy islets confirming impaired insulin secretion. However, in the presence of BL001 the SI of T2D islets increased to 2 whereas the index of healthy islets remained constant. The impact of BL001 was then assessed in the RIP-B7.1 animal model of EAD. Within 8 weeks post vaccination, 60% of vehicle-treated animals developed hyperglycaemia whereas only 20% of BL001-treated animals exhibited high blood glucose. Autoreactive T-cells were detected in normoglycaemic BL001/vaccinated mice confirming that these animals mounted a proper immune response. Histological analyses performed on pancreas sections from normoglycaemic BL001/vaccinated animals revealed marginal insulitis as compared to hyperglycaemic vaccinated mice. These results suggest that BL001 treatment blunted the diabetes-prone detrimental immune response by regulating local inflammation.
Conclusion: Activation of LRH-1 using the small synthetic agonist BL001 rescues function of cultured human T2D and prevents development of hyperglycaemia in RIP-B7.1 mice, a process that appears to partly involve modulation of the local immune response. We thus provide proof-of-concept that LRH-1 is an attractive druggable target for the treatment of diabetes.
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Insulin receptor internalisation and Erk signalling require caveolin-1 in pancreatic beta cells
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Background and aims: Type 2 diabetes is associated with defects in insulin receptor (InsR) signalling and a loss of functional beta-cells. The overall goal of this study was to understand the mechanisms by which insulin transmits its signals in beta-cells. It has been shown that internalization of the activated InsR complex is necessary to initiate full insulin signalling, but this process in beta-cells remains poorly understood. Our aim was to determine the proteins

involved in InsR internalization and elucidate their roles in InsR signalling, with a particular focus on Caveolin-1 (Cav1) which has been implicated in InsR traffic in other cell types.
Materials and methods: The InsR was labelled with monomeric fluorescent proteins in a non-critical linker region between the furin-like region and the transmembrane domain. InsR trafficking in MIN6 beta-cells was monitored using confocal microscopy and fast live cell total internal reflection microscopy (TIRFM). Super-resolution stimulated emission depletion (STED) microscopy was applied to resolve the organization of membrane nanodomains harbouring the InsR. InsR signalling was addressed by western blotting. In vivo effects of Cav1 deficiency were examined using systemic Cav1 knockout mice.
Results: TIRFM in living cells revealed that Cav1 is recruited to InsR membrane domains immediately before receptor internalization. To investigate the role of Cav1, we compared the plasma membrane content of the InsR in cells expressing Cav1 mutants of the Y14 tyrosine phosphorylation site. MIN6 cells over-expressing dominant negative Cav1-Y14F showed defective InsR internalization, with significant accumulation of InsR at the plasma membrane. Conversely, cells expressing the phosphomimetic Cav1-Y14D mutant had excess InsR internalization into large vesicles, also strongly suggesting a requirement for Cav1 in InsR internalization. Insulin stimulated Erk phosphorylation was decreased in MIN6 cells expressing the dominant negative mutant Cav1-Y14F. Conversely, Erk phosphorylation was enhanced in cells expressing Cav1-wt and Cav1-Y14D. Interestingly, insulin stimulated Akt phosphorylation was not affected by the overexpression of wild-type or mutant Cav1. siRNA mediated knock-down of Cav1 confirmed the requirement of Cav1 in Erk but not Akt signalling. These results were further validated by the analysis of Erk translocation in beta-cells of pancreatic sections obtained from Cav1 deficient mice. Interestingly, these mice display a significantly reduced beta-cell mass compared to littermate controls. These findings support a role for Cav1 in the mitogenic effects of insulin-induced Erk activation on beta-cells.
Conclusion: We identified Cav1 as a key protein in InsR trafficking and signalling. We discovered that Cav1 phosphorylation is specifically required for InsR internalization and the subsequent activation of Erk signalling. Our data support a model wherein Akt mediated InsR signalling occurs from receptors located at the plasma membrane and is therefore independent of Cav1 mediated InsR internalization. We demonstrated that Cav1-mediated InsR signalling plays a critical role in the maintenance of adult beta-cell mass. Together, our data provide new molecular insights into the mechanisms of insulin receptor trafficking in pancreatic beta-cells.
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Protection from diabetes in mice with genetically engineered K-cells expressing modified proinsulin
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Background and aims: Several cell sources have been investigated for the development of surrogate insulin producing pancreatic beta-cells. The majority of candidate cell types do not have the machinery for proinsulin processing and meal regulated insulin secretion. Enteroendocrine K-cells line the intestinal epithelium and produce glucose-dependent insulinotropic polypeptide (GIP) and may be a good candidate for engineering to restore endogenous insulin production. In beta-cells, proinsulin is fully processed into mature insulin via prohormone convertases (PC) 1/3, PC2 and carboxy-peptidase E. However, unlike beta-cells, it has been shown that the majority of K-cells do not express PC2. The aim of this study was to optimize the processing of proinsulin in K-cells using a genetically modified insulin gene.
Material and methods: We generated transgenic mice with a transgene consisting of a rat GIP promoter linked to a modified proinsulin gene. Using a PCR based mutagenesis strategy, we changed the P4 residue in the proinsulin PC1/3 site from methionine to lysine to enhance PC1/3 cleavage at this site, and changed two residues in the PC2 site to resemble a PC1/3 cleavage site. Mice harbouring this transgene and their non-transgenic littermate controls were monitored for glucose homeostasis and body weight over one year, along with circulating GIP and insulin. An insulin tolerance test, oral glucose tolerance test and intraperitoneal glucose tolerance test were performed. Food intake, respiratory exchange rate, physical activity, and energy expenditure were assessed using metabolic cages. Lipid levels were profiled by measuring cholesterol, triglyceride and free fatty acid in collected plasma. The proportion of fat/lean tissues was evaluated with dual-energy X-ray absorptiometry. Protection from diabetes was assessed following injection with the pancreatic beta-cell toxin streptozotocin (STZ).
Results: Transfection experiments in gut cells (STC-1) with this GIP/Ins construct and Western blots of transgenic gut tissue validated proinsulin processing to mature insulin. Transgenic mice had equivalent body weight and insulin sensitivity to non-transgenic littermates but maintained significantly lower fasting blood glucose levels and dramatic improvements in glucose tolerance following either intraperitoneal or oral glucose. GIP levels were significantly lower in transgenic versus non-transgenic mice whereas insulin levels were ~50-fold higher, unchanged by STZ, and protected transgenic mice from STZ-induced diabetes. Isolated islets of transgenic animals were equally responsive to glucose stimulation but released less insulin at basal glucose concentrations compared to littermate islets. The transgene had no impact on body weight, fat/lean ratio, metabolic cages parameters and circulating levels of cholesterol, triglyceride and free fatty acids.
Conclusion: Our studies demonstrate simple genetic alterations to improve proinsulin processing and further support K-cells as candidate surrogate beta-cells for insulin replacement.
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OP 29 Genetics of renal and cardiovascular complications

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Genome-wide association studies for renal complications of type 1 diabetes

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Background and aims: Diabetic nephropathy (DN) is a major complication of diabetes and the leading cause of end-stage renal disease (ESRD) and there is evidence of a considerable heritable component. Hence we performed a meta-analysis of four genome-wide association studies (GWAS) of DN in type 1 diabetes (T1D) to identify genetic determinants of DN.
Materials and methods: DN was defined as microalbuminuria, macroalbuminuria or ESRD, whereas controls were defined as T1D patients with normoalbuminuria and T1D duration at least 15 years. GWAS data were available from four cohorts: FinnDiane (N=3,435), Eurodiab (N=812), Scania Diabetes Registry (SDR; N=513) and Cambridge (N=400), with ~2.4 million single nucleotide polymorphisms (SNPs) imputed with HapMapII CEU reference panel. Using logistic regression adjusted for age at onset of T1D, sex and duration of T1D, we studied the association of these SNPs with DN (2,563 cases and 2,597 controls in total) and with three additional case definitions: microalbuminuria (879 cases), the combination of macroalbuminuria and ESRD (1,743 cases), and ESRD alone (822 cases; Cambridge excluded due to low number of ESRD cases). Meta-analysis was performed using the fixed effects model.
Results: We identified several suggestive associations ($P<10^{-5}$). rs11123857, intronic in *NPAS2,* was associated with DN (OR=1.26, $P=7.3\times10^{-7}$) and microalbuminuria (OR 1.35, $P=4.8\times10^{-6}$). *NPAS2* is involved in the circadian rhythm pathway. Variants in *AFF3* were associated with ESRD (rs13405175, OR=2.04, $P=9.0\times10^{-7}$), as reported also in a recent meta-analysis including both the FinnDiane and the SDR studies. rs476865 between the *EN1* and *INSIG2* (insulin induced gene 2) was associated with the combined macroalbuminuria and ESRD phenotype (OR=0.69, $P=8.5\times10^{-7}$); SNPs in the same intergenic region have been suggestively associated with multiple traits including creatinine and LDL cholesterol. rs12640850 between the *RAPGEF2* and *FSTL5* was associated with microalbuminuria (OR=1.45, $P=1.2\times10^{-6}$), only 13kb away from a SNP earlier associated with VLDL cholesterol. We also examined SNPs previously reported for DN from GWAS or candidate gene studies, but only the association in *AFF3* remained significant after adjustment for multiple testing (four phenotypes and 35 SNPs, $P<0.0003$). However, this cannot be considered independent replication since the previous report of association at this locus included a subset of the same data.
Conclusion: We identified several putative loci for DN in T1D, but replication in independent studies is still required to confirm these findings. Imputation with 1000G reference panel may reveal more loci and help fine-map the implicated regions. Furthermore, functional analysis will be undertaken in SUMMIT to explain the affected biological pathways.
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The Haptoglobin 1 allele correlates with corpus callosum hyperintensities in individuals with type 1 diabetes

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Background and aims: Although numerous prospective investigations have confirmed a lower risk of coronary artery disease (CAD) among individuals with diabetes carrying the Haptoglobin (Hp) 1-1 genotype, we recently reported a direct association between Hp 1-1 and stroke incidence in a cohort of type 1 diabetes (T1D). Given previous evidence in patients with hypertension of a correlation between Hp and extent of cerebral deep white matter lesions, we conducted neuroimaging studies to evaluate the presence of differences in brain white and gray matter volume by Hp genotype in our T1D population.
Materials and methods: Participants from the Epidemiology of Diabetes Complications (EDC) study, a prospective investigation of childhood onset T1D, attending the 24 year EDC study follow-up were recruited for a neuroimaging study during 2010-2012. A brain Magnetic Resonance Imaging (MRI) was completed using a Siemens 12-channel head coil in a 3T Siemens Tim Trio MR scanner in 112 eligible and willing participants (mean age, 48.7 and diabetes duration, 41 years).
Results: Among the 112 eligible and willing to participate, data on Hp were available for 96, of which 15.6% and 39.6% were homozygous for the Hp 1 and Hp 2 allele, respectively. Despite the small sample size, a trend toward increased total white matter hyperintensities volume (WMH) was observed with an increasing number of Hp 1 alleles (p-trend=0.03, Table 1). This association appeared localized in the corpus callosum (p-trend=0.004), especially the posterior part (p-trend=0.006). This association, though attenuated, remained significant for the total corpus callosum (p-trend=0.04) after adjustment for diabetes duration, but again differences in the posterior (p-trend=0.02) appeared stronger than in the frontal portion (p-trend=0.11). In separate models in a restricted sample with complete covariate data, further allowing for waist to hip ratio, HbA1c, systolic blood pressure and HDL cholesterol did not change these findings. No significant differences by Hp were noted in gray matter areas.
Conclusion: Despite being inversely related to CAD risk in T1D, the Hp 1-1 genotype correlates with WMH localized in the inter-hemispheric connecting fibers of the corpus callosum in this population. WMH are common in adults >65 years but rare in younger groups. The presence of WMH in the brain of middle aged adults with T1D may thus indicate an accelerated aging process, which appears linked to the presence of the Hp 1 allele. Further, including mechanistic, studies on the role of the Hp genotype in cerebrovascular disease are needed to shed light on the pathophysiology of these associations and possible therapeutic interventions.

Table 1. Total white matter volume (voxels) and corpus callosum by Hp

White matter areas	Hp 1-1 (n=15)	Hp 2-1 (n=37)	Hp 2-2 (n=35)	p-value	p-trend*
White matter hyperintensities x10^4	12.527 (7.372, 24.582)	8.990 (6.144, 15.164)	8.946 (5.279,13.312)	0.19	0.03
Posterior corpus callosum x10^4	1.16 (0.21, 4.29)	0.92 (0.12, 2.29)	0.29 (0.02, 0.77)	0.04	0.006
Frontal corpus callosum x10^4	2.63 (1.34, 5.06)	1.86 (1.09, 3.12)	1.41 (0.78, 1.98)	0.05	0.01
Corpus callosum x10^4	3.92 (2.28, 8.45)	2.74 (1.33, 6.12)	2.01 (0.97, 2.98)	0.03	0.004

Data are presented as median (interquartile range) * Logarithmically transformed variables were used

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Known SNPs in ADAMTS7, the 9p21 region and UBE2E interact with type 2 diabetes status to modify the risk of coronary artery disease in large populations

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Background and aims: Patients with type 2 diabetes (T2D) are 3-4 times more likely to suffer from coronary artery disease (CAD) than individuals without T2D. Despite diabetic status contributing to the risk of CAD, no

overlapping loci between CAD and T2D have been identified by the largest genetic association studies to date. In this study, we aimed to: identify loci that modify the risk of CAD in patients with T2D; assess whether known CAD loci have a different effect on CAD risk in patients with T2D; and evaluate the influence of known T2D loci on CAD risk in non-diabetic individuals compared to patients with T2D.
Materials and methods: Summary statistics for 2,295,146 (directly typed and imputed) SNPs from 16,942 patients with T2D (6,022 CAD cases and 10,920 CAD free controls) and 28,727 non-diabetic individuals (10,892 CAD cases and 17,835 CAD free cases) were combined in a fixed effects meta-analysis and stratified by T2D status. The effects of 45 known CAD SNPs on CAD risk were tested for an interaction with T2D status and the effects of 61 known T2D SNPs on CAD were also tested for different effects in non-diabetic individuals compared to patients with T2D.
Results: The meta-analysis of SNP effects on CAD in patients with T2D identified associations in ADAMTS7 at genome wide significance that were represented by two independent SNPs previously reported for CAD. Rs11072811 (OR=1.17, effect allele frequency=0.53, proxy for rs7173743, p=3.9E-11) and rs11634042 (OR=1.15, effect allele frequency=0.58, proxy for rs4380028, p=5.7E-08) were associated with CAD in patients with T2D: rs11072811 had a smaller effect on CAD risk in non-diabetic individuals (OR=1.08, p=1.2E-02) when compared to its effect in patients with T2D, and this interaction with T2D status was nominally significant (p=3.5E-02). Atherosclerosis is a major underlying cause of CAD and is accelerated in patients with T2D. The meta-analysis of SNP effects in non-diabetic individuals also identified associations in the 9p21 region with CAD at genome wide significance. The signal was represented by rs1556516 (OR=1.17, effect allele frequency=0.48, p=2.9E-10) which is a proxy for the well-known CAD SNP rs1333049. Allelic effect sizes at this locus were smaller in patients with T2D (OR=1.10, p=3.9E-03), and the interaction with T2D status was nominally significant (p=4.2E-02). Investigation of the known T2D-risk loci revealed that the major allele (frequency=0.89) of rs7612463, in UBE2E2, decreased the risk of CAD in patients with T2D (OR=0.86, p=7.4E-03) but increased the risk of CAD in non-diabetic individuals (OR=1.11, p=3.7E-02). This interaction was significant after Bonferroni correction (p=7.2E-04).
Conclusion: This study suggests that known CAD SNPs in ADAMTS7 and 9p21, and known T2D SNPs in UBE2E may differentially modify CAD risk based on T2D status.
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ADAMTS17 interacts with smoking status to increase the risk of lower extremity arterial disease in non-smokers and also increases the risk of LEAD in patients with diabetes
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Background and aims: Lower extremity arterial disease is a major complication of type 1 and type 2 diabetes mellitus (DM) with smoking being an exacerbating risk factor. To date few large genetic association studies have been conducted for LEAD therefore the aim of this study was to identify genetic loci associated with LEAD in those with and without DM and to consider the impact of smoking on any genetic associations.
Materials and methods: LEAD cases were defined based on a history of lower limb amputations not due to trauma, vascular laboratory confirmed diagnoses, LEAD associated corrective procedures and medication to treat claudication; the control group excluded any individuals that conformed to the case definition. We performed fixed-effects meta-analysis of allelic effects, from 2381275 directly typed and imputed SNPs, estimated in an analysis of 2774 LEAD cases and 17941 controls. Analyses were also stratified by DM status (n= 1582 LEAD cases and 7715 controls with DM) and smoking status regardless of DM status (n=1904 LEAD cases and 17754 controls who ever smoked).
Results: A combined meta-analysis of patients with DM and non-diabetic individuals identified rs10757269, in CDKN2A/B, previously associated with LEAD as the most strongly associated SNP (OR=1.16, effect allele frequency=0.45 and p=1.62E-06), and replicated a previously reported association of rs1051730 (OR=1.16, effect allele frequency=0.66 and p=2.62E-05) in CHRNA3, an established locus for nicotine dependence, with LEAD. Other suggestive signals (p < 1E-07) were detected in CADM2, HIBADH, LPHN2, KAT2B, GAS7 and BMPR1A. A sub-group analysis of patients with DM found that rs10757269 CDKN2A/B was also associated with LEAD and had a similar allelic effect (OR=1.16 and p=3.46E-04) as overall, but rs1051730 was not associated with LEAD (OR=0.97, effect allele frequency=0.66 and p=5.24E-01. Suggestive associations were observed, in the meta-analysis of patients with DM, in or near gene regions plausibly associated with LEAD because of their roles in lipid metabolism and blood coagulation - LIPG, CTNNA3, LPHN2, LRP2 and LAPTM4B (p < 1E-07). The smoking interaction analysis revealed a significant interaction (p=8.79E-07) for rs12593235 in ADAMTS17, that increased the risk of LEAD in never smokers (OR=1.58, effect allele frequency=0.40, effect allele frequency=0.40, 95% CI (1.43 - 1.74), p=2.31E-07) when compared to its effect in ever smokers (OR=0.98, effect allele frequency=0.40, 95%CI (0.90 - 1.05), p=6.45E-01). The same risk allele was nominally significant for an increased risk of LEAD in patients with DM (OR=1.11, effect allele frequency=0.40, 95%CI (1.02 - 1.20), p= 3.41E-02) regardless of smoking status. Rs12593235 is part of a large quantitative trait locus that has been shown in other studies to interact with smoking status to increase blood pressure in non-smokers.
Conclusion: In this study we have detected signals in loci possibly associated with LEAD with some differences between the diabetes only and overall analyses. This would indicate that distinct genetic mechanisms underlie the development of LEAD in individuals who smoke and those that have diabetes. Additional analyses that include more individuals will be used to investigate this hypothesis.
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Genome-wide association study for circulating levels of soluble receptor for advanced glycation end-products (sRAGE)
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Background and aims: sRAGE levels have been found to be decreased in chronic inflammatory diseases including atherosclerosis, diabetes, renal failure and the aging process and is considered as a valuable vascular biomarker. However, the genetic determinants of the circulating sRAGE levels are not fully characterized.
Materials and methods: To identify loci associated with circulating sRAGE we combined genome-wide association study data from CARDS participants (n=594), genotyped using the Perlegen 6 SNP set and data from the GoDARTS type 2 diabetes cohort genotyped on Affymetrix Mapping 6 (n=348) and Illumina OmniExpress (n=440) platforms. Imputation of genotype at loci not directly typed was done to the HapMap II dataset using IMPUTE2. sRAGE was measured using the Quantikine Immunoassay (R&D Systems). We tested for an association of both directly typed and imputed SNPs with sRAGE using multiple linear regression implemented in SNPTEST adjusted for age, sex and BMI. Conditional logistic regression analysis in SAS 9.2 was used to test for the independence of associations found.
Results: 22 SNPs reached the genome-wide significance level of $p<10^{-8}$, all of which lie within 500kb of the *RAGE* locus. These markers were tagged by two SNPs, rs2070600 (MAF=5%, P=8.25E-18, beta= -0.14(±0.02)) the *Gly82Ser* variant in *RAGE* on chromosome 6 and rs9272346 an autoimmunity associated variant in the *HLA-DQA1* region (MAF=42%, P= 7.30E-09, beta= -0.05 (±0.01)). These markers were associated with sRAGE levels independently of each other. After adjustments for rs2070600, age, sex, and BMI, rs9272346 was significantly associated with sRAGE levels (P=0.0003). Together variation at these two loci explained 12% of the variance in circulating sRAGE. We note that both these SNPs (rs2070600 $MAF_{European}$=5% vs. $MAF_{African}$=10%; rs9272346 $MAF_{European}$=42% vs. $MAF_{African}$=48%) show ethnic variation in a direction that is consistent with the previously reported much lower total sRAGE in those of African Caribbean origin versus European populations.
Conclusion: We report a novel tagging variant rs9272346 in the *RAGE-HLA-DQA1* region independently associated with sRAGE levels and confirm the known association of sRAGE levels with the *Gly82Ser* variant (rs2070600) in the *RAGE* gene. Variation in the frequencies of these tagging markers, as seen in the HapMap samples may explain the ethnic differences in circulating sRAGE levels.
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Circulating microRNAs and micro/macrovascular complications of type 1 diabetes

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Background and aims: MicroRNAs (miRNAs) are small non-protein-encoding RNAs that post-transcriptionally regulate gene expression via suppression of target mRNAs. Specifically, miRNAs bind through canonical base pairing to a complementary site in the 3' untranslated region of their target mRNAs and can direct the degradation or translational repression of these transcripts. MiRNAs are critically involved in many biological processes in health and diseases and there is preliminary evidence of a role of miRNAs in cardiovascular diseases and diabetic complications. MiRNAs are also present in the circulation in a remarkably stable form. Circulating miRNAs have been investigated in myocardial injury, coronary artery disease, and heart failure. In addition, a recent study has shown that loss of miRNA-126 is a charactheristic feature of patients who will develop type 2 diabetes. Vascular complications are major causes of morbidity and mortality in patients with type 1 diabetes (DM1); however, a systematic analysis of circulating miRNAs in DM1 patients with vascular complications has not yet been performed.
Materials and methods: We performed a cross-sectional nested case-control study from the EURODIAB Prospective Complications Study. A total of 531 DM1 patients, diagnosed at <36 years of age, were studied. Cases (n=363) were defined as those with one or more complications of diabetes and control subjects (n=168) were those with no evidence of any complication. Total RNA was extracted from pooled serum samples (10 µl/subject) from either cases or controls (profiling step) as well as from individual serum samples (n=531) (validation step) using the Triazol reagent. Synthetic C. elegans miR-39 was added to all samples prior to RNA extraction. Total RNA was reverse transcribed and pre-amplified. The Human TaqMan miRNA Arrays Card A (Applied Biosystem) was used to perform expression profiling of 376 miRNAs. Values were normalised using the external control c.ele-miR-39 and the endogenous controls U6 snRNA. Differentially expressed miRNAs with Ct values ≤35 were identified.
Results: MiRNA expression profiling identified 41 miRNAs expressed in pooled serum samples from DM1 patients. Among them 25 miRNAs were differentially expressed in DM1 patients with micro/macrovascular complications. The difference in miRNA expression between cases and controls was 2 to 10 fold for miR-106a and miR-16; 10 to 20 fold for miR-29a, miR-126, miR-146a, miR-17, miR-222, miR-223, miR-320, and miR-484; and over 20 fold for the other miRNAs (miR-155, miR-134, miR-145, miR-150, miR-191, miR-197, miR-885-5p, miR-92a, miR-342, let-7e, miR-24, miR-574-3p, miR-483-5p, miR-486-3p, miR-486-5p). Validation of profiling results using individual case/control sample is currently ongoing.
Conclusion: These results show that miRNA are detectable in serum samples from DM1 patients and that the expression of circulating miRNAs differs in DM1 with and without vascular complications.
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OP 30 Insulin signalling and myokines

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Deletion of both Rab-GTPase activating proteins TBC1D1 and TBC1D4 in mice eliminates insulin- and AICAR-stimulated glucose transport

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Background and aims: Previously, the Rab-GTPase activating proteins TBC1D1 and TBC1D4 (AS160) were shown to regulate GLUT4 translocation in response to activation of AKT and AMP-dependent kinase. However, knockout mice lacking either Tbc1d1 or Tbc1d4 displayed only partially impaired insulin-stimulated glucose uptake in fat and muscle tissue. The aim of this study was to determine the impact of the combined inactivation of Tbc1d1 and Tbc1d4 on glucose metabolism. Therefore, we characterized energy- and substrate metabolism of Tbc1d1/Tbc1d4- double-deficient (dbKO) mice.
Materials and methods: Tbc1d1-deficient mice (RCS.B6.SJL-Nob1.10) were crossbred with conventional Tbc1d4 knockout mice to yield double-deficient mice on a C57BL/6J background. We analyzed males fed a standard chow. Substrate utilization was determined by indirect calorimetry with 10-week-old mice (TSE systems). Glucose, insulin and AICAR tolerance were investigated after i.p. injection into 12-16 wk old mice. Ex vivo glucose uptake and fatty acid oxidation (FAO) were performed with intact isolated Extensor digitorum longus (EDL) and soleus muscle by measuring 3H-palmitate oxidation and 3H-2-DOG (deoxyglucose) uptake. 14C-2-DOG uptake was determined in isolated adipose cells. GLUT4 protein abundance was determined by Western blot.
Results: Tbc1d1/Tbc1d4- double-deficient (dbKO) mice showed a tendency towards lower body weight and reduced fat mass. Postprandial glucose levels were decreased (Con: 7.31 ± 0.18 mmol/l vs. dbKO: 6.34 ± 0.24 mmol/l; $p<0.05$) but fasting glucose levels were unchanged. Glucose and AICAR tolerance were reduced (AUC Con: 124.94 ± 7.10 vs. dbKO: 162.98 ± 9.28; $p<0.05$ and Con: 266.75 ± 27.01 vs. dbKO: 365.52 ± 40.89; $p<0.05$). DbkKO mice displayed reduced respiratory quotient (RQ) during the dark phase (Con: 1.00 ± 0.01 vs. dbKO: 0.97 ± 0.01; $p<0.05$). Glycolytic EDL muscle of dbKO mice showed substantially increased FAO in the basal state (Con: 1.14 ± 0.12 pmol/(mg*min) vs. dbKO: 2.54 ± 0.60 pmol/(mg*min); $p<0.05$) and no further increase was observed after stimulation with AICAR. In contrast, FAO in soleus muscle was not different between the genotypes. GLUT4 protein expression in dbKO mice was substantially reduced in skeletal muscle and WAT (~50% of Con). Basal glucose uptake was normal in muscle of dbKO mice (EDL 1.74 ± 0.20 and soleus 2.07 ± 0.17 nmol/mg/20 min). However, insulin- and AICAR-stimulated glucose uptake was completely abolished (Ins Con; dbKO: EDL 5.37 ± 0.44; 2.75 ± 0.38; soleus 4.46 ± 0.54; 2.28 ± 0.34; AICAR Con; dbKO: EDL 3.98 ± 0.50; 2.59 ± 0.32; soleus 4.32 ± 0.56; 2.36 ± 0.32 nmol/mg/20 min).
Conclusion: Our results show that both TBC1D1 and TBC1D4 are required for insulin- and AICAR-stimulated glucose uptake in skeletal muscle. As indicated by the elevated RQ and increased FAO in skeletal muscle, dbKO mice compensate for the severe impairment in peripheral glucose utilization by increased use of lipids as fuel.

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Role for SREBP-1c in inducing skeletal muscle insulin resistance via repressing IRS-1 transcription and promoting inflammatory response

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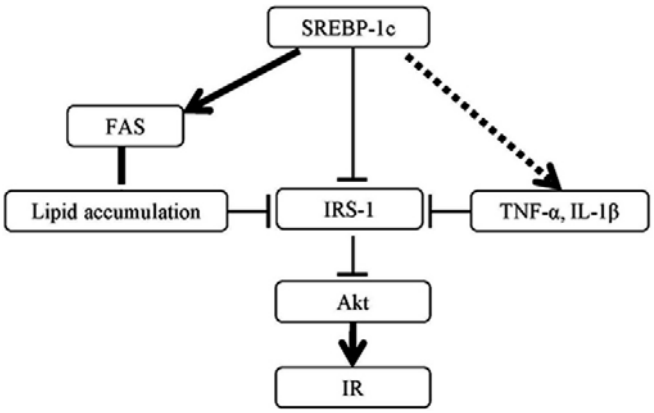
Background and aims: Sterol regulatory element binding protein 1c (SREBP-1c) is a master regulator of lipogenesis. Insulin receptor substrate-1 (IRS-1) is a key insulin signaling molecular in skeletal muscle. Little information is available on the specific role of SREBP-1c activation on IRS-1 associated insulin signaling pathway in skeletal muscle. This study was aimed to determine

the regulatory effect and molecular mechanisms of SREBP-1c on IRS-1-associated insulin signaling in myotubes.
Materials and methods: Adenovirus vectors expressing SREBP-1c were tranfected into rat primary and L6 myotubes to study the regulatory effects of SREBP-1c activation on transduction of IRS-1-associated insulin signaling and expressions of inflammatory cytokines. Luciferase reporter analysis, electrophoretic mobility shift assay, and chromatin immunoprecipitation assay were used to detect the direct binding site of SREBP to the IRS-1 promoter.
Results: We found that accumulation of SREBP-1c decreased gene and protein levels of IRS-1 and suppressed insulin-stimulated p-IRS-1(Tyr608) and p-Akt (Ser473) activation in a dose dependent manner. Concomitantly, SREBP-1c induced gene expressions of fatty acid synthase (FAS) and promoted expressions of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) with increasing titers of transfection. *In vitro* and *in vivo* interaction assays showed that SREBP-1c directly inhibited IRS-1 transcription activity through non-SRE domain at a location of -200 to +50bp of the promoter.
Conclusion: In this study, we report a new mechanism for insulin resistance in lipotoxicity that a high level of SREBP-1c, a pivotal transcription factor regulating de novo lipogenesis, can result in muscular insulin resistance through a direct effect of suppressing IRS-1 transcription, as well as an indirect effect of promoting the release of inflammatory cytokines. Our study highlights reciprocal relationships between metabolic pathways and suggests that SREBP-1c could be one pathogenesis involved in skeletal muscle insulin resistance.



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Potent anti-inflammatory properties of adiponectin on the skeletal muscle
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Background and aims: Adiponectin (ApN) is an adipokine, which is decreased in the metabolic syndrome, thereby playing a key role in the pathogenesis of these disorders. We have previously shown that ApN exerts important anti-inflammatory effects on skeletal muscle in mice exposed to acute inflammation (LPS injection) or metabolic stress (obesogenic diet). The aim of this work is to test the potency of the anti-inflammatory properties of ApN by using a model with severe and sustained inflammation.
Materials and methods: Mdx mice (a mouse model of Duchenne muscular dystrophy, where persistent inflammation worsens the consequences of the genetic deficiency) were crossed with transgenic mice that over-express adiponectin (Tg-ApN mice) in order to generate mdx-Tg-ApN mice. Different markers of inflammation/oxidative stress (TNF-α, IL-1β, NF-κB, CD68, CD3, peroxiredoxin 3/5 (PRDX3/5), 4-hydroxynonenal (HNE)) were studied and quantified by immunohistochemistry, ELISA and western blot. In vivo functional tests were also carried out to determine the global force of mice. Finally, the extent of muscle damage was quantified by Evans Blue Dye (EBD) injection following an eccentric exercise.
Results: Compared to WT mice, muscles of mdx mice presented significant increases (+ 10 to 15 fold; p<0.001) in the expression of pro-inflammatory factors (TNF-α, IL-1β, NF-κB) and oxidative stress markers (PRDX3/5, HNE) as well as a massive infiltration of macrophages and T lymphocytes, as shown by CD68 and CD3 immunolabeling respectively. All of these abnormalities were drastically reduced in mdx-Tg-ApN mice (- 60 to 75 % vs mdx; p<0.001). In addition, mdx-Tg-ApN mice exhibited higher global muscular force (+ 50 % vs mdx; p<0.01) along with a significant decrease in EBD content in their muscle fibers (- 50 to 60 % vs mdx; p<0.001).
Conclusion: Adiponectin proves to be an extremely potent anti-inflammatory agent that protects muscle against injury. These anti-inflammatory properties of ApN are of interest in the metabolic syndrome as well as in other diseases where inflammation plays a triggering or worsening pathogenic role.
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Fractalkine is a novel myokine which protects myotubes from TNF-alpha induced insulin resistance
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Background and aims: Fractalkine has been shown to be secreted by adipose tissue and associated with type 2 diabetes. We have previously demonstrated that its mRNA expression was increased in skeletal muscle cells treated with TNF-alpha. Nevertheless, fractalkine secretion, regulation and action on skeletal muscle remain to be studied. The aims of our study were to identify the physiological source of fractalkine and to evaluate the impact of fractalkine on human skeletal muscle cells with or without induction of insulin resistance by TNF-alpha.
Materials and methods: Fractalkine levels were measured in plasma from 5 healthy males performing 3h of bicycling exercise at 50% VO2 max. and in skeletal muscle biopsies for mRNA and protein. In 9 healthy subjects performing 2 h of one legged knee extensor exercise, muscle biopsies were obtained at 0, 2, 5 and 24h from both resting and exercising leg. Plasma fractalkine was also measured in 10 healthy males who had received 4h TNF-alpha infusion or placebo on 2 different days. Human skeletal muscle biopsies were collected from abdominal muscle and cells were cultured in order to measure the impact of fractalkine on 3H-2-deoxyglucose uptake (60 min) and insulin signalling with or without induction of insulin resistance by TNF-alpha (20 ng/ml for 1 or 24h).
Results: Plasma fractalkine increased rapidly after the beginning of exercise, reaching a maximum after 3h of bicycling (1.4+/-0.4 μg/l; p<0.01) and returning to basal levels by 24h. Using a model of one legged knee extensor exercise, fractalkine mRNA in the exercising leg muscle was increased 6.6+/-1.8-fold (p<0.01) and protein expression 4.2+/-0.8-fold (p<0.01) when compared to the resting leg. Moreover, human volunteers who received TNF-alpha infusion showed an increase of plasma fractalkine after 2h, with a maximum after 4h (8.2+/-0.8 μg/l; p<0.001). In order to explore autocrine action, human primary myotubes were treated with recombinant fractalkine for 24h and then with TNF-alpha to induce insulin resistance. Myotubes pretreated with fractalkine showed an increase of basal glucose uptake (120+/-18 vs. 190+/-8 cpm/mg protein/min; p<0.05). Nevertheless, fractalkine protected myotubes from the impact of TNF-alpha on glucose uptake after insulin stimulation (140+/-31 for 1h TNF-alpha vs. 220+/-12 fractalkine + TNF-alpha and 146 +/-25 for 24h TNF-alpha vs 245 +/-9 for Fractakine + TNF-alpha; p<0.05). Fractalkine pretreatment (25 ng/ml for 24h) also protected against the negative impact of TNF-alpha on AS160, Akt, and IRS-1 phosphorylation after insulin stimulation (10 min, 100 nmol/l). Moreover, TNF-alpha (24h) induced phosphorylation of ERK, mTOR and NFkB was prevented in human skeletal muscle cells pretreated with 25 ng/ml of fractalkine for 48h.
Conclusion: Our study using human subjects shows that fractalkine is expressed by skeletal muscle and regulated by exercise and TNF-alpha infusion. We further show that fractalkine protects human skeletal muscle cells from TNF-alpha induction of insulin resistance, indicating a possible autocrine role in the exercising state in addition to the previously suggested endocrine role following release from adipose tissue.

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G-CSF, a novel fatty acid-dependent myokine
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Background and aims: We recently identified *CSF3* as a palmitate-responsive myocyte gene encoding G-CSF. Yamada et al. and others showed elevated

G-CSF plasma levels upon exercise, however they did not specify its origin. Since elevated saturated fatty acid concentrations contribute to proinflammatory processes and insulin resistance in muscle cells, we intended to further analyze the stimuli and pathways inducing *CSF3* in human myotubes and C_2C_{12} cells.
Materials and methods: Protein levels were measured by ELISA and mRNA concentrations by RT-PCR.
Results: The strongest induction of *CSF3* was seen in human myotubes treated with the saturated fatty acids palmitate and stearate ($p<0.05$), while the unsaturated fatty acids palmitoleate, oleate and linoleate showed weak or no effects. Compared to other fatty acid-induced myokines like *TNF* (10-fold) and *IL6* (2.2-fold), *CSF3* showed the strongest response to palmitate (133-fold). Co-incubation of myotubes with palmitate and the unsaturated fatty acids oleate or linoleate blunted *CSF3* induction. This was also evident at protein level. This might reflect a protective effect of unsaturated fatty acids. Since palmitate induces the expression of several myokines, we analyzed their effect on *CSF3* expression. Only TNF-α induced a significant increase in *CSF3* expression (12-fold, $p<0.05$) while IL-6, IL-8, IL-15 and MCP-1 did not have any effect. TLR4 inhibition with TAK-242 in human myotubes and down regulation in C_2C_{12} cells blunted the palmitate effect on *CSF3*, while down regulation of TLR2 did not show any effects. Inhibition of the stress kinases JNK, MEK1/2 and p38 did blunt the palmitate effect on *CSF3* expression as well. Treatment of human myotubes with 0.1ng/ml G-CSF for five days revealed mitogenic properties of this protein (WST, 1.4-fold, $p<0.05$).
Conclusion: In conclusion, we describe G-CSF as a novel saturated fatty acid-dependent myokine of potential auto/paracrine relevance.
Supported by: German Centre for Diabetes Research

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Evidence against a beneficial effect of irisin in humans
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Background: Irisin has been described as a new hormone that participates in the regulation of energy consumption. Irisin was published to be released in mouse muscle upon exercise in a PGC1-α-dependent fashion and to increase energy expenditure in adipose tissue. The main body of evidence was generated in mouse, but the high interest in irisin is clearly fuelled by hope that the principle can be transferred to humans.
Materials and methods: Genomic DNA, mRNA, and expressed sequence tags of FNDC5 were analysed using bioinformatic methods. HEK293 cells were transiently transfected with human and mouse expression vectors, FNDC5 protein expression was analysed via Western blot. Human pre-adipocytes were differentiated into adipocytes in the presence of recombinant irisin, FNDC5, and BMP7. Expression of genes was analysed using quantitative RT-PCR.
Results: The analysis of genomic DNA and mRNA data show that FNDC5, the gene coding for the precursor of irisin, is present in rodents and most primates, but shows a deleterious mutation of the conserved start codon ATG to ATA in human. According to the current UniProt entry Q8NAU1 this is potentially a non-canonical start site and could still lead to full length FNDC5, that could be proteolytically cleaved to release irisin. Hence we tested the ability of the human transcript to be translated into protein. We cloned mouse and human FNDC5 with the naturally occurring ATA and additionally mutated it to ATG. In HEK293 cells the human expression vector with ATG as start codon produced similar amounts of full length protein compared to mouse FNDC5. In contrast, the human transcript with ATA as potential start codon was translated from the downstream in-frame ATG (represented by NP_715637) with strongly reduced efficiency. To test the proposed activity of irisin on human cells we isolated preadipocytes from primary human subcutaneous AT and differentiated these cells to mature adipocytes in the presence of recombinant FNDC5, irisin, or BMP7 as positive control. Further analysis confirmed that BMP7 potently induced a brown-fat-like gene program in cultured adipocytes. BMP7 treatment during differentiation induced an increased expression of the general differentiation marker for adipogenesis, PPARγ (3.6-fold). Notably, UCP1, known as a brown/brite marker, was even more strongly enhanced (6.4-fold). Additionally, the mRNA expression of TCF21, which is a marker for white AT, was significantly reduced after BMP7 treatment. Neither recombinant FNDC5 nor recombinant irisin had an effect on PPARγ, UCP1 or TCF21 mRNA expression.
Conclusion: FNDC5 in humans should be annotated as a transcribed pseudo-gene. A shorter protein version is translated only with low efficiency, but this protein has lost the signal protein and almost 50% of the irisin sequence. Could irisin nevertheless be a potential drug-target in humans, as the loss of irisin is in an evolutionary context a recent event and the downstream regulatory pathways might still exist? To verify this hypothesis we treated human pre-adipocytes with irisin or recombinant FNDC5 but this had no effect on browning. Thus in humans irisin does not exist and we conclude that the function of irisin proposed for mice is not observable in humans.

OP 31 Glucose down the drain

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Dapagliflozin-induced weight loss impacts 24 week HbA_{1c} and blood pressure levels

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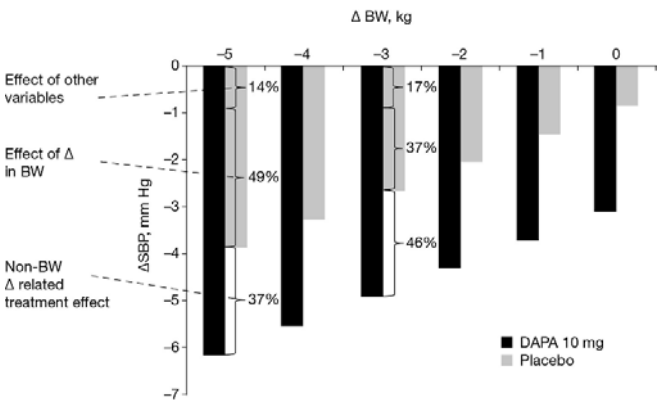
Background and aims: The majority of patients with type 2 diabetes (T2DM) are overweight or obese. Weight loss in patients with T2DM has been shown to improve glycaemic control, lipid profile and blood pressure (BP) and is recommended by EASD, ADA and IDF. It is not clear if comparatively small weight losses would contribute significantly to improvements in cardiovascular risk factor profile. In this study we investigated, by linear regression analyses, the extent to which changes in HbA1c and BP after 24 weeks of treatment with the sodium glucose co-transporter 2 inhibitor dapagliflozin (DAPA) were dependent on the simultaneously attained weight loss.

Materials and methods: Data were pooled from 7 studies evaluating DAPA 10 mg either as monotherapy or in combination with an antihyperglycaemic medication (metformin, sulphonylurea, thiazolidinedione, insulin). The investigated covariates were: baseline (BL) age, BL HbA1c, BL estimated glomerular filtration rate, BL total body weight (BW), BL seated systolic and diastolic blood pressure (SBP, DBP), gender, change in weight, smoking status, antihypertensive medication use at BL, treatment assignment, and possible treatment covariate interactions.

Results: Pooled demographic and BL characteristics were balanced between the DAPA group (N=1066: men, 47.9%; mean age, 55 years; mean HbA1c, 8.3%, mean BW, 88.7 kg) and the placebo (PBO) group (N=988: men, 49.6%; mean age, 56 years; mean HbA1c, 8.3%; mean BW, 88.1 kg). Over 24 weeks, greater reductions from BL in adjusted mean [95% CI] BW were observed with DAPA (-2.29 [-2.53 to -2.05] kg) compared with PBO (-0.27 [-0.51 to -0.03] kg). In addition, over 24 weeks there were also greater adjusted mean reductions with DAPA compared with PBO in HbA1c levels (DAPA -1.10 [-1.15 to -1.04] %; PBO -0.60 [-0.66 to -0.54] %), SBP (DAPA -4.72 [-5.67 to -3.76] mm Hg; PBO -1.04 [-1.93 to -0.16] mm Hg) and DBP (DAPA -2.36 [-2.95 to -1.77] mm Hg; PBO -0.57 [-1.11 to -0.02] mm Hg). Linear regression was used to estimate the contribution of the change in weight to the change in HbA1c, SBP and DBP. For the reduction in HbA1c, the β-value (HbA1c (%) per kg) of the weight change variable was estimated to be 0.028 (P<0.0001) and it is estimated that an overall weight loss of 3 kg and 5 kg would contribute to 8.0% and 13%, respectively of the total reduction in HbA1c for DAPA. For the reductions in SBP and DBP, the β-value (mm Hg per kg) estimates for the weight change variable were 0.61 (P<0.0001) and 0.25 (P<0.0001), respectively. With an overall weight loss of 3 kg and 5 kg there is estimated to be an approximately 37% and 49% contribution of weight loss to the total reduction in SBP for DAPA (Figure) and 32% and 44% contribution of weight loss to the total reduction in DBP for DAPA.

Conclusion: The results from this pooled analysis indicate that there is an added benefit of weight loss during DAPA treatment that contributes to overall changes in HbA1c and particularly to changes in blood pressure after 24 weeks of treatment.



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Supported by: AZ and BMS

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Canagliflozin demonstrates durable glycaemic improvements over 104 weeks compared with glimepiride in subjects with type 2 diabetes mellitus on metformin

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Background and aims: Canagliflozin (CANA) is a sodium glucose co-transporter 2 (SGLT2) inhibitor developed for the treatment of patients with type 2 diabetes mellitus (T2DM). The 104-week efficacy and safety of CANA compared with glimepiride (GLIM) were assessed in this randomised, double-blind, Phase 3 study, which represents the longest follow-up of CANA treatment to date.

Materials and methods: Subjects with T2DM on metformin (MET; N = 1,450; mean age, 56.2 years; HbA_{1c}, 7.8%; fasting plasma glucose [FPG], 9.2 mmol/L; body mass index [BMI], 31.0 kg/m^2; estimated glomerular filtration rate [eGFR], 90.2 mL/min/1.73 m^2) received CANA 100 or 300 mg or GLIM (up to 6 or 8 mg/day) during a 52-week core period, followed by a 52-week extension (n = 1,050).

Results: At Week 104, both CANA doses reduced HbA_{1c}, FPG, body weight, and systolic blood pressure (BP) compared with GLIM. Both CANA doses were also associated with increases in high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) that were stable from Week 26 to Week 104, with smaller increases in triglycerides relative to GLIM. Fewer subjects had hypoglycaemia events with CANA 100 and 300 mg than with GLIM (7%, 8%, 41%, respectively). The coefficient of durability (rate of HbA_{1c} rise from Week 26 to Week 104) was lower with CANA (0.16% both doses) than with GLIM (0.37%). (Table) Overall incidences of adverse events (AEs) were 73%, 78%, and 78% with CANA 100 and 300 mg and GLIM, and incidences of serious AEs were 10%, 10%, and 14%; discontinuation rates due to AEs were low across groups. Genital mycotic infection rates were higher in the pooled CANA group than the GLIM group (women, 15% vs 3%; men, 9% vs 2%). Higher rates of osmotic diuresis-related AEs (6%, 7%, 2%) and urinary tract infections (11%, 9%, 7%) were seen with CANA 100 and 300 mg compared with GLIM. Rates of these AEs were generally lower in the 52-week extension period versus the 52-week core period. A larger decrease in eGFR was seen with GLIM (6%) than with CANA (~1-3%) at Week 104.

Conclusion: In summary, CANA showed durable glycaemic improvements compared with GLIM and was generally well tolerated in subjects with T2DM on MET over 104 weeks.

Table. Summary of Efficacy Endpoints at Week 104 (mITT, LOCF)

Parameter*	CANA 100 mg (n = 483)	CANA 300 mg (n = 485)	GLIM (n = 482)
HbA_{1c} change, %	−0.65 (0.04)	−0.74 (0.04)	−0.55 (0.04)
Difference vs GLIM	−0.09 (−0.20, 0.01)	−0.18 (−0.29, −0.08)	
Coefficient of durability, %	0.16 (0.03)	0.16 (0.03)	0.37 (0.03)
Difference vs GLIM	−0.21 (−0.29, −0.12)	−0.21 (−0.30, −0.13)	
% of subjects reaching HbA_{1c} <7.0%†	42.5 (2.3)	50.2 (2.3)	43.9 (2.3)
Difference vs GLIM	−1.4 (−7.9, 5.1)	6.3 (−0.2, 12.9)	
FPG change, mmol/L	−1.1 (0.1)	−1.3 (0.1)	−0.6 (0.1)
Difference vs GLIM	−0.5 (−0.7, −0.3)	−0.7 (−0.9, −0.4)	
Body weight % change	−4.1 (0.2)	−4.2 (0.2)	0.9 (0.2)
Difference vs GLIM	−5.1 (−5.6, −4.5)	−5.2 (−5.7, −4.6)	
Systolic BP change, mmHg	−2.0 (0.6)	−3.1 (0.6)	1.7 (0.6)
Difference vs GLIM	−3.7 (−5.2, −2.3)	−4.8 (−6.2, −3.4)	
Triglycerides % change	4.5 (2.7)	7.9 (2.6)	13.9 (2.6)
Difference vs GLIM	−9.4 (−16.0, −2.9)	−5.9 (−12.5, 0.6)	
HDL-C % change	9.4 (0.9)	10.1 (0.9)	0.8 (0.9)
Difference vs GLIM	8.6 (6.4, 10.7)	9.3 (7.1, 11.5)	
LDL-C % change	11.1 (2.1)	14.2 (2.1)	6.3 (2.1)
Difference vs GLIM	4.9 (−0.4, 10.1)	8.0 (2.7, 13.2)	
LDL-C/HDL-C % change	4.3 (2.3)	5.3 (2.3)	7.7 (2.3)
Difference vs GLIM	−3.4 (−9.1, 2.3)	−2.4 (−8.1, 3.4)	
Non–HDL-C % change	6.3 (1.3)	10.3 (1.3)	6.1 (1.3)
Difference vs GLIM	0.3 (−3.0, 3.6)	4.2 (0.9, 7.5)	

mITT, modified intent to treat; LOCF, last observation carried forward; LS, least squares; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. *LS mean (SE) change from baseline using ANCOVA and GLIM-subtracted LS mean (95% CI) for all parameters except for % of subjects reaching HbA_{1c} <7.0%; †% (SE) and GLIM-subtracted % (95% CI) of subjects reaching HbA_{1c} <7.0%.

Clinical Trial Registration Number: NCT00968812
Supported by: Janssen Research & Development, LLC

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The sodium glucose co-transporter-2 (SGLT2) inhibitor empagliflozin improves glycaemic control in patients with type 1 diabetes: a single-arm clinical trial

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Background and aims: Adjunctive therapy to basal-bolus insulin regimens may provide an added strategy to improve glycemic control in patients with type 1 diabetes.
Materials and methods: In an 8-week single-arm open-label proof of concept pilot study of the SGLT2 inhibitor empagliflozin 25 mg po QD in subjects receiving optimized standard of type 1 diabetes care, we evaluated the efficacy on glycemia and rates of hypoglycemia compared to a 2-week placebo run-in period. Other outcome measures were urinary glucose excretion (UGE), insulin requirement, and anthropometrics.
Results: We recruited 42 normoalbuminuric patients (28 insulin pump, 14 multiple daily injection) with mean±SD age 24±5 years, A1c 8.0±0.9%, and fasting plasma glucose (FPG) 10.0±4.8 mmol/L. 40 patients completed 8 weeks of treatment with empagliflozin and the mean A1c decreased by 0.4% to 7.6±0.9% (p<0.0001). FPG decreased non-significantly to 8.6±3.1 mmol/L (p=0.06). Symptomatic hypoglycemia (<3.0 mmol/L) declined from 0.12 to 0.04 events per day (p=0.005) and mean daily insulin dose declined from 55±20 to 46±19 U/day from the placebo run in period (p<0.0001). The mean UGE increased from 19±19 at baseline to 134±61 g/day at 8 weeks (p<0.0001). Weight decreased by 2.7 kg from 72.6±12.7 (p<0.0001) and waist circumference decreased by 3.8 cm from 82.9±8.7 (p<0.0001). Apart from hypoglycemia, polyuria (79%) and thirst (74%) were the most frequent adverse events. Two subjects were withdrawn after the early occurrence of diabetic ketoacidosis (one in the setting of gastroenteritis, the other in the setting of insulin pump failure).
Conclusion: In patients with type 1 diabetes, empagliflozin as adjunctive to insulin therapy was generally well-tolerated and associated with improvement in glycemic control, a reduction in rates of hypoglycemia and reduced insulin requirement and body weight. A randomized clinical trial of the efficacy of adjunctive empagliflozin in type 1 diabetes is warranted.
Clinical Trial Registration Number: NCT01392560
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Luseogliflozin, a selective SGLT2 inhibitor, added on to glimepiride for 52 weeks improves glycaemic control with no major hypoglycaemia in Japanese type 2 diabetes patients

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Background and aims: Luseogliflozin, a highly selective sodium glucose cotransporter 2 inhibitor, reduces plasma glucose by increasing urinary glucose excretion. The insulin-independent mechanism of luseogliflozin would be expected to be beneficial in combination with other oral antidiabetic drugs. The aim of current study was to assess the efficacy and safety, including the risk of hypoglycemia, of luseogliflozin administered with glimepiride for 52 weeks.
Materials and methods: Japanese patients with type 2 diabetes mellitus (T2DM), who has not been sufficiently controlled (HbA1c : 6.9-10.4%) by glimepiride monotherapy over 12 weeks, received luseogliflozin once daily for 52 weeks. This study consisted of double-blind period (week 0-24) to assess mainly the safety comparing with placebo and open-label period (week 24-52). In double-blind period, a total of 221 patients were randomized to receive placebo (N = 71) or luseogliflozin 2.5 mg (N = 150). Then, in open-label period, all patients received luseogliflozin 2.5 mg (patients who had insufficient glycemic control received 5 mg) and 141 patients received luseogliflozin for 52 weeks. Change from baseline (BL) in glycemic and other efficacy endpoints were assessed. Adverse events (AEs) were monitored throughout the study.
Results: HbA1c was significantly decreased by luseogliflozin compared with placebo (-0.88%) at week 24, and was significantly decreased from BL (-0.63%) at week 52. Fasting plasma glucose (FPG) and body weight (BW) were also significantly decreased by luseogliflozin compared with placebo at week 24, and was significantly decreased from BL at week 52 (Table). In addition, systolic/diastolic blood pressure, plasma lipid (triglyceride, HDL-cholesterol), fasting insulin, plasma C-peptide, insulin sensitivity (HOMA-R) and intact proinsulin significantly improved compared with BL at week 52. In patients who received luseogliflozin for 52 weeks, incidences of AEs, AEs leading to discontinuation and serious AEs were 81.3%, 2.7%, 6.0%, respectively. Most AEs were mild in severity. Incidences of hypoglycemia observed in double-blind period were 4.2% with placebo and 8.7% with luseogliflozin. Incidence of hypoglycemia with patients who received luseogliflozin for 52 weeks was 10.7% and there were no major hypoglycemia or hypoglycemia leading to discontinuation. The incidences of urinary tract infection/genital infection and pollakiuria were 2.7% and 2.8%, and every event was mild in severity.
Conclusion: Add-on therapy with luseogliflozin for 52 weeks significantly ameliorated glycemic control and reduced BW, and was well tolerated with no major hypoglycemia in Japanese patients with T2DM poorly controlled by glimepiride monotherapy.

Table Summary Efficacy Endpoints at Week 24 (LOCF) and Week 52

	Double-blind period		Throughout the study
	Placebo	Luseogliflozin	Luseogliflozin
	Week 24 (N = 71)	Week 24 (N = 150)	Week 52 (N = 141)
HbA1c, %			
Baseline (mean)	8.01	8.07	8.10
LS mean change from baseline (95% CI)	0.40** (0.2, 0.6)	-0.50** (-0.6, -0.4)	-0.63** (-0.8, -0.5)
Difference vs Placebo (95% CI)	—	-0.88* (-1.0, -0.7)	—
FPG, mg/dL			
Baseline (mean)	148.2	151.1	151.3
LS mean change from baseline (95% CI)	18.9** (12, 26)	-16.8** (-21, -12)	-22.4** (-27, -18)
Difference vs Placebo (95% CI)	—	-34.2* (-41, -27)	—
BW, kg			
Baseline (mean)	65.3	66.4	66.5
LS mean change from baseline (95% CI)	0.2 (-0.2, 0.6)	-1.4** (-1.6, -1.1)	-2.2** (-2.5, -1.9)
Difference vs Placebo (95% CI)	—	-1.5* (-2.0, -1.0)	—

*: P < 0.001 vs Placebo
1-sample t-test **: P < 0.001 vs Baseline
LOCF: last observation carried forward

Clinical Trial Registration Number: JapicCTI-111507

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Efficacy and safety of tofogliflozin administered for 52 weeks as monotherapy or combined with other oral hypoglycaemic agents in Japanese patients with type 2 diabetes

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Background and aims: Tofogliflozin, a highly selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, reduces blood glucose and body weight by inhibiting renal glucose reabsorption and promoting urinary excretion of excess blood glucose in patients with type 2 diabetes mellitus (T2DM). The characteristics of tofogliflozin, which include high selectivity toward SGLT2, a short half-life and an insulin-independent mode of action, mean that it exerts sustained efficacy with low hypoglycemic risk and can be used in combination with any existing T2DM therapy. This study was undertaken to verify the efficacy and safety of tofogliflozin administered for a long period of time to Japanese patients with T2DM with inadequate glycemic control on diet/exercise therapy alone or on diet/exercise therapy plus one oral hypoglycemic agent (OHA).
Materials and methods: The study was designed as a randomized, open-label, parallel-group comparison. Tofogliflozin 20 or 40 mg was orally administered once daily for 52 weeks to 191 patients as monotherapy (MONO) and to 593 patients as combination therapy (COMBO) (sulfonylurea + glinide, n=190; dipeptidyl peptidase-4 inhibitor, n=103; biguanide, n=101; thiazolidine, n=101; and α-glucosidase inhibitor, n=98). The primary endpoint was the change in HbA1c from baseline to the end of treatment.
Results: With tofogliflozin monotherapy, the change in HbA1c from baseline to week 52 of treatment was −0.7% (both 20 mg and 40 mg), and the changes in body weight were −3.1 kg (20 mg) and −3.4 kg (40 mg). With tofogliflozin combination therapy, changes in HbA1c from baseline to week 52 of treat-

ment were −0.8% and −0.9%, and the changes in body weight were −2.5 kg and −3.0 kg, in the 20 mg and 40 mg groups, respectively. Weight loss reached maximum values at 24 weeks, and these were maintained until the end of study. Moreover, reduced systolic and diastolic blood pressure, improved HOMA-R and Matsuda index, reduced abdominal circumference, increased levels of adiponectin, HDL-C, and decreased levels of uric acid, alanine aminotransferase and γ-glutamyl transferase were secondarily observed. Regarding safety, the incidences of adverse events in the 20 mg and 40 mg groups were 76.6% and 86.6%, respectively (MONO), and 86.3% and 84.7%, respectively (COMBO). Adverse drug reactions occurring at an incidence ≥5% in either the monotherapy or combination therapy group included increased blood ketone bodies, pollakiuria and thirst. The incidence of hypoglycemia was higher in patients receiving tofogliflozin in combination with sulfonylurea/glinide 11.9%(20mg) and 8.1%(40mg) than in all other groups. There were no clinically significant adverse events in any treatment groups.

Conclusion: In Japanese patients with T2DM, tofogliflozin administered once daily was sustainably effective for glycemic and body weight control used in monotherapy or combination therapy with other OHAs throughout the 52-week study period, and additional beneficial effects were also observed. Tofogliflozin at doses of 20 mg and 40 mg was also well tolerated.

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Favourable gastrointestinal and genitourinary safety profile of LX4211 added-on to metformin in a Phase 2b study

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Background and aims: LX4211 is a dual inhibitor of SGLT1 and SGLT2, the major glucose transporters in the gastrointestinal (GI) tract and kidney. It has shown a favorable GI and genitourinary (GU) safety profile in healthy subjects and Phase 2 studies of patients with type 2 diabetes.

Materials and methods: In a 12-week dose ranging study, 299 patients with inadequately controlled T2DM on metformin were randomly assigned to LX4211 (75 mg qd, 200 mg qd, 200 mg bid, or 400 mg qd) or placebo (PBO). The GI and GU tolerability and overall safety of LX4211 were evaluated.

Results: In the safety population, the incidences of diarrhea and nausea were comparable between all LX4211-treated patients and PBO. Diarrhea was 7.2 vs 6.7% and nausea was 6.8 vs 5.0% in all LX4211-treated patients vs PBO, respectively. Diarrhea was not dose-dependent or related to initiation of dosing. Nausea and diarrhea were generally mild and resolved without treatment. Constipation and vomiting were less common with LX4211 vs PBO; constipation 3.4 vs 6.7%, and vomiting 0.4 vs 5.0%. Urinary tract infections occurred evenly among treatment groups. Vaginal yeast or bacterial infections were seen only with LX4211; they were mostly mild, their incidence was low, and none led to therapy discontinuation. There were no deaths, treatment related serious AEs, or episodes of hypoglycemia reported.

Conclusion: LX4211 combined with metformin was safe and well-tolerated in this study. Studies of greater exposure duration are needed to characterize long term safety of LX4211.

Overview of GI and GU Adverse Events

AE of Interest	75 mg qd n=57	200 mg qd n=60	200 mg bid n=60	400 mg qd n=59	Placebo n=60
Diarrhoea, number (%)	2 (3.5)	6 (10.0)	4 (6.7)	5 (8.5)	4 (6.7)
Severity					
Mild	2	3	2	4	3
Moderate	0	3	2	0	0
Severe	0	0	0	1	1
Nausea, number (%)	5 (8.8)	3 (5.0)	2 (3.3)	6 (10.2)	3 (5.0)
Severity					
Mild	5	3	2	4	2
Moderate	0	0	0	1	1
Severe	0	0	0	1	0
Vomiting, number (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7)	3 (5.0)
Constipation, number (%)	1 (1.8)	5 (8.3)	1 (1.7)	1 (1.7)	4 (6.7)
Urinary Tract Infection[a, b], number (%)	1 (1.8)	1 (1.7)	1 (1.7)	1 (1.7)	1 (1.7)
Genital Tract Infection[b], number (%)	0 (0.0)	3 (5)	2 (3.3)	3 (5.1)	0 (0.0)
Vulvovaginal mycotic infection	0	1	1	1	0
Vaginal infection	0	1	0	1	0
Vaginitis bacteria	0	0	1	0	0
Vulvovaginal candidiasis	0	0	0	1	0
Vulvovaginitis	0	1	0	0	0
Balanitis	0	0	0	0	0

[a] Includes 1 fungal infection in the 200 mg bid group
[b] All patients with AE of urinary tract infection or genital tract infection were female

Clinical Trial Registration Number: NCT01376557

OP 32 Hypoglycaemia: balancing glucose control

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Lower rates of overall, nocturnal and severe hypoglycaemia during maintenance treatment with IDegAsp vs biphasic insulin aspart 30 in patients with type 2 diabetes mellitus: a meta-analysis

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Background and aims: IDegAsp is a soluble co-formulation of 70% insulin degludec (IDeg) and 30% insulin aspart (IAsp) that provides ultra-long-acting basal insulin and rapid-acting bolus insulin in one injection. The aim of this *post-hoc* meta-analysis was to compare hypoglycaemia rates for IDegAsp vs biphasic insulin aspart 30 (BIAsp 30).

Materials and methods: This patient-level meta-analysis included the two phase 3a, randomised, treat-to-target trials in which twice-daily IDegAsp and BIAsp 30 were compared in patients with type 2 diabetes (T2D; IDegAsp: n=504; BIAsp 30: n=364). Both trials were 26 weeks and open-label; insulin was given with breakfast and the main evening meal. HbA_{1c}, fasting plasma glucose (FPG), bodyweight and insulin dose was analysed using a linear model; hypoglycaemia was analysed using a negative binomial model. Confirmed hypoglycaemia was defined as PG <3.1 mmol/l or severe (requiring assistance); nocturnal confirmed hypoglycaemia had an onset 00:01-05:59.

Results: Treatments were similar in terms of end-of-trial HbA_{1c} (7.06 vs 7.07%, estimated treatment difference [ETD] IDegAsp-BIAsp 30: 0.00 [-0.11; 0.10], p=0.96). IDegAsp was associated with a greater reduction from baseline in FPG (ETD: -1.12 mmol/l [-1.38; -0.85], p<0.0001) and less weight gain (ETD: -0.50 kg [-0.88; -0.11]; p=0.012). Daily insulin doses at end-of-trial were lower for IDegAsp than BIAsp 30 (0.9 vs 1.1 U/kg; estimated dose ratio: 0.84 [0.80; 0.89]; p<0.0001). For the full trial period, rates of overall confirmed hypoglycaemia were lower (by 19%; p=0.03) with IDegAsp than BIAsp 30 (table) as were rates of nocturnal hypoglycaemia (by 57%; p<0.0001). Few severe hypoglycaemic episodes were reported; IDegAsp was associated with a numerically lower rate of severe hypoglycaemia vs BIAsp 30 (p=0.27). For the maintenance period (defined as after 16 weeks of treatment when glucose targets and insulin doses were near final levels), the rate of severe hypoglycaemia was lower (by 84%; p=0.0061) for IDegAsp (table). Similarly, differences in rates of overall and nocturnal confirmed hypoglycaemia (IDegAsp vs BIAsp 30) were more pronounced during the maintenance period (table). No apparent differences in standard safety variables were observed for IDegAsp vs BIAsp 30, including major adverse cardiovascular events (4 vs 7 events).

Conclusion: Patients with T2D treated with twice-daily IDegAsp had a significantly greater reduction in FPG, less weight gain, lower insulin use, and significantly lower rates of hypoglycaemia than BIAsp 30. The finding of a significantly lower rate of severe hypoglycaemia during maintenance, when patients have become familiar with IDegAsp, supports the need for additional studies to further explore its efficacy and safety profile.

Meta-analysis of hypoglycaemia		
	Estimated rate ratio (IDegAsp/BIAsp 30)	
	Full trial period	Maintenance period [a]
Overall confirmed hypoglycaemia	0.81 [0.67; 0.98], p=0.0341	0.69 [0.55; 0.87], p=0.0015
Nocturnal confirmed hypoglycaemia	0.43 [0.31; 0.59], p<0.0001	0.38 [0.25; 0.58], p<0.0001
Severe hypoglycaemia	0.61 [0.26; 1.45], p=0.2661	0.16 [0.04; 0.59], p=0.0061

[a] From 16 weeks to end of trial; [ ]: 95% confidence interval

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Retrospective analysis of the burden of drug-induced hypoglycaemia in diabetes (SIMEU study)

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Background and aims: The direct burden of hypoglycemia for people with diabetes and for the National Health System is difficult to determine. Very few data exist on the extent of service use by subjects with diabetes, including frail people with Type 2 disease, after episodes of hypoglycemia.
Materials and methods: We report the interim results of a retrospective analysis of patients' attendance to 38 Italian Emergency Departments (EDs) following 2,889 episodes of referred hypoglycemia in a 18-month period (January 2011-June 2012). Cases were identified in the individual databases of the different centers using a predefined Case Report Form. The centers were part of a network, repeatedly involved in collaborative studies.
Results: After excluding cases of cancer-related cachexia or terminal conditions, 2,675 episodes were identified in subjects with DM (mean age, 71; 51% Males; referred mean blood glucose - when available and measurable - 44 mg/dL). Current drug use was recorded in 2,599 cases. Insulin was present in 64%, alone or coupled with any other treatment (32% and 32% of total cases, respectively). Among non-insulin treated cases (n=882), a variable combination of antidiabetic drugs was recorded. Metformin was present in 55%, sulfonylureas in 62%, repaglinide in 15%, pioglitazone in 2%, GLP-1 agonists in 1%, DPP-4 inhibitors in 2%, acarbose in 4%. In over 80% of non-insulin-related hypo cases, either sulfonylureas or repaglinide were present. Among sulfonylureas, the drug most commonly involved in hypoglycemic events was glibenclamide (61%), followed by glimepiride (22%), gliclazide (14%), gliquidone and glipizide (1%). In 234 cases the hypogycemic event was associated with trauma (insulin, 157), in 39 with motor vehicle accidents (insulin, 25). Out of hospital, hypoglycemia had been self-treated in 18% of cases, was treated by the Emergency personnel in 51%. When in ED, blood glucose was on average 78 ± 53 mg/dL; in ED, hypoglycemia was treated by oral glucose (19%), i.v. glucose (28%), i.m. glucagon (2%). Following visit and/or treatment, 44% of cases were immediately referred to their GPs, 18% were kept under observation for less than 24 hours, 31% were admitted to different medical units, 7% refused admission; 6 patients died in ED. When admitted, median length of stay was 8 days (interquartile range, 6) and death occurred in 77 cases (9% of total admitted cases).
Conclusion: Hypoglycemic events generate a significant burden to persons with diabetes and are an important cost for the National Health system, not limited to subjects treated with insulin. Outcome is poor in many cases, reflecting the frailty of most patients, exacerbated by hypoglycemia.
Clinical Trial Registration Number: CE-Bologna, 175/2012/O/OssN

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The effect on insulin analogues on the risk of severe hypoglycaemia in patients with type 1 diabetes and recurrent severe hypoglycaemia: the HypoAna trial

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Background and aims: Insulin therapy in type 1 diabetes is limited by the risk of severe hypoglycaemia. Insulin analogues are developed to improve insulin treatment of diabetes in respect to glycaemic control and avoidance of hypoglycaemic episodes. The effect of insulin analogues on glycaemic control is documented in large trials, while their effect on the frequency of severe hypoglycaemia is less clear, especially in patients with recurrent severe hypoglycaemia. The trials comparing the effect of insulin analogues and human insulin's in type 1 diabetes have invariably excluded patients at high risk of severe hypoglycaemia from participation, i.e. those with recurrent severe episodes or hypoglycaemic unawareness. In clinical practice, however, many patients at high risk of severe hypoglycaemia have changed treatment regimen from human insulin to insulin analogues based on a non-evidence-based belief in a potential of analogues to reduce occurrence of severe hypoglycaemia. In the HypoAna Trial we therefore tested whether a treatment regimen based on insulin analogues is superior to human insulin in reducing the rate of severe hypoglycaemic episodes in patients at high risk of severe hypoglycaemia.
Materials and methods: The study was a two-year investigator-initiated, prospective, randomised, open, blinded endpoint (PROBE) trial including 159 patients with type 1 diabetes and two or more episodes of severe hypoglycaemia in the preceding year. Subjects were randomized to treatment with basal-bolus therapy based on analogue (aspart/detemir) and human (regular/NPH) insulin in a balanced cross-over design. Primary endpoint was the number of episodes of severe hypoglycaemia defined by need for treatment assistance from others. A blinded adjudication of endpoints according to Whipple's triad and severity was applied.
Results: Treatment with the analogue-based insulin regimen resulted in a 31% (12-49%) rate reduction (p=0.007) in the intention-to-treat analysis and a 30% (95% CI: 10%-49%) rate reduction (p=0.012) in the per-protocol analysis corresponding to an absolute reduction of 0.5 episodes per patient-year. The reduction was primarily due to reduction in the rate of nocturnal severe hypoglycaemia. There was no difference between the treatments in markers of severity of the reported episodes. The result was obtained despite maintenance of baseline glycaemic control (HbA1c: 8.0±1.0% (64±11 mmol/mol) (mean±SD)) throughout both treatment arms. The number of patients needed to treat to avoid one episode of severe hypoglycaemia was approximately two patients in one year.
Conclusion: In patients with type 1 diabetes and recurrent severe hypoglycaemia treatment with insulin aspart/detemir resulted in a clinically significant reduced rate of severe hypoglycaemia at the same level of glycaemic control as compared to treatment with human insulin.
Clinical Trial Registration Number: NCT00346996
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Exercise-related hypoglycaemia occurs at similar frequency with insulin degludec and insulin glargine

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Background and aims: During exercise, increased glucose requirements as well as increased insulin sensitivity can lead to hypoglycaemia in patients with diabetes. Exercise intensity, dose timing and the individual pharmacodynamic properties of insulin can affect the risk of hypoglycaemia. Insulin degludec (IDeg) is a new basal insulin with an ultra-long and stable glucose-lowering effect and this property might be expected to alter the susceptibility to exercise-related hypoglycaemia.
Materials and methods: We investigated exercise-related hypoglycaemia in seven randomised, open-label, treat-to-target, clinical trials with IDeg vs insulin glargine (IGlar) once daily. Trials were of 26 or 52 weeks in type 1 (T1D; n=1116) and type 2 diabetes (T2D; n=3598). Patients reported hypoglycaemic episodes in their subject diaries and were instructed to note any relation to exercise (based on their own judgment). Confirmed hypoglycaemia was defined as plasma glucose <3.1 mmol/L or severe episodes requiring assistance. Nocturnal hypoglycaemia was defined as any episode between midnight and 6:00AM.
Results: The proportion of patients experiencing ≥1 confirmed hypoglycaemic episode related to exercise was similar with IDeg and IGlar for T1D and T2D (table). In both arms, more exercise-related hypoglycaemia occurred in patients with T1D than with T2D.
Conclusion: In conclusion, the data suggest no increased risk of self-reported exercise-related hypoglycaemia with IDeg compared with IGlar.

	T1D		T2D basal-bolus therapy		T2D basal-oral therapy	
	IDeg	IGlar	IDeg	IGlar	IDeg	IGlar
Total n subjects	801	315	753	251	1734	860
Total n subjects with confirmed hypoglycaemic episodes	769	303	609	206	779	380
Total n subjects with ≥1 exercise-related confirmed hypoglycaemic episode (% of n with confirmed hypoglycaemic episodes)	614 (80%)	242 (80%)	311 (51%)	106 (51%)	163 (21%)	83 (22%)

Trials: T1D: NN1250-3770, NN1250-3583 (basal–bolus therapy), T2D: NN1250-3586, NN1250-3668, NN1250-3672, NN1250-3579 (all basal ± oral), NN1250-3582 (basal–bolus therapy). Safety analysis set: subjects exposed to ≥1 dose of trial product.

Supported by: Novo Nordisk

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A prospective, randomised study in patients with type 1 diabetes on the need for additional carbohydrates with standardised physical activity to prevent hypoglycaemia

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Background and aims: Patients with type 1 diabetes treated with intensified insulin regimens tend to develop hypoglycaemia with physical activity, and need to compensate for the glucose-lowering effect with additional carbohydrates or with reductions in insulin doses. It is not known, which category, timing or quantity of carbohydrate is necessary to prevent hypoglycaemia. We wanted to study this question with a prospective cross-over design in patients with type 1 diabetes on intensified insulin regimens.
Materials and methods: 24 subjects with type 1 diabetes (age 41 ± 12 years; 11 women, 13 men; body-mass-index 26.5 ± 4.7 kg/m2; diabetes duration 16 ± 11 years; HbA1c 9.1 ± 1.5 %; insulin dose 0.64 ± 0.22 IE/kg per day; 1.7 ± 1.5 symptomatic hypoglycaemic episodes per week; estimated hypoglycaemia awareness threshold 60 ± 15 mg/dl) participated. In randomized order, the volunteers participated in one afternoon session without and three afternoon sessions with a standardised physical activity (4 km walk lasting approximately 60 min) beginning at 2 p.m. (a) without additional carbohydrates, (b) with 10 g carbohydrates, or (c) 20 g carbohydrates, each at the start of and 30 min after commencing physical activity (muesli bar). Blood glucose was determined at 2:00 p.m., 2:30 p.m., 3:00 p.m., 6:00 p.m., and 8:00 p.m. (laboratory-based method). Hypoglycaemic episodes and hypoglycaemia-induced additional carbohydrate intake were monitored between 2:00 and 6:00 p.m., i.e. during and after physical activity. Statistical analysis: Repeated-measures ANOVA.
Results: During and after physical activity, independent from the regimen of preventive carbohydrate replacement, blood glucose uniformly fell from approximately 175 mg/dl at 2 p.m., i.e. 1-2 h after lunch, to approximately 100-110 mg/dl at 2:30 p.m. Later, it increased again with larger quantities of additional carbohydrates ($p = 0.0036$ for 40 vs. 0 g). With preventive administration of carbohydrate, less additional carbohydrate needed to be administered due to intercurrent hypoglycaemic episodes (20 g: $p = 0.017$; 40 g: $p = 0.015$). During and after physical activity hypoglycaemic episodes were often asymptomatic and were only detected by frequent blood glucose measurements.
Conclusion: The amounts of carbohydrates employed for the prevention of hypoglycaemia induced by physical activity in our prospective study protocol turned out to be too small, and their influence to elevate blood glucose was observed too late. For the prevention of hypoglycaemic episodes induced by physical activity of intermediate intensity and one hour's duration more than 40 g carbohydrates need to be recommended, and the initial step should be the administration of more rapidly absorbed carbohydrates. Further studies are warranted to define the optimum recommendations for type 1 diabetic patients.
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Which algorithms best reduce the risk of hypoglycaemia during exercise for patients with type 1 diabetes on pump therapy?

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Background and aims: In T1 diabetic (T1D) subjects on pump therapy, there is no consensus regarding the adjustment of insulin doses in the event of physical activity (PA). Taking into account the time of exercise relative to meals, we tested several adjustments of insulin doses according to the intensity of PA.
Materials and methods: Twenty T1D patients with HbA1c < 9% and practicing leisure PA were included. $VO2_{max}$ was recorded for all subjects on entering the study. The subjects performed 4 physical tests at least 48 hours apart (30-min cycling sessions on an bicycle ergometer) 3h after lunch (basal tests), in random order, at 2 levels of PA (moderate and intensive, i.e. 50% and 75% $VO2_{max}$), with 2 options each time regarding reduction in basal rate (BR): BR reduction of 50% or 80% in the case of 50% $VO2_{max}$ and of 80% or 100% in the case of 75% $VO2_{max}$. Each time, BR was reduced during the exercise period and the following 2 hrs, except for the last one, as the pump was switched ON at the end of the exercise period. A resting test served as a reference. Blood glucose and insulin levels were measured during the exercise (or rest) period and over the following 2 hrs. On each test day, patients wore an iPro2 device from lunchtime until breakfast the next day. We focused on the occurrence of hypoglycemic events recorded on the CGM system.
Results: Patients (11 men, BMI 24±5 kg/m2, age 45±12 yrs, HbA1c at baseline 7.9±0.7%, diabetes duration 17.6±10 yrs, on pump therapy for 5 years, practicing 4.3 hrs/week of PA) performed 137 physical tests between 02/2011 and 02/2013, 100 basal tests and 37 prandial tests. For moderate PA, the comparison revealed that an 80% BR reduction led to fewer hypoglycemic events than a 50% reduction, with no concomitant increased occurrence of hyperglycemic events. For intensive PA, the adjustment that led to the fewest hypoglycemic events was switching the pump OFF during PA and ON at the end of PA. In all cases, the nadir of BG values occurred at the end of the exercise session (30 min). The reduction in glycemia was 72 mg/dl for both 50% $VO2_{max}$ sessions and of 67 and 72 mg/dl respectively for BR-100% and BR-80% during the 75% $VO2_{max}$ sessions.
Conclusion: To prevent the occurrence of hypoglycemic events with moderate PA, BR-80% + 2hrs could be suggested to begin with then adjusted if necessary. For intensive PA, the solution of turning the pump OFF during exercise and turning it ON at the end of exercise could be proposed first. In all cases, the action of insulin on board must be kept in mind, even with the pump OFF. These results should incite healthcare providers to advise patients to reduce their BR at least 30 min before the start of exercise in order to prevent hypoglycemia.
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OP 33 Mitochondrial metabolism

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Participation of CDK4 in the regulation of mitochondrial metabolism and energy homeostasis

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Background and aims: Much has been studied about the participation of CDKs, in cell growth, proliferation and development, whereas little attention has been paid to their implication in metabolic pathways. Interestingly, Cdk4 inactivation *in vivo* led to marked metabolic phenotypes. We are currently testing several hypotheses that point to the participation of Cdk4 in key steps of energy metabolism, including: 1) mitochondrial biogenesis and function; 2) lipid oxidation; and 3) glycolysis.
Material and methods: Yeast-two hybrid experiments were conducted using CDK4 as bait, and screening a library of human protein fragments. Differentiated brown adipose tissue (BAT) cells were used to study the effects of CDK4 inhibition on oxygen consumption. Undifferentiated primary MEFs were used to perform fatty acid oxidation assays. Oxygen consumption and fatty acid oxidation experiments were carried out using an XF24 seahorse analyzer. C57/Bl6 wild-type mice (n = 3 to 5 per experiment) were used to evaluate the effects of the treatment with the CDK4/6 inhibitor PD-0332991 on mitochondrial gene expression. PD-0332991 or the vehicle were administered daily by oral gavage. Mice were fasted over-night prior to organ harvest. All statistical tests were two-tailed t-tests.
Results: Our experiments point towards an implication of CDK4 in mitochondrial metabolism and mitochondrial function, in glucose and fatty acid degradation and in the TCA cycle. First of all, yeast two-hybrid experiments using CDK4 as bait identified the mitochondrially encoded Cytochrome C oxidase subunit II (MT-CO2) as a CDK4 partner. Second, electron microscopy imaging in liver and BAT sections from $Cdk4^{-/-}$ mice showed that the absence of Cdk4 leads to an increase in mitochondrial size and number, as well as to a decrease in the size and the number of lipid droplets. Moreover, our preliminary experiments using PD-0332991, a chemical inhibitor of CDK4 kinase activity, also suggest that CDK4 is an anabolic regulator, since BAT cells treated with this compound exhibit a modest (1.2 fold) but significant (p=0.0001) increase in oxygen consumption. *In vivo,* 3 days of treatment with PD-0332991 caused a 1.5-2 fold increase in the expression of numerous mitochondrial genes, like PGC1a (p=0.05), its target genes TFAM (p=0.016) and NRF1 (p=0.011), the mitochondrial complex II subunit SDHA (p=0.019), and the mitochondrial complex I subunits Ndufs8 (p=0.033) and Ndufc1 (p=0.015), in BAT. When fatty acid oxidation (FAO) was measured with the seahorse flux analyzer in MEFs from $Cdk4^{+/+}$, $Cdk4^{-/-}$ and $Cdk4^{R24C/R24C}$ (from mice carrying a gene encoding for a CDK4 protein that is resistant to the inhibition by Ink4 family members), we observed that the deletion of Cdk4 led to an increase in FAO whereas a constitutively active Cdk4 drove a significantly decreased FAO. At the level of oxygen consumption, we observed a two-fold increase when comparing $Cdk4^{+/+}$ and $Cdk4^{-/-}$ MEFs. This increased mitochondrial was correlated to an increase in PGC1a protein expression.
Conclusion: Overall, our results implicate that CDK4 has an anabolic role in energy metabolism that can very well be independent of E2f1 transcriptional activity.

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Dissociation between mitochondrial dysfunction and insulin resistance in adipose-specific PGC-1beta knockout mice

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Background and aims: White adipose tissue (WAT) plays a crucial role in the regulation of glucose homeostasis owing to its endocrine and lipid storage functions. In addition, compelling evidence suggest that adipocyte mitochondrial function is a crucial determinant of whole body insulin sensitivity. In support of this view, mitochondrial dysfunction has been observed in WAT of insulin-resistant individuals and a variety of rodent models for type 2 diabetes. Consistent with a reduced mitochondrial function, clinical studies have shown that patients with insulin resistance or type 2 diabetes have decreased mitochondrial mass in WAT. Furthermore, WAT is the main target of thiazolidinediones (TZDs), which exert their insulin sensitizing effects by promoting de novo adipogenesis and, presumably, also by stimulating mitochondrial biogenesis and function. Although the action of TZDs on mitochondrial gene expression has been attributed to their capacity to increase the expression of the PGC-1α coactivator, we have recently shown that PGC-1α is not required to sustain basal or rosiglitazone-induced mitochondrial biogenesis in WAT, suggesting that other factors may play a more relevant role in the control of mitochondrial gene expression in white adipocytes. Here, we address the role of PGC-1β in the function of WAT and its contribution to energy and glucose homeostasis.
Materials and methods: To study the function of PGC-1β in WAT, we have generated a new mouse model in which the gene encoding for PGC-1β has been ablated specifically in adipocytes by homologous recombination (PGC1β-FAT-KO mice). Mice were fed a high fat diet to induce insulin resistance and then treated with either vehicle or 10 mg/Kg of rosiglitazone for 15 days.
Results: Genome-wide expression profiling analysis of WAT from PGC1β-FAT-KO mice reveals that PGC-1β function is mostly restricted to the regulation of a specific subset of mitochondrial genes involved in ATP production (i.e. OxPhos system, TCA cycle). Accordingly, WAT of PGC1β-FAT-KO mice exhibits decreased mitochondrial protein content and oxidative capacity. In addition, we have found that rosiglitazone-induced expression of mitochondrial genes and activity is severely blunted in WAT of PGC1β-FAT-KO mice. This is in contrast with our results in mice lacking PGC-1α in WAT, in which mitochondrial biogenesis and function were normally induced by TZDs. Similarly to our in vivo observations, knockdown of PGC-1β but not PGC-1α impairs basal and rosiglitazone-induced mitochondrial gene expression and oxygen consumption. Despite a decrease in mitochondrial function comparable to that observed in WAT of insulin resistant or type 2 diabetic patients, we have found that lack of PGC-1β in mouse adipocytes does not induce tissue-specific or whole body insulin resistance. Moreover, PGC1β-FAT-KO mice normally respond to the insulin-sensitizing effects of TZDs.
Conclusion: Our findings provide evidence that PGC-1 coactivators play differential roles in WAT, being PGC-1β the major regulator of basal and TZD-induced mitochondrial oxidative function. Our results show that mitochondrial dysfunction in WAT is not associated to the development of insulin resistance and also suggest that full mitochondrial oxidative function is not required for the effects of TZDs on insulin sensitivity.
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Lack of PGC-1α is associated with dysregulation of AICAR mediated AMPK regulation of ACC and PDH

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Background and aims: A sedentary lifestyle has been shown to be associated with metabolic inflexibility, defined by an impaired ability to adjust fuel oxidation with changes in fuel availability. Pyruvate dehydrogenase (PDH) is a key enzyme in mammalian oxidative glucose metabolism. In addition, the intracellular energy sensor, AMP activated protein kinase (AMPK), is known to be an important regulatory protein in substrate utilization, and previous studies have indicated that AMPK may regulate PDH. AMPK is activated by muscle contractions, but can also be pharmacologically activated by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR). Lack of peroxisome proliferator-activated receptor γ co-activator (PGC)-1α is known to cause a change in phenotype to a more glycolytic muscle and appear to be associated with metabolic inflexibility. Therefore, skeletal muscle without PGC-1α may be seen as a model for a sedentary lifestyle, and it is possible that AMPK-mediated regulation of substrate utilization potentially via PDH is changed in such muscles. The purpose of this study was therefore to test the hypothesis that in vivo injections with AICAR regulates AMPK signaling and PDH in skeletal muscle, and that this regulation is impaired in muscles lacking PGC-1α.
Materials and methods: Plasma and quadriceps muscles were obtained from littermate wild type (WT) and muscle-specific PGC-1α knockout mice (MKO) 1 hour after injections with either saline or AICAR.
Results: PDH-E1α and cytochrome *c* protein content was lower ($p<0.05$) and hexokinase II protein tended to be lower ($0.05<p<0.1$) in PGC-1α MKO than

in WT mice, whereas GLUT4 protein content was similar in PGC-1α MKO and WT mice. AICAR treatment increased glucose-6-phosphate ($p<0.05$) in WT with no change in PGC-1α MKO mice. Plasma glucose concentration decreased ($p<0.05$) with AICAR in WT mice, but remained unchanged in PGC-1α MKO mice. AICAR treatment increased AMPK phosphorylation similar in skeletal muscle from PGC-1α MKO and WT mice, but while AICAR increased ($p<0.05$) ACC phosphorylation in WT mice, no change was evident in skeletal muscle from PGC-1α MKO mice. AICAR induced in WT mice an increase ($p<0.05$) in PDH-E1α site 2 phosphorylation without an associated change in PDHa activity, and in PGC-1α MKO mice a dephosphorylation ($p<0.05$) of PDH-E1α together with decreased ($p<0.05$) PDHa activity indicating regulatory dysfunction in acute metabolic regulation when PGC-1α is lacking.
Conclusion: *In vivo* AICAR-mediated PDH regulation seems to involve dissociation between PDH-E1α phosphorylation and PDHa activity. The blunted ACC phosphorylation and the changed PDH regulation in skeletal muscle from PGC-1a MKO mice in response to AICAR may indicate an impaired ability to increase fatty acid oxidation and an impaired acute regulation of substrate utilization when PGC-1α is lacking. Together these findings may suggest that a sedentary lifestyle, with decreased expression of PGC-1α, leads to metabolic inflexibility in part through dysregulation of ACC and PDH.

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Effects of the mtAtp-8 OXPHOS mutation on susceptibility to diet-induced lipoinflammation in conplastic B6-mt^{FVB} mice
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Background and aims: The conplastic mouse strain B6-mt^{FVB} with a mutation in the ATP synthase complex of the OXPHOS system shows an increased mitochondrial ROS production in various tissues and a pathological mitochondrial phenotype. Feeding a high fat diet induced an impaired glucose tolerance, insulin resistance and ultimately premature death after high fat diet in comparison to the B6-mt^{AKR} control strain. As lipoinflammation plays a pathogenetic role for nonalcoholic fatty liver disease (NAFLD) it was the aim of this study to investigate the effect of HFD on (a) hepatic triglycerides, (b) gene expression of proinflammatory cytokines in adipose tissue and, (c) protein level of fetuin A after 3 and 12 months in conplastic B6-mt^{FVB} mice.
Materials and methods: B6-mt^{AKR}(AKR) control and B6-mt^{FVB} (FVB) mice were fed high fat diet (HFD, 60 % fat) or control diet (CD, 10 % fat) after weaning. After 3 or 12 months animals were killed and tissues were collected for different assays. Expression of proinflammatory cytokines (IL-6, IL-1β, TNFα, INFγ) and adipokines (adiponectin, resistin, leptin) in adipose tissue was quantified by RT-qPCR. Hepatic triglyceride content was determined by photometric assay. Fetuin A serum levels were quantified by ELISA.
Results: AKR mice showed increased proinflammatory cytokines (IL-6 3-fold; $p<0.001$, IL-1β 4-fold; $p<0.01$, TNFα 4.6-fold; $p<0.001$) in adipose tissue after 3 months HFD. Only IL-6 expression was 4-fold increased in FVB mice after HFD ($p<0.001$). Gene expression of proinflammatory cytokines (TNFα, IL-1β) increased at the age of 12 months irrespective of strain and diet. After 3 months of HFD adiponectin gene expression were not different between the FVB and AKR. However, after 12 months of HFD adiponectin expression was 30 % lower in FVB mice than in AKR mice. HFD resulted in a 6.4-fold higher resistin expression in FVB mice compared to AKR HFD ($p<0.05$). Hepatic triglyceride content was 15 % higher in both strains after 3 months of HFD ($p<0.01$) whereas after 12 months of HFD a 47 % increase ($p<0.05$) could be observed in AKR compared to FVB mice. Leptin gene expression was 2.3-fold increased after 3 months of HFD in the FVB strain (2.3-fold; $p<0.05$) but did not show a difference to the AKR strain after a prolonged HFD for 12 months. In the AKR strain serum fetuin A serum levels showed an age-dependent increase in response to a HFD while in FVB mice fetuin A levels were not affected by the diet.
Conclusion: The *Atp8* mutation in the ATP synthase resulted in an increased susceptibility to HFD at the age of 3 months. The reduced life span of the FVB strain in response to HFD does not result from significant lipoinflammation but may be modulated by adiponectin and resistin levels. Our data indicate that mtDNA variations in the OXPHOS system negatively affect life span after metabolic stress irrespective of proinflammatory cytokines from adipose tissue. Importantly, hepatic triglyceride content appears not to be the predisposing factor for increased mortality of B6-mt^{FVB} mice in response to HFD.
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Effects of the mt-*Cytb* mutation in the B6-mt$^{129S1/SvlmJ}$ strain on metabolism, ROS defence and OXPHOS proteins after high fat diet
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Background and aims: Polymorphisms in the mitochondrial cytochrome b (*Cytb*) gene of the oxidative phosphorylation system (OXPHOS) have been associated with obesity in young adults. To elucidate this mitochondrial phenotype, we generated the conplastic mouse strain B6-mt$^{129S1/SvlmJ}$ which carries a mitochondrial DNA mutation in the *Cytb* gene coding for a subunit of Complex III of the respiratory chain. It was the aim of this study to investigate the effects of a long term HFD on metabolic parameters, gene expression of antioxidative enzymes and protein expression of the respiratory chain.
Materials and methods: B6-mt$^{129S1/SvlmJ}$ (mt129) were fed high fat (HFD, 60% fat) or control diet (CD, 10% fat) directly after weaning. Metabolic characterization comprised serum insulin, leptin, glucose tolerance and insulin sensitivity after 3 and 6 months of diet. Gene expression of Catalase, Superoxide dismutase 1 (SOD) and 2, uncoupling protein 2 (UCP) and mammalian target of Rapamycin (mTOR) were quantified by real-time PCR analyses. The protein expression of OXPHOS complexes were measured by western blot analysis in liver tissue.
Results: Mean blood glucose levels after 3 and 6 months of HFD or CD were not significantly different. Serum insulin levels in the mt129 were 4 times higher after 3 months (3.5 ±0.8 vs. 0.9 ± 0.1 µg/l; $p<0.05$) and 3-fold higher after 6 months of HFD (7.6 ± 1 vs. 2.5 ± 0.5 µg/l; $p<0.001$). mt129 showed a pronounced increase of serum leptin levels after 3 months of HFD (130 ±16 ng/ml vs. 34 ± 20 ng/ml). HFD for 3 and 6 months resulted in an impaired glucose tolerance and insulin sensitivity ($p<0.05$) in the mt129 strain. In liver tissue gene expression for the mitochondrial antioxidative enzyme SOD2 showed a significant twofold increased. Cytosolic SOD 1 and catalase gene expression were not affected by HFD in mt129 mice. Also UCP2 (2-fold, $p<0.05$) and mTOR (1.5-fold, $p<0.01$) were upregulated in HFD fed animals indicating compensatory mechanisms to the mitochondrial phenotype. The protein expression levels of complex III showed no differences while protein expression of complex I was significantly downregulated ($p<0.05$) at all investigated time points.
Conclusion: In line with the observation in obese adults we could demonstrate that long term high fat diet in *Cytb* mutant B6-mt$^{129S1/SvlmJ}$ mice leads to impaired glucose tolerance and insulin resistance in response to metabolic stress. The reduced complex I expression in the B6-mt^{129} strain could protect these mice against potential mitochondrial ROS production after metabolic challenge by nutrients. This view is supported by the observation that HFD induced antioxidative defense mechanisms (SOD 2) and OXPHOS uncoupling (UCP2) counteracting ROS production. The B6-mt^{129} could be an interesting model to study mitochondrial mechanisms that confer a high risk of obesity in early adulthood.
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Hepatic mitochondrial function in human obesity: association with insulin sensitivity and oxidative stress
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Background and aims: Hepatic energy metabolism can be lower in patients with overt type 2 diabetes, but it remains controversial whether alterations of hepatic mitochondrial oxidative capacity precede insulin resistance and occur in states such as human obesity. The present study assessed hepatic mitochondrial function in obese humans compared to healthy lean controls, and its association with insulin sensitivity and markers of systemic and hepatic oxidative stress.
Materials and methods: Sixteen non-diabetic obese patients scheduled for bariatric surgery (age 43±10 years, body mass index 51±9 kg/m^2) and seven non-diabetic controls (46±10 years, 26±2 kg/m^2) underwent euglycemic-hy-

perinsulinemic clamps with infusion of deuterated glucose to assess whole-body and hepatic insulin sensitivity. During surgery, liver samples were obtained and maximal mitochondrial respiratory capacity was measured in both intact tissue and isolated mitochondria using ex vivo high-resolution respirometry. Reactive oxygen species (ROS) production was measured in isolated mitochondria with the Amplex Red method, while lipid peroxidation in serum and liver was assessed by measuring thiobarbituric acid reactive substances (TBARS).
Results: Obese participants presented markedly lower insulin sensitivity (M-value 3.3±1.5 vs. 6.5±2.5 $mg.kg^{-1}.min^{-1}$, p=0.03) and 34% greater hepatic mitochondrial respiratory capacity for pyruvate and glutamate oxidation than controls (59±14 vs. 39±10 $pmol.s^{-1}.mg^{-1}$, p=0.006). They also tended to have higher maximal capacity for glutamate oxidation in isolated mitochondria (638±87 vs. 335±200 $pmol.s^{-1}.mg^{-1}$, p=0.06). In addition, obese subjects featured higher hepatic levels of hydrogen peroxide (973±181 vs. 618±201 pmol/min/mg protein), higher TBARS in liver tissue (114±21 vs. 82±30 μmol/mg protein) and in serum (36±13 vs. 27±10 μmol/mg protein) than controls (all p<0.05). Across all subjects, hepatic ROS production correlated positively with mitochondrial beta oxidation (r=0.68, p=0.04) as well as with pyruvate and glutamate respiration (r=0.83, p=0.006), while serum TBARS correlated positively with hepatic maximal glutamate oxidation (r=0.47, p=0.05). Furthermore, hepatic maximal glutamate oxidation correlated positively with hepatic insulin sensitivity (r=0.82, p=0.001).
Conclusion: Our study provides direct evidence that hepatic mitochondrial oxidative capacity is upregulated in non-diabetic obese humans and associates with augmented hepatic and systemic oxidative stress. This upregulation could be due to a transitional hepatic adaptation to lipid overload, which might precede impaired hepatic energy metabolism in overt type 2 diabetes.
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OP 34 Impact of treatment and genetic susceptibility to comorbidities and mortality

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Cardiovascular mortality in type 2 diabetes patients with incident exposure to long-acting insulin analogues
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Background and aims: To investigate the impact of insulin glargine exposure on cardiovascular mortality in type 2 diabetes (T2DM) patients with incident insulin initiation.
Materials and methods: All consecutive diabetes patients aged over 40 years, residing in a major urban area were screened at their first diabetes outpatient visit between 01/01/2001-12/31/2008 (n=79869). Exclusion criteria were insulin treatment at screening (n=14752), no insulin treatment until 12/31/2008 (n=55795), <6 months of glucose-lowering treatment alone before insulin initiation (n=1154), insulin prescription before glargine became available (04/17/2003, n=1761), age <40 or ≥80 years at first insulin prescription (n=406), and <6 months of insulin treatment following insulin initiation (n=1011). A total of 4990 subjects (58.4% females) were followed-up for death until 12/31/2011, based on death certificate and using data from the National Institute of Statistics. Baseline was defined at 6 months after insulin initiation. Data on mean dose of oral antidiabetics at insulin initiation, time between screening and insulin exposure, gender, age, time-dependent oral and insulin treatment (by each available active substance) during follow-up were available. Adjusted time-dependent competing risk regression analysis, with daily updates of treatment modalities was performed. Simultaneously use of cumulative exposure and ever exposed term of the available treatment options, a "fixed" cohort, cumulative exposure limited to that attained one year prior to death (minimizing the reverse causation), and a propensity score analysis completed the evaluation.
Results: Mean baseline age was 62±9 years, with a mean follow-up of 4.7±1.9 years (23179 person-years). During 23179 person-years exposure time, there were 521 cardiovascular deaths and 366 deaths from other causes (competing events). Glargine cumulative dose exposure (10,000 IU increments) significantly lowered overall cardiovascular mortality risk, subhazard ratio (SHR) 0.977 (95% CI 0.960-0.993, p=0.006), but not specifically for myocardial infarction, SHR 0.961 (95% CI 0.922-1.002, p=0.064) and stroke, SHR 0.974 (95% CI 0.929-1.021, p=0.266). Glargine cumulative time exposure (one year increments) significantly lowered overall cardiovascular, SHR 0.963 (95% CI 0.944-0.981, p<0.001), and myocardial infarction mortality risk, SHR 0.945 (95% CI 0.899-0.994, p=0.028), but not that for stroke SHR 0.973 (95% CI 0.927-1.020, p=0.257). Cumulative exposure limited to that attained one year prior to death showed a glargine cumulative exposure time SHR 0.940 (95% CI 0.919-0.960, p<0.001) and cumulative dose SHR 0.954 (95% CI 0.934-0.974, p<0.001) as regards cardiovascular mortality. "Fixed" cohort analysis showed no significant cardiovascular mortality hazard associated with baseline glargine exposure. Propensity score analysis did not significantly changed original results.
Conclusion: Both cumulative dose and cumulative time exposure to insulin glargine were associated with a lower risk of cardiovascular mortality. The effect was mostly driven by myocardial infarction end-point, with no significant contribution for stroke. These data support the concept of macrovascular benefit for basal analog insulin use in type 2 diabetes.

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Combination therapy with metformin plus sulfonylureas versus metformin plus DPP-4 inhibitors and risk of all-cause mortality
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Background and aims: The aim of this study was to evaluate the comparative risk of all-cause mortality for patients exposed to dual therapy with metfor-

min and sulfonylureas (SUs) compared with metformin and DPP-4 inhibitors (DPP-4i).
Materials and methods: Retrospective data were extracted from the Clinical Practice Research Datalink (CPRD): a data resource comprising approximately 10% of all patients treated in primary care in the United Kingdom. Patients with type 2 diabetes initiated with dual regimens comprising metformin with either a SU or a DPP-4i between 2007 and 2012 were selected, regardless of where these regimens were used in the natural history of the disease. The primary endpoint was all-cause mortality. Time to all-cause mortality was compared using Cox proportional hazards models. In addition to the main comparative analysis adjusting for key covariates within the model, two additional sensitivity analyses were performed. Firstly, a matched-cohort study comparing those treated with SUs (exposed) versus DPP-4i (non-exposed) using the following matching criteria at baseline: age (±2 years), gender, diabetes duration (±1 year), BMI (±3 kg/m2), serum creatinine (±10 μmol/L) and HbA1c (±1%). Secondly, patients were also matched by propensity score predicted by the same candidate variables.
Results: In the main analysis, 27,251 patients were prescribed metformin in combination with SUs, and 5,215 were prescribed metformin in combination with a DPP-4i. 3,454 patients were included in each arm of the direct matched cohorts and 4,703 in each arm when propensity matched. With respect to all-cause mortality; the hazard ratio was significantly increased for metformin+SUs compared with metformin+DPP-4i for those matched directly (adjusted hazard ratio [aHR]=2.314; 95%CI 1.348—3.973), for all subjects (aHR=1.265; 0.900—1.779), and for those matched on propensity score (aHR=1.691; 1.135—2.519; see table).
Conclusion: There was a consistent reduction in mortality for patients prescribed metformin in combination with DPP-4i versus metformin in combination with SUs. These data should be considered when initiating combination therapy with metformin.

Table | Events, crude rates and adjusted hazard ratios for all-cause mortality: metformin plus sulfonylurea versus metformin plus DPP4 inhibitors

	Cohort	Patients	Events	Crude rates (pkpy)	aHR	95% CI		p-value
All subjects	SU	27,251	818	16.9	1.265	0.900	1.779	0.176
	DPP-4i	5,215	50	7.6				
Directly matched	SU	3,454	71	10.1	2.314	1.348	3.973	0.002
	DPP-4i	3,454	18	4.1				
Propensity matched	SU	4,703	71	10.8	1.691	1.135	2.519	0.010
	DPP-4i	4,703	41	6.9				

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Association between first-line monotherapy with sulfonylurea versus metformin and risk of all-cause mortality
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Background and aims: The aim of this study was to evaluate the comparative risk of all-cause mortality for patients exposed to first-line diabetes monotherapy with either sulfonylureas or metformin.
Materials and methods: Retrospective data were extracted from the Clinical Practice Research Datalink (CPRD): a data resource comprising approximately 10% of all patients treated in primary care in the United Kingdom. Patients with type 2 diabetes initiated with first-line, glucose-lowering regimens between 2000 and 2012 were selected. The primary endpoint was all-cause mortality. Time to clinical endpoints was compared using Cox proportional hazards models. In addition to the main comparative analysis adjusting for key covariates within the model, two additional sensitivity analyses were performed. Firstly, a matched-cohort study comparing those treated with sulfonylureas (exposed) versus metformin (non-exposed) using the following matching criteria at baseline: age (±2 years), gender, year of index exposure, diabetes duration (±1 year), BMI (±3 kg/m2), serum creatinine (±10 μmol/l) and Hba1c (±1%). Secondly, patients were matched by propensity score predicted by the same candidate variables.
Results: In the main analysis, 76,811 patients were prescribed metformin monotherapy (mean follow-up 2.9 years), and 15,687 sulfonylureas (mean follow-up 2.9 years). 2,048 patients were included in each arm of the direct matched cohorts and 8,836 in the propensity matched. With respect to all-cause mortality, using all three analytical approaches the hazard ratio was significantly increased for sulfonylureas compared with metformin: adjusted HR=1.580 (95% CI 1.483—1.684) for the main analysis, 1.902 (1.733—2.088) for those matched on propensity score and 1.338 (1.051—1.704) for the directly matched analysis (see table).
Conclusion: Mortality was significantly increased in patients prescribed sulfonylureas as first-line, glucose lowering mon—therapy, compared with metformin monotherapy. Whilst residual confounding and confounding by indication may remain, this study indicates that treatment with first-line monotherapy with sulfonylureas should be reconsidered.

Table 1 | Events, crude rates and adjusted hazard ratios for all-cause mortality: sulfonylurea versus metformin first-line monotherapy

	Cohort	Patients	Events	Crude rates (pkpy)	aHR	95% CI		p-value
All subjects	SU	15,687	2,172	44.6	1.580	1.483	1.684	<0.001
	Met	76,811	3,209	13.6				
Directly matched	SU	2,048	145	23.5	1.338	1.051	1.704	0.018
	Met	2,048	123	16.3				
Propensity matched	SU	8,836	1,121	41.3	1.902	1.733	2.088	<0.001
	Met	8,836	739	22.9				

Supported by: Bristol-Myers Squibb

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Myocardial infarction in subjects using anti-diabetic and/or anti-depressant agents compared to non-users: a nationwide register study in Sweden
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Background and aims: We prospectively explored the gender- and age-specific risk of developing myocardial infarction (MI) in patients treated with anti-diabetic and/or anti-depressant drugs compared to subjects with no pharmaceutical treatment for diabetes or depression in a nationwide register study.
Materials and methods: A cohort of all Swedish residents born between 1925 and 1963, n= 3 738 524, were followed in an individual-level register study by cross-linkage of three nationwide registers. The prescription and dispensing of anti-diabetics, anti-depressants and anti-hypertensive drugs were used as markers of disease. Subjects with prior MI before the start of the study period were excluded from analyses. We categorised all subjects as having "diabetes" or "depression" according to the different drug classes prescribed and dispensed in Swedish pharmacies. Thus, all subjects were categorised as; 1) diabetes and depression combined, 2) depression only, 3) diabetes only or, 4) neither depression nor diabetes. The cohort was followed over a period of three years, 2008-2010. All participants were redefined and categories were updated every year. Data were analysed using binary logistic regression. The outcome was first fatal or non-fatal MI event. Present hypertension was used for adjustment.
Results: During follow-up, 44 298 subjects suffered a first MI of which 15 782 (36%) were females and 28 516 (64%) were males. Of the total number of MIs, 16 569 were fatal which comprised 37% of all MIs in both females and males respectively. Compared to women without diabetes or depression, the Odds ratio (OR) for MI was 7.1 (95% CI: 6.1-8.2) for women with both diabetes and depression in the age category 45-64 years. The corresponding OR for men with diabetes and depression was 2.8 (2.5-3.2) compared to men without diabetes or depression. The OR for females, 45-64 years, with diabetes alone was 5.0 (4.4-5.6) compared with females without diabetes or depression. In the same age category, the corresponding OR for males with diabetes alone was 2.5 (2.3-2.6) compared with men without diabetes or depression. The results were virtually unchanged when adjusted for hypertension.
Conclusion: Use of both anti-diabetics and anti-depressants as markers of diabetes and depression was associated with a higher risk for MI compared to diabetes alone. This increased risk was in relative terms most substantial in middle-aged women compared to middle-aged men.

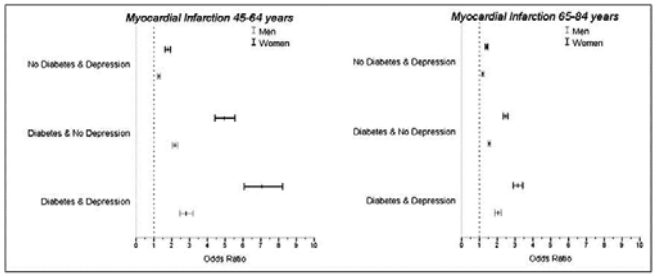

Figure 1. Odds Ratios with 95% CI for myocardial infarction in different groups of treatment categories in relation to subjects with neither treatment for diabetes nor depression stratified for age.

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KCNJ11 E23K polymorphism increases susceptibility for cardiovascular mortality in patients with type 2 diabetes

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Background and aims: Type 2 diabetes is caused by interactions between genetic and environmental factors. Diabetes and its complications are considered as a major cause of morbidity and mortality. Genome-wide association studies have identified more than 60 genetic variants associated with type 2 diabetes and/or glucose and insulin traits, but their effect on total mortality or cardiovascular (CVD) mortality has not yet been studied. The aim of this study was to explore whether these variants were also associated with total or CVD-mortality in patients with type 2 diabetes.
M**aterials and methods:** Discovery analyses included 30-36 common genetic variants in total of 3,610 patients from the local Malmö Scania Diabetes Registry (SDR), the Malmö Diet and Cancer Study (MDCS) and the Diabetes Alliance for Research in England (DARE) cohorts. Replication of KCNJ11 rs5219 (E23K) for association with total and CVD mortality was performed in the Botnia, the Diabetes Genetics Initiative (DGI) and the Steno studies. The Cox proportional hazards model was used to estimate relative genotype and phenotype effect on the risk of total and CVD-mortality.
Results: In the combined analyses of the SDR, MDCS and DARE cohorts, carriers of the T-allele (K-allele) of KCNJ11 rs5219 had increased total mortality rates (42.0% vs 39.5%; $HR_{additive}$ 1.10, 95% CI 1.15-1.20, p = 0.020), particularly CVD-related mortality (44.5%vs 39.5%, HR 1.26, 95% CI 1.10-1.44, p = 0.0006). In the meta-analyses of discovery and replication studies in up to 4,608 patients with type 2 diabetes, 820 of whom were cases of CVD-related deaths, carriers of the T-allele had a 1.21-fold increased risk for CVD-mortality (95% CI, 1.10, 1.34, p=0.00011).
Conclusion: We demonstrated that the KCNJ11 E23K variant is associated with increased risk of CVD-mortality in patients with type 2 diabetes, and, thus, seems to be a common denominator in the pathogenesis of type 2 diabetes and cardiovascular complications.
Supported by: Grants from the Swedish Research Council

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Diabetes, incretin therapy, pancreatitis and pancreas cancer: meta-analyses

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Background and aims: Concerns have been raised on the potential association between incretin-related therapies for type 2 diabetes and the risk of developing pancreatitis. Most recently, the debate has extended to the potential for the potential incretin-induced pancreatitis resulting in an increased risk of pancreas cancer.
Materials and methods: Meta-analyses were undertaken, following PRISMA guidelines, of the potential associations between (i) diabetes and pancreas cancer; (ii) diabetes and risk of pancreatitis; (iii) use of incretin therapies and risk of pancreatitis; (iv) use of GLP-1 RAs and risk of pancreatitis; (v) use of DDP-4 inhibitors and risk of pancreatitis. The aim was to shed light on the presence, strength and magnitude of this complex series of associations
Results: Patients with diabetes have an increased risk of pancreas cancer [SRR=1.62, 95% CI (1.42, 1.86)] (based on 48 prospective studies) and of being diagnosed with pancreatitis [SRR=1.86, 95% CI (1.59, 2.19)] (9 studies). Pancreatitis, in all its forms and irrespective of having diabetes, bears an increased risk of going on to develop pancreas cancer [SRR=10.52, 95% CI (6.49, 17.06)] (27 studies) although patients specifically with chronic pancreatitis have an even greater risk of pancreas cancer [SRR=13.2, 95% CI (6.43, 27)](9 studies). Patients with diabetes prescribed incretin therapies have a SRR=1.27 (95% CI (0.97, 1.66)) for pancreatitis compared to patients using other anti-diabetic medications. The use of GLP-1 RAs does not appear to have an increased risk of pancreatitis [SRR=1.31, 95% CI (0.85, 2.03)] (13 studies) although the unexplained variability in the individual findings is substantial. Similarly, DPP-4 inhibitors do not appear to have an increased risk of pancreatitis when all studies are considered together [SRR=1.23, 95% CI (0.84, 1.79)](6 studies). SRRs are lower when short-term randomized trials are excluded: GLP-1 RAs [SRR=1.13, 95% CI (0.77, 1.65)](6 studies) and DPP4i [SRR=1.40, 95% CI (0.92, 2.150](4 studies). There are important limitations of these meta-analysis including incomplete data sources, selective populations, short follow-up, inconsistent criteria for diagnosing pancreatitis and, consequently, a large degree of unexplained variability between individual study findings which limit drawing definitive conclusions.
Conclusion: Patients with diabetes have an increased risk of pancreatitis. When all available data are analyzed together, there does not appear to be an increased risk of pancreatitis in incretin-treated patients when compared to patients on other anti-diabetic therapies. However, the poor quality of the data available precludes any definitive conclusions apart from observing that any potential increased risk of pancreatitis associated with incretin therapies will be small. Patients with diabetes have an increased risk of pancreas cancer although whether this is mediated through pancreatitis is an open question. The inability to come to firm conclusions highlights once again the limitations of short-term observational studies. Carefully designed large prospective, population-based registry studies with good follow-up and key clinical information are urgently needed to answer these critical safety questions and provide reassurance to diabetic patients and their clinicians.

OP 35 Diabetes prediction: is stratification the solution?

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Genetic variation near SEPSECS/LGI2 associated with metformin treatment failure

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Background and aims: Metformin is the first line treatment for type 2 diabetes. Unfortunately the glycaemic response to metformin is highly variable between individuals and genetic factors appeared to be involved in this variability. A genome-wide association study (GWAS) by Zhou et al identified a locus near the *ATM* gene associated with metformin treatment response in T2D patients and we were able to replicate this association in our cohort. In addition to the *ATM* locus other loci reached borderline significance in the study by Zhou et al. ($p<10^{-5}$) and await confirmation in other studies. In the current study we aimed to replicate the reported effect of these genetic loci on metformin treatment response in T2D patients.
Materials and methods: Eleven SNPs with a minor allele frequency >5% in the by GWAS identified loci were measured in 600 patients on metformin monotherapy from the Diabetes Care System (n=7500), a large longitudinal cohort of Dutch type 2 diabetic subjects, using a Sequenom assay. Twenty-eight percent of the patients on metformin monotherapy did not reach the treatment target of an HbA1c <53 mmol/l (7%) within one year after initiation of therapy. We assessed if this treatment failure was associated with genetic variation using logistic regression with baseline HbA1c, eGFR and metformin dose as covariates.
Results: We found that rs10007566 a SNP with a minor allele frequency of 42% and located between the *SEPSECS* and *LGI2* genes on chromosome 4 is associated with treatment failure (OR=0.69 95% CI 0.53-0.89, p=0.005). This is in line with the original finding in the GWAS study and a meta-analysis combining our results with the published GWAS results in an OR=0.71 (95% CI 0.61-0.82, $p=5.2*10^{-7}$). Interestingly, *SEPSECS* is required for selenoprotein biosynthesis, a protein that is associated with T2D. There was no significant effect on treatment response observed with the other measured SNPs in our study, however five SNPs showed a trend in the same direction as in the original finding.
Conclusion: Using a large cohort of Dutch patients with type 2 diabetes, we have replicated that genetic variation near the *SEPSECS/LGI2* genes is associated with metformin treatment failure.
Supported by: Dutch Diabetes Research Foundation

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Biomarkers of cardiac stress and risk of microvascular events in patients with type 2 diabetes: results from the ADVANCE trial

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Background and aims: Emerging data indicates that N-terminal pro B-type natriuretic peptide (NT-proBNP) and high sensitivity troponin T (hsTnT) may be clinically useful in the prediction of macrovascular risk. However, whether they link to microvascular disease is not known. We therefore aimed to clarify the association of these cardiac biomarkers with microvascular risk in a cohort with type 2 diabetes.
Materials and methods: The ADVANCE trial (Action in Diabetes and Vascular disease: PreterAx and DiamicroN MR Controlled Evaluation) recruited 11,140 middle-aged type 2 diabetes patients. After 5 years follow-up we conducted a case cohort study in stored samples, measuring plasma NT-proBNP and hsTnT in 439 incident cases of new or worsening microvascular disease (including 283 incident cases of nephropathy and 183 incident cases of retinopathy), and a subcohort of 3423 non-cases.
Results: Median (IQR) concentrations of NT-proBNP in cases (all microvascular) and non-cases were 119pg/ml (46-324pg/ml) and 87pg/ml (34-211pg/ml), respectively, where for hsTnT result were 8ng/L (4-15ng/L) and 5ng/L (1.5-11ng/L). After adjustment for age, sex and treatment the HR (95% CI) for CVD per 1 standard deviation increase in the log-transformed NT-proBNP was 1.63 (1.44-1.84) and for hsTnT 1.67 (1.51-1.85). After adjusting for classical and diabetes-related CVD risk factors, renal function, CRP, and each other, the associations were 1.22 (1.08-1.38) and 1.31 (1.16-1.47) respectively. Estimates for associations with nephropathy and retinopathy were similar in fully adjusted models. In a prediction model including only events from the random subcohort, the baseline c-statistic of 0.731 (0.669-0.763) did not improve on addition of NT-proBNP and hsTNT (p=0.43 and p=0.14 respectively). However, the IDI increased 0.004 (0.001, 0.0082) and 0.00109 (0.0053, 0.0164) for NT-proBNP and hsTnT respectively. The corresponding figures for net reclassification (NRI) in a 3-category model were 0.7% (-3.3%, 4.8%) and 1.2% (-4.5%, 6.3%).
Conclusion: Cardiac biomarkers NT-proBNP and hsTnT were strongly positively associated with risk of microvascular events in ADVANCE, but the associations were largely attenuated by classical risk factors, such that addition of these biomarkers to risk scores does not improve risk prediction beyond these classical risk factors. Cardiac biomarkers have more utility for macrovascular risk prediction.
Clinical Trial Registration Number: NCT00145925
Supported by: NHMRC grant

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Durability of oral hypoglycaemic agents in drug naïve patients with type 2 diabetes: observational study from the Swedish National Diabetes Register (NDR)

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Background and aims: At diabetes onset, early initiation of oral hypoglycemic agents is advocated in order to control glucose levels and prevent long-term complications. Due to the progressive nature of type 2 diabetes, treatment intensification is often required. The aim of this study was to evaluate the durability of initiated mono therapy with metformin, sulphonylureas (SUs)/meglitinides, and non-pharmacological treatment in drug naïve patients, in clinical practice.
Materials and methods: Men and women with type 2 diabetes, non-pharmacologically treated for at least twelve months prior to the study start, initiating continuous mono therapy with metformin, SUs/meglitinides, or continuing non-pharmacological treatment, and registered in the Swedish National Diabetes Register (NDR) July 2005 - December 2011, were included in the study (n=143,642). At least 12 months continuous treatment with the initiated drug was required to be included. Patients were grouped based on glucose-lowering treatment, and the proportion of patients experiencing therapy failure (drug change, addition of other glucose-lowering drugs or cessation of the initial treatment) and mean time to therapy failure were analysed in each group.
Results: Of the total population, 66,076 patients (46%) remained non-pharmacologically treated, 72,146 (50%) were allocated metformin, and 5420 (4%) were allocated SUs/meglitinides. Patients were relatively newly diagnosed in all groups, with mean diabetes duration at baseline ranging between 2.5 years (non-pharmacological treatment) and 4.3 years (SUs). In the non-pharmacologically treated group 7% (n=4349) of the patients experienced therapy failure, while the corresponding proportions for metformin and SUs/meglitinides were 92% (n=66,360) and 86% (n=4674). Time to therapy failure was shortest for patients allocated metformin, 29.2 months (confidence interval (CI): 29.1 - 29.4). For patients on SUs/meglitinides and non-pharmacological treatment mean time to therapy failure were 32.9 months (CI: 32.4 - 33.5) and 33.4 months (CI: 33.2 - 33.6) respectively. Mean HbA1c-levels decreased during the study period in patients using metformin, SUs, and meglitinides, while HbA1c-levels increased in patients on non-pharmacological treatment.
Conclusion: The majority of patients were initiated on metformin at the start of the study, but a rather large proportion remained non-pharmacologically treated despite mean diabetes duration of 2.5 years. Pharmacologically treated patients, in general, were more likely to experience therapy failure, and metformin was associated with the shortest time to therapy failure. These findings, reflecting results from clinical practice, are most likely affected by

factors such as different treatment intensity in the treatment groups and need to be further analysed, including comparisons adjusted for important covariates.

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Estimating the interval between onset and diagnosis of type 2 diabetes from the time-course of retinopathy prevalence

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Background and aims: Type 2 diabetes (T2DM) may remain undiagnosed for years. The time preceding diagnosis has been estimated, on a population basis, at longer than 10 years, by extrapolating the correlation between known diabetes duration after diagnosis and prevalence of diabetic retinopathy (DR). However, those calculations were based upon questionable assumptions: 1) that such correlation is linear, 2) any degree of DR was considered, including very mild lesions observed in 10% of non diabetic individuals, 3) uncertain definition of T2DM, 4) time from onset of diabetes to first appearance of any DR arbitrarily set at 5 years. The aim of this study was to estimate the interval preceding diagnosis of T2DM after correcting the above assumptions.
Materials and methods: 12,074 patients subjected to screening for DR in 1991-2010 (35,545 screening episodes) were stratified into Younger-Onset (YO) if age at diagnosis was <30 or Older-Onset (OO, ≥30) and currently on insulin treatment (IT) or not (NIT). DR was assessed by 2-field retinal photography and considered either as including all lesions from very mild (ETDRS grade 20) to more severe (AnyDR) or moderate (ETDRS grade 35) and more severe (ModDR). The best-fits between known duration of diabetes and prevalence of AnyDR and ModDR were calculated by the coefficient of determination (R2) and Akaike's information criterion. The time preceding diagnosis of T2DM was estimated by adding the extrapolated negative intercept on the horizontal axis of the best-fitting correlation line between known duration of OO-NIT (equivalent to T2DM) and prevalence of ModDR (estimating time from appearance of ModDR to diagnosis) to the positive intercept of the best-fitting correlation between duration of YO-IT (equivalent to T1DM) and appearance of ModDR (estimating time from known onset of diabetes to appearance of ModDR).
Results: There were 7,298 OO-NIT, 1,719 with AnyDR and 685 with ModDR, and 1,725 YO-IT, 756 with AnyDR and 385 with ModDR. In the OO-NIT, a linear model suggested that AnyDR had appeared 8.26 years before diagnosis of T2DM. However, the best-fits were a quadratic model, suggesting appearance of AnyDR 3.89 years before diagnosis of T2DM, and a linear model with ModDR appearing 2.66 years before diagnosis. Appearance of AnyDR following onset of YO-IT could not be timed, whereas a quadratic model suggested appearance of ModDR 3.29 years after onset of YO-IT. The resulting estimate was (2.66+3.29) 5.95 years between onset and diagnosis of T2DM. Applying a standard approach, ie a linear model with AnyDR plus 5 years for appearance of DR, would have resulted in (8.26+5) 13.26 years.
Conclusion: Use of appropriate fitting models and stratification by insulin treatment and DR severity lowered the estimate of unknown duration of T2DM before diagnosis to about 6 years, which appears more realistic than previous estimates.

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A population-based study of diabetes incidence by ethnicity and age: support for the development of ethnic-specific prevention strategies

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Background and aims: As compared with individuals of European ethnic ancestry, a number of ethnic groups experience a higher risk for developing diabetes. This elevated risk may also begin at a younger age, particularly among people of South Asian descent. Current clinical guidelines are not clear about the optimal age at which to initiate screening in high risk populations.
Materials and methods: We conducted a longitudinal, population-based, retrospective cohort study using linked administrative health and immigration records for 592,376 immigrants to Ontario, Canada. We used Cox-Proportional Hazard models to: 1) generate adjusted incidence rates by ethnicity, sex and age; and 2) to determine the age cut-offs at which different ethnic groups experienced equivalent risk of developing diabetes.
Results: Individuals from South Asia had the highest overall incidence rate (age-sex adjusted rate of 15.7 per 1,000 person-years), which was 3.3 times higher than in the Western European group ($p < 0.05$). The risk for developing diabetes among 40 year-old Western European men (3.7 per 1,000 person-years) was roughly equivalent to the risk experienced by South Asian men and women at age 25. For all other non-European ethnic groups, the equivalent risk was experienced between age 30 and 35. For women of Western European origin, the diabetes risk at age 40 (1.7 per 1,000 person years) was lower than observed at any age in South Asian women. These risk differentials persisted despite controlling for income, education, immigration category and time in Canada.
Conclusion: By age 40, there was a marked disparity in diabetes incidence between South Asian and Western European populations - in the order of 5-fold for men and 9-fold for women. High risk ethnic groups display a similar level of diabetes risk between 10 and 15 years younger than individuals of Western European origin. This has practical implications for primary and secondary prevention efforts.

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Long term age-related trajectories of cardiovascular risk factors are affected by cohort effects: the Whitehall II study

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Background and aims: Analysis of long-term changes in cardiovascular risk factors based on sequential measurements can give insight into the process of ageing, but longitudinal analyses may be influenced by cohort effects and secular trends. Our aim was to investigate how age-related trajectories of obesity, blood pressure and lipid levels were affected by year of birth.
Materials and methods: We fitted quadratic age-related trajectories for BMI, waist circumference, systolic and diastolic blood pressure as well as total and HDL cholesterol between the ages of 35 and 80 based on the Whitehall II study, a longitudinal dataset of 10,308 individuals with 36,692 observations obtained during over 25 years of follow up. Year of birth and its interaction term with age and age^2 were added to the models in order to evaluate cohort effects. Smooth kernel distributions were fitted for subgroups of participants aged 57 to 61 at each of four study phases (5 years apart) to visualise the cohort effect.
Results: BMI and waist circumference distributions for the age group 57-61 showed a higher degree of right-skewing with each subsequent phase in both sexes, whereas a right-shift in the distribution was observed for men but not for women. In both sexes distributions of diastolic blood pressure and total cholesterol showed a left-shift with each subsequent phase, whereas HDL-cholesterol showed a slight right-shift. There were marked differences in BMI, diastolic blood pressure and total cholesterol trajectories depending on the year of birth (Figure 1). For any given age, younger birth cohorts had higher BMI and a steeper increase in BMI with age. At any given age over 50 both total cholesterol and diastolic blood pressure were lower in younger birth cohorts. A model that does not take birth year into account (Figure 1, dashed line) underestimated the age-related decrease in total cholesterol in men and in diastolic blood pressure in both sexes.
Conclusion: Lifetime exposure to cardiovascular risk factors differs markedly for people born as little as 5 years apart, with cohort effects of opposite directions and different magnitudes. These effects are likely due to the different age and stage of life at which each subsequent generation experienced the changes in society, diet, exercise, and developments in cardiovascular risk detection and management seen during the past decades. Cohort effects should be taken into account when investigating the effects of ageing in longitudinal datasets.

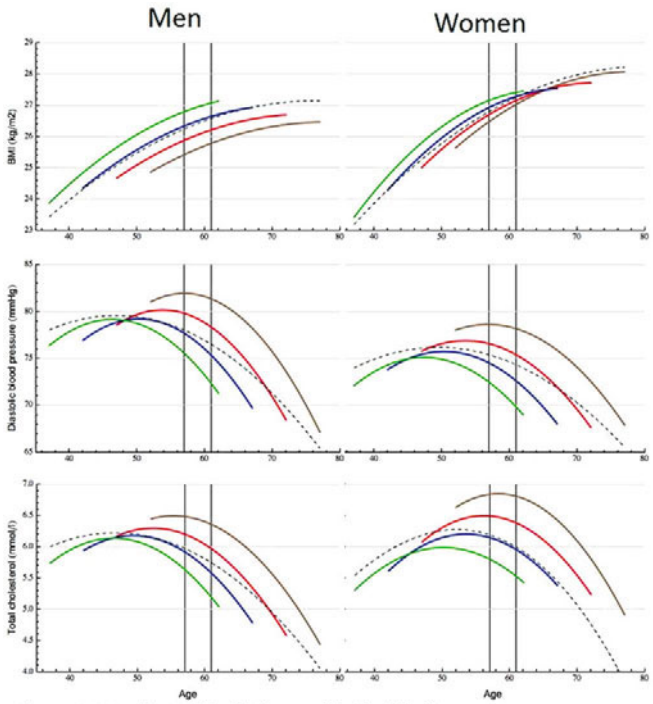

Figure 1. Unadjusted for birth year (dashed line)
Birth years: 1933 (brown), 1938 (red), 1943 (blue), 1948 (green).

Supported by: MRC, BHF, NHLBI, NIA

OP 36 Islet cell development and plasticity

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ARX knockout hESCs show biased pancreatic endocrine specification to unihormonal somatostatin cells

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Background and aims: Humans with a genetic syndrome known as X-linked lissencephaly with abnormal genitalia (XLAG) have null mutations in the gene Aristaless-related homeobox (*ARX*). *ARX* is expressed in the developing and adult brain, heart, skeletal muscle, testis, and pancreas. The pancreatic islets of XLAG patients lack glucagon-positive alpha cells but retain insulin-positive beta cells and somatostatin-positive delta cells. Mice lacking functional *Arx* expression have no alpha cells while beta and delta cells increase in numbers. To model human developmental *ARX* deficient pancreatic phenotypes, we have developed *ARX* knockout human embryonic stem cells (hESCs) and subjected them to pancreatic endocrine differentiation conditions.
Material and methods: *ARX* knockout hESCs (ARXko) were generated by zinc-finger nuclease mediated targeted genomic editing. Two independent clones were differentiated to pancreatic endocrine cells with C-peptide, glucagon and somatostatin release measured by RIA or EIA. Flow cytometry was used to assess the progressive differentiation of hESCs to CXCR4-positive definitive endoderm, PDX1-positive foregut endoderm, and NKX6.1-positive pancreatic progenitors. Final hormone-positive populations and transcription factor expression profiles were assessed by immunofluorescence.
Results: Two independent ARXko clones were isolated with either a 23 or 41 base pair deletion in exon 1 of *ARX*, both of which created a frameshift mutation resulting in a premature stop codon. Both clones retained embryonic morphology and expression of OCT4 and SSEA3. Following a stage-specific differentiation protocol aimed at forming pancreatic endocrine cells, ARXko hESCs were compared to unmodified hESCs (WT). ARXko cells efficiently formed CXCR4-positive definitive endoderm cells (WT: 94±1%, ARXko1: 95±1%, ARXko2: 95±2%). These cells continued developing into PDX1-positive foregut endoderm (WT: 91±3%, ARXko1: 94±3%, ARXko2: 96±1%), which subsequently developed into NKX6.1-positive pancreatic progenitors (WT: 49±3%, ARXko1: 70±2%, ARXko2: 66±4%, $p<0.05$) that co-expressed PDX1 by immunohistochemistry. Continued culture yielded similar fractions of pancreatic endocrine cells in both WT and ARXko (24-28%). Interestingly, examination of the endocrine subpopulations revealed an increase in unihormonal somatostatin-positive cells (WT: 18±2%, ARXko1: 73±4%, ARXko2: 75±2%, $p<0.05$) at the expense of both insulin (WT: 76±1%, ARXko1: 27±4%, ARXko2: 24±2%, $p<0.05$) and glucagon-positive (WT: 40±9%, ARXko1: 4±1%, ARXko2: 5±1%, $p<0.05$) cell lineages in ARXko cells.
Conclusion: While XLAG and ARXko hESCs endocrine populations both contain a near complete absence of glucagon-positive cells, we found a dramatic increase in unihormonal somatostatin-positive cells at the expense of insulin-positive cells, which were more common in XLAG pancreas samples compared to differentiated ARXko hESCs. The approximately 3-fold decrease in insulin-positive cells in the ARXko hESCs suggests a specific deficit of the current *in vitro* culture system that favours unihormonal somatostatin-positive cells. Thus ARXko hESCs warrant further study into the human pancreatic endocrine cell fate specification process with the goal of understanding the mechanisms that distinguish the beta and delta-cell lineages.
Supported by: CIHR, JDRF, Stem Cell Network

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Efficient generation of definitive endoderm from human embryonic stem cells by GSK3 beta inhibition and nodal signalling

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Background and aims: The generation of insulin-producing cells from embryonic stem cells (ES cells) requires the stepwise transfer of developmental principles to in vitro differentiation protocols. It is accepted that treatment of human ES cells with high concentrations of activin A by activation of the Nodal/Tgf-beta pathway is the biochemical reason for differentiation of ES

cells into cells reminiscent of definitive endoderm (DE). However, the thesis that Nodal/Tgf-beta is solely responsible for in vitro differentiation of ES cells into DE appears as a simplification of developmental mechanisms in particular when compared to early in vivo events during gastrulation. Consequently, the differentiation protocols used so far have not yet yielded DE-like cells with 100% purity. The inhibition of the GSK3 beta was recently discovered as a promising method to generate DE-like cells in vitro. We analyzed the role of the GSK3beta inhibitor CHIR-99021 in a comparative study against a classic endoderm differentiation protocol.
Materials and methods: Human ES cells (Hues8) were differentiated with a control protocol comprising the treatment with wnt3a (25 ng/ml) and activin A (100 ng/ml) for 3 days and a protocol designed to initiate a primitive-like stage by a 24 h GSK3 beta inhibition with 5 μM CHIR-99021 (Chir) followed by a 48 h treatment with activin A (100 ng/ml). The differentiation into DE was monitored by flow cytometry of the DE-surface markers CXCR4 and CD49e, qPCR, and immunofluorescence staining.
Results: The control protocol yielded 22±4% CXCR4/CD49e-positive cells and was inferior to cells in which Chir was added initially for 24 h followed by a further 48 h treatment with activin A (57±8%). Analysis of typical DE-related genes revealed an expression pattern similar to developmental kinetics with T and MIXL1 expressed after induction with wnt3a and activin A for 24 h from where it decreased. SOX17 and FOXA2 were induced and reliably detectable after 3 days of differentiation. In Chir/activin A treated cells T expression was 17.3 fold higher expressed and MIXL1 7.4 fold higher after 24 h. Upon change of the medium to activin A, both genes were downregulated and SOX17 and FOXA2 mRNA transcripts were detectable 3.6 and 6 fold higher (for FOXA2 and SOX17, respectively) compared to control cells. In immunofluorescence stainings, Chir/activin A treatment resulted in predominantly brachyury-positive cells after the 24 h GSK3 beta inhibition with distinct outgrowth of SOX17/FOXA2 double-positive cells later in the differentiation. SOX17/FOXA2 double-positive were not detected before day 2 from where they increased until near homogeneity at day 3. Generally the wnt3a/activin A protocol was less efficient, thereby confirming the flow cytometry results. A dilution of activin A after the induction with Chir showed that activin A concentrations can be significantly lowered to 25-50 ng/ml to yield the same results.
Conclusion: Our analyses revealed the inductive effect of CHIR-99021 which directs human ES cells into cells reminiscent of the primitive streak independent of the Nodal/Tgf-beta pathway. By addition of low doses of activin A, these cells can be effectively differentiated into definitive endoderm. This demonstrates that human ES cell differentiation into DE is initially Wnt-pathway dependent and subsequently Nodal/Tgf-beta dependent.
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NADPH oxidase promotes pancreatic endocrine cell differentiation via mediation of SOX9
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Background and aim: NADPH oxidase is a pivotal reactive oxygen species-generating enzyme, involved in organogenesis, regeneration and differentiation. Nonetheless, the potential role of NADPH oxidase in regulating endocrine cell specification remains unclear. This study was aimed to investigate the expression and function of NADPH oxidase in endocrine cell differentiation.
Material and methods: Mouse embryonic pancreata were dissected at indicated embryonic-days to assess relevant gene and protein expression by real-time PCR and immunohistochemistry. In the *ex vivo* experiments, pancreata were dissected at e12.5 from mouse embryos and cultured for three days with or without diphenyleneiodonium (DPI), a NADPH oxidase inhibitor, to evaluate gene expression.The potential mechanism involved was further studied using an *in vitro* human cell model of pancreatic progenitor cells (PPCs) by Western blot analysis.
Results: Our real-time PCR results showed that *Nox4* and *p22^{phox}*, the subunits of NADPH oxidase, were expressed during embryonic pancreas development and peaked at e15.5, then subsequently declined and disappeared in neonatal pancreas. Meanwhile, NOX4 was detected along with beta-cells, acinar-cells and ductal-cells at e15.5 shortly after secondary transition. However, its expression was decreased in acinar-cells and ductal-cells at e17.5, and persisted along with high level of insulin in endocrine cells, as evidenced by immunocytochemistry; NOX4 also had a high expression at e15.5 within NGN3-expression cells, the progenitors specific for the fate of endocrine cell lineage. On the other hand, *ex vivo* DPI treatment reduced the expression of *Ngn3* (87.8%, ***p<0.001), *Pax4* (91.07%, ***p<0.001), *Nkx6.1* (89.2%, ***p<0.001), insulin (99.64%, ****p<0.001), and glucagon (99.67%, ***p<0.001) at the dosage of 1μM in the cultured embryonic pancreas. Further study showed that *Id2* and *Hes-1*, the suppressor of *Ngn3*, were not affected by DPI while Sox9 (it binds to the promoter region of *Ngn3*) were reduced both in mRNA and protein levels. Furthermore, DPI decreased NGN3 expression in human PPCs while its overexpression of SOX9 abolished the effect exerted by DPI.
Conclusion: Our data suggest that NADPH oxidase enhances pancreatic endocrine cell differentiation, which is probably mediated via the expression of SOX9.
Supported by: The Research Grants Council of the Hong Kong Special Administrative Region

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Contribution of Ins$^+$GLUT2$^-$ progenitor cells to endocrine pancreas regeneration after streptozotocin ablation in the neonatal mouse
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Background and aims: A renewable source of insulin-producing β-cells in the pancreas is required for patients with diabetes, yet evidence suggests that "all" new β-cells originate from pre-existing β-cells. However, the contribution of progenitor cell source to β-cell mass is less well defined, especially within the context of induced regeneration, such as after administration of the β-cell toxin, streptozotocin (STZ). Neonatal rodents exhibit the ability to regenerate some of their endocrine pancreas after STZ ablation, but this response is mitigated when STZ is administered in adulthood. As a glucose mimetic, STZ accesses the β-cell via the Glucose Transporter-2 (GLUT2). A rare sub-population of β-cells, Ins$^+$GLUT2$^-$ cells, have been identified as pancreatic multi-potential progenitor cells, found in adult mouse and human islets. We have recently shown a significantly higher population of these Ins$^+$GLUT2$^-$ cells in neonatal (postnatal day7, P7) extra-islet β-cell aggregates (<5 β-cells, BCA) than in intact islets, which are proliferative and can differentiate into multiple lineages. We hypothesized that because of the numerous Ins$^+$GLUT2$^-$ progenitor cells, neonatal rodents are able to regenerate their β-cell mass after STZ administration because these cells are spared exposure to the toxin.
Materials and methods: Neonatal RIPCreER;Z/AP transgenic mice were used to tag a proportion of β-cells with a human placental alkaline phosphatase (HPAP) reporter, by administration of Tamoxifen at P5. 100mg/kg STZ was given at P7, and EdU at P8 to track cell proliferation. Cells were followed during β-cell regeneration, and pancreata collected between D7-D30 for immunofluorescence.
Results: Glycaemia was affected during acute injury, and recovered, although not to pre-STZ levels. HPAP tagged ~35% of insulin-expressing (β-) cells in islets, which was slightly lower in BCA. After STZ, the proportion of HPAP$^+$ β-cells in islets displayed a significant increase at P14 vs control mice (p<0.05), indicating that some β-cell regeneration in the islet occurred by self-duplication. However, response in the BCA was blunted as compared to the islet. There was a significant decrease in HPAP$^+$ β-cells in BCA at P21, possibly indicating that these cells had developed from BCA into small islets. β-cell mass decreased after STZ administration in islets by P14 (p<0.01, P7 vs P14), concomitant with overt diabetes; in contrast, there was a non-significant decrease in β-cell mass in the BCA, indicating that many of these cells were spared STZ-mediated death. Ins$^+$GLUT2$^-$ cells decreased in number from P7 to P30 in control mice, although interestingly there was an increase in Ins$^+$GLUT2$^-$ cell numbers between P7 and weaning at P21, which decreased thereafter. After STZ treatment, there were a higher number of Ins$^+$GLUT2$^-$ cells at P14 than in control mice; by P30, the cell population decreased in both groups.
Conclusion: These data indicate that Ins$^+$GLUT2$^-$ cells contribute to endocrine pancreas regeneration in the neonatal mouse after STZ administration, and represent a possible cell target for intervention in the human during diabetes progression.
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Foxo1 ablation increases endocrine progenitor cells in the adult pancreas
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Background and aims: Deterioration of β–cell function and decrease of β–cell mass are the sine qua non of diabetes. Previously, we showed that Foxo1 gain-of-function in pancreatic β–cells suppresses proliferation of mature β–cells under physiological condition, in response to insulin resistance and to Glp-1 analogs, but the role of Foxo1 in β–cell differentiation and function remains unclear.
Materials and methods: To address this question, we generated conditional knockouts of Foxo1 driven by Pdx1-cre, Neurogenin3-(Neurog3)cre and Insulin-(Rip)cre, resulting in somatic deletion of Foxo1 in all pancreatic cells (Pdx1-cre), in endocrine progenitors (Neurogenin3-cre), or in terminally differentiated β–cells (Rip-cre).
Results: We report that conditional inactivation of Foxo1 in pancreatic precursors, but not in endocrine progenitors, results in 2-fold increase in pancreas size of 6-month-old mice. Immunohistochemical studies and FACS analyses show that islets and ductal epithelium from adult Pdx1-Foxo1, but not Neurog3-Foxo1 knockout mice are enriched with the pancreatic precursor cell marker Sox9, but are not hyper-proliferative. These results indicate that the increase in pancreas size is likely due to an expanded number of pancreatic precursors. In addition, we illustrate the role of Foxo1 in β–cell development. We found a 5- to 10-fold expansion of β–cell mass in 12-month-old Pdx1-Foxo1 knockouts, and in 12- to 15-month-old Neurog3-Foxo1 knockouts, but not in Rip-Foxo1 knockouts. Quantitative mRNA analysis, FACs analysis and immunohistochemistry demonstrate the presence of Neurog3-positive cells in adult mice, a highly unusual finding, since these progenitor cells are normally restricted to prenatal life. This result was also confirmed when crossing the Pdx1-Foxo1 knockout mice with Neurog3-Gfp reporter. Proliferation rates of β–cells in Pdx1- and Neurog3- Foxo1 knockouts are insufficient to account for the increase in β–cell mass. In addition, lack of Foxo1 in mature β–cells does not affect β–cell replication nor β–cell mass.
Conclusion: Thus these data indicate that the enlarged β–cell mass is due to an increase in Neurog3 progenitors rather than β–cell self-duplication. Together these data provide evidence for a critical role of insulin/growth factor signaling through Foxo1 in β–cell differentiation and β–cell function.
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LKB1 and AMPK differentially regulate pancreatic beta cell fate
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Background and aims: Inactivation in beta cells of the tumour suppressor Liver kinase B1 (LKB1/STK11) or the downstream enzyme AMP-activated protein kinase (AMPK) exerts dramatic, but opposing, effects in vivo. Thus, loss of LKB1 causes hyperplasia and loss of normal cell polarity whereas AMPK deletion inhibits insulin secretion. To examine the molecular mechanisms involved we have explored the transcriptional landscape of islets inactivated selectively in the beta cell for either kinase.
Materials and methods: Mice null for LKB1 or both AMPK catalytic (α1, α2) subunits were generated by Ins2- (beta cell and brain), Ins1Cre- (beta cell), or Pdx1CreER- (adult beta cell) mediated deletion of flox'd alleles. Islets were isolated from four 12 week old mice per genotype. After 24 h culture at 11 mM glucose RNA was extracted (RNAEasy) before deep sequencing (RNASeq) on a Hiseq 2000 platform (Illumina, San Diego, CA, USA) or microarray analysis (Affymetrix mouse Gene 2.0 array). Enrichment against functional gene categories was performed using the Functional Annotation Clustering algorithm of DAVID (version 6.7) or Gene Set Enrichment Analysis using mouse MSigDB gene sets.
Results: Consistent with the differing effects of deleting either kinase in beta cells, overlap between LKB1 and AMPK-regulated genes was limited (80/1034 mRNAs up-regulated and 32/980 down-regulated by deletion with Ins2Cre). mRNAs up-regulated by LKB1 deletion, but unaffected by AMPK inactivation, included those encoding neuron- (Dlgap2, Pdyn, Nptx2, Astn1, Cartpt, Mt3) and liver- (Fbp2, Iyd and Alb) enriched genes. mRNAs affected by deletion of either kinase included mcoln3, Th, Lgi (down-regulated), Kcna2, Kcnq2 (up-regulated). Examination of gene classes revealed that endoplasmic reticulum function (mboat1, clstn2) was enhanced by LKB1, but not AMPK, deletion. Conversely, AMPK, but not LKB1 deletion, decreased expression of lysosomal and peroxisomal genes (Hpse, Il4i1) but increased that of G-protein coupled receptors (Ptgit, Galr1) and GTP-binding proteins (Arhgap20, Rap1Gap).
Conclusion: An important function of LKB1 is to repress non-beta cell programs in beta cells, since its absence leads to the de-repression of alternate programs leading to neuronal and hepatic characteristics. This action of LKB1 is largely independent of AMPK which, conversely, maintains degradative pathways whilst restricting specialised functions such as receptor signalling.
Supported by: IMIDIA (EU-FP7), Wellcome Trust, MRC (UK), DRWF, DUK

OP 37 Insulin in type 2 diabetes: earlier? dangerous? stronger?

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The effect of early intensive diabetes treatment on long-term glycaemic control in newly diagnosed type 2 diabetes: multicentre randomised parallel trial

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Background and aims: Early intensive insulin therapy in newly diagnosed type 2 diabetes has been reported to improve pancreatic beta-cell function and facilitate long term glycaemic control. However, it is unclear whether those results are by virtue of the effect of elimination of glucotoxicity or of insulin therapy itself. We performed a multicenter randomized trial to compare the long term effects of early short-term intensive diabetes treatment modalities: intensive insulin therapy (IIT) versus combined oral antidiabetic therapy (COAD).
Materials and methods: Eligible newly diagnosed 97 patients (aged 25-70 years, HbA1c 8-12%, drug naïve) were randomised to IIT group (50, multiple daily insulin therapy with glargine and glulisine) or COAD group (47, glimepiride and metformin) for early intensive diabetes treatment in eight diabetes centers of Korea between 2007 and 2009. Early intensive treatment was performed in outpatient clinic setting according to the protocols and was stopped after achieving HbA_{1c} < 7% or total inulin requirement < 10U/day or 12 weeks of treatment duration. Patients were then followed-up every 1-3 months for 2 years on diet/exercise alone or rescue drug therapy as a protocol in case of HbA1c more than 8%. Primary endpoint was long term glycaemic control and remission (HbA_{1c}<7% without drug) rate at 2 year after early intensive therapy. Analysis was per protocol.
Results: After early intensive treatment, the mean HbA_{1c} significantly decreased from 10.1±1.0 to 6.8±0.7 % in IIT and 10.0±1.2 to 6.6±0.6 % in COAD group with no significant difference between two groups. In IIT group, mean HbA_{1c} was reduced below 7% at 8 weeks but at 12 weeks in COAD group. During the 104 weeks of follow-up period, both groups maintained good glycemic control with mean HbA_{1c} around 7% ($P = 0.093$, linear mixed model analysis IIT vs. COAD), but the proportion of patients with HbA_{1c} < 7% was significantly higher in IIT groups through the follow-up period (68.1%-88.6% in IIT vs. 47.1-65.0% in COAD). In IIT group, 58.1% at 1 year and 51.4% at 2 year maintained glycaemic control with diet/exercise alone, but 33.3% and 21.4%, respectively in COAD group. Remission rate at 104 weeks was significantly higher in IIT groups than in COAD group ($P = 0.022$).
Conclusion: Early intensive diabetes treatments with IIT or COAD in newly diagnosed type 2 diabetes were effective on long glycaemic control, but IIT was more favorable on the remission rate and maintenance of optimal glycaemic control than COAD.
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Exogenous insulin and risk of all-cause mortality in type 2 diabetes: a dose-response association

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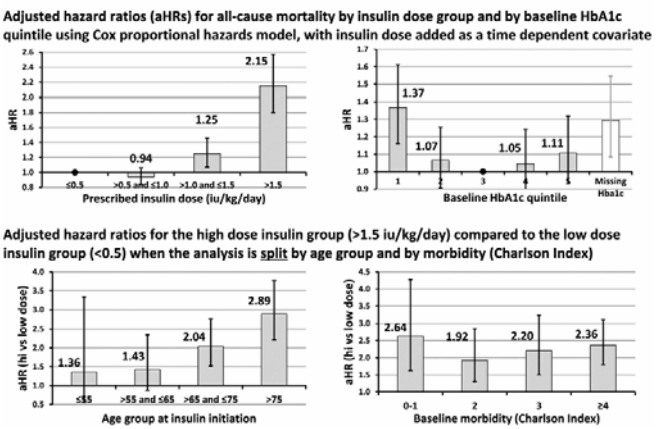
Background and aims: Along with a clinical and biological rationale, epidemiological data have emerged that raise questions about the widespread use of exogenous insulin in people with type 2 diabetes. In the absence of specifically designed randomised trials, here we explore the hypothesis that if insulin increases the risk of death in these people, there should be a dose-response association.
Materials and methods: Data were from a 10% sample of the UK population available from the Clinical Practice Research Datalink. Using strict data quality criteria, subjects with type 2 diabetes were selected if they had progressed to treatment with insulin. The first prescription of insulin was used as the index date. Annual, average, weight-based, prescribed insulin dose was estimated by attributing a quantity to recorded prescriptions. Our outcome was all-cause mortality, and the risk of death was evaluated in Cox models that accounted for common risk factors. Dose was evaluated as a time-dependent covariate. Prescribed dose categories were set at ≤0.5, >0.5 and ≤1.0, >1.0 and ≤1.5, and >1.5 iu/kg/day using the lowest dose as the referent.
Results: We identified 8,414 people exposed to an insulin-only regimen and receiving at least two consecutive prescriptions for insulin. Of these, 7,538 patients had complete insulin dose (with an average dose ≤4iu/kg/day), weight and time-to-event information; 2,193, 3,818, 1,013 and 514 patients were allocated to the ≤0.5, >0.5 and ≤1.0, >1.0 and ≤1.5, and >1.5 iu/kg/day insulin-dose groups, respectively, in year 1. The overall mean age at baseline was 64.6, 64.7, 64.4 and 64.9 years; 63%, 55%, 51% and 47% were males; and average HbA1c at baseline was 9.2%, 9.7%, 9.9% and 9.6% for each increasing dose category, respectively. The risk of death increased in the lowest HbA1c quintile and above 1.0 iu/kg/day (figure) in a generally increasing pattern. The pattern of association with dose was consistent in a number of phenotypic subgroups such as those with good glucose control at baseline. In comparison with the low-dose insulin group (≤0.5iu/kg/day), adjusted hazard ratios for the high-dose group (>1.5iu/kg/day) were 1.36 (0.56-3.34), 1.43 (0.87-2.35), 2.04 (1.52-2.75) and 2.89 (2.22-3.77) for patients aged ≤55, >55 and ≤65, >65 and ≤75, and >75 at baseline.
Conclusion: There was a dose-response association between insulin and the risk of death in subjects with type 2 diabetes. Those progressing to insulin therapy in older age and with good glucose control were at particularly high risk.

Figure



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IDegLira, a novel fixed-ratio combination of insulin degludec and liraglutide, is efficacious and safe in subjects with type 2 diabetes: a large, randomised phase 3 trial

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Background and aims: IDegLira is a novel fixed-ratio combination of insulin degludec (IDeg) and liraglutide (Lira). The aim of this trial was to evaluate the safety and efficacy of IDegLira compared to its individual components, Lira or IDeg alone, in subjects with type 2 diabetes (T2D).
Materials and methods: In this 26-week randomised, parallel three-arm, open-label trial in subjects with T2D inadequately controlled on metformin ± pioglitazone, IDegLira was added OD and compared to IDeg or Lira (Victoza®1.8 mg) added alone. IDegLira and IDeg were titrated to the same fasting glucose [FPG] (4-5 mmol/L [72-90 mg/dL]). Of 1,663 adults (mean age: 55 yrs, diabetes duration: 6.6 yrs, BMI: 31.2 kg/m^2), 834 were randomised to IDegLira, 414 to IDeg, and 415 to Lira.

Results: The primary endpoint, HbA_{1c}, decreased by 1.9% from 8.3% to 6.4% with IDegLira. This decrease was significantly greater than with IDeg (-1.4% to 6.9%) or Lira (-1.3% to 7.0%) (Table). More subjects on IDegLira achieved an HbA_{1c} of <7% (81%) or ≤6.5% (70%) compared to IDeg (65%; 48%) and Lira (60%; 41%). At 26 weeks, mean FPG was similar for IDegLira (5.6 mmol/L [100 mg/dL]) and IDeg (5.8 mmol/L [104 mg/dL]) and significantly higher for Lira (7.3 mmol/L [131 mg/dL]). Nine-point glucose profiles showed significantly lower mean prandial increments with IDegLira and Lira compared to IDeg. IDegLira resulted in a mean weight reduction of -0.5 kg and a 32% lower rate of hypoglycaemia than IDeg. Few subjects reported hypoglycaemia with Lira. Gastrointestinal (GI) side effects with IDegLira were less than with Lira (nausea: 8.8% vs. 19.7%; vomiting: 3.9% vs. 8.5%).

Conclusion: IDegLira substantially improves glycaemic control in subjects with T2D with a low risk of hypoglycaemia, weight gain or GI complaints.

Table. Key results with IDegLira vs. IDeg or Lira alone

	IDegLira vs. IDeg Estimate [95% CI]	p-value	IDegLira vs. Lira Estimate [95% CI]	p-value
HbA_{1c} change (%-points)	−0.47 [−0.58 ; −0.36]	<0.0001	−0.64 [−0.75 ; −0.53]	<0.0001
FPG change (mmol/L)	−0.17 [−0.41 ; 0.07]	NS	−1.76 [−2.00 ; −1.53]	<0.0001
Weight change (kg)	−2.22 [−2.64 ; −1.80]	<0.0001	2.44 [2.02 ; 2.86]	<0.0001
Hypoglycaemia[§]	0.68 [0.53 ; 0.87]	0.0023	7.61 [5.17 ; 11.21]	<0.0001

[§]Hypoglycaemia: PG < 3.1 mmol/L [56 mg/dL] and/or requiring assistance, Rate ratio: IDegLira/Comparator;
p-value: two-sided test at a 5% significance level.

Clinical Trial Registration Number: NCT01336023
Supported by: Novo Nordisk

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New insulin glargine formulation: glucose control and hypoglycaemia in people with type 2 diabetes using basal and mealtime insulin (EDITION I)

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Background and aims: Insulin glargine 100 U/mL (Gla-100) is widely used in people with type 2 diabetes (T2DM). A new insulin glargine formulation, 300 U/mL (Gla-300), has a more prolonged and flatter pharmacokinetic profile than Gla-100. The phase 3 EDITION I study compared the efficacy and safety of Gla-300 *vs* Gla-100 in people with T2DM using basal plus mealtime insulin.

Materials and methods: In a multicentre, open-label study, 807 people [mean age 60.0 yr (SD: 8.6 yr), duration of T2DM 15.8 yr (SD: 7.5 yr), body mass index 36.6 kg/m^2 (SD: 6.4 kg/m^2), HbA_{1c} 8.15% (SD: 0.78%), total insulin dose 1.2 U/kg (SD: 0.47 U/kg), basal insulin dose 0.67 U/kg (SD: 0.25 U/kg)] were randomized (1:1) to Gla-300 (n=404) or Gla-100 (n=403), administered once daily in the evening, while continuing mealtime insulin. The dose was titrated seeking fasting plasma glucose 4.4-5.6 mmol/L. Primary endpoint was change in HbA_{1c} from baseline to month 6, and first secondary endpoint was percent of people with ≥1 severe or confirmed (≤3.9 mmol/L) nocturnal hypoglycaemia from month 3 to month 6.

Results: Gla-300 was non-inferior to Gla-100 for change in HbA_{1c} [least squares mean change -0.83% (SE: 0.06) in both groups; difference -0.00% (95% CI: -0.11 to +0.11)]. No relevant differences were seen for the other measures of glucose control such as fasting plasma glucose, 8-point self-monitored plasma glucose profiles and pre-injection plasma glucose. Fewer people using Gla-300 experienced severe or confirmed nocturnal hypoglycaemia during months 3-6 (first secondary endpoint: 36.1% *vs* 45.5%; relative risk 0.79 (95% CI: 0.67 to 0.94; p=0.0070). Occurrence of any hypoglycaemic event (% of people with at least one event) during study period was numerically lower in the Gla-300 group than in the Gla-100 group. No between-treatment differences in adverse events were seen.

Conclusion: In people with T2DM, Gla-300 was as effective as Gla-100 in control of blood glucose and was associated with a 21% reduction in severe or confirmed nocturnal hypoglycaemia from month 3 to month 6. Gla-300 was well tolerated.

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OP 38 Hypertension: towards an individualised assessment

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Frailty and the relationship between blood pressure and mortality in elderly patients with type 2 diabetes (ZODIAC-34)

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Background and aims: Several studies have found an inverse association of blood pressure with cardiovascular and all-cause mortality in elderly patients with type 2 diabetes mellitus (T2DM). Frailty has been suggested as an explanation for this consistent finding. Unfortunately, measures of frailty were lacking in previous studies. We aimed to investigate whether adjustment for frailty influences the inverse relationship of blood pressure with mortality.
Materials and methods: Patients aged older than 60 years were selected from two cohorts (1998 and 2001) of the prospective observational ZODIAC study (n=1232). Frailty was defined as a score lower than 80 on the subscale 'physical functioning' of the RAND-36 questionnaire. Data on frailty were available for 791 patients (64%). After a follow-up time of 10 years, Cox regression analyses were performed to evaluate the association of the different measures of blood pressure with cardiovascular and all-cause mortality. Analyses were performed in strata according to the level of frailty ('physical functioning' score <80 and ≥80) and were repeated for the oldest elderly (>75 years). Age, gender, smoking (yes or no), BMI, duration of diabetes, serum creatinine level, macrovascular complications (yes or no), albuminuria (yes or no) and total cholesterol-HDL ratio were selected as possible confounders.
Results: Frailty was highly prevalent in our study group; 573 out of 791 patients (72%) fulfilled the criterion of frailty compared to 229 out of 260 elderly patients (88%). During follow-up, 370 (47%) patients died, of which 161 patients (44%) from cardiovascular causes. The results of the Cox regression analyses are presented in table 1. Higher blood pressure was independently associated with increased cardiovascular mortality in non-frail subjects in the total study group. An inverse relationship was observed between diastolic blood pressure and all-cause mortality for frail subjects. For the oldest elderly, systolic and diastolic blood pressure were inversely related to all-cause mortality in frail subjects. Even in this age category, a positive association was observed between systolic blood pressure and all-cause mortality for non-frail subjects.
Conclusion: Frailty modifies the relationship between blood pressure and mortality in elderly patients with T2DM. These results underline the importance of considering the level of frailty when determining individual target values. Also, trials in old age should always contain frailty indicators in order to facilitate translating the results of studies into daily practice.

Table 1. Hazard ratios and their 95% confidence intervals of blood pressure for mortality.

	Total group		**>75 years**	
	All-cause mortality	*CVD mortality*	*All-cause mortality*	*CVD mortality*
Non-frail subjects				
SBP	1.11 (0.98-1.24)	1.22 (1.02-1.43)	1.45 (1.06-1.86)	1.30 (0.82-1.80)
DBP	1.35 (1.08-1.63)	1.73 (1.30-2.17)	1.72 (0.98-2.15)	1.51 (0.65-2.45)
Frail subjects				
SBP	0.96 (0.91-1.01)	0.96 (0.88-1.04)	0.89 (0.82-0.96)	0.93 (0.83-1.04)
DBP	0.88 (0.77-0.99)	0.88 (0.71-1.05)	0.72 (0.58-0.87)	0.83 (0.60-1.06)

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Resistant hypertension: clinical correlates and association with complications in subjects with type 2 diabetes

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Background and aims: The phenotype of resistant hypertension (RH) has not been characterized in subjects with type 2 diabetes. This analysis was aimed at assessing the independent correlates of RH and the association of this condition with complications in subjects with type 2 diabetes.
Materials and methods: We used the baseline data from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study, including 15,773 patients consecutively visiting 19 Diabetes Clinics in years 2007-2008. RH was defined as systolic and/or diastolic blood pressure values not on-target (i.e.>130 and 80 mmHg, respectively) in subjects on ≥3 antihypertensive agents or on-target but using >4 drugs (n=2,363; 15% of the whole RIACE cohort). Patients on-target with 2 drugs (Ctr1; n=1,369) and 3 drugs (Ctr2, n=803) served as control groups. GFR was estimated using the simplified MDRD equation, albuminuria was measured by immunonephelometry or immunoturbidimetry, retinopathy was evaluated by fundus examination, and cardiovascular disease (CVD) was adjudicated based on hospital discharge records.
Results: No clinically significant differences among groups were observed for age, diabetes duration, HbA_{1c}, BMI, waist and lipid profile. Subjects with RH had higher albuminuria, lower eGFR and, hence, higher prevalence of chronic kidney disease (CKD) than control groups (56.3% vs 41.9% in Ctr1 and 46.1% in Ctr2; p<0.0001). Likewise, rate of advanced retinopathy, but not of CVD, was higher in individuals with RH. Logistic regression analysis showed that RH was independently associated with age (1.020 [1.012-1.027]), waist (1.026 [1.020-1.032]), micro (1.379 [1.201-1.583]) and macroalbuminuria (2.131 [1.644-2.762]), eGFR 30-59 ml/min/1.73m^2 (1.269 [1.053-1.529]) and <30 ml/min/1.73m^2 (1.527 [1.007-2.318]), and advanced retinopathy (1.271 [1.048-1.542]). Moreover, RH was associated with a higher risk of Stages 1-2 (1.510 [1.282-1.778]), nonalbuminuric Stages 3-5 (1.366 [1.135-1.645]), and particularly albuminuric Stages 3-5 (1.954 [1.594-2.395]) CKD. No independent correlation was found between RH and CVD, either total or by vascular bed.
Conclusion: In subjects with type 2 diabetes, RH is associated with age, waist, and microangiopathy, especially albuminuric CKD, but not with macroangiopathy. These data suggest a strict bi-directional relationship between RH and renal damage.
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Supported by: Fo.Ri.SID, DEM Foundation, Eli-Lilly, Takeda, Chiesi, Boehringer

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Antihypertensive treatment in patients with type 1 diabetes according to the stages of diabetic nephropathy

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Background and aims: Intensive treatment of elevated blood pressure (BP) has been shown to prevent or delay the progression of kidney disease in type 1 diabetes. While previous studies have established the prevalence of resistant hypertension (RH) in the general hypertensive population, the rate is unknown in patients with type 1 diabetes with respect to different stages of diabetic nephropathy. Therefore, we estimated the prevalence of resistant hypertension, as well as the effectiveness of antihypertensive treatment in patients with type 1 diabetes stratified by stage of nephropathy.

Materials and methods: The Finnish Diabetic Nephropathy Study (FinnDiane) data (N=2,857; mean age 38.1 ± 11.8 years, 51.7 % men, mean duration of diabetes 22.3 ±11.9 years) were linked to the Drug Prescription Register to obtain all purchases of antihypertensive drugs 6 months before the baseline visit. Based on their urinary albumin excretion rate (AER) patients were divided into four nephropathy groups: normoalbuminuria, microalbuminuria, macroalbuminuria and end-stage renal disease (EASD). We used treatment targets published by The American Diabetes Association (ADA 2000). RH was defined as failure to reach blood pressure goals (BP <130/85) despite the use of ≥3 antihypertensive drugs of different classes (one of which is diuretic).
Results: In patients with normal AER 13.6% were on antihypertensive treatment and of them 25% had BP on target. The corresponding figures were 60.1% and 30.9% for the microalbuminuria group, 90.4% and 20.5% for the macroalbuminuric patients, as well as 90.1% and 9.7% for patients with ESRD. In the normalbuminuric group 56.5% and in the microalbuminuric group 62.2% were taking only one antihypertensive drug, although the patients had not reached the BP targets. In general, the prevalence of RH was 8.6% for all patients and 21.7% for the drug treated patients. When the patients were stratified by nephropathy status the prevalence of RH was 1.2% in patients with normal AER, 4.2% in microalbuminuric, 28.1% macroalbuminuric patients and 30.6% in the ESRD group. The prevalence of RH of the drug treated patients was 8.0%, 7.0%, 31.1% and 33.9%, respectively.
Conclusion: The number of patients who had not reached the BP treatment targets and the prevalence of RH increased with the severity of diabetic nephropathy. Our data suggests that there is urgent need for improvement of antihypertensive treatment, and especially in those with diabetic nephropathy.

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Prognostic impact of treatment resistant hypertension on clinical events during 2-year follow-up in outpatients with diabetes and hypertension: results of the 3A Registry

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Background and aims: Aim of this study was to determine the incidence of treatment resistant hypertension (TRH) and prognostic value of TRH on cardiovascular events during 2-year follow-up in a large number of hypertensive diabetic outpatients in Germany.
Materials and methods: The non-interventional 3A Registry included hypertensive outpatients in whom the physician had decided to initiate or modify antihypertensive therapy in patients not achieving blood pressure (BP) control or where a modification was required for other reasons. 2512 subjects (28.9%) of the total cohort had type 2 diabetes. We defined TRH at baseline as BP > 140/90 mm Hg (for diabetic *and* non-diabetic patients) despite treatment with at least 3 antihypertensive drugs including a diuretic.
Results: Out of a total of 8697 patients with a 2-year follow up, 2772 (32%) had TRH, of whom 1170 had diabetes (46.6% of all diabetic patients) and 1602 had not diabetes (26% of all non-diabetic patients), respectively. Thus, TRH was more frequent in diabetics (p<0.001). The incidence of clinical events during the 2 year follow-up period is depicted in table 1.

Table 1

	Treatment resistant hypertension (N=2772)	Diabetic Ss. (N=1170) BP > 140/90 mmHg	Non-diabetic Ss. (N=1602) BP > 140/90 mmHg	P-Value
Age (years)	68.3	69.6	67.2	<0.001
No. of drugs	4.3	4.5	4.1	<0.001
Events during 2-year follow-up				
Death (%)	3.6	4.4	2.9	<0.05
Myocardial infarction (%)	0.9	1.3	0.6	0.07
Stroke (%)	1.1	1.4	0.8	0.15
MACCE (%)	4.8	6.2	3.8	0.01

Conclusion: In this real life analysis patients with TRH had a higher prevalence of diabetes. Treatment resistant hypertension, if coexisting with diabetes, was associated with an increased incidence of cardiovascular events during follow-up. Therefore, in particular diabetic patients with TRH need better BP control to improve their outcome.
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OP 39 Lipids: prediction and functional studies

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Association of hypertriglyceridaemia with complications in subjects with type 2 diabetes

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Background and aims: Atherogenic dyslipidemia, i.e. high triglyceride (TG) and low HDL cholesterol levels, contributes to the increased morbidity and mortality for cardiovascular disease (CVD) in subjects with type 2 diabetes. We assessed whether raised TG levels are associated with an increased burden from renal, retinal and CVD complications in patients with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study.
Materials and methods: Subjects from the RIACE (n=15,773) were divided in 4 groups depending on whether they had plasma TG levels below (NTG) or above (HTG) 150 mg/dl and were or not on statin therapy, which reduces TGs by up to 40%. TGs were assessed by standard colorimetric methods, estimated GFR (eGFR) was calculated using the simplified MDRD equation, albuminuria was measured by immunonephelometry or immunoturbidimetry, retinopathy was evaluated by fundus examination, and CVD was adjudicated based on hospital discharge records or specialist visits.
Results: HGT subjects, either with or without statin, had higher HbA_{1c}, BMI, waist, non-HDL cholesterol and albuminuria, and lower HDL cholesterol and, only for subjects on statin, eGFR, as compared with NTG individuals (P<0.001). HTG patients, particularly if on statin, had higher prevalence of chronic kidney disease (CKD), especially albuminuric, than NTG subjects. In contrast, CVD and (advanced) retinopathy were more prevalent in subjects on statin than in those not, independently of TG levels. Logistic regression analysis with backward variable selection confirmed that HTG without or with statin was independently associated with a higher risk of CKD, Stages 1-2 (1.227 [1.081-1.393] and 1.246 [1.090-1.426]), and Stages 3-5 albuminuric (2.003 [1.654-2.427] and 2.667 [2.213-3.214]) and nonalbuminuric (1.535 [1.294-1.820] and 1.838 [1.553-2.175]). NTG with statin therapy was associated only with the latter. Conversely, in NTG and HTG subjects, statin treatment was independently associated with a higher risk of CVD, total (2.893 [2.621-3.195] and 2.705 [2.399-3.049]) and by vascular bed (coronary, cerebrovascular and peripheral), but not of any or advanced retinopathy.
Conclusion: HTG is associated with all CKD phenotypes independently of statin treatment, whereas the latter correlates with CVD independently of TG levels. These data point to a possible role of HTG in the development of CKD and to the importance of treating atherogenic dyslipidemia.
Clinical Trial Registration Number: NCT00715481
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Relationship between serum cholesterol efflux capacity and glucose tolerance status in Japanese-Americans

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Background and aims: Serum cholesterol efflux has been suggested to be a key mechanism of the anti-atherogenic function of high-density lipoprotein (HDL). Although dysfunctional HDL is reported to be present in patients with diabetes, it remains unclear whether serum cholesterol efflux capacity is impaired in cases of newly diagnosed glucose intolerance. We therefore hypothesized that serum cholesterol efflux capacity as an HDL function may be impaired in subjects with glucose intolerance, causing a residual risk of atherosclerosis. To demonstrate this, we compared serum cholesterol efflux capacity according to the glucose tolerance status and assessed the relationship between serum cholesterol efflux capacity and glucose intolerance.
Materials and methods: We measured the capacity of whole-serum to mediate cholesterol efflux from human THP-1 macrophages in a cohort of 439 Japanese-Americans living in Los Angeles (186 men, 253 women, mean age 59.2±14.7 years old). Glucose tolerance status was ascertained in individual subjects (normal glucose tolerance: NGT, impaired glucose tolerance: IGT and diabetes mellitus: DM) by 75g oral glucose tolerance test. We then compared serum cholesterol efflux capacity depending on category of glucose tolerance status by using age and sex adjusted analysis of covariance for comparison. Next, we defined study subjects by combining the IGT and DM group to form a glucose intolerance group and assessed the relationship between serum cholesterol efflux capacity and glucose intolerance by using multiple regression analysis after adjusting for age and sex.
Results: Serum cholesterol efflux capacity was 33.2±6.1% in the NGT group, 31.4±6.0% in the IGT group and 31.5±6.2% in the DM group, respectively. We found no significant difference among the three groups (P=0.061). In all subjects, we found significant positive associations between serum cholesterol efflux capacity and each of serum total cholesterol (T-Cho) (β=0.127, P=0.012), HDL-C (β=0.107, P=0.034) and apolipoprotein-AI (apo-AI) (β=0.102, P=0.047), and negative associations between serum cholesterol efflux capacity and each of C-reactive protein (CRP) (β=-0.129, P=0.010) and glucose intolerance (β=-0.125, P=0.012) after adjustment was made for age and sex. Moreover, the association between serum cholesterol efflux capacity and glucose intolerance remained significant even after further adjustment was made for each of T-Cho (model 1: β=-0.128, P=0.010), HDL-C (model 2: β=-0.113, P=0.024), apo-AI (model 3: β=-0.116, P=0.021), T-Cho, and CRP (model 4: β=-0.103, P=0.041).
Conclusion: Serum cholesterol efflux capacity tended to be reduced in subjects with IGT or DM than in subjects with NGT, although the difference was not significant. However, glucose intolerance was found to be independently and negatively associated with cholesterol efflux capacity. This finding may suggest that an impaired anti-atherogenic function in HDL may lead to increased risk of atherosclerotic disease in subjects with glucose intolerance.

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Influence of HDL cholesterol levels on endothelial progenitor cells function in patients with type 2 diabetes mellitus

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Background and aims: High-density lipoprotein (HDL) levels are inversely associated with coronary heart disease. The mechanisms responsible for this beneficial effects are still controversial. Additional atheroprotective actions of HDL have been claimed on top of positive effects of reverse cholesterol transport. Individuals with high HDL-cholesterol (HDL-C) levels show increased endothelial progenitor cells (EPCs) colony forming units and HDL-C is believed a strong determinant of EPCs number and function.
Materials and methods: To gain further insights on the effect of HDL-C we have characterized in vitro function of circulating EPCs of type 2 diabetic subjects (T2DM) with low (34.7±7.9 mg/dl; N=57, M/F 70/30%; age 61.8±7.8 years; BMI 30.6±4.7 kg/m2; HbA1c 7.3±1.0%) and high (74.4±12.9 mg/dl; N=48, M/F 46/54%; age 65.8±8.3 years; BMI 27.3±5.6 kg/m2; HbA1c 7.3±0.8 %) HDL-C. After an overnight fast, venous blood was drawn in EDTA tubes and processed within 2-hrs from sampling. Peripheral blood mononuclear cells (PBMCs) were fractionated using Biocoll density-gradient centrifugation; after 3-day culture, non-adherent cells were discarded and adherent cells positive for Dil-ac-LDL/Lectin dual fluorescent staining were identified as EPCs. After 5-7 day culture in EBM-2 medium, adherent cells were evaluated for viability/proliferation (MTT assay), senescence (beta-galactosidase activity detection), migration (modified Boyden chamber using VEGF as chemoattractant), adhesion capacity (on fibronectin-coated culture dishes) and ROS production (ROS-sensitive fluorescent probe CM-H2DCFDA). Data

have been analyzed as a function of HDL levels, as well as anthropometric features and glycaemic control.
Results: EPCs cells from low HDL-C T2DM had mean 26% lower viability as compared to high HDL-C T2DM (100±30 vs. 74±29; p=0.0001). Four hours H2O2 exposure impaired cell viability to a similar extent in both groups (100±27 vs. 69±25 in high HDL, p<0.0001; 100±24 vs. 81±19 in low-HDL, p=0.007). EPC senescence was comparable in the two groups (100±28 vs. 98±22; p=0.774) and was not significantly modified by exposure to H2O2. There was no difference in the migration capacity between the two groups (100±38 vs. 110±35; p=0.442), while cell adhesion was 25% impaired in low compared to high HDL-C (100±23 vs. 75±15; p=0.0001). Finally, ROS production was slightly (12%) higher in low- (n=27) than in high-HDL (n=18, +12±29; p<0.426). These findings persisted after adjustment for gender, age, BMI and HbA1c.
Conclusion: Our data suggest that in type 2 diabetic subjects HDL-C is a determinant of circulating EPCs function contributing through this mechanism to CV protection.

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Hypoxia exacerbates the up-regulatory effect of electronegative LDL on cardiomyocyte intracellular triglyceride accumulation
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Background and aims: In diabetes, the development of cardiomyopathy is linked to increased myocardial neutral lipid content, in particular to myocardial triglyceride accumulation. Plasma lipoproteins are a source of neutral lipids for cardiomyocytes. By overexpressing certain lipoprotein receptors, these cells have increased capacity to take up neutral lipids from lipoproteins under hypoxic conditions. Electronegative LDL [LDL(-)], a subfraction of LDL with oxidative characteristics, is increased in subjects with diabetes mellitus. Among other features, LDL(-) is characterized by increased content of triglyceride (TG) and non-esterified fatty acids (NEFA). Our aim was to study the capacity of LDL(-), compared to native LDL (nLDL), to alter intracellular cholesteryl ester (CE) and TG content of cardiomyocytes exposed to normoxic and hypoxic conditions.
Materials and methods: LDL(-) was isolated from total LDL by anion-exchange chromatography. HL-1 cardiomyocytes were exposed to increasing nLDL or LDL(-) dose under normoxic (21% O_2) or hypoxic (1% O_2) conditions. Cardiomyocytes were then collected and subjected to thin layer chromatography after lipid extraction.
Results: In normoxic conditions, LDL(-) (0, 50,100 and 200 μg/mL) increased intracellular TG content from 0.25±0.06 to 21.92±1.7 μg TG/mg cell protein. Hypoxia promoted intracellular TG accumulation to 11.28±1.18 μg TG/mg cell protein, in absence of LDL. Remarkably, hypoxia strongly exacerbated LDL(-)-derived intracellular TG accumulation to 51.17±1.7 μg TG/mg cell protein. nLDL did not exert any significant effect on intracellular TG accumulation either under normoxic or hypoxic conditions. LDL(-) and nLDL showed similar ability to accumulate cardiomyocyte intracellular CE. This effect was stimulated under hypoxic conditions.
Conclusion: LDL(-) potentiates cardiomyocyte triglyceride accumulation and this effect is exacerbated under hypoxic conditions. These results suggest that electronegative LDL contributes to myocardial steatosis in diabetes and especially in the context of myocardial ischemia.

OP 40 Beta cell function in vivo

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Effects of a small protein and lipid preload on oral glucose tolerance in subjects with normal and impaired glucose tolerance
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Background and aims: In normal subjects, a protein preload has been shown to reduce the AUC of plasma glucose following glucose ingestion by reducing hepatic insulin extraction. In type 2 diabetic patients, a preload of either proteins or lipids has been shown to improve the plasma glucose response to carbohydrate ingestion by slowing gastric emptying and stimulating GLP-1 release. In those studies, the preload was either oil or a protein formula, neither glucose fluxes nor insulin sensitivity or ß-cell function were evaluated and the degree of glucose intolerance was not taken into account. Our aim was to evaluate the effects on glucose fluxes, insulin secretion and peripheral insulin sensitivity of a small mixed protein/lipid preload in subjects with normal (NGT) and impaired (IGT) glucose tolerance.
Materials and methods: Twenty-five volunteers (12 NGT and 13 IGT) were enrolled (13 males, age 38±3 years, BMI 25.7±1.0 kg/m^2). On two separate days, after an overnight fast, subjects were randomised to a preload of either 500 ml of water (control) or Parmesan cheese (50 g) plus a boiled egg with 300 ml of water; 30 min later, they underwent a standard 75 g OGTT. Timed arterialised blood samples were collected to measure plasma glucose, insulin, C-peptide, GLP-1, GIP, glucagon, NEFA and pancreatic polypeptide (PP). Two stable glucose tracers were administered ([6,6-$^2H_2$]glucose *i.v.* and [U-13C]glucose *per os*) to measure ingested glucose appearance (RaO) and endogenous glucose production (RaE). Three major components of ß-cell function, namely ß-cell glucose sensitivity (ßGS), rate sensitivity (ßRS) and potentiation (POT) were evaluated by modeling insulin secretion (estimated from C-peptide deconvolution) and plasma glucose values according to Mari and Ferrannini. Insulin sensitivity was estimated using the OGIS method and AUC of glucose clearance.
Results: In healthy subjects, the mixed protein/lipid preload decreased plasma glucose peak levels at 60 min into the OGTT (6.3±0.2 *vs* 7.7±0.3 mmol/L, $p<0.001$). In the IGT group, it decreased 2-h plasma glucose levels from 8.9±0.3 to 7.8±0.4 mmol/L ($p<0.01$) as well as the whole glucose profile (AUC, $p<0.00001$). In both groups, this improvement in glucose tolerance, which was proportional to the degree of its derangement (AUC *vs* AUC change, r=0.60, $p<0.002$), was associated with an enhancement of ßRS ($p<0.02$), a reduction in oral glucose appearance (RaO, $p<0.01$ only in IGT), a leftward shift of ßGS ($p<0.002$), a small increment in insulin sensitivity as estimated by both OGIS ($p<0.05$) and by tracer-determined glucose clearance ($p<0.04$). Endogenous glucose production similarly declined during the two OGTTs in both groups ($p<0.006$). In neither group were the glucagon, NEFA, PP and GLP-1 responses affected by the preload, while GIP secretion was increased ($p<0.001$) in both.
Conclusion: A small mixed protein/lipid preload significantly improves glucose tolerance, especially in subjects with IGT. This effect results from the combination of a reduction in glucose absorption and a minor improvement in both insulin secretion and peripheral insulin sensitivity. Simple dietetic recommendations have the potential to produce a significant reduction of postprandial hyperglycaemia in pre-diabetic patients.

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Metabolic shift with impaired perfusion of pancreas in type 2 diabetes: positron emission tomography study

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Background and aims: Glucolipotoxicity and impaired pancreatic blood flow are suggested mechanisms behind β-cell exhaustion in obesity, leading to type 2 diabetes mellitus. The present study was conducted to reveal whether these phenomena exist in humans and if so, their clinical relevance. Positron emission tomography (PET) offers a non-invasive method of studying organ metabolism and blood flow. We have previously validated the use of fluorine-18 labeled radiotracers for the measurement of pancreatic metabolism.
Materials and methods: A total of 47 morbidly obese subjects (20 NGT, 10 IGT, 17 T2DM) with BMI 43±4kg/m^2 and 25 age-matched healthy subjects participated in the study. Pancreatic glucose uptake, fatty acid uptake and perfusion were studied after an overnight fast using [^{18}F]FDG, [^{18}F]FTHA, and [^{15}O]H_2O with PET or PET-CT. Moreover, abdominal MRI and standard OGTT were performed. Regions-of-interest (ROIs) were drawn on head, body, and tail of pancreas. Pancreatic fat index was calculated from in- and outphase MR images. Glucose and fatty acid uptake were calculated using fractional uptake rate (FUR) and blood flow using one-tissue compartment model. The total splanchnic flow was calculated using validated gut compartment model.
Results: As compared to healthy subjects, obese ones had elevated pancreatic fatty acid uptake (1.1±0.3 *vs.* 0.6±0.2μg/100g/min, p<0.0001), lower glucose uptake (1.6±0.5 *vs.* 2.1±0.5μg/100g/min, p<0.03) and perfusion (1.1±0.3 *vs.* 1.7±0.6ml/ml/min p<0.001), and had significantly higher pancreatic fat index (18±21 *vs.* 2.2±5.7, p=0.01). Between obese subgroups no difference was found in previous parameters. Pancreatic blood flow correlated with total splanchnic flow (r=0.35, p=0.04), and both of these parameters were independently associated with higher glucose area under the OGTT curve (r=-0.45, p<0.01 and r=-0.38, p=0.02 for pancreatic and splanchnic blood flow, respectively). Obese subjects were insulin resistant (IR_{HOMA} 0.8±0.9 *vs.* 0.2±0.2, p=0.002), and had higher plasma free fatty acid levels (0.8±0.2 *vs.* 0.5±0.2mmol/l, p=0.0002). No differences were found in GLP-1 levels between the groups but subjects with type 2 diabetes had higher plasma GIP levels compared to controls (22±14 *vs.* 14±6.1ng/ml p<0.01).
Conclusion: This study shows that in obesity the pancreatic metabolism is shifted from glucose to fatty acid utilization in parallel with impairment in organ blood flow and triglyceride accumulation. The association between pancreatic and splanchnic blood flow and mean incremental glucose links blood flow with the regulation intermediary metabolism.
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Pancreatic islet cell function before and after Roux-en-Y gastric bypass in glucose tolerant individuals

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Background and aims: Roux-en-Y gastric bypass (RYGB) increases postprandial insulin and glucagon secretion. This change in pancreatic islet cell function is in part explained by altered extrinsic regulation from gut hormones, in particular an exaggerated glucagon-like peptide-1 (GLP-1) meal response. To investigate the influence of changes in intrinsic islet cell regulation, we comprehensively assessed postoperative insulin and glucagon secretion in response to intravenous stimulation including the responsiveness of the islet cells to GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) during stable hyperglycaemia, and compared the results with concomitant changes in response to an oral glucose load.
Materials and methods: Eleven severely obese glucose tolerant individuals (6 women and 5 men; age 40.9 ± 3.3 (SEM) years; preoperative BMI 41.6 ± 1.5 kg/m2) underwent 3 hyperglycaemic clamps (60 min; plasma glucose 9 mmol/L) with bolus injection of arginine at 45 min and primed co-infusion of GLP-1 (1.0 pmol/kg/min), GIP (1.5 pmol/kg/min) or saline, before, 1 week and 3 months after RYGB. In addition, an OGTT was performed before and 3 months after surgery. Acute insulin response to glucose from 0-10 min (AIRg), second phase insulin response from 20-40 min (2nd phase), acute insulin response to arginine (AIRarg), as well as glucagon response to glucose (iAUCglucagon) and arginine (AGRarg) were calculated from the clamp data. Insulinogenic index (IGI) and incremental insulin and glucagon responses were calculated from the OGTT data.
Results: In response to the hyperglycaemic saline clamps, insulin stimulation and glucagon suppression were largely unaltered after RYGB, except for a transient increase in AIRg and AGRarg at 1 week (AIRg: before 9.9 ± 1.1 pmol kg-1 min-1, 1 week 11.3 ± 1.5 P = 0.035, and 3 months 10.1 ± 1.4 P = 0.905; 2nd phase: 4.6 ± 0.6 pmol kg-1 min-1, 3.8 ± 0.5 P = 0.082, and 4.7 ± 0.7 P = 0.941; AIRarg: 47.0 ± 7.7 pmol kg-1 min-1, 33.7 ± 1.9 P = 0.145, and 38.2 ± 3.3 P = 0.210; iAUCglucagon: -270 ± 65 pmol/L min, -414 ± 87 P = 0.104, and -286 ± 53 P = 0.675; and AGRarg: 49.8 ± 10.4 pmol/L, 69.2 ± 14.2 P = 0.009, and 44.6 ± 9.3 P = 0.095). GLP-1 and GIP infusions stimulated insulin responses to glucose and weakly inhibited glucagon release preoperatively, and these effects were unchanged after surgery. In response to the OGTTs, insulin secretion increased and hypersecretion of glucagon was seen at 3 months (IGI: before 3.73 ± 0.63 pmol kg-1 min-1 mmol/L-1 and 3 months 5.90 ± 1.06 P = 0.045; incremental insulin response: 881 ± 68 pmol kg-1 and 1321 ± 100 P = 0.005; incremental glucagon response: -530 ± 174 pmol/L min and 584 ± 75 P < 0.001). Further, GLP-1, but not GIP, secretion during the OGTT increased after RYGB and the glucose profile was altered with a substantial reduction in 2 h plasma glucose.
Conclusion: After RYGB, changes in insulin and glucagon secretion are linked to the oral, but not intravenous, route of delivery, and the insulinotropic and glucagonostatic actions of the incretin hormones are unaltered, highlighting the importance of the gut for the changes in pancreatic islet function.
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Beta cell function and insulin sensitivity after Roux-en-Y gastric bypass in patients with type 2 diabetes and normal glucose tolerance

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Background and aims: Roux-en-Y gastric bypass (RYGB) improves glucose metabolism within days after surgery. We examined changes in beta-cell function after intravenous and oral challenges as well as changes in peripheral and hepatic insulin sensitivity after RYGB to elucidate the physiological mechanisms responsible for the improved glycemic control.
Materials and methods: We studied 10 obese subjects with type 2 diabetes (T2D) (BMI 38.1±1.8 kg/m^2 mean ± sem) and 10 obese subjects with normal glucose tolerance (NGT) (BMI: BMI 40.2±0.8 kg/m^2) before, 1 week, 3 months and 1 year after RYGB using intravenous glucose glucagon tests (GGTs) and 4 hour hyperinsulinemic (40 mU/m^2/min) euglycemic (5.5 mmol/l) clamps with [6,6-$^2H_2$]-glucose co-infusion. OGTTs (75 g in 5 min) were performed before, 3 months and 1 year after surgery. Acute insulin response (AIR=ΔC-peptide$_{0\text{-}max}$) to GGT and insulinogenic index (IGI = ΔC-peptide$_{0\text{-}30}$/ΔGlucose$_{0\text{-}30}$) from OGTT were used to assess beta-cell function while peripheral and hepatic insulin sensitivity (HISI) were estimated as Rd$_{Clamp}$/Insulin$_{Clamp}$ and $10^5\times$(Ra$_{Basal}\times$Insulin$_{Basal}$)$^{-1}$, respectively.
Results: Glycemic control improved after RYGB in patients with T2D despite cessation of all anti-diabetic medication from the time of surgery (HbA1c T2D: pre 7.0±0.3%, 3 mo 5.9±0.2 p<0.05, 1y 5.8±0.2 p<0.05, NGT: 5.4±0.1, 5.3±0.1 p=ns, 5.3±0.1 p=ns). Insulin secretion after the intravenous challenge was unchanged after RYGB in patients with T2D (AIR: pre 1463±279 pM, 1 wk 1502±440 p=ns, 3 mo 1799±396 p=ns, 1y 1572±395 p=ns) and decreased in NGT at 3 months and 1 year (AIR: 4117±772, 3040±668 p=ns, 3167±748 p<0.01, 3022±804 p<0.05). In response to the oral glucose, insulin secretion increased in T2D after RYGB (IGI: pre 189±60 pM/mM, 3 mo 341±60 p<0.01, 1y 266±55 p<0.05) and was unchanged in NGT (IGI: 788±79, 747±119 p=ns, 770±93 p=ns). Hepatic insulin sensitivity improved equally in both groups

from 1 week postoperatively with further improvements at 3 months and remained elevated at 1 year (HISI T2D: pre 8±2, 1 wk16±6 p<0.01, 3 mo 22±4 p<0.01, 1y 21±6 p<0.01; NGT: 9±1, 16±2 p<0.01, 25±3 p<0.01, 33±8 p<0.01), whereas peripheral insulin sensitivity was unchanged at 1 week but improved at 3 months and 1 year in the two groups (Rd/I T2D: pre 14±2 μg/kg_{ffm}/min/pmol/l, 1 wk 16±3 p=ns, 3mo 25±3 p<0.01, 1y 26±7 p<0.01; NGT: 22±3, 18±2 p=ns, 30±3 p<0.01, 33±5 p<0.01).

Conclusion: Insulin secretion increases after RYGB, but only in patients with type 2 diabetes and only in response to an oral challenge. In patients with NGT, insulin secretion after an intravenous challenge is decreased after RYGB, possible as an adaptation to improved insulin sensitivity. Hepatic insulin sensitivity is equally improved in patients with type 2 diabetes and NGT already from 1 week after surgery while peripheral insulin sensitivity is not increased until 3 months postoperatively in either group. Thus, after RYGB the progressive improvement in glycemic control in patients with type 2 diabetes can be explained by a fast improvement in hepatic insulin sensitivity, a slower increase in peripheral insulin sensitivity and an improved beta-cell function in response to an oral challenge.

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OP 41 Short- and long-term impact of nutrition

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The effect of frequency of meals on beta cell function in subjects with type 2 diabetes

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Background and aims: The aim of our study was to compare the effect of six vs. two meals a day with the same caloric restriction on β-cell function in subjects with type 2 diabetes (T2D).

Materials and methods: In a randomized, crossover study, we assigned 54 patients with T2D to follow two regimens of a hypocaloric diet (-500 kcal/day), each for 12 weeks: six meals a day (A), and two meals a day, breakfast and lunch (B). The diet in both regimens had the same macronutrient and energy content. All subjects were examined at weeks 0, 12 and 24. Insulin secretory rate (ISR) and β-cell function were assessed during the standard meal tests. ISR was calculated by C-peptide deconvolution, and β-cell function was quantified with a mathematical model that describes ISR as a function of absolute glucose levels (insulin secretory tone and glucose sensitivity), and of glucose rate of change (rate sensitivity), as well as a potentiation factor. Insulin sensitivity (OGIS) was calculated, which quantifies glucose clearance per unit change of insulin. Hepatic fat content was measured by the proton magnetic resonance spectroscopy performed by 3T MR scanner (Magnetom - Trio Siemens). For statistical analysis, 2x2 crossover ANOVA was used.

Results: Insulin secretion at the reference level and glucose sensitivity increased (p<0.05) comparably in both regimens. OGIS increased in both regimens (p<0.01), more in B (+8.2; 95% CI +3.4 to +13.1 ml.min−1m−2 in A vs. +21.0; 95% CI +16.1 to +26.0 ml.min−1m−2 in B; p<0.01). Body-mass-index (BMI) decreased in both regimens (p<0.001), more in B (-0.82; 95% CI -0.94 to -0.69 kg.m-2 in A vs. -1.23; 95% CI -1.4 to -1.17 kg.m-2 in B; p<0.001). Hepatic fat content decreased in response to both regimens (p<0.001), more in B (-3.4; 95% CI -3.8 to -3.1 % in A vs. -4.2; 95% CI -4.5 to -3.8% in B; p=0.03). Changes in glucose sensitivity and OGIS correlated negatively with changes in hepatic fat content (r=-0.28; p=0.02 and r=-0.47; p<0.001, respectively). After adjustment for changes in BMI the correlations were no longer significant.

Conclusion: Two meals a day led to a greater decrease in BMI and hepatic fat content and a greater increase in OGIS compared to six meals a day. Insulin secretion at the reference level and glucose sensitivity increased comparably in both regimens. The association between changes in glucose sensitivity, OGIS and HFC was dependent on changes in BMI. Our data suggest that eating fewer larger meals may be more beneficial than more frequent meals during the day for patients with T2D.

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The effect of carbohydrate intake on glycaemic control in patients with type 1 diabetes treated with insulin pumps

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Background and aims: Modern regimens for flexible insulin therapy in Type 1 Diabetes advocate dietary freedom, whereby patients vary insulin doses according to anticipated carbohydrate (CHO) intake. However, there is concern that large variations in CHO intake and in particular high CHO meals may still be detrimental to glucose control and differences in CHO intake may underlie variations in glycaemic outcome between patients and between national diabetes education programmes. We explored the relation between CHO intake and glycaemic control from the routine data downloads of a cohort of insulin pump treated patients with type 1 Diabetes.

Materials and method: Data involving insulin doses, CHO intake and finger-prick blood glucose (BG) measurements were collected from pump downloads. Those from all adult patients using continuous subcutaneous insulin

infusion (CSII) at a single centre were considered (n=467). Inclusion criteria included CSII duration of ≥6 months, at least 2 weeks data from a Medtronic download with bolus wizard usage of ≥80% and ≥3 finger-prick BG tests per day, since January 2011. 148 patients' downloads met the criteria. HbA1c values were obtained concurrent to the download. In a subset of 105 patients, individual mealtime data including pre- and post-meal BG, CHO intake and insulin bolus were collected (4129 meals in total).

Results: Our cohort was 65.5% women, of mean (±SD) age 43 (±13) years and duration of CSII 48 (±30) months, of whom 92.7% had completed a structured education course. Mean HbA1c was 7.8% [62mmol/mol] (±0.93) and range was 5.2-11.0% [33-97mmol/mol]. Mean daily CHO intake was 166g (±71) and median (IQR) was 158g (109-206). There was no association with HbA1c for daily CHO intake (r^2=0.033), mean CHO content per meal (r^2=0.039) or number of meals per day (r^2=0.137). Inter-day variability of daily CHO intake did not affect HbA1c (r^2=4.335E-4) or variability of daily mean BG (r^2=0.004). There was no relationship between meal CHO content and change in BG for post-prandial BG (PPG), either 1-3 or 4-7 hours later. When pre-prandial BG was within target (and no correction dose required), there was no significant association between meal CHO content and the percentage of 1-3 hour (p=0.275) or 4-7 hour (p=0.414) PPG readings that fell within the categories <4, 4-10 and >10mmol/L. For meals containing <40, 40-79 and >79g of CHO, 67, 57 and 59% of 1-3 hour PPG readings were within 4-10mmol/L, respectively. For 4-7 hour PPG readings, meals containing <40, 40-79 and >79g of CHO resulted in 65, 65 and 62% of PPG readings within 4-10mmol/L, respectively. There was a significant association between pre-prandial BG and PPG, both 1-3 (n=1993, p<0.001) and 4-7 (n= 1502, p<0.001) hours later, as determined by chi-squared tests.

Conclusion: These data suggest that at least following structured education and using best technology for insulin delivery (pumps and bolus calculators), people with type 1 diabetes can accommodate a flexible diet with variable, sometimes high, CHO intake, without detriment to their glucose control. Pre-meal BG is a major determinant of post-meal BG, regardless of meal CHO content, suggesting a very important role for correct basal insulin replacement between meals. These data support dietary freedom in well-educated and well-motivated patients.

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Effect of whey protein concentrate on blood glucose, insulin and C-peptide serum levels following a high glycaemic index breakfast in patients with type 2 diabetes

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Background and aims: Whey proteins have insulinotropic effects and reduce postprandial glycemia in healthy subjects. The mechanism is not known, but insulinogenic amino acids and the incretin hormones seem to be involved. To evaluate whether supplementation with whey protein concentrate (WPC) administered before consumption of a high glycemic index (high-GI) breakfast may decrease the postprandial glucose and increase posprandial serum insulin and C- peptide in patients with type 2 diabetes.

Materials and methods: The protocol included 15-controlled type 2 diabetic patients (9 male), duration of known diabetes 7.9 ± 6.0 years, who attended the laboratory after an overnight fast on 2 separate occasions. At the first visit, 15 type 2 diabetic subjects were randomized to consume either 50 gm WPC dissolved in 250 ml water sweetened with artificial sweetener (WPC) or 250 ml water sweetened with artificial sweetener (placebo). Twenty minutes later, subjects were served a high-GI breakfast (3 slices or 90 gr white bread + 15 gm jelly). Venous blood samples were drawn 15 min prior to consuming WPC or P, 15 min after to consuming it, then15 min after breakfast and thereafter every 30 min until 180 min after breakfast. Glucose, insulin and C- peptide were measured at each time point and area under the curve (AUC) was calculated. At the second visit, the same procedure was repeated, with each subject now crossed over to the opposite treatment (WPC to P and P to WPC).

Results: The subjects were aged 64 ± 5.5 years; BMI 26.9 ± 4.6 kg/m2; and HbA1C 6.7 ± 0.7%. AUC for blood glucose was significantly reduced in WPC vs. P: 745.66 ± 169.98 mg/dl*min vs. 1026.90 ± 291.90 mg/dl*min respectively, p< 0.001. AUC for insulin was significantly greater in WPC than P condition: 367.90 ± 218.15 mIU/ml*min vs. 178.95 ± 112.22 mIU/ml*min respectively, p< 0.003. AUC for C peptide was significantly greater in WPC than P: 40.45 ± 13.32 ng/ml*min vs. 28.44 ± 9.94 ng/ml*min respectively, p< 0.009.

Conclusions: Consumption of WPC short time prior to consuming a high glycemic index breakfast reduces glucose while simultaneously increasing insulin and C-peptide Whey protein may represent an adjuvant treatment for patients with type 2 diabetes.

Clinical Trial Registration Number: NCT01571622

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Dietary sodium intake and incidence of diabetic complications in Japanese patients with type 2 diabetes: analysis of the Japan Diabetes Complications Study (JDCS)

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Background and aims: Many guidelines recommend that patients with (T2DM) should reduce their dietary intake of salt. However, the relationship between dietary sodium intake and incidence of diabetic complications in patients with T2DM has not been explored. Therefore, we investigated these relationships in our nationwide prospective cohort comprised of Japanese with T2DM.

Materials and methods: The present analysis was conducted as part of JDCS, a multicenter prospective study on the incidence of and risk factors for macro- and microvascular complications among Japanese patients with T2DM from outpatient clinics in 59 universities and general hospitals. Eligible were previously diagnosed patients with T2DM aged 40-70 y whose HbA1C levels were ≥6.5%. After excluding non-responders to a dietary survey, 1588 patients were analyzed. Baseline dietary intake was assessed by the Food Frequency Questionnaire based on food groups. Primary outcomes were times to cardiovascular disease (CVD), overt nephropathy, and diabetic retinopathy. Hazard ratios (HRs) for dietary intake were estimated by Cox regression adjusted for age, gender, body mass index (BMI), HbA1C, diabetic duration, serum lipids, lipid-lowering agents, smoking, energy intake, and physical activity.

Results: Mean daily dietary sodium intake in quartiles ranged from 2.8 to 5.9 g and mean energy intake ranged from 1470 to 2010 kcal. HbA1C, BMI, triglycerides, and systolic blood pressure were well controlled. During the 8-y follow-up, HR for CVD in patients in the 2nd, 3rd, and 4th quartiles of sodium intake compared with the 1st quartile were 1.70 (95% confidence interval, 0.98 to 2.94), 1.47 (0.82 to 2.62), and 2.07 (1.21 to 3.90), respectively (trend p <0.01). This association remained substantially unchanged even after further adjustment for systolic blood pressure and antihypertensive agents. In addition, among patients who had high HbA1c levels ≥9.0%), HR for CVD in patients in the top vs. bottom tertile of sodium intake was dramatically elevated compared with patients with HbA1c levels <9.0% (1.54 (0.80 to 2.99) and 10.29 (2.02 to 52.30), respectively, interaction term, P=0.03). There were no significant associations of sodium intake with overt nephropathy and diabetic retinopathy.

Conclusion: Findings suggested that high dietary sodium intake is associated with elevated incidence of CVD in patients with T2DM and is a possible risk co-factor increasing the risk in patients with HbA1c levels ≥ 9.0%. It is suggested that dietary salt restriction as medical nutritional treatment would be useful to prevent complications of diabetes in patients with T2DM.

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OP 42 SIRT1: the good guy?

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AAV8-mediated Sirt1 overexpression in the liver prevents high-carbohydrate diet induced NAFLD

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Background and aims: Non Alcoholic Fatty Liver Disease (NAFLD), is characterized by an aberrant lipid liver accumulation and is strongly related with insulin resistance and the metabolic syndrome. It is well known that caloric restriction (CR) is an important method to reduce lipid accumulation. Recent studies demonstrate that Sirt1 is one of the key genes involved in numerous effects during CR. Sirt1 is a protein deacetylase that acts by up-regulating the oxidative metabolism and by down-regulating processes like lipogenesis and inflammation. We therefore hypothesize that an increase in hepatic Sirt1 expression may protect the liver from diet-induced NAFLD. Thus, adeno-associated viral (AAV) vectors that overexpress Sirt1 specifically in the liver were injected to mice fed with a high carbohydrate diet.
Materials and methods: AAV8 vectors encoding the murine Sirt1 gene under the control of the hAAT (human α1 antitrypsin) liver specific promoter were generated. AAV8-hAAT-null (CT mice) or AAV8-hAAT-Sirt1 (Sirt1 mice) vectors were injected to three-month old C57Bl6 male mice at a dose of $5x10^{11}$vg/mice by tail vein injection. One week later the high carbohydrate diet was administered. During the diet, body weight and food intake were measured weekly and an insulin tolerance test was performed. At week 15, mice were euthanized for tissue sampling.
Results: During diet, no differences were observed in body weight gain and food intake between the Sirt1 and CT mice. However, a slight amelioration during the ITT was detected in the Sirt1 mice, suggesting that insulin sensitivity was improved. As expected, Sirt1 protein overexpression (3 fold change, $p<0.05$) was only detected in the livers of the AAV8-hAAT-Sirt1 injected animals. Sirt1 mice showed a reduction in the liver triglyceride content (-43%, $p<0.05$) and a reduction in the liver weight compared with CT mice. This reduction could be explained by the rise in liver PGC1α, Sirt3, Sirt6, LCAD and VLCAD mRNA levels. Moreover, an increase in hepatic PGC1α protein levels was also observed in Sirt1 mice. MAC-2 immunohistochemistry in liver showed a reduction in macrophage infiltration in Sirt1 mice that was corroborated by a reduction in CD68 and F4/80 mRNA levels. Furthermore, Sirt1 mice showed a reduction in epididymal WAT (eWAT) adipocyte size and a decrease in leptin expression levels. The mRNA levels of cytokines like MCP-1 and iNOS and also the HIF1α levels were diminished in the eWAT of Sirt1 mice, suggesting a reduction in adipose tissue inflammation and hypoxia. On the other hand, Sirt1 mice showed a trend to accumulate less triglycerides in the skeletal muscle (-30%, $p=0.09$), suggesting that ß-oxidation activity could be up-regulated. In agreement, UCP2, PPARδ and PDK4 mRNA levels were increased in Sirt1 mice. Furthermore, the ratio AMPK-P/AMPKtotal was also increased in the skeletal muscle of Sirt1 versus CT mice.
Conclusion: All these data suggest that long-term AAV8-mediated hepatic Sirt1 overexpression prevents the development of NAFLD induced by a high carbohydrate diet and improve whole body metabolism.
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MicroRNA-34a in obesity reduces NAD^+ levels and SIRT1 activity by directly targeting NAMPT and SIRT1 genes

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Background and aims: SIRT1 is an NAD^+-dependent deacetylase that connects nutrient availability to homeostatic responses by modulating the acetylation status and activity of transcriptional regulators and enzymes. Importantly, NAD^+ bioavailability critical for SIRT1 activity were decreased in obesity and the aged.
Materials and methods: To determine whether NAMPT is a direct target of miR-34a, the wild type (WT) or a mutated sequence of the predicted miR-34a site in the NAMPT 3'UTR was inserted into a luciferase reporter and then checked luciferase activity. To explore the effect of miR-34a on NAMPT and SIRT1 in vivo, miR-34a or anti-miR34a were overexpressed in lean mice or HFD mice via tail vein injection of adenoviral vectors.
Results: We show that hepatic microRNA-34a (miR-34a), highly elevated in obese mice and steatosis patients, reduces NAD^+ levels by inhibiting both NAMPT, the rate-limiting enzyme for NAD^+ biosynthesis, and SIRT1 expression. Hepatic verexpression of miR-34a reduced NAMPT/SIRT1/NAD^+ levels and increased acetylation of SIRT1 target transcriptional regulators, PGC-1α, SREBP-1c, and NF-κB, resulting in obesity-mimetic transcriptional and metabolic outcomes. Conversely, antagonism of miR-34a in dietary obese mice increased NAMPT/SIRT1/NAD^+ levels and alleviated steatosis, inflammation, and glucose intolerance. Anti-miR-34a-mediated increases in NAD^+ levels and lipid-lowering gene expression patterns were largely attenuated when NAMPT was downregulated in hepatocytes from dietary obese mice.
Conclusion: This study highlights an unexpected role of miR-34a in reducing NAD^+ levels, revealing therapeutic options of targeting miR-34a for SIRT1-related diseases of obesity and aging.

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Lack of SIRT1 attenuated the effect of Exenatide to ameliorate liver steatosis in mice

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Background and aims: Growing evidence suggests that Glucagon-like peptide 1 (GLP-1) receptor agonist Exenatide could relieve hepatocyte steatosis both in vivo and in vitro. Our previous study found that lack of SIRT1 (sirtuin 1) could lead to liver steatosis in mice. It prompted us to wonder whether GLP-1 receptor agonist improving liver steatosis is mediated by SIRT1.
Materials and methods: In this study, 7-8 weeks male SIRT1 heterozygous knockout mice with C57BL/6J background ($SIRT1^{+/-}$) and their wild type littermates mice (WT) were randomly divided into WT+chow diet, WT+HFD(high fat diet), and $SIRT1^{+/-}$+HFD groups and fed for 12 weeks. After the diet induction, animals were randomly divided into WT+chow diet, WT+HFD+saline, WT+HFD+Exenatide, $SIRT1^{+/-}$+HFD+saline and $SIRT1^{+/-}$+HFD+Exenatide groups for another 8 weeks. Systemic evaluations were as follows: body weight, fasting blood glucose, cholesterol and triglyceride both in serum and liver was measured. Livers were collected and weighed, and histology study was carried out such as HE and Oil-Red O staining. Western blot were used to detect protein expression in the liver.
Results: The results showed that the body weight, fasting blood glucose, liver weight, cholesterol and triglyceride level both in serum and in liver were significantly decreased after Exenatide treatment compared with saline control in WT+HFD group. However, no statistically changes were observed in $SIRT1^{+/-}$+HFD group after Exenatide treatment compared with $SIRT1^{+/-}$+HFD+saline group. Meanwhile, significant reduction of hepatic lipid accumulation was shown in WT+HFD+Exenatide group according to the histology results, but after heterozygous knockout of SIRT1, no difference was observed in the liver histology between $SIRT1^{+/-}$+HFD+Exenatide and $SIRT1^{+/-}$+HFD+saline groups. Western blot indicated the expression of SIRT1 was up-regulated, both SREBP-1 and PNPLA3 were significantly decreased in WT+HFD+Exenatide group as compared with WT+HFD+saline group. However, none of the difference above was observed in $SIRT1^{+/-}$+HFD group after Exenatide treatment compared with $SIRT1^{+/-}$+HFD+saline group.
Conclusion: Our data suggested that GLP-1 receptor agonist Exenatide could improve hepatic steatosis through up-regulating SIRT1 expression and then inhibit lipogenesis, while lack of SIRT1 attenuated the effect of Exenatide to ameliorate liver steatosis in C57BL/6J background mice.
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High-fat diet-induced impairment of skeletal muscle insulin action is not prevented by SIRT1 overexpression

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Background and aims: SIRT1 has been implicated in the regulation of skeletal muscle metabolism in response to changes in nutrient availability, though its role in the modulation of skeletal muscle insulin action remains to be completely defined. Previous studies have demonstrated that SIRT1 expression decreases under insulin-resistant conditions, such as those induced by a high-fat/hypercaloric diet (HFD). Furthermore, pharmacological activation of SIRT1 reverses HFD-induced impairments in insulin sensitivity. Thus, the purpose of this study was to determine whether constitutive activation of SIRT1 in mouse skeletal muscle prevents the deleterious effects of HFD on skeletal muscle insulin sensitivity.
Materials and methods: Mice with muscle-specific overexpression of SIRT1 (mOX) and their wildtype (WT) littermates were fed a low-fat diet (LFD; 10% calories from fat) or a HFD (60% of calories from fat) for 12 weeks beginning at 10 weeks of age. Magnetic resonance imaging and indirect calorimetry were used to measure body composition and energy expenditure, respectively. Basal and insulin-stimulated (0.36 nmol/L [60 μU/mL]) glucose uptake were measured using a 2-deoxyglucose uptake assay (2DOGU) in isolated soleus and extensor digitorum longus (EDL) muscles.
Results: SIRT1 protein abundance was ~50-300-fold higher in soleus and EDL muscles from mOX vs. WT mice. As expected, HFD increased body weight and percent body fat by 30% and 300%, respectively, while there was no effect of genotype on these parameters. In addition, energy expenditure was not affected by diet or genotype, though HFD significantly increased the contribution of fat to total EE. Importantly, 12 weeks of HFD decreased insulin-stimulated glucose uptake in WT soleus (LFD: 0.29±.04 vs. HFD: 0.14±0.03 μmol/20 min/g muscle, p<0.05) and EDL (LFD: 0.25±.04 vs. HFD: 0.13±0.02 μmol/20 min/g muscle, p<0.05), and interestingly, this impairment was not prevented in mOX soleus (LFD: 0.25±.05 vs. HFD: 0.09±0.02 μmol/20 min/g muscle, p<0.05) or EDL (LFD: 0.25±.04 vs. HFD: 0.10±0.03 μmol/20 min/g muscle, p<0.05). These impairments in insulin action were paralleled by decreased insulin-mediated activation of Akt and its downstream substrate, glycogen synthase kinase 3β.
Conclusion: The present results demonstrate that upregulation of SIRT1 activity in skeletal muscle does not prevent HFD-induced impairments in skeletal muscle insulin action. Thus, the improvement in muscle insulin sensitivity in rodent models of insulin resistance following treatment with a pharmacological activator of SIRT1, likely occurs secondary to SIRT1 activation in tissue(s) other than skeletal muscle.
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OP 43 Defining strategies for the treatment of type 2 diabetes

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Inadequate plasma adiponectin response characterises non-responsiveness to pioglitazone in individuals with impaired glucose tolerance: results from the ACT NOW study

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Background and aims: Several studies have shown that thiazolidinediones (TZDs) delay/prevent onset of diabetes in subjects with IGT. However, a significant number of IGT individuals fail to respond to TZDs. We examined factors that predict lack of response to Pioglitazone in ACT NOW.
Materials and methods: ACT NOW is a randomized double-blind, placebo-controlled study to test whether pioglitazone (PIO, 45 mg/day) can prevent/delay development of type 2 diabetes mellitus (T2DM) in persons with IGT (n= 602, FPG =105, 2-h PG [OGTT]=168 mg/dL). Indices of insulin secretion and insulin sensitivity were derived from plasma glucose, insulin, and C peptide concentrations during OGTT; plasma adiponectin was measured before and at study end (median = 2.4 yrs).
Results: Diabetes developed in 50 PLAC-treated vs. 15 PIO-treated subjects (hazard ratio=0.28: 95% CI= 0.16,-0.49; p<0.001). Of the 213 PIO-treated subjects who completed the study, 101 (47%) reverted to NGT (responders) and 112 (53%) either remained IGT or developed diabetes (non-responders, p<0.005 vs PLAC). 2-h PG was slightly higher in non-responders (172 vs 167 mg/dl, p=0.03); there was no difference in FPG, Matsuda index (MI) of insulin sensitivity, and plasma adiponectin between responders and non-responders at baseline, while indices of beta cell function ($\Delta I_{0\text{-}120}/\Delta G_{0\text{-}120}$ x MI [3.57 ± 0.2 vs. 2.90 ± 0.2, p=0.002], AIR [415± 39 vs 312±27, p=0.03] and disposition index from IVGTT [908± 69 vs 684±72, p=0.02]) were lower in non-responders compared to those who reverted to NGT. Plasma adiponectin increased by approximately 2.5 fold (12± 0.7 to 32±2.0 μg/ml) in non-responders; however, a much greater ~4 fold (11± 0.5 to 46± 4.0 μg/ml) increase was observed in responders (p<0.001). The increase in adiponectin correlated with improvement in FPG (r=0.322, p<0.001), 2-h PG (r=0.281, p<0.001), Matsuda index (r=0.526, p<0.005), and $\Delta I_{0\text{-}120}/\Delta G_{0\text{-}120}$ x MI (r=0.306, p<0.005). When categorized into quartiles of change in plasma adiponectin concentration, individuals in the highest quartile had a markedly lower FPG and 2-h PG, and higher Matsuda insulin sensitivity index and $\Delta I_{0\text{-}120}/\Delta G_{0\text{-}120}$ x MI versus those in the lowest quartile (all p<0.01).
Conclusion: Inadequate rise in adiponectin and markedly reduced β-cell function (IS/IR index) characterize non-responsiveness to PIO in IGT subjects. In individuals with poor adiponectin response to PIO, additional pharmacologic agents may be considered
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The efficacy of metformin-based OAD is not inferior to insulin glargine in newly diagnosed type 2 diabetes mellitus patients with severe hyperglycaemia after short-term intensive insulin therapy

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Background and aims: In newly diagnosed type 2 diabetic patients with severe hyperglycemia, insulin therapy should be strongly considered from the outset. However, once the metabolic state becomes stabilized, should insulin treatment be continued or transferred to an oral anti-diabetic drug (OAD) therapy is unclear. An open, parallel, randomized trial was conducted to compare the efficacy and safety of metformin-based OAD with glargine in newly

diagnosed type 2 diabetic patients with severe hyperglycemia after short-term insulin therapy.

Materials and methods: All subjects were hospitalized and treated with intensive insulin therapy for 10-14 days. At discharge, patients were randomized into the glargine group (n=21) and the metformin-based OAD group (n=26) for a six-month intervention. Glargine and OAD doses were adjusted aiming for a fasting plasma glucose concentration of 4.4-7.2mmol/l. After the six-month intervention, the glargine group was changed to metformin-based OAD therapy while medication of the OAD group remained unchanged. These patients continually followed up for another six months. Subjects received an oral glucose tolerance test after the intensive insulin therapy, at the end of both the 6th and 12th month. The primary outcome was the HbA_1c change. The secondary outcomes included β-cell function, insulin sensitivity, hypoglycemia episodes, weight change, treatment satisfaction and quality of life.

Results: Of the 108 patients who were eligible, 47 newly diagnosed type 2 diabetic patients with severe hyperglycemia were randomized. HbA_{1c} was reduced by a similar amount in the two treatment groups at the end of the six-month intervention (glargine: 11.81±1.70 vs. 6.48±0.79%; P<0.001; OAD: 11.71±1.89 vs. 6.16±0.52%; P<0.001). At the end of the 12th month, HbA_{1c} was still kept at a comparable and near optimal level in the metformin-based OAD group and the glargine group (6.25±0.60 vs. 6.42±0.72%, P=0.45). HOMA-β increased similarly in the two groups (glargine: 2.78±0.77 vs. 3.90±0.64; P<0.001; OAD: 2.96±0.77 vs. 3.54±0.58; P=0.02) after the six-month intervention. There were no significant differences between the two groups in insulin sensitivity improvement, hypoglycemia episodes, weight change, treatment satisfaction and quality of life after the six-month intervention.

Conclusion: The effects of metformin-based OAD therapy on glycemic control and β-cell function improvement are not inferior to glargine in newly diagnosed type 2 diabetic patients with severe hyperglycemia after short-term intensive insulin therapy.

Table . Parameters of glycemia and insulin secretion before and after the six-month intervention

	Before intervention		After intervention	
	OAD	Glargine	OAD	Glargine
Number (male : female)	26 (16 : 10)	21 (15 : 6)	24 (14 : 10)	20 (14 : 6)
HbA_{1c} (%)	11.71±1.89	11.81±1.70	6.16±0.52*	6.48±0.79*
No.(%) of subjects with HbA_{1c} ≤7.0%	—	—	22 (91.7%)	16 (80.0%)
No.(%) of subjects with HbA_{1c} ≤6.5%	—	—	22 (91.7%)	14 (70.0%)
IGI	2.35±0.65	1.92±0.95	2.99±0.90*	2.92±1.09*
HOMA-β	2.96±0.77	2.78±0.77	3.54±0.58*	3.90±0.64*
Matsuda index	1.75±0.52	2.00±0.69	1.84±0.51	1.60±0.48
HOMA-IR	2.87±0.56	2.71±0.67	2.58±0.58	3.07±0.48#
DI_{HOMA}	2.41±0.49	2.37±0.67	3.26±0.44*	3.12±0.59*
$DI_{Matsuda}$	1.41±0.41	1.38±0.37	2.00±0.37*	1.92±0.45*

IGI: insulinogenic index, HOMA-β: homeostasis model assessment of β-cell function, HOMA-IR: homeostasis model assessment of insulin resistance; DI: disposition index. IGI, HOMA-β, Matsuda index, HOMA-IR and DI were natural logarithm transformed before analysis. *, P<0.05 compared with baseline; #, P<0.05 between two groups.

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Initiation of new injectable treatment introduced after anti-diabetic therapy with oral-only regimens (INITIATOR) study: an interim analysis

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Background and aims: Patients with T2DM failing oral antidiabetic drugs (OADs) may initiate injectable therapy with insulin or a glucagon-like peptide 1 (GLP1) analogue. However, real-world data are limited. The INITIATOR study is an observational longitudinal cohort study using both medical chart and health claims data from two of the largest US commercial health insurers, aimed at providing a comprehensive understanding of patients' characteristics, treatment patterns, and associated outcomes. This analysis reports the interim results of the study.

Materials and methods: T2DM patients aged ≥ 18 years, previously on OADs only with HbA1c ≥ 7%, who initiated either insulin glargine SoloSTAR® pen (GLA) or GLP1 analogue liraglutide (LIRA) between 2010-2011 were included. Patients were enrolled in one of the two health insurance plans, with continuous healthcare coverage during the 6 months before (baseline), and 12 months after initiation (follow-up). Health claims and medical chart data from eligible enrolled patients were extracted by OptumInsight™ (OI) and HealthCore® (HC) from respective health plans. Differences in baseline characteristics, treatment patterns, clinical outcomes at follow-up and change in costs from baseline to follow-up were assessed descriptively.

Results: A total of 1,417 patients were included. At baseline, patients had a mean age of 53.2 years and were on 2 OADs. Compared to patients initiating LIRA, patients initiating GLA had poorer health status, as indicated by higher Charlson Comorbidity Score, and higher HbA1c, but lower weight (Table). After 1 year follow-up, treatment persistence was higher for GLA users than LIRA users. Despite higher HbA1c at entry, GLA patients had experienced greater HbA1c reduction but also slight weight gain, while LIRA patients lost weight. Although rates were low, hypoglycaemia was more common in GLA patients. A significant increase in diabetes-related cost was observed in the LIRA group, but not the GLA group, mainly driven by increasing diabetes drug costs (Table).

Conclusion: The interim analysis from this real world study showed significant baseline differences between T2DM patients initiating LIRA and GLA. Results suggest that GLA and LIRA are being used to treat different patient groups. Although each treatment has clinical advantages, significant healthcare cost increases were observed in the LIRA group, warranting further cost-effectiveness analysis.

	OI[a] GLA (n = 355)	OI[a] LIRA (n = 400)	HC[a] GLA (n = 319)	HC[a] LIRA (n = 343)
Baseline Female, n (%)	151 (42.5)	184 (46.0)	130 (40.8)	171 (49.9)*
Baseline Weight (kg), Mean (SD)	103.00 (24.4)	112.16 (23.5)***	100.36 (24.45)	111.24 (24.26)***
Baseline HbA1c %, Mean (SD)	9.66 (1.83)	8.63 (1.44)***	9.81 (5.38)	8.55 (1.42)***
Baseline Charlson Comorbidity Score	0.92 (1.58)	0.57 (1.11)***	0.94 (1.63)	0.71 (1.32)*
Follow-up Persistence with Medication, n (%)[b]	237 (66.8)	205 (51.3)	214 (67.1)	157 (45.8)
Follow-up HbA1c[c] % Change, Mean (SD)[b]	-1.28 (2.08)	-0.52 (1.61)	-1.42 (5.96)	-0.57 (1.88)
Follow-up Weight[c] Change (kg), Mean (SD)[b]	1.12 (6.01)	-2.02 (6.79)	1.06 (7.89)	-3.08 (8.38)
Follow-up Hypoglycaemia/Severe Hypoglycaemia[d], n (%)[b]	10 (2.82)/ 5 (1.41)	10 (2.50)/ 0 (0)	14 (4.39)/ 7 (2.19)	3 (0.87)/ 2 (0.58)
Change in Diabetes-related Healthcare Cost, Mean ($D)[c]	+$1,258.09 ($19,333.40)	+$956.46 ($8,151.88)*	-$1320.27 (17,561.38)	+$1,146.05 (8,912.2) ***
Change in Diabetes Drug Costs Mean ($D)[c]	+$613.03 ($1,037.37)***	+$955.83 ($932.11)***	-$58.13 (695.58)	+$800.66 (1,005.21) ***

*, P<0.05, ** P<0.01, ***, P<0.001; [a]Data collected from two health insurance plans, associated with OptumInsight™ (OI) and HealthCore® (HC), respectively. [b]Compared descriptively due to differing baseline characteristics. [c]Among patients with = 1 HbA1c result or = 1 weight reading in the follow-up period. [d]Requiring inpatient or ER treatment. [c]Difference in costs between the last 6-months of follow-up and the baseline period, statistical significance denotes change in cost from baseline to follow-up.

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Initial triple combination therapy is more effective and safer than stepwise add on conventional therapy in newly diagnosed type 2 diabetes mellitus

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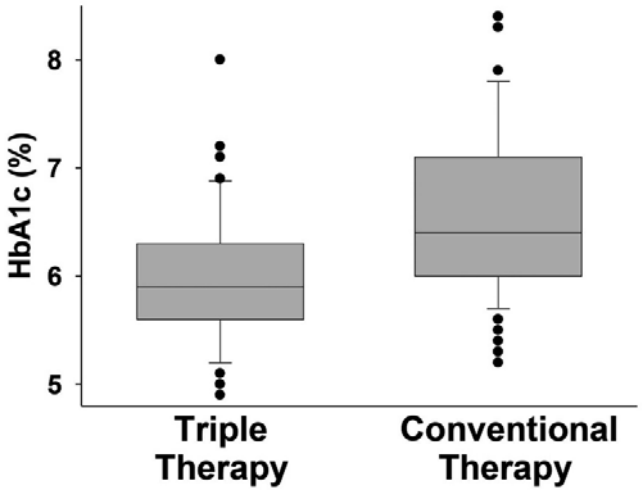
Background and aims: To compare the efficacy and safety of initiating therapy in new onset T2DM with triple therapy (metformin/pioglitazone/exenatide) versus metformin followed by sequential addition of sulfonylurea and basal insulin.

Materials and methods: 144 newly diagnosed T2DM (age = 45±1 y; BMI=36±0.5; HbA_{1c} = 8.6±0.1%; diabetes duration = 5.6±0.5 mo) were randomized to receive metformin (1000→2000 mg/d) plus pioglitazone (15→45 mg/d) plus exenatide (5→10 μg BID) (Triple Therapy, n=71) or escalating dose of metformin (1000→2000 mg/d) followed by sequential addition of sulfonylurea (glipizide, 20 mg/d) and basal insulin to maintain HbA_{1c} < 6.5 (Conventional Therapy, n = 73).

Results: In subjects receiving Triple Therapy, HbA_{1c} decreased from 8.6 to 6.1% at 6 mo and to 5.9% at month 24. In the Conventional Therapy arm, HbA_{1c} declined to 6.3% at 6 mo and subsequently increased to 6.6% at mont 24. More subjects in the conventional arm failed to achieve the treatment

HbA1c goal <6.5% (35 vs 16%, respectively, p<0.0001). Moreover, as the Box plot below demonstrates, >90% of subjects receiving the triple therapy maintained HbA1c <7.0% at 2 years compared to <75% of subjects in the conventional therapy arm. Despite significantly lower HbA_{1c}, subjects in Triple Therapy arm experienced a 13.6-fold lower rate of hypoglycemia compared to subjects receiving Conventional Therapy. Lastly, subjects in the Triple Therapy arm experienced mean weight loss of 1.2 kg compared to mean weight gain of 3.6 kg (p=0.02) in subjects in the Conventional Therapy arm.
Conclusion: The present results demonstrate that antidiabetic therapy targeting the core metabolic pathophysiologic disturbances (insulin resistance and beta cell dysfunction) responsible for hyperglycemia is more effective and safer than therapy simply aimed at lowering the plasma glucose concentration without correcting the underlying metabolic abnormalities present in T2DM.



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OP 44 Hypoglycaemia

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Brain glutamate falls during hypoglycaemia in controls but not in type 1 diabetes mellitus subjects with hypoglycaemia associated autonomic failure

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Background and aims: Patients with type 1 diabetes (T1D) who experience recurrent hypoglycemia (HG) as a result of insulin treatment are at risk for developing hypoglycemia associated autonomic failure (HAAF), the syndrome where the first symptom of HG is unconsciousness due to an impaired counterregulatory hormonal response. The mechanism for HAAF is unknown. Bischoff et al (2006) observed lower glutamate-to-creatine in the occipital cortex at moderate hypoglycemia in controls, but not in patients with T1D and blunted glucose counterregulation. However, it was not clear if this difference was due to diabetes or HAAF. Here we test the hypothesis that glutamate (GLU) levels in the occipital cortex will decrease in response to moderate hypoglycemia in healthy controls and in T1D without HAAF, but not in T1D with HAAF.
Materials and methods: 10 subjects with T1D (3M/7F, age 42±14 yrs.) and 5 healthy controls (1M/4F, age 32±4 yrs.) completed the study. Subjects underwent a 2-step hyperinsulinemic euglycemic hypoglycemic clamp in a 7 Tesla scanner; blood glucose was first maintained at 90 mg/dL, then dropped to ~50 mg/dL and maintained at this level. Epinephrine was measured during hypoglycemia. T1D with mean epinephrine < 100 pg/ml were categorized as having HAAF. ^{1}H MR spectra were collected from a 22x22x22 mm^3 occipital cortex voxel using STEAM (TE = 8 ms). CNS metabolites were quantified using LCModel.
Results: High spectral quality was consistently achieved, allowing the quantification of 13 neurochemicals including aspartate, ascorbate/vitamin C, total choline, total creatine, GABA, glutamine, glutamate, glutathione, myo-inositol, lactate, total N-acetylaspartate, scyllo-inositol and taurine with mean CRLB ≤ 20 %. During HG, GLU dropped in controls and T1D without HAAF, but not in T1D with HAAF (see table). In addition, myo-inositol and scyllo-inositol increased in all groups and aspartate increased and lactate decreased in T1D without HAAF (p≤0.03) during HG vs. euglycemia.
Conclusions: We found brain GLU significantly drops in controls and in T1D without HAAF, likely due to glutamate oxidation as demonstrated in healthy rodent brain during HG, but not in T1D with HAAF. This suggests higher glucose and/or alternative fuel availability to the brain in HAAF, eliminating the need to oxidize glutamate. Interestingly, lactate utilization and aspartate increase were also observed in the healthy rodent brain in response to hypoglycemia.

Epinephrine and GLU responses during HG

Group	Epinephrine response durng HG (pg/ml)	GLU during Euglycemia (μmol/g ww)	GLU during HG (μmol/g ww)	% GLU drop	p for GLU drop
Control (n=5)	403 ± 139	8.8 ± 0.3	8.4 ± 0.3	3.7	0.003
T1D with HAAF (n=5)	35 ± 27	9.0 ± 0.4	8.8 ± 0.4	2.0	0.06
T1D w/o HAAF (n=5)	218 ± 80	9.1 ± 0.6	8.6 ± 0.7	5.9	< 0.001

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Myocardial glucose metabolism: impact of GLP-1 in healthy men during normo- or hypoglycaemia

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Background and aims: Glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists provide beneficial cardiovascular effects by protecting against ischemia and reperfusion injury. Type 2 diabetes mellitus (T2DM) patients have reduced glycolysis in the heart. Thus, a mechanism of enhancing myocardial energetic efficiency by increasing glucose availability and utilization reducing beta-oxidation and increasing cardioprotection may exist. Of interest, GLP-1 seems to increase myocardial glucose uptake (MGU) in dogs but not in subjects with T2DM. Thus, our aim was to assess the effects of the GLP-1 receptor (GLP-1r) activation on myocardial glucose metabolism in healthy subjects during normo- and hypoglycemia.
Materials and methods: We conducted two randomized, double-blinded, placebo-controlled cross-over studies including ten healthy men with normoglycemia and eight healthy men with hypoglycemia in order to assess the effect of GLP-1 on myocardial glucose metabolism using positron emission tomography to determine glucose uptake (MGU) during pituitary-pancreatic normo- and hypoglycemic clamps (plasma glucose 4.5 and 3.0 mM) and 18fluoro-deoxy-glucose (FDG) as tracer.
Results: GLP-1 did not affect MGU (during normoglycemia: 0.15 +/- 0.04 and 0.16 +/- 0.03 micromol/g/min, P=0.46 and during hypoglycemia: 0.16 +/- 0.03 and 0.13 +/- 0.04 micromol/g/min, P=0.14). However, regression analysis revealed that the effect of GLP-1 was negatively correlated to baseline MGU in both normo- and hypoglycemia, (P = 0.007 and 0.02, respectively) and positively correlated to the level of insulin resistance in the hypoglycemia study, P=0.04. Except from a GLP-1 mediated increase in glucagon levels and increased glucose infusion rates during hypoglycemia, no differences in circulating hormones and metabolites (catecholamines, free fatty acids, insulin, growth hormone, cortisol and P-glucose) were found.
Conclusion: While GLP-1 does not seem to enhance MGU during normo-nor hypoglycemia, the GLP-1 induced effect on MGU seems to be positive in subjects with low baseline MGU and negative in subjects with high baseline MGU regardless of glycemia level. In the hypoglycemia study the most insulin resistant subjects gained positive effect as expected from the literature.
Clinical Trial Registration Number: NCT00418288 NCT00256256

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Glucose-dependent insulinotropic polypeptide protects against hypoglycaemia in type 1 diabetes

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Background and aims: Significant glucagon releasing properties of glucose-dependent insulinotropic polypeptide (GIP) during insulin-induced hypoglycaemia have been demonstrated in healthy individuals and patients with type 2 diabetes. Thus, GIP could potentially increase the glucagon counter-regulatory response also in patients with type 1 diabetes. Glucagon-like peptide 1 (GLP-1) receptor agonists are being explored as add-on treatment to insulin in patients with type 1 diabetes. Owing to inhibitory effects on glucagon secretion demonstrated in healthy individuals and patients with type 2 diabetes, GLP-1 could potentially reduce the glucagon counter-regulatory responses in patients with type 1 diabetes. We sought to evaluate the effects of GIP and GLP-1 on counter-regulatory glucagon responses and endogenous glucose production in patients with type 1 diabetes.
Materials and methods: Ten male subjects with type 1 diabetes (C-peptide negative after 5 g-*iv* arginine test, age: 26±1 years (mean±SEM); BMI: 24±0.5 kg/m^2; HbA_1c: 7.3±0.2%) were studied on 3 separate days. In a randomised double-blinded cross-over study, saline or supraphysiological doses of GIP (4 pmol×kg^{-1}×min^{-1}) or GLP-1 (1 pmol×kg^{-1}×min^{-1}) were administered *iv* for 2 hours. The first hour plasma glucose was lowered by exogenous insulin infusion, thereafter insulin infusion was stopped and plasma glucose was followed for another hour.
Results: GIP infusions elicited larger glucagon responses during the second hour of the study (1.7±0.3 (GIP) vs. 0.4±0.2 (GLP-1) vs. 0.7±0.1 (saline) nmol/l×min, $p<0.0001$). There was no significant difference in glucagon responses between GLP-1 and saline days. During GIP infusions significantly less glucose was needed to keep identical glucose excursions and plasma glucose above 2 mmol/l (156±35 (GIP) vs. 234±41 (GLP-1) vs. 214±56 (saline) mg×kg^{-1}, $p<0.05$). There was no significant differences in glucose infusions between GLP-1 and saline days. Insulin levels were similar on all study days. Hypoglycaemia symptoms score and cognitive tests during hypoglycaemia did not differ significantly between days.
Conclusions: Our results suggest that GLP-1 receptor agonists do not reduce counter-regulatory responses to hypoglycaemia in patients with type 1 diabetes and that exogenous GIP could be exploited in preventing hypoglycaemia in C-peptide negative patients with type 1 diabetes.
Clinical Trial Registration Number: NCT01739283
Supported by: Novo Nordisk Foundation and Beckett Fonden.

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DPP-4 inhibition protects from insulin-induced hypoglycaemia: results from a novel graded hyperinsulinaemic hypoglycaemic clamp in mice

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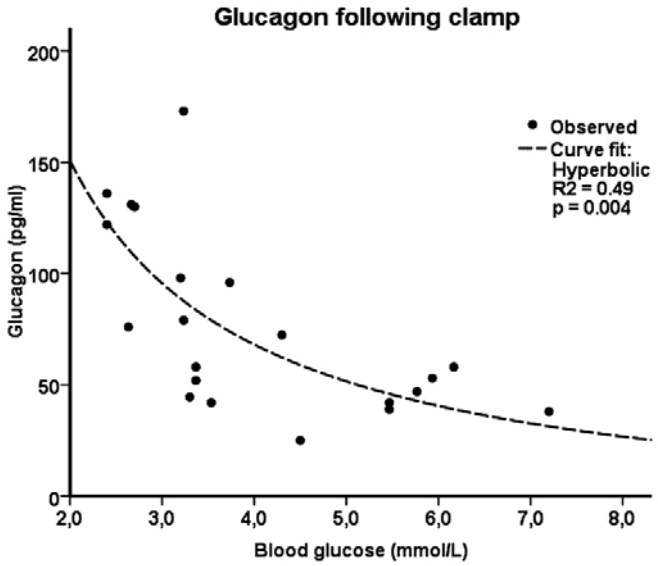
Background and aims: Glucose lowering therapies in type 2 diabetes (T2D) may increase the risk of hypoglycemia, which is associated with acute unpleasant consequences and increased long-term risk for cardiovascular diseases. A major defense mechanism to combat hypoglycemia is glucagon counter-regulation; however, this mechanism is compromised in some patients with T2D. Therefore, there is a need to develop glucose-lowering therapies which sustain the endogenous counter-regulations to hypoglycemia. To enable such development, we have developed a graded hypoglycemic clamp technique in mice for careful and detailed examination of insulin-induced hypoglycemia and its counter-regulation *in vivo*. The aim of this study was to use this novel technique in combination with a meal challenge to explore the influence of DPP-4 inhibition, a relatively novel treatment strategy for T2D associated with a low risk for hypoglycemia, on hypoglycemia and glucagon counter-regulation.
Materials and methods: Anesthetized C57BL/6 mice were simultaneously infused with 15 mU/kg/min of insulin and variable rates of glucose to target a steady state blood glucose of 2.5, 3.5, 4.5 or 6 mmol/L for 30 min between min 60 and 90 of the graded hyperinsulinemic hypoglycemic clamp. To explore the influence of DPP-4 inhibition, mice were given a 0.285 kcal mixed meal with or without the DPP-4 inhibitor NVPDPP728 (NVP) 45 min prior to infusion followed by a hypoglycemic clamp targeting a steady state glucose of 2.5 mmol/L. Glucose and glucagon levels were measured and glucose infusion rate (GIR) during the clamp was estimated.
Results: Steady state glucose levels obtained were 2.7±0.1, 3.4±0.1, 4.4±0.1 and 6.0±0.3 mmol/L. This resulted in a hypoglycemia-dependent and gradual increase in glucagon levels with decreasing glucose. The relation between steady state glucose and glucagon counter-regulation was a highly significant inverse hyperbolic regression (see Figure). The method revealed a clear difference between glucagon levels after clamp at 2.5 or 3.5 mmol/L of glucose (131.9±10.0 and 61.8±8.7 pg/ml respectably, p=0.0022). After a mixed meal with NVP, there was a partial resistance to hypoglycemia, thus, GIR was only 0.6±0.5 mg/kg/min after NVP compared to 4.9±0.9 mg/kg/min (p=0.0095) in controls, even though blood glucose was not suppressed below 3.2±0.4 mmol/l after NVP compared to 2.6±0.1 mmol/L (p=0.04) in controls. Glucagon did not differ between the NVP treated group and control after the hypoglycemic clamp (64.3±9.1 and 83.3±12.0 pg/ml respectably, p=0.25).
Conclusion: We developed a novel *in vivo* technique in mice to enable exploration of glucose-lowering therapies on hypoglycemia and glucagon counter-regulation. Using this, we demonstrate a hypoglycemia-dependent gradual increase in glucagon counter-regulation within the hypoglycemic range along an inverse hyperbolic regression. Finally, we show that DPP-4 inhibition partially protects against severe hypoglycemia.



OP 45 Brown fat: feel the heat?

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The novel adipokines BMP4 and BMP7 promote browning of primary human adipose-derived stem cells

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Background and aims: White adipose tissue (AT) is an energy storage depot and a real endocrine organ, while brown AT is specialized in energy dissipation and has recently been shown to be present in adult humans. UCP1-expressing adipocytes, with a different origin from classical brown adipocytes, have been found in white AT. These "beige" adipocytes may represent a therapeutic target to fight obesity. Bone morphogenetic proteins (BMPs) have been shown to play an important role in adipogenesis. While BMP4 induces stem cell commitment to the white adipocyte lineage, BMP7 promotes brown adipogenesis. We have previously demonstrated that BMP4 and BMP7 are secreted from human AT and represent novel adipokines. Thus, we aimed to investigate the role of BMP4 and BMP7 in the white-to-brown shift in human adipose-derived stem cells (hASCs).
Materials and methods: hASCs were isolated from human subcutaneous adipose tissue from lean or moderately overweight females. hASC were chronically challenged with BMP4 or BMP7 (50 ng/ml) during the differentiation period (14 days). Lipid accumulation was assessed by Oil Red O staining. PPARγ, C/EBPα, UCP1 and Tcf21 expression was analysed by PCR. Mitochondrial biogenesis was studied with an OXPHOS antibody by Western Blot.
Results: Lipid accumulation was increased in BMP4 and BMP7 treated hASCs (2.5 and 2.6-fold, respectively), as assessed by Oil Red O Staining. Chronic BMP4 and BMP7 treatment increased PPARγ (4.0 and 3.9 fold, respectively) and C/EBPα (7.7 and 6.1-fold, respectively) mRNA levels. Surprisingly, both BMP4 and BMP7 increased UCP1 expression (6.2 and 7.7-fold, respectively). Moreover, both adipokines significantly decreased 50% the levels of the white-specific marker Tcf21. Interestingly, the ability to induce UCP1 in response to BMP4/7 was highly variable between donors. Thus, donors with the strongest UCP1 upregulation displayed higher levels of the recently identified "beige" adipocyte marker CD137. Moreover, BMP4/7 treatment upregulated the mitochondrial OXPHOS complexes in a subset of donors, as determined by Western Blot.
Conclusion: In summary, we showed for the first time that not only BMP7, but also BMP4 is able to induce a white-to-brown transition in hASCs. Therefore, the novel adipokines BMP4 and BMP7 arise as potential therapeutic targets to counteract obesity in humans in an auto/paracrine manner.

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Role IGF1R in the regulation of brown fat function and energy balance

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Background and aims: Excess of visceral adiposity and overweight is associated with the development of metabolic disorders such as type 2 diabetes, hyperlipidemia, and fatty liver and cardiovascular disease. Fat storage results from an imbalance between food intake and energy expenditure. White adipose tissue (WAT) is the main energy storage organ in the body, whereas brown adipose tissue (BAT) is an essential component of non-shivering thermogenesis and energy expenditure by dissipating energy as heat through uncoupled respiration mediated by uncoupling protein 1, a BAT specific mitochondrial protein. Insulin-like growth factor 1 (IGF1) has an important role in brown adipocyte proliferation and also in its adipogenic and thermogenic differentiation together with insulin itself, BMP-7 signaling and sympathetic innervations through beta-adrenoreceptors. However, the implication of IGF1 receptor (IGF1R) and its signaling in the mechanisms controlling the regulation of BAT thermogenesis and energy expenditure and how its dysregulation could affect BAT functionality, remains to be investigated. Thus, the aim of this work was to assess the role of IGF1R in BAT development and thermogenesis and their potential metabolic complications.
Material and methods: We have generated BAT-specific knockout mice of IGF1R (BATIGF1RKO) to eliminate IGF1R expression and signaling in brown adipocytes. Whole-body glucose and insulin tolerance, circulating lev-

els of important mediators, regulation of insulin and BMP-7 signaling pathways, brown fat cell differentiation, BAT functionality, energy expenditure and body fat mass/body weight were studied.
Results: BATIGF1RKO mice displayed higher fasting hyperglycemia and whole-body white fat mass upon ageing. However, KO mice showed a decrease in brown fat mass. Histological analysis of BAT showed a deteriorated morphology in BATIGF1RKO as compared to wild-type mice (WT), which is correlated with a lower basal core temperature. BAT from IGF1RKO mice showed a decrease in gene expression of thermogenic related genes *vs.* controls. Accordingly, BATIGF1RKO mice showed worse thermogenic capacity and exhibited a less oxidative phenotype than WT littermates. Finally, IGF1R deletion in fetal brown adipocytes assessed the essential role played by IGF1R in BAT differentiation and function.
Conclusion: Overall, our data identify IGF1R as an important negative regulator of brown fat activity, key to the maintenance of thermogenesis and body weight, which may have important therapeutic implications for the treatment of obesity and associated metabolic disorders.
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Presence of brown adipocytes in white adipose tissue and its role in obesity resistance and insulin sensitivity
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Background and aims: Brown adipose tissue (BAT), characterised by uncoupling protein 1 (UCP1) expression, has recently been described as metabolically active in humans. It induces an increase in energy expenditure by increasing thermogenesis. Lou/C rats which originate from the Wistar strain are resistant to obesity induced by either age or a high fat diet. In inguinal white adipose tissue (WATi) of Lou/C rats, we previously demonstrated the presence of ectopically expressed UCP1, as well as of an overexpression of the β3-adrenoreceptor which controls UCP1 expression. The aim of the present study was to investigate the role of UCP1 in the metabolic regulation of Lou/C rats, including the control of body weight gain and the improvement of insulin sensitivity.
Materials and methods: A β3 agonist treatment (CL-316243, s.c, 1mg/kg/day) was administered in 3 month-old Wistar and Lou/C rats for 2 weeks. Several metabolic parameters were measured. In particular, glucose and cold tolerance, fat mass repartition, overall and tissue-specific insulin sensitivity, as well as energy expenditure were determined.
Results: The treatment induced lower food efficiency, a decreased fat volume (Wistar control =19.08 cm^3±1.3, Wistar treated =7.91±0.6, Lou/C control =4.83±0.9, Lou/C treated =2.27±0.2 $p<0.001$) and enhanced energy expenditure (W=7.09±0.1 kcal/h/$kg^{0.75}$, Wt=9.04±0.1, L=8.68±0.05, Lt=11.23±0.3, $p<0.001$) in both strains. It also similarly increased overall insulin sensitivity (GIR: W=22.3±1.1, Wt= 32.6±0.8, L=31.8±1.3 Lt=40.6±0.5, $p<0.001$), as determined using the euglycemic hyperinsulinemic clamp technique. However and interestingly, muscle glucose uptake, measured with labelled 2-deoxy-glucose, was unaltered by the treatment in both groups of animals. On the contrary, insulin-stimulated glucose uptake in different white adipose tissue depots was highly increased by the β3 adrenoceptor treatment in the Lou/C group only (L=1.6±0.3 ng gluc/mg tissues, Lt=19.7±1.2, $p<0.001$), an increase that correlated with the expression of UCP1 in these tissues. Regarding this gene, the treatment induced its overexpression in the Lou/C group only. In this group, such overexpression was observed in WATi (164 fold increase, $p<0.01$) and was even more marked in epididymal white adipose tissue (WATe) (28864 fold increase, $p<0.01$). These results were confirmed by Western blot analysis. In BAT, UCP1 increased to a similar extent in Wistar and Lou/C rats in response to the treatment (3 fold increase in both groups, $p<0.001$).
Conclusion: A β3 adrenoceptor treatment has similar beneficial effects in Wistar and Lou/C rats on food efficiency through a stimulation of energy expenditure, as well as on overall insulin sensitivity. However, the treatment-induced increase in white adipose tissue insulin sensitivity is more marked in Lou/C than in Wistar rats, as a likely result of UCP1 overexpression in this tissue. This could be one of the mechanisms responsible for the maintenance of the lean phenotype in Lou/C rats.
Supported by: FNS, DIABAT

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18F-fluorodeoxyglucose uptake in brown adipose tissue during insulin-induced hypoglycaemia in non-diabetic adults
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Background and aims: Hypothermia has been associated with acute hypoglycaemia since the earliest use of insulin to treat diabetes. In humans, the capacity to generate heat resides principally in two organs: skeletal muscle and brown adipose tissue (BAT). Hypoglycaemia inhibits muscle shivering and hence suppresses muscle thermogenesis. However, an unexplained persistence in hypoglycaemia induced heat production has been noticed. Hypoglycaemia is characterised by acute sympatho-adrenal activation causing profuse catecholamine secretion which stimulates BAT activity. This study aimed to investigate the effects of hypoglycaemia on the activity of BAT in adult humans to elucidate the counterintuitive increase in heat production that occurs during hypoglycaemia.
Materials and methods: Nine healthy lean male volunteers (age range 19-30 years; body mass index range 20-23 kg/m2) underwent hyperinsulinaemic glucose clamps (insulin infusion rate 1.5 mU/kg/min) on two separate occasions in a cold environment (17-18°C) followed by a 18F-FDG PET-CT scan as a measure of BAT activity. Arterialised blood glucose level was maintained at 4.5 mmol/L for 30 minutes, and either continued at this level (euglycaemia condition) or lowered to 2.5 mmol/L (hypoglycaemia condition) over 20 minutes. An hour after the start of the glucose clamp, when the subjects during the hypoglycaemic condition were hypoglycaemic for at least 15 minutes, 200 MBq of 18F-FDG was administered intravenously and exposure to the specific glycaemic condition was continued for a second hour. The maximal standardized uptake values (SUVmax), defined as the activity in Bq/ ml within the region of interest (cervical, supraclavicular, superior mediastinal fat depots and muscle) divided by the injected dose in Bq/g (body weight), were determined. Counterregulatory hormones were measured at baseline, 50, 70, 90 and at 110 minutes. Results were analysed by means of the non-parametric Wilcoxon signed-rank test and Spearman's rank correlation coefficient.
Results: BAT activity was observed in all volunteers. No difference was observed in the standardised uptake value SUVmax measured during either condition: euglycaemia (median (range) 4.2 g/ml (1.0-7.7); hypoglycaemia 3.1 g/ml (2.2-12.5); (p=0.7). Further, no difference was found for the SUVmax in skeletal muscle between the conditions: 4.1 (2.2-6.5) vs. 3.4 (1.7-5.5) g/mL (p=0.3). A greater fall in temperature was observed during hypoglycaemia when compared to euglycaemia 80 min from baseline (after 40 min of hypoglycaemia): -0.1 (-0.5-0.8) °C during euglycaemia vs. -0.3 (-1.1-0.1) °C (p=0.02) during hypoglycaemia and this difference continued at 90, 100 and 110 min after. No correlations existed between the SUVmax of 18F-FDG in BAT and levels of counterregulatory hormones.
Conclusion: This study shows that there is a similar amount of 18F-fluorodeoxyglucose uptake in brown adipose tissue during hypoglycaemia when compared to euglycaemia. It is therefore not likely that brown adipose tissue activity is a major determinant of the increased heat production that occurs during hypoglycaemia.
Clinical Trial Registration Number: NTR2744

OP 46 Beta cells under attack

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Pro-apoptotic Bax and Bak control beta cell death and early ER stress signalling under glucolipotoxic stress

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Background and aims: Loss of functional pancreatic beta-cells is a critical event in the pathogenesis of diabetes and mounting evidence suggests that chronic endoplasmic reticulum (ER) stress contributes to beta-cell dysfunction and apoptotic death. Two core pro-apoptotic proteins from the Bcl-2 family, Bax and Bak, mediate the execution of mitochondrial apoptosis and have also been suggested to regulate aspects of ER physiology and the unfolded protein response (UPR) induced by ER stress. In this study we aimed to determine the relative importance of Bax and Bak in beta-cell death under glucolipotoxic stress and examine their putative involvement in beta-cell ER stress signaling.
Materials and methods: We established a mouse line in which single or combined knockout of Bax and/or Bak can be induced in pancreatic beta-cells by crossing $Bax^{flox/flox}$:$Bak^{-/-}$ mice with Pdx1-CreER mice. Ablation of islet Bax was achieved by injecting all animals with 3 mg/40 g of tamoxifen for 4 consecutive days. Bax and Bak single knockout (SKO), double knockout (DKO), and wild-type (WT) islet cells were assayed for total cell death by kinetic image analysis of propidium iodide (PI) incorporation following treatment with 1.5 mmol/L palmitate (lipotoxicity), palmitate in combination with 25 mmol/L glucose (glucolipotoxicity), and the chemical apoptosis inducer staurosporine (1 μmol/L). To examine ER stress, Chop and spliced Xbp1 were assayed by real-time PCR in islets exposed to either glucolipotoxicity or the chemical ER stress inducer thapsigargin (0.1 μmol/L).
Results: Tamoxifen administration lead to >80% Bax knockout in $Bax^{flox/flox}$ islet cells (87±0.03%, p<0.05). No significant differences in islet function were observed among all genotypes under physiological conditions, with respect to *in vivo* glucose tolerance and *in vitro* analysis of insulin content and glucose stimulated insulin secretion. Kinetic cell death analysis demonstrated the expected protection of single and double Bax-Bak knockout beta-cells from mitochondrial apoptosis induced by staurosporine compared to WT littermate controls (cell death at the 36h time-point: WT 78±6%, Bax SKO 44±5%, Bak SKO 42±8%, Bax-Bak DKO 26±5%, p<0.05) . Bax-Bak DKO beta-cells were partially protected from cell death induced by glucolipotoxicity (Bax-Bak DKO 69±4% relative to WT 100%, p<0.05), while single Bax or Bak knockout beta-cells showed no significant protection compared to WT littermate controls. ER-stress induced by either thapsigargin or glucolipotoxicity revealed a time-dependent increase in Chop expression, with no significant differences between the genotypes. Interestingly, spliced Xbp1 was significantly increased in Bax-Bak DKO islets under early stages of ER stress compared to WT littermate control islets (12h thapsigargin: Bax-Bak DKO 4.1-fold±0.3, WT 2.6-fold±0.3 relative to DMSO control, 24h glucolipotoxicity: Bax-Bak DKO 2.1-fold±0.3, WT 1.2-fold±0.1 relative to 25 mmol/L glucose control, p<0.05).
Conclusion: Together, our data suggests that Bax and Bak have individual roles in mediating mitochondrial apoptosis induced by staurosporine but may be redundant in the execution of beta-cell death under chronic glucolipotoxicity. Furthermore, our findings show that Bax and Bak regulate early activation of the UPR in the beta-cell, suggesting these core apoptotic proteins play important roles in pancreatic beta-cell stress signaling upstream of their canonical roles regulating apoptosis execution.
Supported by: CDA Operating Grant and CFRI Establishment Award

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The transcription factor JunB protects pancreatic beta cells from lipotoxicity by induction of the ER stress response gene XBP1 and AKT signalling

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Background and aims: Environmental factors such as diets rich in saturated fats contribute to the loss of pancreatic β-cells in type 2 diabetes. JunB, a member of the AP1 transcription factor family, promotes β-cell survival in models of type 1 diabetes. We have previously shown that GLP-1 agonists and induction of cellular cAMP protect β-cells from lipotoxicity partially via JunB induction. In this study we interrogated the molecular mechanisms involved in JunB-mediated β-cell protection from lipotoxicity.
Materials and methods: Apoptosis was detected by Hoechst 33342/propidium iodide staining, mRNA expression by qPCR, protein expression or phosphorylation by Western blot. Gene silencing was done by siRNA and overexpression by adenovirus infection. Affymetrix microarray analysis was performed in JunB-deficient INS-1E cells.
Results: JunB knockdown (KD) potentiated palmitate-induced apoptosis in INS-1E cells (from 26 ± 1 to 38 ± 3%, p<0.05), primary rat β-cells (from 24 ± 2 to 33 ± 3%, p<0.05) and dispersed human islets (from 31 ± 3 to 43 ± 5%, p<0.05), while its overexpression protected from lipotoxicity (from 26 ± 2 to 18 ± 2% apoptosis, p<0.05). By array analysis we found that JunB KD downregulates the endoplasmic reticulum (ER) stress response gene XBP1 (by 50 ± 7%, p<0.05) and inhibits AKT signalling. We therefore investigated whether these pathways are involved in JunB-mediated β-cell protection. XBP1 overexpression rescued cell viability in JunB-deficient cells (from 41 ± 2% in JunB KD cells to 27 ± 2% in JunB KD and XBP1 overexpressing cells, p<0.05). Downstream of JunB, c/EBPδ mediates the induction of XBP1 since: 1) the XBP1 promoter has a c/EBP binding site; 2) JunB siRNA decreased c/EBPδ expression (by 62 ± 2%, p<0.05) and 3) c/EBPδ KD decreased palmitate-induced XBP1 expression (by 52 ± 6%, p<0.05). JunB KD decreased AKT activation (by 50 ± 7%, p<0.05) and activated the proapoptotic Bcl2 protein BAD via its dephosphorylation (48 ± 9%, p<0.05). BAD KD reversed lipotoxic β-cell death potentiated by JunB siRNA (from 39 ± 6% in palmitate-treated JunB-depleted cells to 27 ± 5% in palmitate-treated cells double KD for JunB and BAD, p<0.05). Interestingly, XBP1 links JunB and AKT signaling since XBP1 KD also reduced AKT phosphorylation (by 56 ± 7%, p<0.05). We next investigated whether the XBP1-AKT axis mediates the previously shown JunB-dependent protection of β-cells by GLP-1 agonists. Forskolin, an inducer of intracellular cAMP, increased AKT phosphorylation (by 2.3 ± 0.5 fold, p<0.05) and this was partially prevented by JunB or XBP1 KD (by 32 ± 5% and 44 ± 1%, respectively, p<0.05) or by inhibition of XBP1 expression using the IRE1 inhibitor 4μ8C (by 46 ± 7%, p<0.05). β-Cell protection from lipotoxicity by forskolin (from 28 ± 1 to 19 ± 0% apoptosis, p<0.05) was abrogated by chemical inhibition of AKT.
Conclusion: JunB modulates the β-cell ER stress response and AKT signaling via the induction of XBP1. The activation of the JunB gene network and the crosstalk with the ER stress and AKT pathways constitutes a crucial defense mechanism mediated by GLP-1 agonists to protect β-cells from lipotoxicity. These findings elucidate novel β-cell protective signal transduction in type 2 diabetes.
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Involvement of Pannexin-2 in the regulation of pancreatic beta cell function and apoptosis

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Background and aim: We recently constructed biological protein networks relevant for islet function in Type 1 Diabetes (T1D) based on genome-wide association data, protein interaction data and human islet gene expression profiles. Pannexin2 (Panx2), a membrane protein primarily described in the central nervous system, emerged in one of these functional networks. Recent studies illustrate that proteins of the Pannexin family are involved in immune functions and cell death signalling in neurons. However, little is known about the expression of Panx2 in pancreatic islets and the potential functional role of Panx2 in beta cells remains to be addressed. Aim: To evaluate the expression and function of Panx2 in beta cells and to investigate whether Panx2 might be involved in deleterious beta cell processes seen in T1D.
Methods and results: We found that Panx2 transcripts were present in both insulin-secreting INS1 cells, in primary rodent beta cells and in human pancreatic islets, quantified by realtime-PCR. In addition, Panx2 transcripts were significantly downregulated in INS1 cells and primary rat beta cells after stimulation with pro-inflammatory cytokines (IL-1β + IFNγ) known to induce beta cell failure in T1D. RNAi knockdown experiments in INS1 cells and primary rat beta cells (~70% knockdown efficiency) demonstrated that knockdown of Panx2 aggravates cytokine-induced apoptosis as quantified by ELISA detection of cytosolic histone-DNA complexes and by Hoechst/PI stainings, respectively. In line with this, we found that knockdown of Panx2 in

INS1 significantly increased cytokine-induced caspase 3/7 activity compared to control cells. Islets isolated from Panx2 -/- mice showed significantly increased levels of apoptosis compared to age-matched wild-type islets, quantified by the cytosolic histone-DNA complexes. Moreover, glucose-stimulated insulin secretion was significantly decreased in Panx2 knockout islets compared to controls.
Conclusion: The data obtained indicates that Panx2 may have important anti-apoptotic properties in beta cells and that differential regulation of Panx2 could influence beta cell failure in T1D.

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Contribution of long non-coding RNAs to beta cell dysfunction in type 1 diabetes
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Background and aims: Recent transcriptome analysis revealed that the vast majority of the mammalian genome is transcribed and produces different classes of non-coding RNAs, including a large number of long non-coding RNAs (lncRNAs). Only very few of these lncRNAs have been functionally characterised, and little is known about their role in pancreatic β-cells. The aim of this project is to identify and functionally characterise the lncRNAs differentially expressed in pathophysiological conditions that favour the development of Type 1 Diabetes (T1D).
Materials and methods: The expression of lncRNAs in the mouse β-cell line MIN6 exposed to proinflammatory cytokines (IL-1β, TNFα, IFNγ), a treatment that mimics the conditions occurring during the development of T1D, was determined by microarray. Differential lncRNA expression was confirmed by quantitative real-time PCR. The changes induced by cytokines were subsequently verified in primary mouse islets and in islets derived from NOD mice, a well-known model of T1D. The lncRNAs of interest were overexpressed in MIN6 cells to investigate their impact on cell survival and insulin secretion.
Results: MIN6 cells were found to express approximately 15000 previously annotated non-coding RNA transcripts. About 470 of them were up-regulated and 224 were downregulated. The changes in four upregulated lncRNAs (lncRNA-1, lncRNA-2, lncRNA-3, lncRNA-4) were confirmed by qPCR. These lncRNAs were almost undetectable in untreated cells and their expression raised 209±75 (p=0.0471), 3.8±1.1 (p=0.0402), 1072±319 (p=0.0215), 148±39 (p=0.0062) fold respectively, in the presence of cytokines. Similar results were obtained with primary mouse islets where lncRNA-1 increased 36±9 (p=0.0044) fold, lncRNA-2 3.6±0.6 (p=0.0028), lncRNA-3 520±214 (p=0.0412) and lncRNA-4 26.2±4.7 (p=0.0007). The expression of all these genes was mainly triggered by INFγ but was further potentiated by IL-1β and TNFα. The expression of lncRNA-1, -3 and -4 was also increased in islets of NOD mice concomitantly with the development of insulitis and T1D. LncRNA-1 and lncRNA-2 overexpression in MIN6 cells increased apoptosis, whereas lncRNA-3 and lncRNA-4 overexpression triggered apoptosis only in combination with either IL-1β or TNFα. LncRNA-3 and -4 overexpression did not affect insulin secretion.
Conclusion: Our study shows that lncRNAs are substantially modulated by exposure to proinflammatory cytokines, a treatment that mimics the pathophysiological conditions associated with the development of T1D. Indeed, some of these lncRNAs, display analogous changes in the islets of prediabetic NOD mice. Overexpression of these lncRNAs sensitises β-cells to apoptosis, suggesting that they may contribute to β-cell failure during the initial phases of T1D.
Supported by: FNS, EFSD/Lilly Fellowship

OP 47 Metabolic programming and reprogramming

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Genes associated with obesity or type 2 diabetes display altered levels of DNA methylation in human adipose tissue in response to exercise
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Background and aims: Obesity is a predictor for type 2 diabetes (T2D), which in combination with genetic and life-style factors suggests a central role for adipose tissue in the pathogenesis of T2D. Environmental factors potentially alter the epigenome and recent studies point towards epigenetic mechanisms to be involved in the regulation of genes important for glucose metabolism and the pathogenesis of T2D. Here we describe the DNA methylation pattern in candidate genes for T2D and obesity, in human adipose tissue from healthy men before and after a six months exercise intervention.
Materials and methods: A supervised exercise intervention was performed in 23 men with a mean age of 37.4 years and BMI of 27.8 kg/m^2 at inclusion. Subcutaneous fat biopsies were obtained before and after the intervention. DNA methylation was analysed using Infinium HumanMethylation450 BeadChip (Illumina), containing 485,577 probes (99% RefSeq genes). mRNA was analysed using HumanGene 1.0ST array (Affymetrix). DNA methylation data before vs. after the six month exercise intervention was analysed using a paired non-parametric test, whereas a paired t-test was used to compare the mRNA expression. False discover rate analyses were used to correct for multiple testing.
Results: The clinical and metabolic outcomes of the exercise intervention was a significant decrease in waist circumference, waist/hip ratio, diastolic blood pressure and resting heart rate, whereas a significant increase was seen for VO_{2max} and HDL. For the 476,753 CpG sites analysed on the Infinium HumanMethylation450 BeadChip and passing quality control, we found a total of 17,975 individual CpG sites, corresponding to 7,663 unique genes, with altered levels of DNA methylation in adipose tissue after the exercise intervention (q<0.05). Additionally, among all 476,753 analysed CpG sites, 1,351 sites mapped to 53 genes suggested to contribute to obesity, and 1,315 sites mapped to 39 genes suggested to contribute to T2D based on previous genome-wide association studies. We found 24 CpG sites located within 18 of the candidate genes for obesity with a difference in DNA methylation in adipose tissue in response to the exercise intervention (q<0.05). Additionally, two of those genes (*CPEB4* and *SDCCAG8*) showed concurrent inverse change in mRNA expression after exercise (q<0.05). Among the T2D candidate genes, 45 CpG sites in 21 different genes were differentially methylated (q<0.05) in adipose tissue before vs. after exercise. Of note, 10 of these CpG sites mapped to *KCNQ1* and 6 sites mapped to *TCF7L2*. A simultaneous change in mRNA expression was seen for four of the T2D candidate genes where mRNA expression decreased while DNA methylation increased in adipose tissue in response to exercise (q<0.05).
Conclusion: We present a link between exercise and altered adipose tissue DNA methylation in candidate genes for obesity or T2D. This study highlights the dynamic feature of DNA methylation, described using a genome-wide analysis in human adipose tissue before and after exercise.
Supported by: T Nilssons, Thurings, Söderberg and Påhlsson Foundation, EFSD/Lilly

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Methylation of SCD gene promoter and LINE-1 repeat region are associated with weight gain: a study of primary prevention of diabetes
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Background and aims: In the last years, several studies are emerging showing the role that epigenetic modifications could have in the etiology of complex human diseases, such as Type 2 Diabetes Mellitus, obesity, etc. Epigenetic changes are dynamics and are known to vary with cell age, development and differentiation. These epigenetic processes may also be affected by envi-

ronmental factors (diet and exercise). DNA methylation is one of the most common epigenetic marks studied. DNA methylation measured in LINE-1 elements (long interspersed nucleotide element-1) has been correlated with global DNA methylation. On the other hand, Stearoyl CoA desaturase (SCD) is a key enzyme in the synthesis of monounsaturated fatty acids from saturated fatty acids. Variations in SCD activity may be involved in various processes that can lead to diseases such as obesity or other related metabolic disorders. The aim of this study was to analyze whether the patterns of global and specific DNA methylation are involved in the different response to dietary intervention program in a population-based cohort study.
Materials and methods: Cohort study (prospective) and intervention with control group. Subjects: After an OGTT, those subjects with a carbohydrate metabolism disorder were selected (200 control subjects and 200 intervention group). Intervention program consisted of intensive program with regular controls to achieve goals for dietary habits, exercise and weight within the Mediterranean dietary pattern. After a year of nutritional intervention, a blood sample was taken at baseline and after an OGTT in both cohorts. Methods: We collected phenotypic, anthropometric, biochemical and nutritional information of all subjects. DNA methylation was quantified by pyrosequencing technology. Previously DNA was extracted from peripheral blood sample and treated with bisulphite. Global methylation was measured by LINE1 elements and specific methylation was measured from a region of Stearoyl Coa Desaturase (SCD) gene promoter.
Results: DNA methylation patterns (LINE and SCD promoter) were similar at the beginning of the study in both populations, whereas after a year these profiles were significantly greater in control group than in intervention group ($p<0.001$). We observed intra-individual change in global DNA methylation in both groups (Control: 64.2% *vs* 66.9%, $p<0.001$; Intervention group: 64.1% *vs* 63.6%, $p<0.001$) whereas SCD promoter methylation only changed in control group (1.4% *vs* 2.4%, $p<0.001$). Global DNA methylation was statistically lower in women in both baseline ($p=0.018$) and after of the intervention program ($p<0.001$). Regarding to weight gain, subjects from intervention group lose more weight than control group (-2.5 ± 4.8 kg vs -0.7 ± 3.5 Kg; $p=0.001$). We found a significant association between weigh gain and SCD promoter methylation levels in the intervention group ($p= 0.013$) in a linear regression model (adjusted for age, sex and physical activity).
Conclusion: Intra individual changes in global DNA methylation vary over time and these levels are different according to gender. Changes in DNA methylation of SCD promoter gene could be involved in the different response to dietary intervention program in a population-based cohort study.
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Intrauterine undernutrition alters patterns of DNA methylation in the next generation offspring through the male line
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Background and aims: Human and experimental data show that malnutrition during early development may increase diabetes risk into the second generation offspring. These transgenerational effects might be mediated by epigenetic mechanisms. We have previously reported a mouse model of intrauterine growth restriction (IUGR) by reducing to 50% global caloric availability to pregnant females. IUGR mice developed metabolic syndrome with ageing. Strikingly, the diabetic phenotypes progressed to the next generation offspring (IUGR-F2) through the paternal lineage, despite IUGR-F2 mice had not been exposed to nutritional stress during their development. Here we aim to test: *a)* whether *in utero* malnutrition influences the epigenome in the next generation offspring and *b)* whether such transgenerational effects are mediated by epigenetic modifications inherited through the gametes (sperm).
Materials and methods: Here we analyzed global DNA methylation patterns in liver samples from control and IUGR-F2 mice (385K, Roche-NimbleGene). Next, we validated positive *loci* identified in the array by PyroSequencing (liver and sperm).
Results: In utero undernutrition altered the methylation of 1,207 regions in livers from IUGR-F2 mice. Bioinformatics analysis showed enrichment in loci belonging to the Wnt ($P= 0.01$) and Hedgehog signaling pathways ($P= 0.04$). Remarkably, no statistic enrichment in loci belonging to metabolic pathways was observed. Next, we confirmed a set of positive candidate genes from the array (Wnt2b, Wnt7a, Wnt9a, Sfrp1 and Gsk3b) by Pyrosequencing. These epigenetic modifications might be inherited from their progenitors (IUGR-F1) through the germ line or develop as a consequence of progressive metabolic dysfunction. To address this, we analyzed DNA methylation of the candidate loci in sperm samples from IUGR-F1 mice. Strikingly, the epigenetic signatures for Wnt2b and Wnt7a were already present in sperm samples from IUGR-F1 male mice, thus suggesting that early nutritional stress may cause transgenerational epigenetic inheritance of epigenetic marks.
Conclusion: Early malnutrition may induce epigenetic modifications in cells from the germ line. Next, these alterations remain stable during gametogenesis and can be inherited into the following generation offspring thus influencing disease risk.
Supported by: EFSD/ Novo Nordisk., MINECO

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Repeated measurements of 25-hydroxyvitamin D and vitamin D-binding protein throughout pregnancy and risk of type 1 diabetes in the offspring
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Background and aims: We have previously reported that low maternal levels of 25-hydroxyvitamin D (25-OH D) in late pregnancy was associated with higher risk of type 1 diabetes in the offspring, whereas a Finish study did not find this relation with early pregnancy samples. No previous study in this context have to our knowledge observed Vitamin D binding protein (VDBP), which is known to increase during pregnancy. We aimed to test whether levels of repeated measurements of 25-OH D and VDBP throughout pregnancy differed from women whose offspring later developed type 1 diabetes compared to controls in a case control study nested within a large cohort.
Materials and methods: Serum samples drawn at 1-4 time points in pregnancy were analysed for total 25-OH D and VDBP in 113 women delivering a child who developed type 1 diabetes during childhood (cases), and from 221 randomly selected control women. Both cases and controls were part of a prospective cohort of 29,072 pregnant women who gave birth in Norway 1992-94. Cases were identified by linking the cohort of women and their offspring to The Norwegian Childhood Diabetes Registry. Linear mixed models with case/control status as the independent variable, and logistic regression with case/control status as dependent variable were used for statistical analyses.
Results: Estimated mean difference in 25-OH D between case- and control women was 5.75 nmol/l (95% CI 1.25-10.24), p=0.012, and in VDBP 0.36 µmol/l (95% CI 0.04-0.67), p=0.025. In third trimester of pregnancy lower maternal levels of 25-OH D was associated with increased risk of type 1 diabetes development in children, OR 2.68 (95% CI 1.16-6.16), test for trend; p=0.013 for lowest vs. highest quartiles of 25-OH D. The association was still present after adjustments for VDBP. In first and second trimester, no such associations were found. The mean levels of 25-OH D and VDBP significantly increased during the progression of pregnancy in both cases and controls, $p<0.001$. Mean increase in 25-OH D and VDBP pr week of gestation tended to be slightly lower in case mothers compared to controls (p=0.003 and p=0.025 respectively) but the interaction between case/control status and week of gestation at blood sampling was not statistically significant.
Conclusion: In this first study of repeated measures of 25-hydroxyvitamin D and vitamin D binding protein during pregnancy and risk of type 1 diabetes in offspring, we found inverse significant associations with both, and differences tended to be greater towards late pregnancy.
Supported by: Oslo Diabetes Research Centre and Helse Sør-Øst, Norway

OP 48 Mechanisms of atherogenesis

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In vivo inhibition of Nuclear Factor of Activated T cells (NFAT) leads to atherosclerotic plaque regression in diabetic IGF-II/LDLR$^{-/-}$ApoB$^{100/100}$ mice

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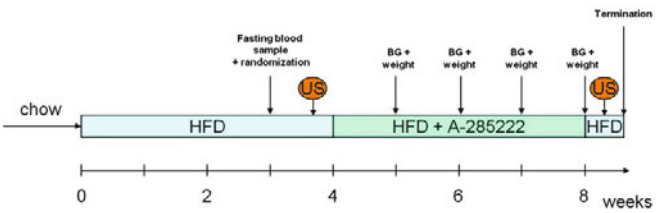
Background and aims: Despite the vast clinical experience linking diabetes and atherosclerosis, it is still unclear what molecular mechanisms are involved. We recently showed that hyperglycemia activates the transcription factor NFAT in the arterial wall. Here we tested whether NFAT is involved in diabetes-driven atherosclerosis and explored the potential benefit of therapeutic inhibition of NFAT.
Materials and methods: A recently developed mouse model of type 2 diabetes, generated by the crossbred of the LDL receptor deficient mice that synthesize only apolipoprotein B100 (LDLR$^{-/-}$ApoB$^{100/100}$) and transgenic mice over-expressing insulin-like growth factor-II (IGF-II) in pancreatic β cells was used. Mice have hyperglycemia, mild hyperinsulinemia and develop calcified and complex atherosclerotic lesions. Young (4 months, n=10) and old (12-16 months, n=17) diabetic IGF-II/LDLR$^{-/-}$ApoB$^{100/100}$ mice were studied. Four weeks after the introduction of a high fat Western diet, mice were treated with daily i.p. injections of the NFAT blocker A-285222 (0.29 mg/kg body weight) or vehicle (saline) for 4 weeks. Body weight, fasting blood glucose and total plasma cholesterol and triglycerides were measured before, during and after treatment. Brachiocephalic and aortic plaque size was assessed non-invasively by ultrasound biomicroscopy before and after treatment. Histological determination of plaque size was performed after termination.
Results: Non-invasive ultrasound measurements revealed that *in vivo* treatment with the NFAT blocker A-285222 significantly reduced plaque size in the brachiocephalic arteries of young mice, both compared to plaque size in vehicle treated control mice (0.0405 ± 0.0056 mm^2 vs. 0.0818 ± 0.016 mm^2; *p=0.0218) and to the same animals prior treatment (0.0405 ± 0.0056 mm^2 vs. 0.0643 ± 0.0063 mm^2; *p=0.0182). No significant effects were observed in the brachiocephalic arteries of old mice. In the ascending aortas of both young and old mice, a trend towards decreased plaque progression was observed after NFAT inhibition. Results from the histological assessment at termination is in line with the ultrasound data, revealing also decreased brachiocephalic plaque size in old mice, which was not evident by ultrasound.
Conclusion: Our results provide evidence for the involvement of NFAT in the development of macrovascular complications in diabetes. Inhibition of NFAT signaling in diabetic IGF-II/LDLR$^{-/-}$ApoB$^{100/100}$ mice not only limits the progression of atherosclerosis in the brachiocephalic artery, a particularly disease prone vascular segment in mice, but more importantly, it leads to atherosclerosis plaque regression.



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Circulating myeloid calcifying cells have anti-angiogenic activity via thrombospondin-1 overexpression

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Background and aims: We have recently identified myeloid calcifying cells (MCCs) as a subpopulation of human monocytes with circulating procalcific potential, characterized by co-expression of osteocalcin (OC) and bone alkaline phosphatase (BAP). Herein, we tested the angiogenic activity of MCCs.
Materials and methods: Human OC+BAP+ MCCs were quantified in diabetic and non diabetic patients by flow cytometry. An in-depth proteomic investigation of MCCs based on FACS, protein extraction and digestion, iTRAQ labelling, peptide fractionation and double mass spectrometry analysis on MALDI-TOF/TOF and LTQ-Orbitrap was performed on human OC+BAP+ MCCs and control OC-BAP- cells. Angiogenesis was assayed in vitro using the Matrigel tubulization assay and in vivo using the Matrigel plug assay and hind limb ischemia. Thrombospondin-1 neutralization was achieved using a blocking antibody.
Results: The proteomic analysis of MCCs identified and quantified more than 700 proteins and revealed pathways activated in OC+BAP+ MCCs compared to OC-BAP- cells. Among proteins referable to angiogenesis, the thrombospondin-1 pathway was markedly upregulated in MCCs vs control cells. Using in vitro and in vivo angiogenesis assays, we found that freshly isolated MCCs and cultured MCCs display an anti-angiogenic function by means of both paracrine activity (conditioned medium) and altered spatial localization in co-cultures with endothelial cells. Treatment with a neutralizing antibody against thrombospondin-1 restored the angiogenic activity of OC+BAP+ MCCs toward normal values, without affecting the function of OC-BAP- monocytes, and abolished the anti-angiogenic effects of MCCs conditioned medium.
Conclusion: These data indicate that circulating MCCs exert an anti-angiogenic activity by virtue of their overexpression of thrombospondin-1. The study also highlights the successful identification and validation of a pathogenic pathway by a gold standard proteomic analysis of blood cells.

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Myeloid calcifying cells promote atherosclerotic calcification in apolipoprotein-E knock-out mice

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Background and aims: Several cell types contribute to atherosclerotic calcification. Myeloid calcifying cells (MCCs) are a monocyte subpopulation expressing osteocalcin (OC) and bone alkaline phosphatase (BAP), with ability to calcify in vitro and in vivo. Herein, we tested whether MCCs promote atherosclerotic calcification in vivo.
Materials and methods: Human OC+BAP+ MCCs were quantified in diabetic and non diabetic patients by flow cytometry. In mice, MCCs were characterized by cell culture, flow cytometry and in vivo calcification assay. Migration of human OC+BAP+ MCCs and control OC-BAP- cells was assessed using a modified transwell system. Induction of calcification was studied by transplanting GFP+ OC+BAP+ MCCs and control OC-BAP- cells into young and old ApoE-/- mice sacrificed 24 and 2 weeks later respectively. The pro-calcific potential of MCC conditioned medium was tested on cultured smooth muscle cells.
Results: Circulating MCCs levels were about 2-fold increased in diabetic versus non-diabetic subjects. We show that OC+BAP+ cells had a selective transendothelial migration capacity, suggesting that MCCs are prone to enter the vessel wall. The spleen of C57Bl/6 mice contained OC+BAP+ cells with a phenotype similar to human MCCs, a high expression of adhesion molecules, and capacity to calcify in vitro and in vivo. Injection of GFP-expressing OC+BAP+ cells into 8- or 40-week ApoE-/- mice led to more extensive calcifications in atherosclerotic areas after 24 or 2 weeks, respectively, compared to injection of control OC-BAP- cells. Tracking of the GFP signal identified a

small numbers of injected cells within atherosclerotic areas, suggesting a paracrine effect. Conditioned medium of OC+BAP+ cells promoted calcification of vascular smooth muscle cells in vitro significantly more than conditioned medium of control OC-BAP- cells.
Conclusion: Murine OC+BAP+ cells correspond to human MCCs and promote atherosclerotic calcification in ApoE-/- mice, prevalently through paracrine activity and modulation of resident cells.

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Selenoprotein P as a diabetes-associated hepatokine that impairs angiogenesis by inducing VEGF resistance in vascular endothelial cells

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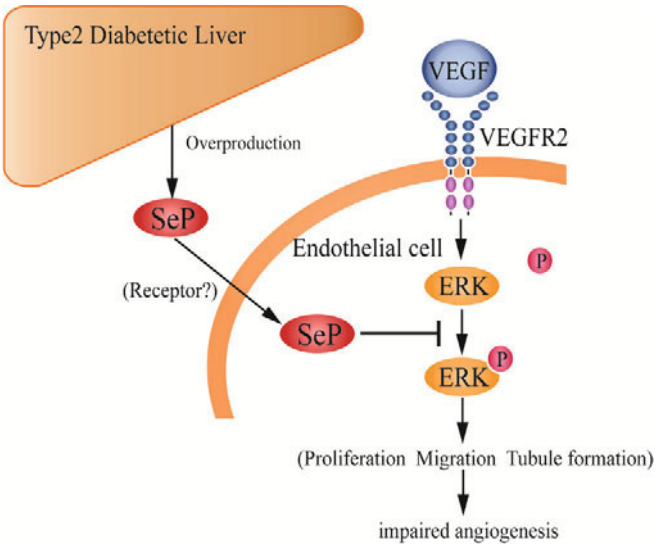
Background and aims: Impaired angiogenesis is associated with the development of various vascular complications in people with type 2 diabetes. A defect of vascular endothelial growth factor (VEGF)-related signal transduction has been postulated for the diabetic dysregulated angiogenesis, but its molecular mechanism is not fully understood. We have recently identified selenoprotein P (SeP), encoded by the *SEPP1* gene in human, as a liver-derived secretory protein that causes insulin resistance in type 2 diabetes. We reported that levels of serum SeP and hepatic expression of *SEPP1* are elevated in type 2 diabetes approximately twice. Here, we investigated the effects of SeP on VEGF signaling and angiogenesis.
Materials and methods: (i) Human vascular endothelial cells (HUVECs) were pretreated with physiological concentrations of SeP (5 μg/ml as the normal group, 10 μg/ml as diabetes) to examine its effects on angiogenic ability. After 2 hr of starvation, HUVECs were stimulated with VEGF (20 ng/ml) for 15 min. The effects of SeP on VEGF signal transduction was investigated by western blotting. (ii) Mice with hepatic SeP over-expression were made using a hydrodynamic injection technique. We evaluated wound healing of the mice. (iii) SeP-heterozygous knockout mice were operated with hindlimb ligation as an ischemic event. The recovery of blood flow after ischemia was evaluated by using a laser-Doppler perfusion scanner. Two weeks after ischemia, angiogenesis was quantified by CD31 staining of lower limb tissue in the mice.
Results: (i) SeP inhibited VEGF-stimulated cell proliferation by 26%, tubule formation by 28%, and migration by 30% in HUVECs. SeP inhibited VEGF-induced phosphorylation of VEGFR2 and ERK 1/2 in HUVECs, whereas phosphorylation of p38MAPK and Akt (Ser473) was not affected. (ii) In the mice overexpressing *Sepp1*, wound closure was impaired by 23% in 7 days after injury. (iii) *Sepp1*-heterozygous knockout mice exhibited a 1.7-fold acceleration of blood flow recovery and a 2.6-fold increase of vascular endothelial cell numbers in the ischemic hindlimb area.
Conclusion: The present study indicates that diabetes-associated hepatokine SeP, elevated in type 2 diabetes, impairs angiogenesis in vitro and in vivo by inducing VEGF resistance in endothelial cells. It might be possible that SeP affects VEGF-stimulated reactive oxygen species (ROS) generation required for VEGF signal transduction. We are researching the effect of SeP to ROS burst caused by growth factors including VEGF. Furthermore, the identification of SeP receptor(s) in endothelial cells would provide more insight into the molecular mechanism and a novel therapeutic target for treatment of impaired angiogenesis in type 2 diabetes.



PS 001 Managing and monitoring diabetes: who does well and who doesn't?

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Metformin - a therapeutic option for prevention of type 2 diabetes in persons with normal glucose tolerance, metabolic syndrome and hyperinsulinaemia

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Background and aims: Metabolic syndrome (MS) is a risk factor for type 2 diabetes (T2D) and cardiovascular disease (CVD). Hyperinsulinaemia is the earliest stage in the development of T2D and a strong CV risk factor. Metformin is a first line therapy for T2D and reduces the risk of T2D in individuals with impaired fasting blood glucose and impaired glucose tolerance. The aim of the present study was to establish the effect of metformin on cardiometabolic risk factors in persons with metabolic syndrome, normal glucose tolerance and hyperinsulinaemia.
Materials and methods: 52 individuals (32 females, 20 males) of mean age 40.1±14.2 yrs with MS (IDF Definition), normal glucose tolerance during oral glucose tolerance test (OGTT) defined as fasting plasma glucose < 6.1 mmol/l and 2-h plasma glucose < 7.8 mmol/l and hyperinsulinaemia- fasting serum insulin > 25 mIU/l and/or 2- h post glucose challenge serum insulin > 3 times from baseline (IRMA, reference range 2-25 mIU/l) were included in a prospective one year observational clinical study. Body weight, body mass index, waist circumference, serum lipids, blood pressure, OGTT and homeostasis model assessment of insulin resistance (HOMA-IR) at three month intervals were followed. Metformin was applied at an initial dose of 500 mg titrated to the maximal 3 g, the mean dose was 2.55±0.2 g daily.
Results: Body weight (BW), body mass index (BMI) and waist circumference (WC) decreased significantly at 6 month of metformin treatment and this effect continued to the end of the observation and it was no more pronounced at 1 year compared to the 6 month. BW 97.5±18.5 kg decreased to 89.8±16.9 kg at 6 month (p=0.028) and to 85.3±16.7 kg at 1 year (p=0.001), BMI 32.3±5.2 kg/m^2 decreased to 29.9±4.8 kg/m^2 at 6 month (p=0.012) and to 28.4±5.0 kg/m^2 at 1 year (p=0.0004), WC 102.8±14.3 cm decreased to 95.3±12.2 cm at 6 month (p=0.004) and to 90.9±12.5 cm at 1 year (p<0.0001). The significant effect of metformin on triglycerides (TG), LDL cholesterol (LDL-C), systolic blood pressure (SBP) and diastolic blood pressure (DBP) was observed at 9 month. At 1 year of metformin treatment TG reduced from 2.60±1.74 mmol/l to 1.44±0.53 mmol/l (p=0.0004), LDL-C reduced from 3.61±0.76 mmol/l to 2.74±0.94 mmol/l (p=0.001), SBP reduced from 131±18 mmHg to 119±12 mmHg (p=0.0006) and DBP reduced from 85±11 mmHg to 76±6 mmHg (p<0.0001). HDL-C significantly increased at 1 year of metformin treatment from 1.10±0.34 mmol/l to 1.44±0.28 mmol/l (p=0.0005). Fasting serum insulin 24.1±15.3 mIU/l decreased significantly at 6 month 17.2±8.8 mIU/l (p=0.012) and at 1 year was 11.6±6.0 mIU/l (p<0.0001), 1-h post glucose insulin (PGI) 136.8±67.6 mIU/l decreased significantly at 3 month 94.9±52.2 mIU/l (p=0.009) and at 1 year was 54.5±51.4 mIU/l (p<0.0001), 2-h PGI 62.6±43.2 mIU/l decreased significantly at 3 month 45.1±26.6 mIU/l (p=0.04) and at 1 year was 27.6±21.6 mIU/l (p<0.0001), 3-h PGI 23.2±11.7 mIU/l decreased significantly at 6 month 13.3±7.1 mIU/l (p<0.0001) and at 1 year was 9.7±5.8 mIU/l (p<0.0001). HOMA-IR 5.74±3.77 was significantly reduced at 6 month of metformin treatment 4.01±2.13 (p=0.001) and at 1 year was 2.58±1.40 (p<0.0001).
Conclusion: Metformin reduces cardiometabolic risk factors and restores physiological insulin secretion in normal glucose tolerant persons with metabolic syndrome and hyperinsulinaemia and could be applied for prevention of type 2 diabetes and cardiovascular disease.

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Reasons for non-intensification of therapy in type 2 diabetes patients uncontrolled by oral monotherapy in general practice in France: the DIAttitude study

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Background and aims: The ENTRED 2007 study, initiated by the French Health Authority, showed that in T2D patients, intensification of therapy as recommended by national guidelines, was imperfectly followed. The DIAttitude non-interventional study (2010-2012), aimed to describe therapeutic management by French general practitioners (GPs), of T2D patients treated with oral hypoglycaemic agents (OHA) and to identify the major reasons for not intensifying treatment.
Materials and methods: The DIAttitude study included three parts and all aimed to characterize treatment in orally treated T2D patients, who were enrolled by GPs, members of a representative panel of office-based physicians. The first part of DIAttitude analyzed retrospective data from 17 493 orally treated T2D patients. The second part was a non-interventional cross-sectional study. The third part presented here, was a 1-year longitudinal follow-up of the T2D patients treated by OHA monotherapy. The criteria for treatment intensification was defined by two consecutive HbA1c ≥ 7% separated by at least 3 months. Major reasons for non-intensification were recorded every 3 months by the GPs.
Results: In total, 937 patients treated by OHA in monotherapy were included; they were enrolled by 198 GPs, and were followed over 1 year. Among the 916 patients having at least two HbA1c values available, 175 (19%) required treatment intensification: men 61%; mean age 67 years; mean duration of diabetes 8 years; mean HbA1c 7.7%; 45% had HbA1c between 7.0 and 7.5%, 55% over 7.5%; at least one macrovascular complication 23%; at least one microvascular complication 29%; overweight or obese 84%; OHA at inclusion: biguanides 49%, sulfonylureas 39%, other 12%; uncontrolled for more than 12 months 37%. There was no treatment intensification for 81 of the 175 patients (46%). Their baseline characteristics were similar to those requiring treatment intensification, except they had lower HbA1c levels: between 7.0 and 7.5% there were 58% *vs* 45%, for HbA1c over 7.5%, 42% *vs* 55%. Reasons for non intensification were reported for 76 patients: 49% current HbA1c satisfactory, 21% lifestyle and dietary advice the priority, 9% other reasons related to the patient, 8% medical priority other than diabetes, 8% HbA1c decreased since previous visit, 5% decision postponed until next visit. The distribution of these patterns was constant during follow-up.
Conclusion: Therapeutic inertia remains common in T2D patients treated by OHA in monotherapy in France: 46% patients who met the criteria for treatment intensification during a 1-year period did not have additional treatment, while 38% of these patients required intensification for at least one year. The most common reason was GP-defined acceptable HbA1c (49%), in spite of 42% having ≥ 7.5%. Data are available with a 6.5% HbA1c threshold which obviously strengthens the finding of therapeutic inertia.
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C-peptide levels not affected by duration of diabetes in a telemedicine based long-term follow up programme

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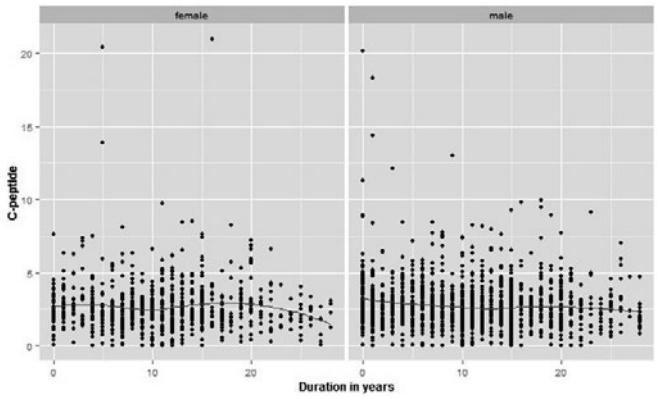
Background and aims: In the natural history of progression of type 2 diabetes the initial hyperinsulinaemia culminates in near total decline in the beta cell function. The eventual loss of beta cell function and associated insulin secretary deficiency is more rapid with higher HbA_{1c} levels. In our center, subjects registered under telemedicine based long term follow up care Diabetes Tele Management System (DTMS*) maintains near normal metabolic targets being followed up frequently via a multi disciplinary trained diabetes team. The follow up program via the telephone or internet as published elsewhere precludes physical visits to the hospital and has been proven cost effective and helps maintain HbA_{1c} targets without significant hypoglycaemia. We hypothesized the correlation between C-peptide levels, duration of diabetes,

HbA1c and the effects of a long term follow up program on these parameters if subjects maintain desirable glycaemic targets.
Materials and methods: A cross-sectional analysis of 2076 T2DM patients aged above 20 years was performed. 37% were female. Mean age was 51.7 year SD 11.9 and C-peptide mean was 2.725 SD 1.7.A1c mean was 8.8 SD 2.1.
Results: 829 patients with duration of follow-up more than 1 year and 1247 patients with duration of follow-up less than 1year were included in the analysis. Age and gender distribution were similar among the two groups. Group with more than 1 year follow up (M=8.4, SD-1.8) had significantly lower A1c compared to new patients with less than 1 year follow-up (M=9.1%, SD=2.2), t(2009) = 7.25, p < .0001. Regression analysis also indicated that A1c tends to be lower with higher C-peptide (R2=0.01,F(4,819)=2.7,p=0.02). Linear regression also showed no significant change of C-peptide levels with duration under care.(Fig.1)
Conclusion: In patients on long term follow up and low HbA1c value when compared with newly enrolled patients, there was no decline in C-peptide levels in DTMS® based follow up care. Maintaining near normal glycaemic targets in type 2 diabetes patients may help preserve the beta cell function even after decades of onset of diabetes.



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Accuracy of self-measured blood glucose (SMBG) in 11317 adult diabetes patients based on Clark Error Grid, Parkes Error Grid, ISO 15197 and Bland-Altman-plot

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Background and aims: Accuracy of SMBG is essential for correct adjustment of insulin dose, for early detection of hypoglycaemia and for adequate assessment of metabolic control. In this study, the agreement between patient SMBG and simultaneous laboratory/POCT measurement is analyzed, together with the effect of SMBG accuracy on metabolic control.
Methods and subjects: The Diabetes Quality Management System (DPV) is a standardized, electronic longitudinal documentation for institutions specialized in diabetes care. Since 2004, data from 11317 patients >18 years of age with parallel measurements of SMBG and laboratory blood glucose were documented (most recent patient year). International Organisation of Standardization criteria (ISO 15197, current and new criteria) were used to quantify accuracy of SMBG. In addition, the percentage of values within region A according to Clarke or Parkes Error Grid is given and Bland-Altman-plot is visualized. HbA1c-concentration is stratified among patients with SMBG results as much too high (Q1), too high (Q2), too low (Q3) or much too low (Q4) compared to the laboratory measurement. SAS9.3 was used for data analysis.
Results: Diabetes onset was at a mean age of 46.0±18.8 years and diabetes duration was 10.7±9.9 years. 75.7% of patients had type 2 diabetes and 24.3% had type 1. According to ISO 15197 2003, 96.3% of measurements were within target. No difference was found between type 1 and type 2 diabetes, between males and females, and between patients on insulin injections or insulin pumps. SMBG accuracy was highest during the first year of diabetes (p<0.01). Based on the newly proposed ISO standard, only 92.2 % of measurements were within target (minimum requirement 95%). Clarke or Parkes Error Grid analysis resulted in 96.5% and 96.2% of values in zone A. Bland-Altman-plot shows a symmetric distribution of blood glucose differences, with higher variation for elevated blood glucose values. In insulin-treated patients (n=8449), after adjustment for age, sex, diabetes duration and year of treatment, higher deviation of SMBG from laboratory values (Q1: much too high or Q2: too low) is associated with higher HbA1c values (p<0.02).
Conclusion: In this large, multicenter, heterogeneous group of patients, using different blood glucose monitoring systems, current ISO 15197 standards were met, but not the proposed new specifications. Lower accuracy of SMBG is associated with worse metabolic control in insulin-treated subjects.
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Monitoring and management of type 2 diabetes patients with renal disease: gap between knowledge and real-life practices

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Background and aims: To evaluate management of T2DM in patients with/without CKD included by GP and diabetologists (DB) in a transversal study in 2012 in France.
Materials and methods: 3704 patients were recruited in 2 cohorts (2/3 considered to have renal disease [CKD patients] and 1/3 considered not to [no-CKD patients]) by 813 GP [recruiting 81% of patients] and 155 DB. Regardless of inclusion in CKD/no-CKD cohorts, patients were classified by their actual eGFR status in normal RF [≥ 60 mL/min/1,73m²], moderate RI [30-60] and severe RI [< 30]. After recording patients' data, physicians received a general questionnaire about their practice.
Results: For both DB and GP, CKD vs no-CKD patients were significantly older (71 vs 63 yrs). DB patients had longer disease history (18 & 13 yrs in CKD/no-CKD vs 12 & 8 yrs respectively for GP) and more micro- and macrovascular complications. Mean eGFR were similar in DB and GP patients in both cohorts: 46 vs 49 ml/min/1.73m² in CKD and 91 vs 88 ml/min/1.73m² in no-CKD. In 80 and 88% of GP and DB patients, eGFR was known (CG formula more frequently used by GP -38% vs 18% by DB- while MDRD was used by 75% of DB vs 52% of GP). Proteinuria was more often available in DB than GP patients (82 vs 69%). Mean A1c was higher in DB (7.8%) than GP patients (7.4%) in CKD, as well as in no-CKD patients (7.3 vs 7.1%). Therapeutic management of patients included by DB significantly differed to that of GP, especially in the CKD cohort: DB patients were prescribed less metformin (42%) and more insulin (68%) (no-CKD metformin 88% & insulin 30%) and GP patients more metformin (66%) and much less insulin (34%) (no-CKD 86% metformin & 13% insulin). However, doses of metformin were not reduced in DB or GP CKD patients (mean of 1.9 & 2.1 g/d respectively, with 30 and 33% of the DB and GP patients receiving >2g/d, and 21 and 23% receiving 3g/d). Use of SU by DB patients clearly decreased with RF (26% of CKD and 41% of no-CKD patients), while it remained stable across RF in GP patients (31 and 29% in CKD/no-CKD patients). Conversely, use of glinides by DB patients increased in CKD (24% vs 12% in no-CKD) as well as by GP patients (12% vs 6% in no-CKD). DPP4-i were used by DB in 25 and 46% of CKD/no-CKD patients, while they were more frequently used by GP (38% and 45% respectively). Of note, in patients with severe RI, metformin was still prescribed in 16% of DB and 37% of GP patients. In this population, DB patients were also prescribed SU (6%) and DPP4i (6%) less often than GP patients (respectively 23% and 31%) and insulin more often (88 vs 54%). Decision to decrease/stop metformin at the end of the visit occurred in 85% of DB and 33% of GP patients. In contrast, based on the general questionnaire fulfilled afterwards, both GP and DB declared decreasing metformin doses at a mean threshold of 54 mL/min/1.73m² and stopping its use at 35 and 32 mL/min/1.73m². In patients with severe RI, respectively 15, 4, 52 and 32% of the DB declared using metformin, SU, glinides and DPP4i (vs 14, 19, 15 and 58% of the GP) and a similar % of DB and GP declared using insulin (64%).
Conclusion: While treatment of T2DM was clearly distinct in CKD vs no-CKD patients and by GP vs DB, the use of metformin differed in practice from theoretical knowledge in both GP and DB. Use of eGFR for adapting antidiabetics' prescriptions and doses is insufficient. Prescription adjustment is more likely to be done after DB visit.
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Meta-analysis of studies examining medication adherence to oral antihyperglycaemic agents in type 2 diabetes

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Background and aims: Medication adherence among patients with type 2 diabetes mellitus (T2DM) has been examined in numerous studies showing substantial variation in the estimated medication adherence rates. The purpose of this meta-analysis was to estimate the overall rates of adherence to oral antihyperglycemic agents (OAHAs) for patients with T2DM by combining results of published studies.
Materials and methods: A systematic literature review was conducted to identify articles published in English over the last 10 years evaluating the use of OAHAs for the treatment of T2DM. Databases searched included PubMed/MEDLINE, EMBASE, and The Cochrane Library. Forty observational studies reporting adherence were identified. Two reviewers independently selected studies for inclusion, performed data extraction, and assessed study quality. Of the studies identified, 18 reported at least one relevant endpoint for analysis. Meta-analyses were weighted using the inverse variance method and examined 1) mean adherence rate defined as the average medication possession ratio (MPR) (sum of days' supply for all fills in period / number of days in period) assessed over a period of 6 to 12 months; and 2) proportion of adherent patients defined as those with an MPR ≥80% assessed over a period of 6 to 12 months. The random effects models incorporated a multilevel structure to account for multiple observations reported in some studies. Subgroup and regression analyses were conducted to investigate if study and patient attributes impact adherence. Heterogeneity was assessed for each analysis using I^2 values.
Results: The pooled mean medication adherence rate (SE) across 12 studies was 74.9% (3.2%) (range: 52-88%). The proportion of adherent patients was 67.2% (4.1%) (range: 44.4-89.8%; n=11). Both I^2 values were greater than 90% indicating significant heterogeneity across studies. Meta-regression showed positive associations between mean age and both outcomes. When centering the covariate for mean age, the meta-regression estimated a mean adherence of 74.3% (3.8%) for the baseline mean age of 54.4 years, and a proportion of patients who were adherent of 64.4% (3.2%) for the baseline mean age of 56 years. Analyses restricted to monotherapy regimens, and separately to treatment naïve patients, both produced estimates for the mean adherence rate and proportion of adherent patients that were similar to the overall results. There were variations in the definitions of MPR across studies, both in what it captured and its method of calculation. In some studies MPR was measured for any OAHA in the index period while in others it was limited to the index therapy (or index class). The estimates for mean adherence rate and proportion of adherent patients to the index therapy were 70.1% (4.4%) and 69.6% (4.8%), respectively; versus 81.6% (2.2%) and 65.8% (5.7%) respectively, for any OAHA received.
Conclusion: This meta-analysis supports the notion that medication adherence to OAHAs is suboptimal. Furthermore, adherence to OAHA medication may vary as a function of patient attributes such as age and therapies examined (index therapy only vs. any OAHA). Additional research is needed to further investigate the factors that impact adherence to OAHAs and examine whether differences in adherence could relate to differences in health outcomes and healthcare costs among patients with diabetes.

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How are patients with type 2 diabetes and renal disease monitored and managed in France? Results from the OreDia 2012 survey

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Background and aims: Chronic kidney disease (CKD) is frequent in patients with T2DM. This study aimed to assess in real-life the therapeutic management of T2DM patients with and without CKD.
Materials and methods: 3704 patients were recruited in 2 cohorts (2/3 considered to have renal disease [CKD patients] and 1/3 not to [no-CKD patients]) by 968 Physicians (81% by GPs) in a nation-wide, transversal study from June 2012 to January 2013. Regardless of inclusion in CKD/no-CKD cohorts, patients were also classified by their actual eGFR status in normal renal function (RF) [eGFR ≥ 60 mL/min/1.73m²], moderate renal impairment (RI) [eGFR 30-60 mL/min/1.73m²] and severe RI [eGFR < 30 mL/min/1.73m²].
Results: CKD vs no-CKD patients were significantly older (71 vs 63 yrs; 40% vs 15% ≥ 75 yrs) with longer diabetes history (13 vs 9 yrs; 38% vs 18% ≥ 15 yrs) but similar sex-ratio (62% M) and BMI (29.3 kg/m²). CV risk factors were highly prevalent overall (98 and 90%) driven by hypertension (91 and 71%) and dyslipidaemia (79 and 65%). Diabetic complications were more prevalent in CKD (84 vs 29%) driven by nephropathy (71 vs 4.5%), cardiovascular disease (40% vs 17%) and retinopathy (20% vs 8%). Creatinine levels < 1 yr were available in all patients (mean of 144 and 80 µmol/L, respectively) and results for eGFR in 82% (mean of 49 vs 90 mL/min/1.73m²). 15% of CKD patients had severe RI (vs 0.4% no-CKD) and 66% moderate RI (vs 7.7% no-CKD) with only about 19% of CKD patients with normal RF. Overall 70% of patients were screened for proteinuria and only 21% of CKD (vs 86% of no-CKD) had normal albumin excretion rate. Glycemic control was worse in CKD patients (mean A1C 7.5% vs 7.1%; with 25% of CKD patients ≥ 8% vs 15%). Therapeutic management of T2DM was clearly distinct in the 2 cohorts, with less use of metformin in CKD patients (62% vs 86%) but without a reduction in daily doses (mean of ~2g/d) and with similar proportions treated with high doses (3g/d: 22.5% and 20% respectively of CKD/no-CKD patients). When classified by their actual eGFR status, the groups with moderate and severe RI included 95 and 99% of patients from the CKD cohort. Metformin was used by 86% of patients with normal RF, 63 and 33% of those with moderate and severe RI. For other OADs, a distinct pattern of use was also seen across renal function, except for IAG which were used in about 5% of patients regardless of RF. SU use decreased with RF (32, 31 and 20% respectively in normal RF, moderate and severe RI), while that of glinides increased (8, 14 and 18% respectively in normal RF, moderate and severe RI) and DPP4-i decreased (41, 36 and 25% in normal RF, moderate and severe RI). Patients in the CKD cohort were more frequently treated with insulin (40% vs 16% of no-CKD) when assessed across RF by eGFR, insulin was used in 19, 39 and 61% of patients respectively with normal RF, moderate and severe RI. Treatment was modified at the end of the visit in only 34% of CKD patients, primarily to stop or reduce metformin. However, metformin was stopped in only 40% of the severe RI patients.
Conclusion: In clinical practice, despite a fairly good screening of renal complications in patients with T2DM, RI is insufficiently taken into account for adjustng anti-diabetic treatment.
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Prevalence of inadequate metabolic control: results from a provincial wide survey in 1,461 patients with type 1 diabetes in China

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Background and aims: Type 1 diabetes mellitus (T1DM) requires comprehensive management of glycaemia, blood pressure and blood lipids. However, data of metabolic control status in Chinese T1DM patients is still lacking to date. In this study, we sought to investigate the current status of metabolic control in Chinese T1DM patients, aiming to provide better public health messages.
Materials and methods: This multicenter, cross-sectional study investigated patients with established T1DM between January 1, 2000 and March 31, 2012 from 21 cities in Guangdong, China. Data entry forms were used to collect patients' clinical information, and HbA_{1c}, LDL-C, HDL-C and triglyceride (TG) were measured centrally. HbA_{1c}, blood pressure, blood lipid levels and target levels achieving rates were calculated.
Results: A total of 1,461 patients were enrolled (51.5% female). The age was 29.5 years (20.6-40.0) [median (interquartile range)], duration of T1DM was 3.7 years (1.6 -7.8), age at diagnosis was 24.4 years (15.9-34.9). Proportions of overweight/obese and underweight patients were 11.4% and 23.9%, respectively. Of patients ≥ 18 years, 21% were with abdominal obesity. HbA_{1c} level was 8.7 % (7.1%-11.1%), and 24.7% of patients achieved the age-specific target level. Highest $HbA1_c$ level (10.2%), as well as lowest target level achieving rate (14.2%), were observed in adolescence (13-19 years). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were 113 mmHg (105-122.5) and 71.5 mmHg (66-78.5), respectively. A total of 69.7% patients achieved target blood pressure level, female higher than male (72.4% vs. 67.0%, P=0.04). LDL-C,HDL-C and TG levels were 2.5 mmol/L (2.0-3.2), 1.4 mmol/L (1.1-

1.7) and 1.1 mmol/L (0.8-1.7), respectively, with 55.3%, 75.5% and 76.7% patients achieving the target levels, respectively. Only 16.2% achieved the target levels of both blood sugar and blood pressure, 12.7% achieved the target levels of both blood sugar and blood lipids, and 9.0% achieved all target levels of blood sugar, blood pressure and blood lipids.
Conclusion: There is a giant gap between current status and the target levels. Our data demonstrated urgent need for a more comprehensive management for these patients.
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Sex differences in glycaemic control among people with type 1 diabetes
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Background and aims: There are limited data describing whether control of type 1 diabetes differs between the sexes. Our aim was to investigate this issue using a large international dataset.
Materials and methods: This analysis represents an international collaboration based on data for 142,260 children and adults with type 1 diabetes, defined using the best available criteria for each country, from 12 countries in total (Austria, Denmark, Germany, Italy, Latvia, New Zealand, Norway, Scotland, Slovenia, Sweden, Ukraine, United States) on glycaemic control over the previous 12 to 24 months derived from both population-based registers and clinic databases. Logistic regression was used to generate odds ratios (ORs) and 95% confidence intervals (CI) for HbA_{1c} ≥7.5% (58 mmol/mol) for females compared to males adjusted for age and duration of diabetes within three age strata that broadly represent paediatric (<15 years), young adult (15-29 years) and adult populations (≥30 years).
Results: Proportions of people with HbA_{1c} ≥7.5% (58 mmol/mol) ranged from 64.4% in boys <15 years of age to 74.0% in women of 15-29 years old. In the youngest age strata there was no statistically significant difference in odds of HbA_{1c} ≥7.5% (58 mmol/mol) between boys and girls: adjusted OR 1.05, 95% CI: 1.00-1.10 (n=15304 boys and 14423 girls). In the two older age categories, women had statistically significantly higher adjusted odds ratios (AOR) for HbA_{1c} ≥7.5% (58 mmol/mol) (15-29 years AOR 1.08, 95% CI: 1.02-1.13 (n=19799 men and 17012 women); ≥30 years AOR 1.06, 95% CI: 1.02-1.10 (n=40388 men and 33241 women)).
Conclusion: In this cross-sectional analysis of type 1 diabetes data from several countries females were more likely to have an HbA_{1c} ≥7.5% (58 mmol/mol) than males. Further work is required to investigate explanations for this finding.

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Glycaemic control is not affected by age and gender: results from a large cohort study (EDGE)
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Background and aims: The Effectiveness of Diabetes control with vildaGliptin and vildagliptin/mEtformin (EDGE) study compared the effectiveness and safety of adding vildagliptin or other oral anti-diabetic drugs (OADs) in patients with type 2 diabetes mellitus (T2DM) inadequately controlled by oral monotherapy. We present the post hoc analysis from this large EDGE study that aimed to investigate whether there were changes in glycaemic control across age classes and gender in patients treated with vildagliptin or sulphonylureas as add-on to metformin.
Materials and methods: Patients became eligible only after the treatment decision on whether to give vildagliptin or other OADs (including any sulphonylurea, thiazolidinedione, glinide, α-glucosidase inhibitor or metformin, but excluding any other dipeptidyl peptidase-4 inhibitor or glucagon-like peptide-1 mimetics/analogues) was finalised by the physician. Glycaemic control comparisons across age and gender between the two groups were performed using the analysis of covariance after adjusting for baseline HbA1c and body weight (p values were adjusted for multiple comparisons).
Results: Overall, 24,854 patients were treated with vildagliptin and 10,858 with sulphonylureas, both as add-on to metformin. The adjusted difference in HbA1c (ΔHbA1c) was -0.20 [95% confidence interval (CI): -0.22, -0.18; p<0.001] in favour of vildagliptin (Table). ΔHbA1c within classes of age in the vildagliptin cohort was not statistically significant, except in patients <40 vs. >80 years who showed a stronger reduction in HbA1c of -0.12 (95% CI: -0.24, -0.00; p<0.05). No difference was found in the sulphonylurea group. ΔHbA1c between treatment groups was statistically significant within each class of age. Vildagliptin showed ΔHbA1c vs. sulphonylureas ranging from -0.12 in the elderly (≥70 years) to -0.26 in the younger classes (≤50 years). There was no difference in HbA1c drop between genders [0.00 (95% CI: -0.01, 0.01; p=0.958)] in either treatment groups.
Conclusion: Treatment with vildagliptin or sulphonylureas, combined with metformin, provides effective HbA1c control regardless of age and gender. Under real-life conditions, vildagliptin lowers HbA1c more effectively than sulphonylureas within each class of age.

	Vildagliptin				Sulphonylureas				Vildagliptin vs. Sulphonylureas
Age classes (years)	n	HbA1c baseline	HbA1c Δ ($)	HbA1c Δ ($$)	n	HbA1c baseline	HbA1c Δ ($)	HbA1c Δ ($$)	HbA1c Δ ($$$) (95% CI)
Overall	24,854	8.11 (8.10, 8.13)	-1.15 (-1.17, -1.14)		10,858	8.20 (8.21, 8.26)	-1.03 (-1.06, -1.01)		-0.20 (-0.22, -0.18)***
<40	1,554	8.41 (8.34, 8.49)	-1.40 (-1.47, -1.33)	-0.12 (-0.24, -0.00)*	658	8.61 (8.52, 8.70)	-1.31 (-1.40, -1.21)	0.01 (-0.16, 0.19)	-0.25 (-0.37, -0.14)***
40-<50	4,449	8.33 (8.29, 8.37)	-1.33 (-1.37, -1.29)	-0.08 (-0.19, 0.02)	2,181	8.46 (8.41, 8.51)	-1.17 (-1.22, -1.11)	0.06 (-0.09, 0.22)	-0.26 (-0.32, -0.20)***
50-<60	7,607	8.20 (8.17, 8.23)	-1.21 (-1.24, -1.18)	-0.06 (-0.16, 0.04)	3,409	8.33 (8.29, 8.38)	-1.08 (-1.12, -1.03)	0.05 (-0.10, 0.20)	-0.23 (-0.27, -0.17)***
60-<70	6,753	8.00 (7.97, 8.04)	-1.07 (-1.10, -1.03)	-0.04 (-0.14, 0.06)	2,889	8.09 (8.04, 8.14)	-0.95 (-0.99, -0.90)	0.02 (-0.13, 0.18)	-0.18 (-0.24, -0.13)***
70-<80	3,720	7.81 (7.77, 7.85)	-0.91 (-0.95, -0.87)	0.00 (-0.11, 0.10)	1,410	7.87 (7.80, 7.94)	-0.82 (-0.88, -0.75)	0.00 (-0.16, 0.16)	-0.12 (-0.20, -0.04)***
=80	771	7.80 (7.71, 7.88)	-0.91 (-1.00, -0.81)	Reference	311	7.78 (7.64, 7.93)	-0.77 (-0.92, -0.63)	Reference	-0.12 (-0.29, +0.05)

ANCOVA: HbA1c delta adjusted for baseline HbA1c and weight by age group and treatment.
$ HbA1c delta comparison vs. baseline
$$ HbA1c delta comparison within age classes (reference class =80 y)
$$$ HbA1c delta comparison vildagliptin vs. sulphonylureas
*p value adjusted with Bonferroni test for multiple comparison: * <0.05; ** <0.01; *** <0.001*

Supported by: Novartis Pharma

PS 002 HbA_{1c} measurement and outcomes

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The use of HbA_{1c} to diagnose type 2 diabetes in a multiethnic and diverse socioeconomic population: results from the South London Diabetes study (SOUL-D)

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Background and aims: The authorities providing health care to a large area of South London adopted the WHO's recommendations of an $HbA_{1c} \geq 6.5\%$ as a diagnostic tool for type 2 diabetes (T2DM) in May 2012. The South London Diabetes Study (SOUL-D) has been recruiting people with new onset (<6 months) T2DM since 2008. We aimed to estimate the proportion of SOUL-D participants diagnosed with HbA_{1c} <6.5% prior to May 2012 and whether they are significantly different from participants with a higher HbA_{1c} in terms of demographics and their diabetes status.
Materials and methods*:* This was a cross-sectional study in a population-based cohort of individuals with newly-diagnosed T2DM recruited through 6-monthly searches of 96 primary care databases in South East London. Data, including HbA_{1c} at diagnoses, are collected from the participants and the practice records. Participants diagnosed with HbA_{1c} <6.5% were compared with participants diagnosed with $HbA_{1c} \geq 6.5\%$ for age at diagnosis, gender and race using ANOVA and chi-square analysis. Biomedical data including micro/macrovascular complication and medication status were also compared at baseline and after one year between the groups.
Results*:* 1461 participants (55% male, mean age 55.73 years, SD = 11.15, 37.8% Black African/Caribbean ethnicity) were analysed. 338 (23.1%) had an HbA_{1c} <6.5% at diagnosis. Those with diagnostic HbA_{1c} <6.5% were significantly more likely to be white ($p<0.001$) and had an older mean age of onset (58.2 ± 10.81 vs. 54.4 ± 11.20, $p<0.001$) but there were no significant differences in gender compared to the rest ($p=0.98$). The prevalence of retinopathy (7.4% vs. 11.9%, $p=0.21$), nephropathy (16.7% vs. 13.2%, $p=0.15$), Myocardial Infarction (3.0% vs. 2.3%, $p=0.07$) and Stroke (1.0% vs. 1.7%, $p=0.35$) did not differ significantly before enrolment into SOUL-D, except for a higher proportion of patients with sensory neuropathy diagnosed with an HbA_{1c} <6.5% (9.9% vs. 5.8%, $p<0.05$). At one year, there were no significant differences in complication status ($p>0.05$). The number patients on insulin (6.0% vs. 3.4%) and mean difference in HbA_{1c} (6.8 ± 1.17 vs. $6.9 \pm 1.28\%$) at one year did not reach significance ($p=0.08$ for both).
Conclusion: Nearly a quarter of patients with newly diagnosed T2DM in South London did not have a diagnostic $HbA_{1c} \geq 6.5\%$ by WHO guidance and may not receive T2DM diagnosis in the future. These patients may nevertheless have clinically significant disease, as there were little significant difference in biomedical data between them and those with $HbA_{1c} \geq 6.5\%$ at baseline or at one year. Ethnicity may need to be taken into account when using HbA_{1c} levels to diagnose diabetes.
Supported by: SOUL-D

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Identification of HbA_{1c} cut-off for pre-diabetes from incidence of diabetes in a Japanese population

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Background and aims: Pre-diabetes is an intermediate stage between normal glucose and diabetes. It includes impaired fasting glucose on fasting plasma glucose (FPG) (110-125 mg/dL) and impaired glucose tolerance on 2-hour plasma glucose on a 75g oral glucose tolerance test (140-199 mg/dL). Individuals with pre-diabetes have a high risk of developing diabetes mellitus (DM). However, there is no clear cut-off point for HbA1c to define pre-diabetes. The aim of the study was to identify the cut-offs for HbA1c with respect to incidence of DM on HbA1c.
Materials and methods: We registered participants who underwent general health check up at SSK hospital from February 2006 to January 2007 as baseline and followed them for five years until January 2012. 3,826 subjects who had FPG <126 mg/dl (mean follow-up period 4.0 ± 1.4y) and 2,772 subjects who had HbA1c <6.5% (mean follow-up period 4.0 ± 1.4y) were analyzed respectively. Diabetes was diagnosed if HbA1c ≥6.5% (HbA1c-DM) or FPG ≥126 mg/dl (FPG-DM) at least once during the follow-up period. We assessed the risk of developing diabetes according to different stage of glucose tolerance by survival analysis using the Cox proportional hazards model. Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cut-off point for incidence of diabetes on FPG and HbA1c, respectively.
Results: Of the 3,826 subjects with FPG<126mg/dL, 129 (3.4%) developed FPG-DM while of the 2,772 subjects with HbA1c<6.5%, 74 (2.7 %) developed HbA1c-DM. In the survival analysis, hazard ratios (HRs) for incidence of diabetes increased with deteriorating levels of FPG and HbA1c ($p < 0.001$ for both) after adjustment for age at baseline, sex, body mass index, waist circumference, systolic blood pressure, and total cholesterol. In addition, HRs of incidence of FPG-DM for FPG of 100-109 and 110-125 mg/dl were 7.5 (95% confidence intervals: 4.0 -14 .0) and 66.7 (37.6-118.2) compared with FPG < 100 mg/dl. HRs for incidence of HbA1c-DM for HbA1c of 5.6-5.9% and 6.0-6.4% were1.6 (0.4-6.0) and 77.9 (26.8-226.2) compared with HbA1c <5.6%. Area under the ROC curve for FPG and for HbA1c were 0.92 (0.90-0.95) and 0.92 (0.88-0.96), respectively. Sensitivity and specificity were 88% and 99% for FPG of 100mg and 86% and 91% for HbA1c of 6.0%, respectively.
Conclusion: The optimal cut-off value for HbA1c to define pre-diabetes with respect to incidence of diabetes may be 6.0% in a 5-year prospective Japanese cohort study.

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Phenotypes and biochemical characteristics of new diabetes patients diagnosed based on different criterion: HbA_{1c} recognises people with more advanced diabetic stage

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Background and aims: Gycated haemoglobin A1c (A1C), fasting plasma glucose (FPG) and oral glucose tolerance test (OGTT) are universally accepted diagnostic tools to identify people with previously unknown type 2 diabetes (NewDM). However, each test could recognize people with different risk factors, and the agreement between these three tests is not high enough. We aimed to compare phenotypes and biochemical features of NewDM groups identified based on FGP, OGTT, and A1C criterion.
Materials and methods: Data derived from recently completed cross-sectional population-based Epidemiologic Survey on Diabetes, Obesity, Hypertension and Endocrine Disease in Turkey (TURDEP-II) that was included 26,499 people (age: ≥20 years, response rate: 87%, women: 63%). We used all of the diagnostic tools to diagnose NewDM (A1C: ≥6.5% or FPG: ≥126 mg/dL or OGTT-2hPG: ≥200 mg/dL) and compare their characteristics.
Results: The prevalence of NewDM based on FPG was 4.6%, that of OGTT: 3.8%, and A1C: 3.4%. OGTT was the most frequent test without any agreement with the others (isolated OGTT-based NewDM was 76.4% vs. 16.0% isolated A1C-based, and 9.4% isolated FPG-based NewDM, $p<0.001$). A1C was the most frequent test found in agreement with one of the other two (Combined New DM based on A1C plus FPG or OGTT: 68.5% vs. FPG plus OGTT or A1C: 44.7% vs. OGTT plus FPG or A1C: 17.8%). Agreement of all three tests together was 2.2% within FPG, 2.4% within OGTT, and 2.9% within A1C-based NewDM groups. NewDM based on FPG was more prevalent among men ($p<0.001$); these peoples were younger ($p<0.001$ and more smoker ($p<0.001$); they had higher insulin ($p=0.011$) and IGF1 ($p<0.001$) but lower cystatin C ($p<0.001$) levels. Whereas NewDM based on OGTT criterion identified more women ($p<0.001$), more elderly people ($p<0.001$), more people with hypertension (HT) ($p<0.001$). On the other hand, NewDM based on A1C criterion identified more men and more people in middle age ($p<0.001$); family history of DM ($p=0.045$), heavy alcohol use ($p=0.018$), prevalent HT and obesity ($p<0.001$) was more common among these group of people; they had higher BMI, waist and systolic BP ($p<0.001$), higher diastolic BP ($p=0.002$), higher FPG, OGTT-1hPG, OGTT-2hPG and A1C ($p<0.001$),

moderately higher insulin (p=0.001), higher HOMA-IR, lower IGF1, higher IGFBP3, lower eGFR (MDRD), higher triglycerides, lower HDL-chol and higher nonHDL-chol (p<0.001). Among central obese and less physically active people, NewDM was more prevalent based on all three diagnostic tests (p<0.001 for all).

Conclusion: Our results indicated that each diagnostic method identifies NewDM people with different clinical and biochemical characteristics. A1C based method recognizes people in more advanced stage of DM. Therefore, we recommend that these people should be treated more aggressively than those diagnosed with FPG or OGTT-based criterion. This study was funded by The Turkish Scientific and Technical Research Council (No. 109S166), and The Society of Endocrinology & Metabolism of Turkey.
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Prediction of diabetes mellitus using HbA_{1c}: Does HbA_{1c} more efficiently predict onset than plasma glucose concentration?

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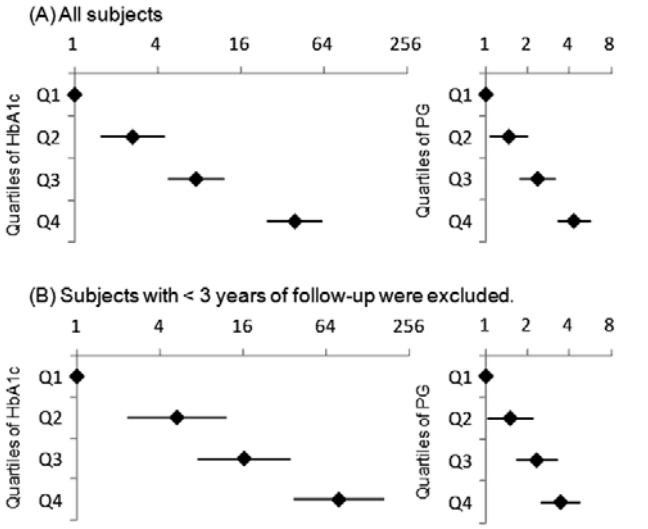
Background and aims: HbA1c concentration is a reliable indicator of blood glucose control in diabetic patients. HbA1c as well as plasma glucose (PG) concentrations are widely used to identify subjects at high risk of developing diabetes mellitus (DM) in Japan. However, there is limited evidence that HbA1c concentrations comparably or more efficiently predict the risk of DM onset in comparison with PG. This paper evaluated the utility of HbA1c in predicting the risk of DM onset and compared it with that of PG.

Materials and methods: The Sado cohort consists of 8,462 inhabitants, comprising 3,526 men and 4,936 women aged 27-92 years, of Sado City located on Sado Island in the Sea of Japan. The baseline data for 761 participants were obtained in 2002, and for the remaining 7,701 participants in 2003. From those participants, 5,044 DM-free subjects aged 40-89 years and comprising 1,875 men and 3165 women, underwent baseline assessment of HbA1c and PG concentrations and were monitored for the onset of DM until 2011 through annual health check-ups organized by Sado City. A subject was considered to have developed DM when he/she declared to have been diagnosed with DM, or when he/she met any of following criteria: 1) fasting PG ≥ 7.0 mmol/L, 2) casual PG ≥ 11.1 mmol/L or 3) HbA1c ≥ 6.5% (NGSP). The follow-up period was determined on the basis of age at diagnosis or the date of blood sampling. Baseline PG and HbA1c concentrations were assessed in quartiles and the association with the risk of DM onset was evaluated using hazard ratios (HRs) and 95% confidence intervals (CIs) derived from sex- and age-adjusted Cox proportional hazards models. For PG, quartile categories were fasting-interval specific.

Results: During the 29,727 person-years of follow-up, 5.9 years per person in average, 539 instances of DM onset were identified. The annual incidence rate was 18.1/1000/year (21.0/1000/year for men and 16.4/1000/year for women). Although both HbA1c and PG were positively associated with the risk of DM onset, the association was much stronger for HbA1c than for PG (Figure A). The sex- and age-adjusted HR (CI) for DM onset for the highest quartile compared with the lowest quartile was 39.3 for HbA1c (24.9-62.1) and 4.3 (3.3-5.7) for PG. When subjects with a <3-year follow-up period were excluded from the analyses, the HR (CI) for the highest vs. the lowest quartiles of HbA1c was elevated to 78.9 (37.0-168.5), although that of PG was decreased to 3.5 (2.5-4.8) (Figure B).

Conclusion: High HbA1c concentrations more closely associate with a high risk of DM onset than high PG concentrations. The longer the follow-up period, the stronger this association will be for HbA1c but not for PG.



Sex- and age-adjusted hazard ratios for the onset of diabetes mellitus according to quartiles of HbA1c and plasma glucose (PG) concentrations

Supported by: JAPF

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Average glucose and respective glycated haemoglobin values in patients with type 2 diabetes: reappraisal of the ADAG study

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Background and aims: The results of ADAG study, recommended by ADA guidelines and widely used for a calculation of HbA1c from average glucose values, were achieved in healthy subjects, patients with type 1 and in only 159 with type 2 diabetes. The aim of the study was to validate those results in a large group of type 2 diabetic patients.

Materials and methods: A prespecified analysis of data from the PROGENS HbA1c study was performed. In 4257 patients with type 2 diabetes (mean age 63.7±9.4 years, mean BMI 30.3±4.5 kg/m2, time from diagnosis 9±5.5 years) treated with premixed insulin with or without oral antidiabetic drugs, HbA1c (using a multitest HbA1c analyser A1cNow+) and average blood glucose from the preceeding 90 days (ABG 90 days), calculated automatically from all values measured by the glucometer, were assessed at visits 2 and 3.

Results: The results of the PROGENS study differ from those from ADAG study by different slope of the regression line (and therefore different glucose values assigned to HbA1c) and broader distribution of the results by lower HbA1c values (Table 1). The probability existence of real HbA1c>7% in patients with ABG 90 days = 140 mg/dl was equal 0.53 (0.51-0.55), with ABG 90 days = 100 mg/dl was still 0.22 (95%CI 0.18-0.25).

Conclusion: Broad use of the results achieved in the ADAG study for the assessment of diabetes control in subjects with no HbA1c value seems to be inappropriate. The probability of wrong assessment of the HbA1c target using only the value calculated from ABG 90 days using ADAG data is high. Therefore HbA1c seems to be necessary for the proper assessment of diabetes control, and average blood glucose estimation as a tool that may replace HbA1c should not be recommended.

Table 1. Comparison of PROGENS HbA1c and ADAG studies

HbA1c [%]	ABG PROGENS (95% CI)	ABG ADAG (95% CI)
6	134,4 (93-175,7)	126 (100-152)
7	149,3 (107,9-190,6)	154 (123-185)
8	164,1 (122,8-205,5)	183 (147-217)
9	179,0 (137,6-220,4)	212 (170-249)
10	193,9 (152,5 -235,3)	240 (193-282)
11	208,8 (182,1-265,2)	269 (217-314)
12	223,7 (195,4-278,9)	298 (240-347)

Supported by: BIOTON SA, Macierzysz, Poland

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Relationship between haemoglobin and glycated haemoglobin A_{1c} in a Japanese population

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Background and aims: The recommendations for the use of hemoglobin A1c (HbA1c) as a diagnostic test is likely increase its use in individuals without a prior history of diabetes. However, HbA1c measurements have several limitations. Plasma glucose molecules attach the hemoglobin (Hb) in erythrocyte through non-enzymatic glycation that results in a small Hb variant of HbA1c. The condition affecting characteristics of Hb, erythrocyte turnover and Hb glycation would influence on HbA1c levels independent from blood glucose levels. Thus, there may be difference in the relation between blood glucose levels and HbA1c values. The HbA1c values are calculated as the ratio of glycated hemoglobin to total Hb, suggesting that Hb may affect HbA1c levels independent from blood glucose levels. Thus, the aim of the study is to assess the relationship between HbA1c and Hb in non-anemic and non-diabetes Japanese population.
Material and methods: Subjects were 31.081 individuals (17,780 men) aged 30 to 75 years old without anemia (men: Hb<13.5 g/dL, women: Hb<11.5 g/dL), Hb with above reference range (men: Hb>17.5 g/dL, women: Hb>15.5 g/dL), self-reported diabetes, fasting plasma glucose (FPG) >=126 mg/dL, HbA1c >=6.5%, or chronic kidney disease (creatinin>1.5 mg/dL) from general health check-up program in Japan. Subjects were classified according to FPG by quintiles (men: <87, 87-90, 91-94, 95-100, >=101 mg/dL, women: <82, 82-85 86-89, 90-94, >=95 mg/dL) and Hb by tertiles (men: <15.1, 15.1-15.7,>=15.8 g/dL, women: <13.1, 13.1-13.7, >=13.8 g/dL), respectively in men and women. Mean HbA1c levels were calculated in FPG quintiles across Hb tertiles. General linear model was used to calculate age- and Hb-adjusted mean HbA1c levels in FPG quintile categories. Multivariate linear regression analysis was performed to analyze factors affecting on HbA1c levels.
Results: HbA1c increased with increase of FPG in both men and women (p<0.001 for both). Subjects with lower Hb had significantly higher HbA1c at given FPG in both sexes (p<0.001). This trend was consistent though FPG quintiles of no-diabetic FPG range (p <0.001), and the difference of mean HbA1c levels at given FPG decreased with deterioration of FPG. Women had lower mean HbA1c levels than men in the same age except for age >=55 years old. Women had higher age-adjusted mean HbA1c levels than men at given FPG across all FPG categories (p<0.001), but had lower age- and Hb-adjusted mean HbA1c levels than men at given FPG across all FPG categories (p<0.001). Older age (beta=0.104 for 10 years, p<0.001), higher FPG (beta=0.306 for 1 mmol/L, p<0.001), women (beta=0.0124, p=0.038) and lower Hb (beta=-0.016 for 1g/dl, p<0.001) were independently related with an increase of HbA1c.
Conclusion: Hb and sex influence on HbA1c values independent from glucose concentrations in non-anemic and non-diabetes Japanese population. This should be considered as one of limitations to use diagnostic criteria for diabetes on HbA1c.
Supported by: Japan Diabetes Society

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'Tracking' of metabolic parameters in patients with diabetes: Are we making a difference?

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Background and aims: The morbidity and premature mortality associated with type 1 and type 2 diabetes (T1D, T2D) can be improved through optimised metabolic control. Indeed this is a major driver for the regular review of diabetic patients. Previous studies have however demonstrated that glycated haemoglobin (HbA_{1c}) levels 'track' within clinic populations. That is, average values for HbA_{1c} rarely improve over time within a given diabetic population. Our aim was to explore secular trends within a large diabetic clinic for 1) average clinic HbA_{1c} , 2) HbA_{1c} within individual diabetic patients, and 3) lipid and BP at a clinic and individual level.
Materials and methods: We used diabetes clinic data from the our university hospital during the period 2008-2013. At each clinic visit, HbA_{1c} and metabolic parameters (cholesterol, triglycerides, creatinine, BP, BMI) are measured. In total, 1051 T1D patients and 2956 T2D patients visited our clinic during the study period. Linear regression was used to evaluate secular trends in mean clinic parameters (using 6 monthly intervals), and to determine predictors of intra-individual mean parameter levels. To further assess intra-individual tracking, we performed a paired t-test to compare differences in values between first and last clinic visits (analyses were restricted to cases in which the time difference between first and last available visits was over one year). All analyses were performed using SPSS.
Results: Secular trends in mean clinic HbA_{1c} and metabolic parameters. In T1D, average clinic values for HbA_{1c} and all metabolic parameters remained constant during the study period, with the exception of cholesterol levels which rose from 4.19 to 4.70 mmol/L (β = 0.081, p<0.001). In T2D, all parameters remained constant except HbA_{1c} which rose from 65.4 to 66.9 mmol/mol (β = 0.047, p<0.001). Predictors of intra-individual mean HbA_{1c} and metabolic parameters. In T1D for all parameters, the patient's individual mean parameter value (averaged out across all of their clinic visits), was predicted by the parameter value at both the first available and last visit (p<0.001 respectively). This was also evident for all parameters in T2D (p<0.001 respectively). Intra-individual tracking. In T1D, there was no significant difference between the parameter value at the first clinic visit and the parameter value at the last visit for all parameters other than systolic BP, which rose from an average of 134 to 137 mmHg (p<0.01). In T2D, HbA_{1c} (mean difference 1.58mmol/mol, p<0.01), triglycerides (mean difference 0.20mmol/L, p<0.001), and BMI (mean difference 0.24, p<0.05) were all lower on the most recent clinic visit. However, both systolic (mean difference 2.54, p<0.001) and diastolic (mean difference 1.61, p<0.05) BP increased.
Conclusion: We demonstrate intra-individual tracking of HbA_{1c} and all metabolic parameters in T1D. This indicates that those patients with high parameter values will continue to suffer from poor control years later, increasing their risk of long-term complications. Our results support other studies of 'glycaemic tracking', but extend it to an intra-individual level, as well as to other metabolic parameters. Further studies are required to determine whether current medical management in these patients is failing, whether there is a biological underpinning to this tracking, and whether regular clinic attendance provides benefits outside the classical parameters used in our study.

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The threshold of glycated haemoglobin and diabetic complications: a community-based screening programme in Shanghai

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Background and aims: To determine the association of HbA1c with diabetic complications and whether such relationship supports the previously proposed diagnositc threshold of diabetes (≥6.3%) in Chinese adults.
Materials and methods: A total of 1996 Chinese subjects were recruited from urban communities in Shanghai and screened for diabetic retinopathy, nephropathy, neuropathy and peripheral arterial disease. Diabetes and impaired glucose regulation were diagnosed with a 75g OGTT. Nephropathy was defined as an elevated morning urine albumin-to-creatinine ratio (30 mg/g or higher). The levels of diabetic retinopathy were identified with nonmydriatic

retinal photographs according to the Diabetic Retinopathy Disease Severity Scale. Peripheral neuropathy were assessed with Michigan Neuropathy Screening Instrument (MNSI) and impaired sensation was defined as MNSI scored over 2. Peripheral aterial disease (PAD) was assessed by measuring an ankle-brachial index (ABI) and defined as ABI ≤ 0.9 with an automatic device that incorporates a sphygmomanometer and 2-way Doppler. Stepwise Logistic regression and receiver operating curve (ROC) were performed to evaluate the risk factors for diabetic complications and the threshold of HbA1c.
Results: Excluding 236 participants with ungradable retinal photographys, 1758 subjects were analyzed, as included 616 with diabetes, 542 with IGR, 936 with hyptertension and 84 with obesity. Retinopathy, nephropathy, neuropathy and PAD were determined in 4.8%, 13.9%,13.9% and 5.3% of the analyzed subjects, separately. HbA1c was the only common risk factor for the four complications(OR for retinopathy: 1.66 [95% CI:1.49-1.86], for nephropathy: 1.35 [1.23-1.48], for neuropathy: 1.29 [1.18-1.42], for PAD: 1.19 [1.03-1.36]). Hypertension contributed to retinopathy (2.07 [1.23-3.49]) and nephropathy(2.26 [1.61-3.17]) . Aging was positively related to nephropathy (1.45 [1.26-1.65]), neuropathy (1.83 [1.60-2.09]) and PAD (1.85 [1.51-2.29]). The area under curve for retinopathy was 0.864 (0.707-0.822), nephropathy 0.644 (0.605-0.683), neuropathy 0.630 (0.590-0.670), PAD 0.622 (0.559-0.684). Based on the ROC and the diagnostic value of the different cut-off points, the observed optimal cut-off point was found to be 6.35% for retinopathy (figure 1).
Conclusion: HbA1c was positively associated with diabetic complications. An HbA1c cut-off point of over 6.3% for identifying diabetic retinopathy was supportive of complementary diagnostic criterion for diabetes in Chinese adults.

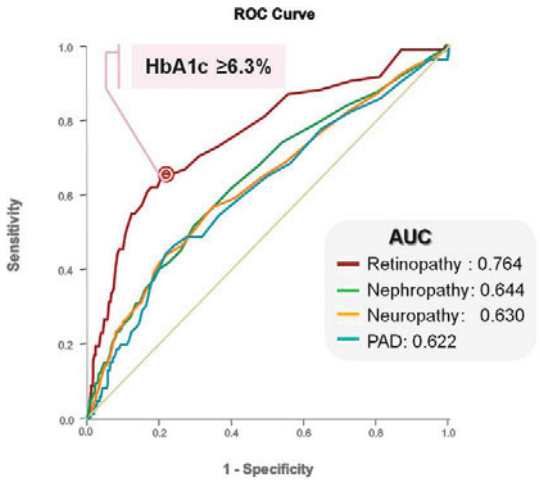

Figure 1. ROC curves for HbA1c (%) and the various diabetic complications

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Seasonal variation in haemoglobin A_{1c} in adult Portuguese patients
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Background and aims: Glycated Hemoglobin (HbA1c) reflects average glycemic control of the last 120 days and is used as a predictor of complications of diabetes mellitus. It was suggested that HbA1c seasonal fluctuations can be directly related to different biological, geographical and cultural parameters. The aim of this study was to evaluate of the HbA1c seasonal variation, starting from assays performed in a large cohort of patients over a period of 5 years (2008 to 2012).
Materials and methods: It was performed a retrospective analysis of all HbA1c assays performed to patients evaluated at a tertiary care university hospital during the period between 1st January 2008 and 31st December 2012. Both patients younger than 18 and HbA1c extreme values (<3% and/or >18%) were excluded.
Results: We obtained 62,384 HbA1c measurements during the period defined for the study. It was observed a cyclic seasonal fluctuation consistently repeated over the 5 years. Higher mean HbA1c levels were found in winter months (January-February), while lower mean HbA1c levels were found in the warm summer months (August-September), with an increasing level tendency from October (p <0,0001, Kruskal-Wallis test). There was a significant HbA1c maximal amplitude value of 0.33% (p <0,0001) between February-September, with mean HbA1c values fluctuations between 6.80% and 7.13%.
Conclusion: Seasonal HbA1c patterns were previously described in other studies and the explanation seems to be multifactorial (seasonal and festivities-related high calorie food intake, secondary insulin resistance, thermal effect on the metabolic axis, among others). The clinical importance of our findings is related to their practical implications, particularly regarding the need to improve the interpretation of HbA1c values. Restriction of HbA1c assay to specific times of the year may reduce the seasonal variation of physiological responses.

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Difference of seasonal variation between glycated albumin and glycated haemoglobin A_{1c}
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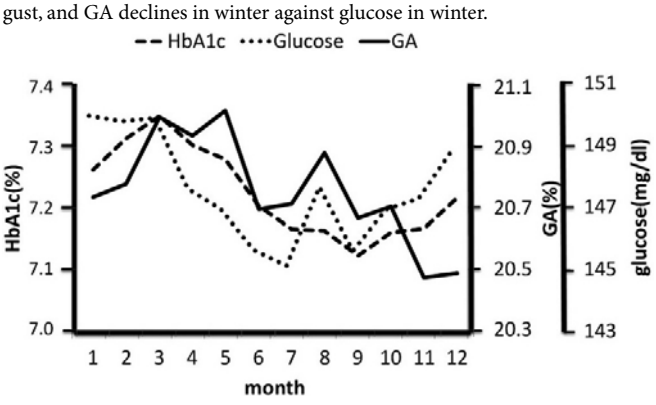
Background and aims: Glycated albumin(GA) is a product of the chemical binding of serum albumin and glucose by non-enzymatic oxidation reaction. It reflects 2-3week glycemic control, and has been used as a glycemic control indicator such as glycated hemoglobin A1c (HbA1c). GA potentially has an advantage compared to HbA1c as its measurement is not influenced by disorders of hemoglobin metabolism. It is a well-known fact that HbA1c has seasonal variations. Considering the same use of both GA and HbA1c, we then presume that GA also has seasonal variations like HbA1c.
Materials and methods: Subjects are all diabetic outpatients of our University Hospital from January 2005 to December 2011, whose HbA1c and GA were examined simultaneously. Casual plasma glucose was also measured simultaneously in the most subjects. HbA1c was measured by HPLC, and GA was measured by an enzymatic method. We analyzed monthly means of plasma glucose, GA, and HbA1c each year with monthly average temperature in Tokyo reported by the Japan Meteorological Agency.
Results: The number of outpatient per month was 1665.6 ± 288.4 (mean ± SD), mean age was 64.2 ± 12.5. Our analysis showed that plasma glucose was recorded highest in January and February, lowest in June and July. It then reascended in August, and declined in September. HbA1c was recorded highest in February and March, and lowest in August and September. GA was highest in March, lowest in November. It also reascended in August, then declined in September. GA tended to have a disjunction with plasma glucose in winter. Plasma glucose and HbA1c showed significant inverse correlations with monthly mean temperature in Tokyo (rs = -0.520, p < 0.001, rs = -0.419, p < 0.001, respectively), but GA did not intercorrelation with temprature.
Conclusion: GA and HbA1c have different seasonal variations from each other. They do not necessarily have same relationship with glucose elevation as below graphs show. HbA1c does not respond to glucose elevation in August, and GA declines in winter against glucose in winter.



PS 003 Prevalence and incidence of diabetes

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Incidence and risk factors of new-onset diabetes mellitus after lung transplantation

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Background and aims: New-onset diabetes mellitus after transplantation (NODAT) is a common complication in renal and liver transplant recipients but has been less extensively studied after lung transplantation. The objective of this study was to examine the incidence of NODAT in our centre and its risk factors and complications.
Materials and methods: We reviewed the medical records of 108 patients who received single or double lung transplants from January 1995 to July 2012. Mean (± SD) age was 52.6 ± 11.4 years. Most patients had chronic pulmonary obstructive disease/emphysema but 9 patients had cystic fibrosis. Immunosuppressive therapy consisted in cyclosporine, azathioprine and methylprednisolone in all patients. Tacrolimus was introduced only if there were contraindications or side effects to cyclosporine. NODAT was diagnosed after at least 3 months post-transplant on the basis of fasting plasma glucose ≥ 7 mmol/l or random glucose ≥ 11.1 mmol/l on two or more separate days in association with symptoms of diabetes or HbA1c ≥ 6.5 %.
Results: Fourteen patients were diabetic before transplantation whereas NODAT was diagnosed in 38 patients (40.4 %). Using Kaplan-Meier estimates, the incidence of NODAT was 30 % after one year and 45 % after 5 years, for a mean duration of follow-up of 4.2 ± 0.5 years. A multivariate Cox regression analysis showed that an age ≥ 50 years and cystic fibrosis were independent risk factors for the development of NODAT (p = 0.032 and p = 0.005 respectively). There was a tendency for patients with NODAT to have lower vitamin D levels (p = 0.096). Other factors such as sex, body mass index, type of immunosuppressive therapy, CMV or hepatitis B immune status were unrelated to NODAT. Main complications included rejection episodes in 46 cases, infections in 89, renal failure in 51 and cerebral vascular accidents in 3. NODAT was significantly more common in infected patients (45 % versus 14 % in non infected patients; p = 0.032). Survival rates were not significantly different between diabetic and non diabetic patients.
Conclusion: NODAT was diagnosed in 40.4 % of patients, a figure comparable with other solid organ transplants. The main risk factors for NODAT were an age ≥ 50 years and cystic fibrosis. NODAT was more frequently associated with infections.

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20 years of type 1 diabetes registry of Catalonia

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Background and aims: Type 1 Diabetes mellitus (DM1) is the most common chronic diseases in childhood and adolescence, particularly in industrialized countries. The Catalan Type 1 diabetes Registry has been recording new cases of type1 diabetes since 1987. Trends in incidence rate during the 24 year period 1989-2010 are described.
Materials and methods: Patients under 30 at diagnosis of DM1, according to WHO criteria, were included in a prospective way. Health professionals and patients association were the primary and secondary sources of ascertainment, respectively. Incidence rates were estimated using Poisson regression models. Completeness of registration was assessed using capture-recapture methodology.
Results: 6310 new cases of DM1: 3713 men, 2597 women under 30 at diagnosis were identified during the period January 1987 - December 2010. There is a similar distribution between the group of 0-14 years (3139) and the group of 15 to 29 years (3171) with a predominance of men in the latter group. The degree of completeness of the registry overall has been estimated at 90.1%. Age and sex -specific rates varied from 11.0 per 100.000 per year (95% CI 9.7-12.3) during 1987-1989 to 8.9 per 100.000 per year (95%CI 7.7-10.2) during 2005-2010. There were no differences between the eight regions of Catalonia.
Conclusion: Incidence rate of Type 1 diabetes in Catalonia is stable. There is no pattern in the geographical distribution in the incidence of type 1 diabetes in Catalonia.

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The incidence of type 1 diabetes mellitus in Romanian children aged 0-17 years increased significantly between 2002 and 2011

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Background and aims: The epidemiology of type 1 diabetes mellitus (T1DM) is a matter of interest worldwide. Its recent time trends in Romanian children aged between 0 and 17 years have not yet been analyzed. The aim of this work was to reveal the evolution, over a 10-year interval, of the incidence of childhood T1DM in Romania.
Materials and methods: The primary source for incident cases of T1DM was the Romanian Childhood Diabetes Register (RCDR), elaborated each year by ONROCAD (Romanian acronym for "Romanian National Organization for the Protection of Children and Adolescents with Diabetes") since 1996. The secondary source was represented by the medical records from Medical Center "Cristian Serban" from Buzias, a reference center for pediatric diabetes, where T1DM children from all over the country are admitted and treated. Demographic data were retrieved from the National Institute for Statistics. The incidence was calculated per calendar year and 100,000 population at risk in age groups 0-4, 5-9, 10-14, 15-17, and 0-17 years. The statistic methods used were chi-square test, chi-square test for trend, and Wilson's procedure for the 95% confidence interval (CI). Statistical significance was set at $p<0.05$.
Results: A number of 3,196 new cases of type 1 DM (1,696 - 53.1% boys, and 1,500 - 46.9% girls), aged less than 18 years, were found in both sources. The primary source detected 3,144 cases, and the secondary source 964 cases (912 patients were diagnosed by both sources), leading to a level of ascertainment was 96.2% The main data regarding the incidence of T1DM in Romanian children, between years 2002 and 2011, are shown in the table. The estimated average incidence of T1DM during the studied interval was 7.35/100,000/year (95% CI 6.58 - 8.12). It was not significantly (p=0.53) higher in boys (7.62/100,000/year, 95% CI 6.53 - 8.71) than in girls (7.08/100,000/year, 95% CI 5.99 - 8.17), leading to a sex ratio for incidence (boys/girls) of 1.08. The incidence in children 0-17 raised extremely significantly ($p<0.0001$) during the studied interval, being 50.5% higher in 2011 as compared to 2002 (mean annual increase 5.61%). This trend was noticed for age groups 0-4, 5-9, and 10-14 years, but not for children aged 15-17 years.
Conclusion: Romania is a country with an intermediate incidence of T1DM in children that showed a continuous increase over a decade. Children aged 10-14 years have the highest probability of developing T1DM. Boys seem to be more predisposed than girls.

Incidence of T1DM (no. of cases/100,000) in Romanian children aged 0-17 years, between 2002 and 2011

Year	0-4 yrs (T)	5-9 yrs (T)	10-14 yrs (T)	15-17 yrs (T)	0-17 yrs (T)	0-17 yrs (B)	0-17 yrs (G)
2002	3.17	7.41	7.34	6.22	6.18	6.95	5.38
2003	3.58	6.74	8.32	5.78	6.31	6.72	5.87
2004	3.54	8.59	8.07	6.49	6.78	7.65	5.88
2005	4.14	7.40	8.68	6.54	6.79	6.94	6.64
2006	3.97	8.94	9.28	6.42	7.25	7.77	6.70
2007	6.22	9.91	10.46	5.88	8.24	8.09	8.40
2008	4.68	9.57	12.09	5.05	8.03	7.35	8.75
2009	5.28	8.97	10.68	3.16	7.29	7.59	6.98
2010	6.35	9.81	10.13	4.22	7.90	8.21	7.58
2011	7.32	10.71	12.40	5.24	9.30	9.28	9.31

Legend: T=total; B=boys; G=girls.

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Undiagnosed diabetes and prediabetes in the Bangladeshi population

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Background and aims: Due to lack of properly organized health care facilities, there is no systematic screening performed for chronic diseases in Bangladesh. Accordingly, a large number of people remains undiagnosed and silently progress towards complications. The present study was undertaken to find out the proportion of undiagnosed diabetes and prediabetes as well as their determinants in a general population of Bangladesh.
Materials and methods: Under a cross-sectional observational design, a total number of 1303, middle aged (range 35-60 years) healthy subjects, responding to open invitation for health check in three districts of Bangladesh, were recruited in the study (male 58.3% and female 41.7%; mean(±SD) age 42.7(±10.0) and 41.3(±8.9) years respectively. Among the participants 46.2% were from rural and 53.8% from urban areas. A two sample oral glucose tolerance test was performed and anthropometric measurements were taken. Glucose was estimated by glucose-oxidase method and triglyceride, total cholesterol and HDL were measured by enzymatic-colorimetric method. LDL was calculated by using Friedewald's formula. Anthropometric, glucose and lipid abnormalities were defined and classified as per WHO criteria. Data were analyzed using statistical Package for Social Program (SPSS) for Windows version 17.
Results: Among the total subjects, type 2 diabetes was 14.3% and impaired glucose regulation was 17.8% (impaired fasting glucose 3.3%, combined impaired fasting glucose - impaired glucose tolerance 3.1% and impaired glucose tolerance 11.5%). The proportion of type 2 diabetes was more than two times higher in urban (18.5%) compared to the rural (8.0%) areas ($p<0.001$). However, the proportion of impaired glucose regulation was only marginally higher in urban (20.1%) compared to the rural (15.4%) areas ($p<0.05$). Type 2 diabetes ($p<0.001$) and impaired glucose regulation ($p<0.01$) subjects were significantly older compared to the normoglycemic. BMI [type 2 diabetes ($p<0.002$); impaired glucose regulation ($p<0.001$)] and WHR [type 2 diabetes ($p<0.001$); impaired glucose regulation ($p<0.01$)] were significantly higher compared to normoglycemic. Pearson correlation analysis showed positive correlation of fasting glucose with age ($p<0.001$), WHR ($p<0.006$), triglyceride ($p<0.001$), cholesterol ($p<0.001$) and LDL ($p<0.001$), and it showed a negative correlation with HDL ($p<0.03$). Multinomial regression showed significant association of type 2 diabetes subjects with WHR ($p<0.002$), triglyceride ($p<0.001$) and area of residence ($p<0.003$); however, the impaired glucose regulation subjects showed significant association only with WHR ($p<0.033$) and triglyceride ($p<0.031$).
Conclusion: Type 2 diabetes and impaired glucose regulation remain undiagnosed in a high proportion of Bangladeshi population and age, central obesity and urbanization are among the major determinants of type 2 diabetes and impaired glucose regulation in this population.

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Undiagnosed type 2 diabetes remains an important health problem disproportionately affecting Irish males: the diabetes mellitus and vascular health initiative (DMVhi)

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Background and aims: Early identification of those with or at greatest risk of developing type 2 diabetes (T2D) is a challenge. Knowledge of local disease burden and epidemiology is important for appropriately targeting healthcare interventions. In the Republic of Ireland, information on undiagnosed T2D is limited and until now has been indirectly estimated or extrapolated from small regional studies. This abstract looks at gender differences in prevalence of undiagnosed T2DM, IGT and IFG in men and women by screening 30 000 people.
Materials and methods: The Diabetes Mellitus and Vascular health initiative (DMVhi) is a prospective, longitudinal study of Irish adults, policy holders in the largest private health insurer, aged 45-75 years without known T2D. At baseline, 29 144 participants completed screening: detailed medical questionnaire; fasting plasma glucose (FPG), lipid profile, blood pressure, weight, height, BMI, waist circumference, and waist:hip ratio were measured. Those with FPG ≥5.6≤6.9mmol/L had an Oral Glucose Tolerance Test (OGTT) performed. Those with a FPG ≥7.0mmol/L had a repeat FPG. Diabetes Risk Score was calculated based on FINRISC.
Results: Abnormal glucose concentrations (T2D, IGT and IFG) were detected in 11.9% of the DMVhi population; prevalence rates were 17.1% in males and 7.8% in females. Prevalence increased with age in both males (10.6%, 18.5%, 21.7% among those aged 45-54, 55-64 and 65-75 years) and females (4.3%, 8.6%, 10.9% respectively). Of the participants with T2D, 69.3% were male, 27.4% exercised ≥5 days/week, 91.4% had a BMI ≥25kg/m^2, 95.3% had abdominal obesity (waist circumference ≥80cm in females, ≥90cm in males), and 46.2% had a family history of diabetes. Risk factors with significant odds ratios of association with T2D included being male, older age, elevated blood pressure, higher BMI, abdominal obesity, family history of diabetes and elevated triglyceride levels. Few gender differences in risk factors were detectable, suggesting that the determinants of disease are similar in males and females. However, proportionally more males than females in our cohort had these risk factors. Compared to DMVhi females, males had a higher BMI (27.5 vs. 26.1 kg/m^2), higher systolic BP (126.4 vs. 123.3 mmHg), more abdominal obesity (79.2% vs. 65.6%), use of anti-hypertensive medication (22.7% vs. 18.9%), consumed alcohol (82.9% vs. 77.3%) and smoked (current and ex-smokers) (47.4% vs. 34.0%).
Conclusion: Undiagnosed T2D and pre-diabetes is an important health problem in Irish adults, particularly in Irish males. A better understanding of why males have a greater prevalence of disease is required. Our data suggest males have proportionally more risk factors for disease than females. Knowledge of risk factors associated with having abnormal glucose from the DMVhi could help focus limited healthcare resources on those at greatest risk of disease development.

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Regional differences in the incidence of known type 2 diabetes mellitus in 45-74 years old individuals: results from five population-based studies in Germany

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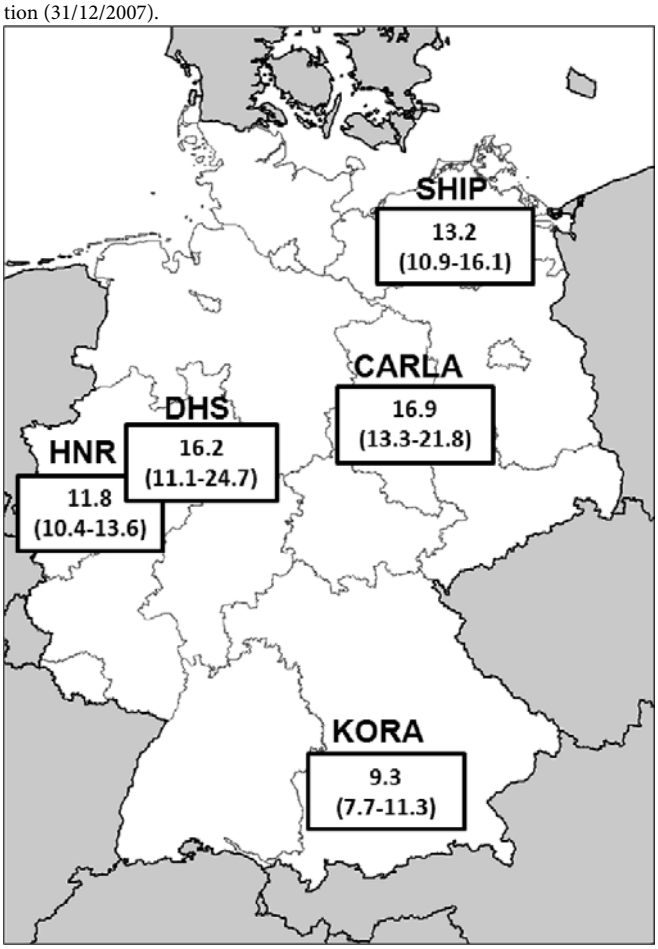
Background and aims: Regional data on the incidence of type 2 diabetes are scarce. Population-based data are paramount to investigate the long-term course of diabetes, for planning in health care and to evaluate the cost-effectiveness of primary prevention. We analysed regional differences in the incidence of type 2 diabetes within Germany.
Materials and methods: Data of participants aged 45 to 74 years at baseline between 1997-2006 (mean follow-up 2.2 to 7.1 years) from five regional population-based studies were included. Information on the incidence of self-reported type 2 diabetes at follow-up was compared. The incidence rates per 1000 person-years (95% CI) and the cumulative incidence (95% CI) from regional studies were directly standardized to the German population (31 December 2007) and weighted by inverse probability weights for losses to follow-up.
Results: Of 8,814 participants, 526 (6.0%) developed type 2 diabetes corresponding to an incidence rate of 11.4 per 1000 person-years (95%CI 10.4-12.4). The regional incidence was highest in the East and lowest in the South of Germany with 16.9 (95%CI 13.3-21.8) vs. 9.3 (95%CI 7.7-11.3) per 1000 person-years, respectively. The incidence increased with age and was higher in men than in women.
Conclusion: Similarly to the previously reported gradient in the prevalence estimates for type 2 diabetes (Schipf et al. 2012) the incidence shows

a Southwest-to-Northeast gradient within Germany. Our results strengthen the hypothesis that the observed differences may partly linked to prevalence differences in common risk factors for type 2 diabetes.

Figure 1: Regional incidence rates (per 1000 person-years) of type 2 diabetes mellitus (45-74 years at baseline) standardized to the German adult population (31/12/2007).



Supported by: Competence-Network-Diabetes of German Federal Ministry of Education & Research

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New estimates of diabetes prevalence in the Netherlands, based on information from 5 million subjects (DUDE-1)

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Background and aims: In 2009, the Dutch National Institute for Public Health and the Environment (RIVM) published an estimation of the prevalence of diabetes in The Netherlands to be around 817,000 in 2011, and a total of 1.3 million subjects known with DM in 2025. This estimate was performed based on five general practitioner registries which contained almost 500,000 members of the general population. Since then, the LOK (Dutch initiative for shared care organisations) has initiated data collection over larger groups of subjects in the years 2010 and 2011, concentrating on subjects with type 2 diabetes (T2DM), treated in shared care groups within a primary care setting. Therefore, it should be possible to make a more precise estimate of the total number of subjects with DM in the Netherlands.
Materials and methods: Collection of data was performed as part of the quality control initiative of the LOK. All shared care groups were invited to voluntarily submit relevant information to the Landelijke Organisatie Ketenzorg (LOK); 66 out of a total of more than 100 did so. Of these 66, 41 delivered sufficient information not only regarding the subjects with T2DM seen within the shared care environment, but also regarding the total amount of subjects known with DM (both T1DM and T2DM) in primary and secondary health care. Data were analyzed as part of the DUDE-initiative (DUtch Diabetes Estimates).
Results: Results are observational and self-reported, which might lead to bias. Still, in the primary care practices of these 41 shared care groups, a total of 5,185,801 members of the total population were known in 2011. Of these, the total amount of subjects known with DM was 276,739 (5.36%), 251,504 of which with T2DM (4.88%). In 2011, the Netherlands had a population of 16,725,902; this translates into 893,000 subjects known with DM (815,000 with T2DM) in 2011, 9.3% more than the RIVM estimate. Using this information as a base for prediction of DM in 2025, the RIVM estimate of 1.3 million will be surpassed by at least 200,000 more prevalent cases, mainly T2DM.
Conclusion: The prevalence of diabetes, especially T2DM, increases even quicker than recently estimated. When this growth remains unchecked, the prediction of the RIVM of 1.3 million subjects with DM in 2025 will be surpassed by at least 200,000. These new results further emphasize the need for both concerted programs for prevention and treatment of DM. It will also mean an extra disease and financial burden for both individuals and the total community when this growth remains unchecked.

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Evaluation of two screening methods for newly diagnosed diabetes in China: a cost-effectiveness study

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Background and aims: To evaluate the performance and cost-effectiveness of two screening methods to identify undiagnosed diabetes at primary care settings among a Chinese population.
Materials and methods: Two screening methods using a fasting capillary glucose (FCG) test or a Chinese diabetes risk score (DRS) at primary care settings followed by diagnostic tests were compared. The performance of FCG and DRS was evaluated by using receiver operating characteristic (ROC) curve analysis. The main economic outcome measures were the total cost of screening per 1000 persons, proportion of undiagnosed diabetes detected, and the cost per undiagnosed diabetes identified from the societal perspective.
Results: Among all participants, 26.6% (2453/9208) had undiagnosed diabetes defined by fasting plasma glucose≥7.0 mmol/l and/or 2hour plasma glucose≥11.1 mmol/l and/or hemoglobin A1c≥6.5%. At the optimal cutoff point of 6.2 mmol/l for FCG and 14 for DRS, the sensitivity was 42.7% and 62.5%, and specificity was 75.4% and 57.0%, respectively. The area under the ROC curve was 67.7% for FCG and 62.4% for DRS (P<0.001). Based on the input costs, the total cost of screening 1000 persons was ¥56000 ($8000) for FCG and ¥77000 ($11000) for DRS. The cost per case identified was ¥491 ($70) for FCG and ¥463 ($66) for DRS, at the optimal cutoff points.
Conclusion: As a first-line screening tool for undiagnosed diabetes, the FCG test performed slightly better than the DRS in primary care settings in China. The non-invasive and layperson-oriented DRS was more cost-effective and feasible.

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Screening for people with glucose metabolism disorders within the framework of the Demojuan project in Barranquilla, Colombia

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Background and aims: In Colombia, many people with type 2 diabetes (T2D) patients remain undiagnosed. The aims of this study were to implement the FINDRISC as a screening tool for disorders of glucose metabolism in five catchment areas of the primary health care system in Barranquilla in Colombia using the FINDRISC during 2011 and 2012, and to describe the risk factors for T2D in the population screened.

Materials and methods: This study was screening opportunistically people aged 34-60 years living in five primary health care catchment areas in Barranquilla, Colombia (Camino El Pueblo, Camino Simon Bolívar, Camino El Bosque de María, COOSALUD and Paso Juan Mina) using the Finnish Diabetes Risk Score (FINDRISC). People with 13 or more FINDRISC points were invited to an oral glucose tolerance test (OGTT).
Results: Out of 14193 participants with the FINDRISC, 35% (n=4915) had a score of 13 points or higher (men 23%, women 40%). Out of 14193 participants with the FINDRISC, 35% (n=4915) had a score of 13 points or higher (men 23%, women 40%). Only 46% of men and 35% of women had a BMI of less than 25 kg/m2. Similarly, central obesity using the WHO criteria (≥102 cm and ≥88 cm) was common (women 70% and men 57%). Only 33% of men and 20% of women reported that they reached the recommended 30 min of physical activity daily. The percentage for daily intake of fruits and vegetables was 32% in men and 34% in women. Approximately 40% of the people had family history of T2D.Hypertension was less common; 16% in men and 24% in women reported to use of antihypertensive drugs. The prevalence of screen-detected T2D was 18% in men and 12% in women, respectively. In both sexes the prevalence of isolated IGT, isolated IFG, and IFG and IGT combined were 8%, 11%, and 8%, respectively. />
Conclusion: Implementing the FINDRISC within the primary health care settings in a large city in Colombia helped to identify people with glucose metabolism disorders and T2D risk factors. The FINDRISC served its function of the initial screening tool well; 65% of the population screened had the score 12 points or under, and could be excluded from further procedures and blood testing. Furthermore, a special emphasis should be given not only to identify people at high risk of T2D as early as possible but also to implement population-wide actions in order to decrease the burden of overweight and obesity as well as to increase physical activity.
Clinical Trial Registration Number: NCT01296100
Supported by: BRIDGES grant Lily Diabetes

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Prevalence of carbohydrate metabolism disorders in patients with acromegaly
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Background and aims: Early carbohydrate metabolism disorders (ECMDs) and secondary diabetes mellitus (DM) are frequently associated with acromegaly. Several factors may be involved in the development of ECMD and/or progression to diabetes mellitus, amongst others the levels of growth hormone (GH) and/or IGF-1, age and gender of the patient, and duration of acromegaly. The aim of this study was to assess the prevalence of ECMDs in patients with acromegaly and compare the results with prevalence of glucose derangements in two population-based surveys (random sample of the adult population and adult population with a high risk of type 2 diabetes). We also analyzed the factors contributing to the development of glucose derangement.
Materials and methods: 97 acromegaly patients: men - 16, women - 81 (age - 56 [47.5-64.5], duration of acromegaly - 12.5 [7.5 - 20.3] years) were evaluated. As in 24 (24.7%) patients DM was diagnosed before acromegaly, an oral glucose tolerance test was done in the remaining 73 patients to reveal asymptomatic DM or ECMD (IGT and IFG). The activity of acromegaly was assessed by the international 2009 consensus criteria. There were two control groups: (1) a random sample of the adult population (2638 participants) and (2) an adult population with type 2 diabetes risk factors (n=2304). The groups had comparable to age and BMI.
Results: DM was diagnosed in 51 (52.2%) patients among 97 examined acromegalics. It was 3.5 times higher than the prevalence of DM2 in the Moscow county random population sample (14.7%). Newly-diagnosed diabetes was revealed in 27 (37%) patients among 73 acromegalics - it is 8.4 times higher than prevalence newly-diagnosed DM2 in general population (4.4%) and 1.5 times higher than prevalence in the high-risk DM2 group (25.2%). ECMDs were in 25 (34.2%) patients among 73 acromegalic patients - it is 3.4 times higher than prevalence ECMD in general population (9.9%) ($p<0.001$) and 1.3 times higher than ECMDs prevalence in the high-risk DM2 group (25.7%) ($p<0.01$). Peak of the DM and ECMD prevalence in acromegalic patients was observed in age interval 50-60 yrs. Acromegalic patients with ECMD or diabetes were older and more obese, and the proportion of women was highest in the diabetes group. Acromegaly duration was longer in those with diabetes. IGF-1 levels (z-score) were higher in subjects with ECMD or diabetes compared to acromegalic patients with normal glucose levels (5.1[3.3-6.6], 4.5[2.3-6.5] and 2.1[0.7-4.4] accordingly, $p=0.002$). Multinomial logistic regression yielded that the severity of glucose derangement was predicted by age, body mass index, female gender and level of IGF-1.
Conclusion: The prevalence of DM and ECMD among acromegalic patients considerably exceeds the prevalence of DM2 and ECMDs in the general population and in the high-risk DM2 group, and its development depends on age, BMI, gender and IGF1 level.

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Antibodies against the Epstein-Barr virus Nuclear Antigen (EBNA-1) in patients with autoimmune diabetes mellitus

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Background and aims: Type 1 diabetes mellitus (T1DM) and latent autoimmune diabetes in adult (LADA) are known as autoimmune types of diabetes mellitus. The etiology of autoimmune diseases is widely studied and environmental factors can contribute to the pathogenesis. The possible role of viral infections is still unclear. Several studies published that Epstein-Barr virus infection is associated with multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis and myasthenia gravis, but no relevant publication was found with diabetes mellitus. The aim of our study was to find a possible relationship between EBV infection and T1DM and LADA.
Materials and methods: Serum samples were collected at the onset of the disease from 52 patients having T1DM with adult onset (30 male/22 female) and 23 patients with LADA (15 male/8 female). The age range was from 18 to 59 years in T1DM group and from 20 to 63 years in LADA group. The diagnosis of T1DM was based on WHO criteria, and LADA was diagnosed if the patient had islet cell antibody (ICA) and/or glutamic acid decarboxylase antibody (GADA); and insulin therapy was not required in the first six months after the diagnosis. Our study included also sex and age matched healthy control subjects (30 male/22 female; 16-58 years and 15 male/8 female; 17-63 years), in whom any type of diabetes mellitus and autoimmune diseases were excluded. The serum concentration of antibodies against the Epstein-Barr virus nuclear antigen (EBNA-1) was determined using a commercial enzyme-linked immunosorbent assay. The anti-EBNA-1 appears 2-4 months after the infection and can be detected during the whole life.
Results: At the diagnosis of diabetes, 94.2% of the patients with T1DM and 100% of the patients with LADA had at least one autoantibody positive either ICA and/or GADA. The median concentrations of fasting glucose and HbA1C were the following in the T1DM and LADA group, respectively: 8.98 and 8.94 mmol/l, 10.17 and 7.75%. The percentage of seropositive subjects was 92.3% vs 84.6% in the T1DM vs control group, and 86.9% vs 73.9% in the LADA vs control group, the difference was not significant between the groups. The median level of anti-EBNA-1 IgG was 165.50 AU/ml in the T1DM group (25% - 75% percentiles: 48.84-370.30 AU/ml), and 91.16 AU/ml in the control group (33.76-179.60 AU/ml). The median level of anti-EBNA-1 IgG was 77.00 AU/ml in the LADA group (30.93-264.50 AU/ml), and 89.71 AU/ml in the control group (8.58-178.71 AU/ml). The statistical analysis demonstrated significant difference in the concentration of anti-EBNA-1 IgG between the newly diagnosed patients with T1DM and healthy subjects ($p<0.01$), but it did not differ between the patients with LADA and healthy subjects. The level of GAD antibody did not correlate with that of anti-EBNA-1 IgG.
Conclusion: Our study demonstrates that anti-EBNA-1 IgG levels measured in the serum of patients with T1DM at the onset of the disease was higher than in the serum from non-diabetic subjects. We did not find the same correlation between patients with LADA and healthy controls. Our result may suggest a different immunological response to EBV infection, which can reflect a difference in the immune and/or autoimmune responses of patients with T1DM and LADA.
Clinical Trial Registration Number: OTKA-NKTH 80842

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The risk of type 1 diabetes mellitus in offspring of immigrants in Sweden

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Background and aims: The epidemic of childhood type 1 diabetes (T1DM) is accelerating in many parts of the world. The lowest incidence of T1DM is reported from China and Venezuela and the highest from Finland, Sardinia and Sweden. Investigating the occurrence of T1DM in immigrants and their offspring offers a unique possibility to explore and delineate the gene-environment interaction for the development of type 1 diabetes. We investigated the risk of developing T1DM in offspring of Swedes and immigrants by specific parental migration background.
Materials and methods: Using Swedish nation-wide data we analyzed the risk of developing T1DM in 3,593,765 female and 3,794,477 male offspring born to native Swedes or immigrants. The subjects were between 0 to 30 years of age and born and living in Sweden between 1969 and 2009. We calculated Incidence rate ratios (IRRs) with 95% confidence intervals (CIs) using Poisson regression models.
Results: Compared with Swedes, children (0 to 14 years of age) and young adults (15 to 30 years of age) with only one parent born abroad had about 30% and 15% to 20% lower IRR for T1DM,respectively, after adjustment for age, calendar period and parental education and regardless of sex. Girls and boys with both parents born abroad had about 40% and young adults had about 25% to 30% decreased risk of TIDM compared with children and young adults of Swedes. These reductions in risks in both children and young adults with immigrant parents retained in all ethnic groups except for offspring of Africans in whom the risk was increased. Compared with offspring of Swedes aged 0-30 years, male and female offspring of mothers or fathers born in Africa had about 20% to 40% higher IRR for T1DM. The increased risk was more pronounced in individuals with parents from Eastern Africa, with a 45% to 60% higher risk compared with offspring of Swedes. With a few exceptions, offspring of any parent born in Asia, Europe (except Northern Europe), Latin America and Northern America (except female offspring to fathers from Northern America) had between 35% to 65% lower IRR compared with offspring of Swedes. Offspring of Finnish immigrants and offspring with parents from other parts of Northern Europe had almost similar risks compared with offspring of Swedes.
Conclusion: Ethnic background and its interaction with environmental factors play an important role in the etiology of TIDM. Further studies on offspring of immigrants from African countries, and in particular from Eastern Africa, might improve our understanding on the etiology of the disease.
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Non-HLA genes and familial predisposition to autoimmune diseases in families with a child affected by type 1 diabetes

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Background and aims: Autoimmunity clusters in individuals and in families. Genetic predisposition could be assumed to be the reason for this clustering but we have recently failed to provide convincing evidence for an increased HLA class II mediated risk in families with clustered autoimmunity. To date, more than 60 loci outside HLA have been associated with type 1 diabetes. As most of these have functions related to the immune system, many of them associate with other autoimmune diseases in addition to type 1 diabetes. Thus, we tested the hypothesis that these non-HLA loci would associate with clustering of autoimmunity in familial type 1 diabetes, in children with multiple autoimmune diseases, or in children with a family history for autoimmune diseases.
Materials and methods: We included 1785 children with type 1 diabetes diagnosed before the age of 15 years (median 7.9, 57% male) from the Finnish Pediatric Diabetes Register. Data on family history of autoimmune diseases in first- and second-degree relatives at diagnosis of the index child were collected with a structured questionnaire. The Sequenom (San Diego, California, USA) platform was used to genotype 40 single nucleotide polymorphisms (SNPs). Statistical analyses were carried out with PLINK v1.07 software package. Three SNPs and 41 individuals failed the missingness tests (call rate >90% and >50%, respectively).
Results: Only 27 children had known multiple autoimmune diseases at diagnosis. This phenotype was associated with rs17388568 (ADAD1, OR 1.74, 95%CI 1.01-3.00) and rs17696736 (C12orf30, OR 0.32, CI 0.30-1.00). Three SNPs; rs2476601 (PTPN22, OR 1.35, CI 1.05-1.74), rs12061474 (PIK3C2B, OR1.31, CI 1.00-1.71), and rs3184504 (SH2B3, OR 0.79, CI 0.63-1.00) associ-

ated with having a first-degree family member with type 1 diabetes (n=180). Having a first-degree family member with another autoimmune disease (n=221) associated with rs9653442 (OR 0.78, CI 0.64-0.96), and with IFIH1 SNPs rs2111485 (OR 0.80, CI 0.64-1.00) and rs1990760 (OR 0.80, CI 0.65-1.00). Being a member of an autoimmune family (>3 autoimmune diagnoses or >2 different autoimmune diseases in the extended family, n=203) associated with rs11711054 (CCR2, OR 0.66, CI 0.52-0.86). Correcting for multiple tests removed statistical significance from all these findings, however.
Conclusion: Some genetic loci reached nominally significant association with clustered autoimmune diseases. PTPN22 was a risk loci for familial type 1 diabetes. These findings did not survive the correction for multiple testing, however. This may be due to the relatively small sample size leading to inadequate power. Larger studies are needed to unravel the possible non-HLA mediated predisposition to clustering autoimmunity. In addition, these findings emphasize the role of environmental factors predisposing to clustering autoimmunity.
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Mortality and having offspring in type 1 diabetes
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Background and aims: Type 1 diabetes is associated with increased mortality compared with the general population, from both acute and long-term diabetic complications. Previous studies have shown that mortality in the general population is higher among women and men who do not have any offspring than among those who have children. Both men and women with childhood-onset type 1 diabetes have fewer offspring than the general population. Our aim was to examine mortality and causes of death among subjects with childhood-onset type 1 diabetes compared to control people, with focus on mortality differences between childless people and those having had offspring.
Materials and methods: The people with diabetes in our study comprise the Finnish DERI cohort (Diabetes Epidemiology Research International). They were all diagnosed with diabetes at 17 years of age or under during 1965-1979 and placed on insulin at diagnosis. 5,162 cases were identified nationwide with an ascertainment rate approaching 100 percent; 2,327 of them were women. Two non-diabetic control persons for each person in the DERI cohort were selected from the database of the national Social Insurance Institution, matched for the year of birth, geographical birth region and gender. This register-based study uses information on the offspring from the national Population Register Centre. All statistical analyses were done using Cox proportional hazards model.
Results: 1,025 people with diabetes and 497 people without diabetes died during the follow-up until the end of 2010. All-cause mortality in people with diabetes was significantly higher than that of control persons, both among men and women ($p>0.01$). All-cause mortality was statistically significantly lower in persons who had offspring ($p<0.01$), among both cases and controls, and in both genders. Among females, having children lowered mortality in a similar way in diabetic persons and controls ($p=0.99$ for interaction). In males, the difference in the shapes of the mortality curves by the number of offspring was statistically significant between diabetic and control people ($p<0.01$ for interaction).
Conclusion: The beneficial effect of having offspring on mortality was observed. It was, however, significantly smaller among men with diabetes than among men in the control group. In women, having offspring was associated with lower mortality in a similar way regardless of the diabetes status. One possible reason for this gender difference is that women with type 1 diabetes are trained and well motivated to achieve better metabolic control during pregnancy and that this motivation may persist also post partum.
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Quality of life, age at onset of type 1 diabetes and metabolic control in a cohort of patients followed for 16 years
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Background and aims: Progressive adaptation to type 1 diabetes (T1DM) is paramount to improve quality of life (QoL) and other psychological dimensions. This study aimed at identifying possible correlations between QoL, Locus of Control (LoC) and clinical variables in patients with T1DM over a 16 year follow up.
Materials and methods: Fifty-nine patients (27 women) with T1DM, age 31.3±2.0, duration of disease 23.0±4.0, part of a cohort of 112 followed since 1996 accepted to participate. Nine patients had completed middle school, 32 high school and 18 had a university degree. HbA1c was 8.8±1.7 at baseline and 8.0±1.2 after 16 years. Patients were divided into those in whom onset of T1DM had been during the first 5 years of life (n=16) or later (n=43). They were also stratified into Worsened (n=7), Stable (n=23) and Improved (n=27), based on whether their HbA1c had increased/decreased by 1 percentage point between baseline and last follow up visit (7.6±1.8 to 9.6±1.9; 7.8±0.8 to 7.7±0.8; and 9.8±1.4 to 7.6±0.8, respectively). QoL was measured by the Diabetes Quality Of Life questionnaire (DQOL), translated into Italian and re-validated. The DQOL includes 46 items that explore 4 dimensions: Satisfaction, Impact, Diabetes Related Worries and Social/Vocational Worries. The LoC was measured by the Peyrot and Rubin specific questionnaire, composed of 18 items that evaluate 3 dimensions of control of disease: Internal Control, Role of Chance, Powerful Others.
Results: Compared with patients who developed T1DM later in life, those in whom onset had been before age 5 had better total DQOL score (69.3±8.3 vs 81.0±18.3, $p=0.022$), due to the Satisfaction dimension (25.0±4.0 vs 30.2±7.8, $p=0.016$), and a tendency to decreased fatalism (Role of Chance, 8.4±3.0 vs 10.5±4.0 $p<0.06$) in adult age. Age of onset higher than 5 and frequent episodes of hypoglycaemia scored the highest ORs (7.6, $p<0.10$ for age of onset and 20.3, $p<0.01$ for frequent episodes of hypoglycaemia) in a multivariate logistic model with HbA1c as dependent variable, dichotomized in 'Worsened' vs all others. All subjects with worsened HbA1c had their diagnosis after 5 years of age and more frequent episodes of hypoglicaemia.
Discussion: Onset of T1DM during the first 5 years of life may result in better QoL and less fatalism in the long term. Presumably, these patients have no memory of disease onset, which may reduce trauma and facilitate adaptation to managing life with diabetes.

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50 years of childhood type 1 diabetes: clinical characteristics of survivors from the Pittsburgh Epidemiology of Diabetes Complications study (EDC)
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Background and aims: More individuals with childhood onset type 1 diabetes are now surviving for 50 years or more after diagnosis. To characterize these survivors, data from the EDC study of childhood onset (<17 years) T1D were examined.
Materials and methods: The EDC study is based on an historical-prospective cohort (n=934) diagnosed between 1950 and 1980 and first seen at Children's Hospital of Pittsburgh within 1 yr of diagnosis. The first examinations and surveys for EDC were conducted between 1986-1988. If they all had lived, 341 would have had a T1D duration of ≥50 yrs by June 1, 2013. Of these, 146 had already died (predeceased) before baseline exam, while 91 died after baseline (1986-1988), and 99 have so far survived.
Results: The predeceased did not differ by age at diagnosis or gender but their mean calendar year of diagnosis was 2 years earlier (1956 vs 1958) than those surviving until baseline ($p<.0001$). As expected, those surviving to ≥50 yrs duration had fewer complications (renal, proliferative retinopathy, distal symmetrical polyneuropathy(DSP), cardiac autonomic neuropathy and coronary artery disease (CAD)) and better anthropometric (weight, BMI), CAD (hypertension, lipids, smoking, white blood cell (WBC)) and diabetes related (HbA1c, insulin dose/kg body weight) baseline risk factors compared to those dying after baseline, except that no differences were seen for gender, weight,

BMI, pulse rate, insulin dose/kg body weight (p values all >0.50), or diastolic blood pressure (p=0.15). Multivariable analyses allowing for age, duration of diabetes and all complications suggested that micro-or-greater-albuminuria (p<0.0001), coronary artery disease (p=0.0006) and distal symmetric polyneuropathy (p=0.01) were all independent predictors in the best fitted model. A similar model for risk factors (excluding renal markers) showed triglycerides (p<0.0001) and WBC (p=0.002) to be the strongest independent predictors. The best fitting combined model of complications and risk factors was comprised of all of these predictors (ie. micro-or-greater-albuminuria, CAD, DSP, triglycerides and WBC).
Conclusion: These results suggest important roles for dyslipidemia and inflammation, in addition to the development of complications per se, for long term survival in T1D, while the role of HbA1c appears to be largely explained by development of these complications and risk factors.
Supported by: NIDDK

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Life expectancy in type 1 diabetes: a Scottish Registry Linkage study
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Background and aims: Historically those with type 1 diabetes mellitus (T1DM) have been reported to have a reduced life expectancy compared to the general population but estimates are seldom based on contemporary data. Given advances in medical care and the availability of nationwide data we sought to determine current life expectancy (LE) in people with T1DM in Scotland.
Materials and methods: The nationwide Scottish Care Information - Diabetes Collaboration database contains data for over 99% of individuals with diabetes in Scotland. Anonymised data extracted from this database was linked with death data from the General Register. Abridged period life tables for those with T1DM in Scotland aged 20 years and older were derived using Chiang's method with 5 year age intervals (plus an open-ended interval for 80 years and older), and a 3 year time interval from 2008-2010, corresponding to latest Scottish general population abridged life tables from the Office of National Statistics (ONS) . 95% Confidence Intervals (CI) for the expected future life time at each age group were derived using Monte-Carlo simulations on the estimated probability of death during each age interval. The period LE estimates for T1DM were compared with interim estimates from the ONS for the general population of Scotland calculated over the same period. These period rather than cohort LE estimates do not attempt to use trends in LE to project further improvements in survival in the future.
Results: 24,971 persons aged 20 years and older were identified as living with T1DM in Scotland at any point in the calendar year period 2008-2010 inclusive, contributing 68,392 person years of follow-up and 1,079 deaths. The population at the mid-point of the study period was 22,592 persons. In those with T1DM, the remaining LE for attained age group 20-24 years was 45.4 years (95% CI: 44.2, 46.5) and 46.6 years (95% CI: 45.2, 47.9) for men and women respectively compared to estimates of 56.5 and 60.9 years respectively for the male and female general populations. The remaining LE at attained age group 65-69 years was estimated at 11.7 years (95% CI: 11.0, 12.6) for men and 12.5 years (95% CI: 11.8, 13.2) for women with T1DM compared to 16.8 and 19.3 for the male and female general population. Overall the difference in life expectancy between those with T1DM versus the general population ranged from 11.1 to 14.3 in men and women respectively at attained age group 20-24 to 5.1 and 6.8 in men and women respectively at attained age group 65-69.
Conclusion: These period LE estimates show that there has been a marked improvement in LE for men and women with T1DM compared with earlier reports. These improvements should now be reflected in life insurance and other relevant policies for those with T1DM. Despite these improvements there remains a substantial gap in LE between the T1DM and general populations that needs to be addressed.
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Excess mortality in early adulthood in a population-based cohort of childhood onset type 1 diabetes
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Background and aims: Recent reports have suggested that mortality in young adults with Type 1 Diabetes (T1DM) has reduced and it is proposed that this is a result of improved glycaemic control and care during childhood. To date however, data have not been available to link population based phenotypic and clinical data during childhood with mortality in young adulthood. The primary aim of this study was to determine the standardised mortality rate for young adults in Western Australia (WA) who had childhood onset T1DM relative to the general population. A secondary aim was to examine the association between demographic and treatment factors during childhood with risk of mortality.
Materials and methods: All children in WA with T1DM aged ≤16 years attend 3-monthly clinics facilitated by Princess Margaret Hospital with clinical records stored in the WA Childhood Diabetes Database (WACDD - established 1987, case ascertainment ≥99%). Records for the full paediatric management period (from diagnosis until transition to adult clinics) for all patients with T1DM from the WACDD born prior to 1st Jan 1993 were extracted and linked to the death registry by the WA Data Linkage Branch. A non-T1DM comparison group matched for date of birth and sex (matched 5:1) were randomly selected from the general population birth registry. Cause of death was determined from the death registry and was based on the International Classification of Diseases coding system. Statistical analyses were completed in R using both univariate and multivariate Cox proportional hazards models (adjusted for birth year) to estimate Hazard Ratios (HR).
Results: Data were available for 1,337 T1DM patients and 6,663 non-T1DM population subjects. Mean age of T1DM diagnosis was 9.5 (SD 4.1) years, mean duration of T1DM was 16.7 (SD 6.4) years and median cohort age at end of follow-up was 26.2 (SD 5.1) years. The overall mortality ratio for the T1DM group (20 deaths observed) relative to the non-T1DM group was 3.32 (p<0.001). The mortality ratio was higher among females (6.53, p<0.001) than males (1.67, p=0.31). Deaths from accidental narcotic overdose, cardiovascular disease and suicide were observed at higher rates (20 times, 7.5 times and 2.5 times respectively) in the T1DM group. Within the T1DM group, factors associated with an increased risk of all cause mortality included a low or middle level socio-economic background, relative to those from a high level socio-economic background (7.6 times, p=0.008; HR 3.7, p=0.1 respectively), a higher mean A1c during paediatric management (HR 1.54, p=0.005 for each 1% increase in mean A1c %) and a history of > 3 severe hypoglycaemic events during childhood.
Conclusion: The mortality rate is 3.3 times higher during early adulthood for those with childhood onset T1DM relative to the general population. Poor glycaemic control, recurrent severe hypoglycaemia and lower socio-economic status are predictors associated with increased risk of mortality. Knowledge of the risk factors for death in young adults with childhood onset T1DM will allow targeted intervention aimed to reduce the mortality risk.
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Associations of genetic variants in thioredoxin (TXN) and mitochondrial thioredoxin reductase (TXNRD2) genes with kidney disease in type 1 diabetes
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Background and aims: Deregulated cellular redox balance is a key factor in the development of diabetic nephropathy and impairment of the thioredoxin (Trx) system has already been demonstrated. This thiol-reducing system comprises Trx (encoded by *TXN),* thioredoxin reductases (the mitochondrial isoform is encoded by *TXRD2*) and a natural Trx inhibitor (encoded by

TXNIP). In the present study we analyzed associations of single nucleotide polymorphisms (SNPs) in *TXN, TXNRD2* and TXNIP with kidney disease in type 1 diabetes patients.
Material and methods: Three SNPs, rs2301242 (promoter region of *TXN*), rs3788319 (promoter region of *TXNRD2*) and rs7211 (3'UTR of *TXNIP*) were genotyped in 448 patients with type 1 diabetes (44.6% with diabetic nephropathy) by real time PCR.
Results: The minor allele A of rs2301242 in *TXN* was associated with established/advanced nephropathy in the overall population (OR 2.30, CI95% 1.11 - 4.81, p=0.0260). The minor allele A of rs3788319 in *TXNRD2* gene was associated with a low estimated glomerular filtration rate (eGFR) in men (p=0.0413) and the genotype AA was associated with established/advanced nephropathy in the overall population (OR 1.50, CI95% 1.04 - 2.16, p=0.0284). No associations were found for rs7211 in *TXNIP*.
Conclusion: The SNPs rs2301242 in *TXN* and rs3788319 in *TXNRD2* modulate the risk for renal disease in the studied population of type 1 diabetes patients and require validation in independent cohorts.
Supported by: FAPESP

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Experimental gene therapy of type 1 diabetes mellitus: dose-dependent effects on glucose levels in blood plasma of mice and rats
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Background and aims: Type 1 diabetes mellitus is associated with a destruction of insulin-producing pancreatic beta cells. In spite of the fact that type 1 diabetes is a multifactor disorder,to date there is no other method of treatment of the disease except insulin therapy. However, treatment with exogenous insulin is not perfect, since it may not always provide glycemic control within the physiological range, because the dose is adjusted empirically, taking into account the hyperglycaemia and disease duration, which leads to serious complications and decompensation. A radical treatment may be provided only by gene therapy after administration of a insulin gene integrated in such a molecular construction that would provide its synthesis in non-specific normal cells which do not produce endogenous insulin.
Materials and methods: Delivery of vectors containing a target gene to the body is the main challenge of gene therapy. We have optimized the system of in vivo delivery of a constructed plasmid vector containing human proinsulin gene, to hepatocytes of animal models using polyplexes. A model of experimental diabetes induced by streptozotocin (STZ) (Sigma, U.S.A.)at a dose 50 mg/kg, has been obtained in C57BL/6j mice and Wistar rats. DNA preparations were injected to liver parenchyma surgically. Transfection efficacy was determined on a flow cytometer, using an Enhanced Green Fluorescent Protein (EGFP). The highest efficacy of transfection was 66%. Glucose content in blood plasma was tested using a test-system "Hemoglan" from ("Norma", Ukraine) and on an analyzer "SUPER GL" (Germany).
Results: Investigations have demonstrated that proinsulin gene transfection led to a decrease in blood glucose levels in C57BL/6j mice and Wistar rats with different duration of STZ diabetes: 16 days (group 1) and 36 days (group 2 of animals). After administration of a plasmid with preproinsulin gene to mice on day 16 and 36 of STZ diabetes development, a decrease was noted, in glycaemia level from 18.78±1.74 to 9.78±1.08 mmol/L, and from 23.63±2.10 to 10.97±2.22 mmol/L (respectively) two weeks after experiment started. Proinsulin target gene administration to rats on day 16 and 36 of STZ diabetes development led to a significant decrease in blood glucose levels from 24.77±1.49 to 8.0±1.79 mmol/L, and from 31.10±1.84 to 13.40±3.67 mmol/L (respectively) two weeks after experiment started. Administration to liver parenchyma of rats with STZ diabetes of different concentrations of DNA preparations(within the range 30 to 40 μg of DNA per 200g of body weight) allowed to determine optimum DNA doses that led to a significant decrease (normalization) of blood glucose levels. It should stressed that a decrease in glycaemia level in mice and rats after a single procedure of gene therapy lasted for 8-10 weeks at most, after which blood glucose levels were increasing.
Conclusion: These results show that regression of diabetes has been achieved after a single course of gene therapy for STZ diabetes in rats and mice. A more pronounced decrease in glucose levels and a higher survival rate in mice was noted when using DNA at a dose 15 μg/g of body weight, compared with the group of mice which were administered with a dose 10μg/20g of body weight.

PS 005 What predicts type 1 diabetes?

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Foetal transfer of preproinsulin through the neonatal Fc receptor protects from type 1 diabetes
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Background and aims: Autoimmune type 1 Diabetes (T1D) results from a failure of immune tolerance towards beta cells, in which preproinsulin (PPI) is the initial target antigen triggering the autoimmune cascade. Since the neonatal Fc receptor (nFcR) is highly expressed in the placenta, we hypothesized that 1) Fc-coupled PPI may be efficiently transferred from mothers to foetuses; and 2) this early PPI introduction may "teach" the immune system to develop tolerance towards PPI before autoimmune epitope spreading, thus preventing subsequent T1D development.
Materials and methods: A PPI-Fc fusion protein was injected i.v. to pregnant G9C8 Cα-/- NOD mice, which are transgenic for a T-cell receptor recognizing the PPI B15-23 epitope. PPI-Fc transfer was documented by in vivo imaging and ELISA assays; its cellular uptake and subsequent effects on pathogenic and regulatory T cells was followed by flow cytometry along with effects on T1D incidence at the adult age.
Results: PPI-Fc was efficiently and selectively transferred to foetuses through binding of the Fc portion to nFcR. One single 100 μg injection of PPI-Fc was sufficient to protect the offspring from subsequent T1D development. This protection was associated with PPI-Fc uptake by migratory dendritic cells, which ferried it to the thymus; with a later decrease in circulating pathogenic CD8+ T cells; and with an increase in regulatory T cells specifically found in pancreatic lymph nodes.
Conclusion: Coupling of PPI with an Fc fragment allows its transfer from mothers to offspring and protects from T1D following a single low-dose administration. PPI-Fc ferrying to the thymus and subsequent changes in pathogenic and regulatory T cells suggest that a central tolerance mechanism may be at play. Since the human nFcR is also expressed throughout life on the intestinal epithelium, the translation of this strategy into clinical trials could be envisaged.
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Lower physical activity in children with persistent islet autoantibodies than in matched controls
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Background and aims: Progression from islet autoimmunity to type 1 diabetes may be slower in physically active persons due to their higher insulin sensitivity or other effects of exercise. Physical activity of high-risk children who have developed persistent islet autoantibodies but remain free of diabetes has not been previously assessed using direct methods, such as accelerometry. Here we compare directly assessed physical activity of islet antibody positive children and genetically comparable autoantibody negative controls.
Materials and methods: Diabetes Autoimmunity Study in the Young (DAISY) has been following since 1993 a cohort of 2547 children at genetically increased risk for type 1 diabetes. Of those, 198 children have developed persistent islet autoantibodies (to insulin, GAD65, IA-2 or ZnT8) and 70 have progressed to diabetes. Of the 97 children with persistent islet autoantibodies who are in active follow-up, but have not yet progressed to diabetes, parents of 62 agreed to participate in this study. Islet antibody-negative DAISY participants were frequency matched to the islet autoimmune group on age and gender and 50 of these were enrolled as controls. Children wore an Actigraph GT3X accelerometer (ActiGraph, Pensacola, FL) for 7 days to record physical activity. Physical activity was classified by counts per minute (cpm) as sedentary (≤400 cpm), light (401-1900 cpm), moderate (1901-3919 cpm) and vigorous (≥3920 cpm).
Results: Children with persistent islet autoantibodies and controls were similar in terms of age, sex, BMI, and waist circumference (TABLE). In mul-

tivariate linear regression models, adjusted for age, sex, BMI and total time recorded, children with islet autoimmunity spent a higher percentage of time sedentary and less time engaged in vigorous physical activity than controls and took fewer total steps and steps per minute.

TABLE. Characteristics and physical activity measures of study participants

Characteristic	IA+ cases n=62	IA- controls n=50	p-value
Age [y]	12.7 ± 3.6	13.0 ± 3.9	0.67
Sex [M]	55%	53%	0.83
BMI [kg/m2]	19.9	21.1	0.20
Waist circ. [cm]	72.4	74.7	0.40
Time sedentary*	85 ± 0.5%	83 ± 0.6%	**0.045**
Time of vigorous activity*	2.9 ± 0.2%	3.6 ± 0.2%	**0.01**
Total steps*	52,561 ± 2,126	59,882 ± 2,354	**0.02**
Steps per minute*	9.3 ± 0.4	10.6 ± 0.4	**0.03**

For the *adjusted models, TABLE presents least square means ± SE.

Conclusion: Children who are persistently positive for islet autoantibodies and at high risk for type 1 diabetes engage in slightly lower physical activity than antibody-negative children of similar age, sex, BMI and genetic susceptibility to diabetes. These differences translated into ~110 extra minutes spent sedentary and 70 fewer minutes spent doing vigorous activity per week. Whether lower levels of activity among some of the autoantibody positive children will accelerate the development of diabetes requires further follow-up of the study participants.
Clinical Trial Registration Number: NIH R01 DK032493

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Prognostic and diagnostic relevance of ZnT8 antibodies in autoimmune diabetes

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Background and aims: Autoantibodies (AAbs) are highly specific and sensitive markers of autoimmune diabetes. Beside GADA, IA-2A and IAA, AAbs against zinc transporter 8 (ZnT8A) are part of autoimmunity in human type 1 diabetes (T1D). A single nucleotide polymorphism in the SLC30A8 gene at aa 325 encoding Arg, Trp and Gln causes the induction of different ZnT8A variants ZnT8RA, ZnT8WA and ZnT8QA, respectively. The present study aimed at differentiating ZnT8A in patients at onset of T1D, LADA and AAb-positive subjects from the general population to examine their diagnostic value and their correlations with the other AAbs.
Materials and methods: Sera from 102 T1D patients at disease onset (median age 13 yr, interquartile range (IQR) 8-20 yr), 97 LADA patients (median age 59 yr, IQR 48-66 yr; median latency to insulin treatment 5 yr, IQR 2-9 yr), 139 AAb-positive schoolchildren from the Karlsburg type 1 diabetes risk study, of those 31 with multiple AAbs as identified by combined screening for GADA, IA-2A and IAA, were analyzed for different ZnT8A variants based on immunoprecipitation of the 35S-Methionine labeled antigens. Additionally, 113 sera from patients with Type 2 Diabetes (T2D; median age at disease onset 49 yr, IQR 35-92 yr) were included.
Results: In T1D patients the ZnT8A prevalence amounted to 51% (n=53), predominantly directed against all three ZnT8 isoforms in 24.5% (n=25). ZnT8WA only were found in 10.7% (n=11), ZnT8RA only in 7.8% (n=8) whereas ZnT8QA only did not appear. In contrast, none of the LADA patients with an overall ZnT8A prevalence of 25.8% (n=25) had reactivity against all three ZnT8 isoforms. Among the single reactivities, ZnT8WA was most frequent with 11.3% (n=11) whereas both ZnT8RA and ZnT8QA only were found in 2.1%. Only 3.5% (n=4) of the patients with T2D had ZnT8A, all directed against ZnT8R only. The prevalence of ZnT8A in high risk schoolchildren with multiple AAbs was 67.7% (n=21), predominantly directed against all ZnT8 isoforms in 32.2% (n=10), similar to T1D patients. In contrast, only 2.8% (n=3) of the schoolchildren at lower risk and with single AAbs were positive for ZnT8A, however two of them progressed to T1D. In T1D, LADA and AAb-positive children ZnT8WA were highly correlated with ZnT8QA (r_S= 0.61-0.94; p<0.0001). In T1D patients and AAb-positives all three ZnT8 isoforms were correlated with IA-2A (r_S= 0.29-0.53; p<0.0001). Additionally, in LADA patients and in AAb-positive children ZnT8WA and ZnT8QA were correlated to a lesser extent with GADA (r_S= 0.21-0.41; p=0.033-p<0.0001).
Conclusion: ZnT8A were detected in all investigated groups but especially in probands with beta cell directed autoimmunity. In patients with T1D and AAb-positive at-risk probands, ZnT8A are directed against multiple variants and AAbs against ZnT8Q only are rare, whereas in LADA patients AAbs against ZnT8W only are most frequent. The strong correlation between ZnT8A and the particular high risk marker IA-2A in patients with T1D and at-risk probands as well as the fact that ZnT8A were detected in schoolchildren previously classified as low risk probands but with later progression to T1D further underlines the importance of these AAb in autoimmune diabetes.

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Levels of 12-HETE are elevated in patients with newly diagnosed but not with established type 1 diabetes

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Background and aims: Global, non-targeted metabolomic screening identified 12-hydroxyeicosatetraenoic acid (12-HETE) as being elevated in serum from patients with newly diagnosed type 1 diabetes (T1DM) relative to controls. 12-HETE has been implicated in inflammatory processes and associated with oxidative stress. It has been extensively linked with pancreatic beta cell dysfunction in both human and mouse islets. This study aimed to determine if this result could be validated in a larger cohort of samples, in a range of disorders of glucose metabolism, using a more specific and quantitative ELISA test, to determine if 12-HETE may be of potential value as a biomarker for diabetes.
Materials and methods: 12-HETE levels were measured in serum from both adult and paediatric patients using a commercially available ELISA. The adult cohort studied included newly diagnosed T1DM (n=8), established T1DM (n=29), newly diagnosed type 2 diabetes (n=34), poorly controlled type 2 diabetes (n=21), impaired glucose tolerance (n=10), controls with BMI ≤ 30 (n=29) and controls with BMI ≥ 30 (n=15). The paediatric cohort included newly diagnosed T1DM (n=14), established T1DM (n=39) and control individuals (n=52).
Results: Significantly raised 12-HETE levels were identified in this study in both adult (2.31 fold, p=0.0022) and paediatric newly diagnosed T1DM specimens (2.60 fold, p=0.0016) compared to controls while levels in established T1DM specimens were similar to levels in control specimens. ROC curve analysis evaluates the power of 12-HETE to distinguish newly diagnosed T1DM patients from control individuals. AUC values of 0.867 for adults and 0.797 for children were calculated for 12-HETE in the T1DM new cohorts analysed here, placing 12-HETE in the average to good range of biomarker evaluation criteria. Sensitivity rates of 100% in adult and 91.7% in paediatric specimens indicate the power of 12-HETE to identify true positive T1DM new patients from control individuals.
Conclusion: We have demonstrated elevated levels of 12-HETE in newly diagnosed T1DM which suggests a potential role for 12-HETE in the inflammatory processes underscoring the condition. Further studies are required to determine possible utility of 12-HETE as a marker of risk of diabetes development in at-risk individuals.
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Relationship of serum cytokines and proinflammatory markers to development of type 1 diabetes

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Background and aims: Elevated tumor necrosis factor α (TNFα), C-reactive protein (CRP), chemerin, fetuin-A, have been reported in insulin resistance patients with type 2 diabetes. It is known that insulin resistance and distur-

bances of insulin secretion play a role in type 1 diabetes. However, it is not entirely clear whether these alterations in adipokine serum concentrations are already present in prediabetic states and in type 1 diabetes. The aim of the study was to investigate whether adipokine profiles (chemerin, fetuin-A, TNFα) and inflammatory markers (CRP) are associated with decreases in insulin sensitivity and beta cell function in first degree relatives of patients with type 1 diabetes (T1D) and their usefulness in the assessment of the risk of development of diabetes.

Materials and methods: The study was conducted in 90 first- degree relatives of the patients with T1D (mean age 27.1±15.48 years; mean BMI 24.6±4.95 kg/m^2) and 60 healthy individuals (mean age 29,9±13,7 years; mean BMI 22,8±2,3 kg/m^2). TNFα , chemerin and fetuin-A concentrations were determined using ELISA method, CRP by turbidimetric method. HOMAIR and HOMA%B indices were calculated using computer calculator from the Website Oxford Centre for Diabetes, Endocrinology and Metabolism.

Results: CRP and TNF-alpha concentrations were significantly higher in the relatives compared to the controls (3.27mg/L±2,48 vs 1.14±1.39; p<0,007; 8.58pg/ml±15.49 vs 0.63±0.79, p<0.005, respectively). We found significantly higher chemerin concentration (0.6848±28.67 vs 0.5112±32.41, p<0.006) and fetuin-A concentration (276.92±30.9 vs 214.66±34.2; p<0.001) in the study group as compared to the healthy controls. Significantly higher HOMAIR (1.16±0.63 vs 0.79±0.34, p<0.002) and significantly lower HOMA%B index (92.84±29.39 vs 114.0±47.06, p<0.015) were found in the relatives as compared to the controls. HOMAIR correlated positively with TNFα (r=0.430, p<0.001), and chemerin (0.732, p<0.0001) and fetuin-A (0.454, p<0.0001) and CRP (r=0.445, p<0.0001). HOMA%B negatively correlated with TNFα (r=-0,431, p<0.0001) and CRP (r=-0,460; p<0.0001).

Conclusion: Alterations in chemerin, fetuin-A, CRP and TNFα are already detectable in first degree relatives of T1D, and may reflect to identify insulin resistance as an early pathogenetic event before T1D development. Fasting levels of chemerin, fetuin-A, CRP and TNFα might be used as biomarkers to identify insulin resistance and decrease of beta cell function in healthy individuals with higher risk of T1D.

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Targeting IL-15 for immunotherapy in autoimmune diabetes

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Background and aims: Inflammatory cytokines promote the development of autoimmune diseases by different mechanisms. Recently, we have also shown that NOD mice lacking IL-15 are protected from T1D due to inefficient activation of diabetogenic CD8+ T cells. Furthermore, inhibition of IL-15 signaling using a mAb to IL-15R beta chain, during the initiation phase of the disease, protected NOD mice from T1D. A greater understanding of the effect of inhibiting IL-15 signaling at different stages of T1D will lay the foundation to develop strategies to target IL-15 signaling in human T1D patients. The aim of this study is to determine whether IL-15 signaling blockade is effective in inhibiting autoreactive T cells after the development of clinical T1D.

Materials and methods: Female NOD mice were treated with anti-IL-15 receptor beta chain (TM-β1) or control antibody corresponding to specific stages of T1D: initation, progression or frank diabetes. The donor splenocytes were extensively phenotyped for T, B and NK cell markers before adoptive transfer to male NOD.Scid recipients. Recipients were monitored for T1D. At sacrifice, pancreata were fixed in formalin for histology and the phenotype of the recovered donor cells was assessed.

Results: We observed that adoptive transfer of splenocytes from diabetic female NOD mice that received anti-IL-15 receptor beta antibody, but not control antibody after the development of T1D did not induce diabetes in NOD.Scid recipients. Treatment with TM-β1 resulted in a decrease in the frequency of CD8+ T cells in the diabetic NOD mice. The reduction was more pronounced in the CD44hi CD62Llo population, which defines the memory CD8+ T cell subset.

Conclusion: Our results suggest that blocking IL-15 signaling in clinically diabetic mice results in the depletion of islet-reactive T cells. These observations are of potential significance in the clinics where islet-specific T cells that are present in circulation compromise the efficient long-term engraftment of transplanted islets.

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Non-diabetic first degree relatives of type 1 diabetics vs recent-onset type 1 diabetics: insulin secretion and insulin sensitivity changes at onset of type 1 diabetes

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Background and aims: Previous studies have reported an important role of impairments in insulin secretion in prediabetes and in the initial phase of type 1 diabetes (T1D), while the data regarding insulin sensitivity levels in previous conditions are still controversial. In addition, the onset of T1D is associated with impairments in the ratio between pro vs anti-inflammatory (Th1 and Th2) T memory cell subsets (characterized with expression of chemokine receptors CXCR3 and CCR4, respectively). However, the relationship between the metabolic and immunological/pro-inflammatory changes at recent onset type 1 diabetes and their first-degree relatives (FDRs) has not yet been clarified. Therefore, the aim of this study was to analyse the changes in (a) insulin secretion, (b) insulin sensitivity levels and (c) percentage of CXCR3+ and CCR4+ T memory cell subsets in peripheral blood in the following groups of subjects: (1) high-risk nondiabetic FDRs of patients with T1D (glutamate decarboxylase antibody (GADA)+, IA2 antibody (IA2A)+) (group A, N=10); (2) low-risk nondiabetic first-degree relatives (FDRs) of patients with T1D (GADA-, IA2A-) (group B, N=34); (3) recent-onset T1D patients (group C, N=24) and (4) healthy, unrelated control subjects (group D,N=18).

Materials and methods: Insulin secretion was evaluated by first-phase insulin response (FPIR) as insulin levels 1+3 min after IVGTT. Insulin sensitivity was tested by using euglycemic hyperinsulinemic clamp method. Total glucose uptake (M value) was calculated on the basis of the amount of glucose infused during steady state period (80-120 min). Plasma insulin levels were determined by RIA. GADA and IA-2A levels were determined by ELISA. The percentages of CXCR3+ and CCR4+ T memory cell subsets were analyzed in peripheral blood by using four-color immunofluorescence staining and flowcytometry.

Results: The levels of FPIR were lower in group A vs B and D (A: 84.56±11.79, B: 121.54±23.39 μU/ml, D: 140.53±38.11, A vs B, D p<0.01, B vs D p=NS), while on all three groups they were significantly higher than in C (9±3.68μU/ml, p<0.001). However, the M value did not vary between groups A, B and D (A: 5.87±0.11, B: 6.58±0.27; D: 6.78±0.28 mg/min/kg, p=NS), but it was significantly lower in C (C: 4.7 ±0.22, C vs A, B,D p<0.05). Simultaneously, the percentage of CXCR3+ T memory cells was found to be higher in group A vs B, C and D (A: 64.27±6.53 vs B: 51.79±6.79; C: 40.19±11.52; D: 53.09±6.29%, p<0.05), while the percentage of CCR4+ T memory cells was significantly lower in groups A, C vs B and D (A: 30.04±3.26 vs B: 41.90±8.59; C: 31.53±9.67; D: 40.90±7.24 %, p<0.05).

Conclusion: Our results have demonstrated that high risk nondiabetic FDRs showed lower levels of FPIR, increases in CXCR3+ Th1 cells and lower levels of CCR4+ Th2 cells subsets without impairments in insulin sensitivity, and in recent-onset T1D similar changes are accompanied with decreased insulin sensitivity. The results imply that initial changes in the course of type 1 diabetes comprise early decline of insulin secretion associated with pro-inflammatory response which does not affect insulin sensitivity while later in the course of the disease insulin sensitivity impairments appear to be primarily due to the marked decrease in insulin secretion.

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Validation of insulin dose-adjusted HbA_{1c} (IDAA1c) in 129 Danish children with new-onset type 1 diabetes

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Background and aims: The aim of the present study is to validate the remission definition as assessed by insulin dose-adjusted HbA1c (IDAA1c) and to investigate the clinical value of IDAA1c (with and without age) as predictor

of stimulated C-peptide (SCP) 12 months after diagnosis in a Danish cohort of children and adolescent with new onset type 1 diabetes (T1D).
Materials and methods: The IDAA1c measure was developed using 251 children aged <16 years from the European Hvidoere Remission Phase Study (1999-2000 Cohort). To confirm the relations in the Hvidoere study 129 newly diagnosed T1D patients aged <17 years from the Danish Remission Phase Study (2004-2005) were followed from onset of T1D. After 1, 3, 6, and 12 months a MMTT (Boost High protein: 33 g carbohydrate, 15 g protein, 6 g fat in Hvidoere; Boost Original: 41 g carbohydrate, 10 g protein, 4g fat in the Danish cohort) was performed and SCP was used as a measure of the residual beta cell function. HbA1c and insulin dose per kg body weight were recorded. The definition of IDAA1c: HbA1c (%) + [4 x insulin dose (units/kg/24H]. IDAA1c ≤ 9 was used to define partial remission. ROC-curves (receiver operating characteristic curve) were calculated to evaluate the predictive value of IDAA1c and age (using the score function from the Hvidoere data) on partial SCP remission (defined as SCP > 300 pmol/l). Additionally, the SCP value after 6 and 12 months in the Danish cohort was regressed on IDAA1C and age.
Results: Partial remission in the Danish cohort occurred in 62,4% at 3 months, 47% at 6 months (45% Hvidoere), 26% at 9 months and 19% at 12 months (18% Hvidoere) after diagnosis with no statistical significant difference (P=0.29) to the proportion of patients in partial remission in the Hvidoere Cohort at the same time points. There was a negative correlation between SCP and IDAA1c at 6 and 12 months with no significant differential effect between the two cohorts (P=0.68). The regression coefficient to IDAA1c is similar in the two cohorts, (Hvidoere: -0.22 vs Danish: -0.21, 6 months; -0.23 vs -0.24 12 months) but the effect of age (0.087 vs 0.18, 6 months; 0.14 vs 0.23, 12 months) is much higher in the Danish cohort compared to the Hvidoere cohort (P<0.0001). Using IDAA1c=9 and age=10 years the predicted C-peptide level is 311 pmol/L in the Hvidoere cohort compared to 428 pmol/L in the Danish Cohort. The ROC curves (6 and 12 months after diagnosis) show a clear predictive power as age and IDAA1c in combination give a more precise prediction of partial remission defined by SCP>300pmol/L in the Danish cohort than age alone. Odds ratio (CI): 6 months: 2.5 (1.8- 3.6); 12 months: 2.4 (1.7-3.2).
Conclusion: We have confirmed the findings for remission defined by IDAA1c≤9 in an independent cohort of children with new onset T1D. A comparable proportion of patients are in remission in the two cohorts despite a higher level of SCP in the Danish Cohort particularly in the adolescent group. As age has a stronger effect on IDAA1c in the Danish cohort, which is most likely due to a different MMTT stimulation, age must be taken into account when using IDAA1c for prediction of SCP in this cohort.

PS 006 Epidemiology: social and environmental factors

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Ethnic disparities in conversion from impaired fasting plasma glucose to type 2 diabetes
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Background and aims: Despite the ethnic differences in prevalence and incidence of type 2 diabetes (DM), the evidence on ethnic disparities in conversion from prediabetes to DM is scarce. We aimed to compare the association of impaired fasting glucose (IFG) with the 10-year cumulative incidence of DM in individuals of South-Asian, African and Caucasian origin.
Materials and methods: We analysed data from a population based sample of 90 South Asians, 190 Africans and 176 Caucasians, who had participated in the population based SUNSET study between 2001 and 2003 and who had also participated in a follow up visit between 2011 and 2012. We excluded those with a missing fasting plasma glucose at baseline, a missing fasting plasma glucose at follow up, or with DM at baseline. Baseline IFG was defined as fasting plasma glucose of 5.7 mmol/L - 6.9 mmol/L. At follow up, DM was defined as fasting plasma glucose ≥ 7.0 mmol/L, HbA1c ≥ 48 mmol/mol (6.5%) or self reported DM.
Results: Of all participants, 41.0% were men and the mean age at baseline was 45.4 ± 6.5. The 10-year cumulative incidence of DM was 18.9% in South Asians, 13.7% in Africans and 4.5% in Caucasians (p<0.05). We found a much stronger association between baseline IFG and incident DM in South Asians than in Africans and Caucasians. This ethnic difference conversion from IFG to DM remained after adjustment for sex, age, baseline BMI and change in BMI over 10 years (odds ratio of 11.1 [3.0-40.8] in South Asians, 5.1 [2.0-13.3] in Africans, 2.2 [0.5-10.2] in Caucasians).
Conclusion: We found a higher 10-year cumulative incidence of DM and a stronger association of DM with baseline IFG in South Asians and Africans than in Caucasians. Our findings do not only emphasize the high risk of DM in South Asians and Africans when compared to Caucasians, but also suggest a more rapid conversion from IFG to DM in South Asians and, to a lesser extent, Africans than in Caucasians.
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Impact of parental socio-economic resources on excess mortality in subjects with childhood onset type 1 diabetes
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Background and aims: Maternal education and other socio-economic factors are known to associate with general health care and child death. The aim of this study was to analyze the possible impact of parental socio-economic resources on mortality in a population based cohort of childhood onset type-1 diabetes (T1D).
Materials and methods: The Swedish Childhood Diabetes Registry (SCDR) has recorded cases of childhood onset T1D (0-14years) since 1 July 1977 with a high level of coverage. A total of 14 827 childhood onset T1D were recorded by 31st Dec 2008. The SCDR was linked to the Swedish Cause of Death Register and the Labor Market Research database. We retrieved data on parental educational level by 31st Dec 2010 and family's (parental) requirement of income support between the years 1990-2010. Mortality data were recorded as of 31st Dec 2010.The age and sex standardized mortality ratio (SMR) was based on national statistics. Cox proportional hazard functions were used for multivariate analyses. 95 % confidence intervals (CI) are given.
Results: A total of 250 deaths (male/female; 167/87) occurred in a total of 357 552 person-years at risk. Mean follow-up was 19.9 years, and maximum was 33.8 years. The overall SMR was 2.3 (CI 1.35 - 3.63). *Parental education*: Crude analyses on the effect of low maternal education (less than college) as compared to high (collage or above) showed an increased mortality for male cases (HR 1.48, CI 1.05-2.09, p=0.025) but not for female cases (HR

1.29, CI 0.78-2.13). Paternal education level had no effect on mortality in a crude analysis (HR 1.17, CI 0.89-1.55). In Cox-models, male sex and higher age at onset predicted increased mortality (p<0.001) together with maternal education (HR 1.35, CI 1.01-1.80, p=0.037). *Familial income support:* In crude analyses the family's requirement of income support (any/none) was associated with an increased risk of death for both men and women (HR 1.87, CI 1.35-2.59, p<0.001 and HR 1.99, CI 1.26-3.18, p=0.003, respectively). In a Cox model adjusting for age at onset and sex, ever having required income support was associated with doubled mortality (HR 1.99, CI 1.52-2.59, p<0.001). *Parental education versus income support*: In a final Cox model including maternal education level together with familial income support, age at onset and sex; ever having required income support was still an independent risk factor while maternal education was not (table 1). Further analyses will be performed stratifying for age at death.

Conclusion: Familial need for income support, in addition to male sex and higher age at onset, seems to be a strong predictor of all cause mortality among childhood onset T1D subjects in Sweden.

Table 1.

COX MODEL	HR (95% CI)	p-value
Male sex	1.83 (1.38-2.42)	0.001
High age at onset	1.47 (1.21-1.78)	0.001
Low maternal education	1.25 (0.93-1.68)	0.145
Family received income support	1.95 (1.49-2.55)	0.001

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Factors associated with sustained poor glycaemic control in Chinese patients with type 2 diabetes in Hong Kong

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Background and aims: We hypothesised that specific clinical, psychological and behavioral factors all contribute to sustained poor glycaemic control in people with type 2 diabetes.

Materials and methods: Amongst 15,622 Hong Kong Chinese patients with type 2 diabetes enrolled in the Joint Asia Diabetes Evaluation (JADE) Program since 2007, 3,648 patients underwent repeat comprehensive assessments(CA) after a median (IQR) follow-up period of 1.36 (1.04-1.99) years. We defined subjects with sustained poor glycaemic control (SUS+ group) as those with A1c≥9% at baseline CA and repeat CA. Factors that were significant in the univariate analysis (p<0.15) were selected to enter multivariate logistic regression analyses to identify predictors for sustained poor glycaemic control.

Results: Amongst 516 patients with A1c≥9% at baseline, 164 (32.4%) remained in the SUS+ group (A1c:10.40±1.25% at baseline CA and 10.30±1.09% at repeat CA). The respective figures were 10.35±1.15% and 7.19±0.92% (p<0.001) in the SUS- group. Compared to the SUS- group, the SUS+ group had longer disease duration, higher body mass index, higher rates of insulin treatment, and lower perceived state of health. On logistic regression analysis, longer disease duration (β=0.052, p=0.017), and use of insulin (β=0.768, p=0.033) predicted sustained poor glycaemic control. On the other hand, higher adherence to balanced diet (β= -0.907, p=0.020), increased physical activity (β= -0.865, p=0.016), increased follow up (β= -1.200, p=0.024), increased use of oral anti-diabetic drugs (β= -1.822, p=0.110), and higher perceived state of health (β= -0.017, p=0.103) were associated with improved glycaemic control.

Conclusion: These results highlight the multidimensional aspects in determining glycaemic control, and emphasize the importance of empowering patients to improve self-management practices. Addressing psychological health may also be particularly important in patients with sustained poor glycaemic control.

Characteristics associated with sustained poor glycemic control

Variable	Parameter		Hazard ratio			P value	R2 %
	Parameter estimate	SEM	Hazard ratio	95% CI			
Disease duration	0.052	0.022	1.053	1.009	1.100	0.017	21.2
Use of insulin(0,no;1,yes)	0.768	0.361	2.155	1.063	4.370	0.033	
Increased use of Oral Anti-diabetic Drug use	-1.816	0.138	0.163	0.017	1.513	0.110	
State of health	-0.017	0.011	0.983	0.963	1.004	0.103	
Adherence to diet (0,no;1,yes)	-0.907	0.390	0.404	0.188	0.867	0.020	
Increased physical activity	-0.865	0.358	0.421	0.209	0.850	0.016	
Increased follow up	-1.200	0.533	0.301	0.106	0.856	0.024	

Variables entered: Age, Body mass index, Disease duration, Follow up time, Use of insulin, Use of oral anti-diabetic drugs,Increased use of oral anti-diabetic drugs, Increased use of insulin,Regular follow up, Increased follow up, State of health, Adherence to diet, Increased physical activity, Increased self monitoring, Increased pain, Increased anxiety.

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Age, glycaemic control and social deprivation independently predict 10-year mortality in a UK type 1 diabetes cohort

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Background and aims: Patients with type 1 diabetes continue to have increased mortality and morbidity despite advances in diabetes care. In order to optimise provision of specialist services with the aim of improving outcomes, this study set out to determine potentially modifiable factors that influence mortality in type 1 diabetes in a defined urban population.

Materials and methods: Retrospective analysis of combined biochemical (HbA1c), demographic (age, sex, ethnicity and socio-economic status) and health resource utilisation data collected over a 10 year period for a cohort of T1D patients (n=1038) attending two inner city London specialist diabetes outpatient clinics. The cohort was defined to include all patients attending the service in 2002 with sequential HbA1c data available for each year from 2002 to 2004 and with on-going follow-up within the clinics until 2010. Baseline glycaemic control was defined based on mean HbA1c for the period 2002-2004. Economic status was determined using the index of multiple deprivation (IMD) a weighted deprivation score derived from a national dataset based on postcode of residence.

Results: At baseline, mean (±SD) age was 41.6 + 12.3 years and duration of diabetes 17.7 ±13.7 years . Mean HbA1c was 8.1 ± 1.4. 37 deaths occurred in the cohort by December 2012 (3.6% total cumulative mortality). Patients who died were significantly older (50.9 + 9.1 years, p <0.001) and had higher mean baseline A1c (9.1+ 1.6%, p<0.001) but did not differ in duration of diabetes (14.7+15.6, p=0.8) For individuals with baseline HbA1c ≥ 9.0% cumulative 10-year morality was significantly increased at 9.0 % (p< 0.001). Patients who died were more likely to be socially deprived, 61% of deceased patients having scores in the upper quintile of the population range (mean IMD score 32.3 ± 11.2 deceased vs 23.6+12.3 alive, p=0.001). In a three step regression analysis, the effects of baseline HbA1c, age and IMD score on mortality were mutually independent.

Conclusion: Glycaemic control and social deprivation are independent risk factors for mortality in type 1 diabetes and identify characteristics of a population that may benefit from targeted interventions aimed at improving health outcomes.

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Does birth weight affect insulin sensitivity and secretion and the prevalence of diabetes in a Japanese general population?

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Background and aims: In Japan, babies with low birth weight (LBW) are increasing and average of birth weight (BW) is decreasing. Recently, LBW has been considered as an independent risk factor for diabetes mellitus (DM) in the studies from Europe and U.S, but there are few data from Asia. The unfavorable intrauterine environment may lead to future insulin resistance (IR) and beta cell dysfunction. Japanese are characterized by a lower BMI but higher percent body fat, than that in Whites, and have lower insulin secretary capacity than Whites, since the impact of birth weight on insulin sensitivity and secretion might be different from previous reports in Europe and U.S. Thus, the aim of this study was to assess the effect of BW on insulin sensitivity and secretion and the prevalence of DM in a Japanese population.
Materials and methods: Subjects were 847 (534 men, mean age 47±7 years) who participated in the health checkups at SSK hospital from February '06 to January'07 and answered the questionnaire about BW. Subjects with diabetic medication (n=20) were excluded when analyzing data for plasma glucose and insulin concentrations. DM was defined as self-reports of diabetes, or if they have fasting plasma glucose (FPG) ≥126mg/dl. BW was divided into three groups: G1; <2500g (LBW), G2; 2500-3000g, G3; 3001-3999g (Normal birth weight divided equally), G4; ≥4000g (high birth weight (HBW)). LBW was defined <2500 according to the summaries from American Academy of Pediatrics. Homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-B) were used to assess IR and insulin secretion. General liner model was used to analyze the association between BW and FPG, and log-transformed value of HOMA-IR or HOMA-B. Multivariate logistic regression model was used to calculate odds ratios (ORs) associated with DM, IR (highest quartile of HOMA-IR) and low insulin secretion (lowest quartile of HOMA-B) in subjects with LBW compared with those with G3 adjusted for age, sex, BMI and family history of DM.
Results: The average BW was 3070±430g, and the prevalence of LBW and HBW were 5.2% and 3.1%. The prevalence of DM was higher in subjects with LBW (9.1%) than those with normal BW(G2: 6.2%, G3: 2.6%), whereas there was no DM in subjects with G4 (HBW). Adjusted means for FPG and log HOMA-IR increased with decreasing BW ($p<0.05$ for both) while there was no significant association between adjusted means for log HOMA-B and BW. Multiple logistic regression analysis showed that the risk of having DM continuously decrease by 46% according to increase of 500g of BW. The adjusted OR of having DM, IR and low insulin secretion in subjects with LBW was 2.3 (95% confidence interval: 1.1-5.1), 3.9 (1.5-10.3) and 1.4 (0.5-3.4) compared with those with G3. In subjects with G4 (HBW), the adjusted ORs for having IR and low insulin secretion were 1.5 (0.9-2.5) and 2.1 (0.7-6.3), respectively, compared with those with G3.
Conclusion: BW was inversely associated with FPG and HOMA-IR, whereas there was no association with HOMA-B. LBW increase the risk of DM, and IR may be the key factor of the association in Japanese population.

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Predictive ability of simple clinical information without blood tests for future incident diabetes: a meta-analysis

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Background and aims: Development of a simple method to predict diabetes mellitus is essential for early detection of persons at high risk of diabetes due to its worldwide growing epidemic and the seriousness of its many complications. This meta-analysis aimed to assess the predictive ability of clinical information without blood testing for future incident diabetes.
Materials and methods: Electronic literature search was conducted for cohort studies that prospectively followed up incident diabetes using MEDLINE (from 1950 to Dec. 3, 2012) and EMBASE (from 1974 to Dec. 3, 2012). Studies had to include at least age, an obesity indicator, and family history of diabetes as clinical information to predict future diabetes and allow calculations of the number of true-positive, false-negative, true-negative, and false-positive cases. Pooled sensitivity, specificity, positive likelihood ratio (LR+), and negative likelihood (LR-) were estimated with a hierarchical summary receiver-operating characteristic model.
Results: Of 15,908 citations retrieved from the two databases, 8 eligible studies (follow-up duration from 5 to 15 y) consisting of 98,191 participants and 9,358 cases were identified. Two studies had samples that included both a derivation and validation study. Finally, 10 datasets were included in this meta-analysis. Point estimates of sensitivity, specificity, LR+, and LR- with 95% confidence interval were 0.70 (0.62-0.75), 0.69 (0.62-0.75), 2.25 (2.00-2.53), and 0.44 (0.38-0.50), respectively (Figure). Definite heterogeneity in study results was not observed through several specified population characteristics such as gender, mean age, and ethnicity. Slightly low LR+ (<2) was observed in 4 studies that had an observation period of <6 years (LR+=1.94) and in 4 studies that used the oral glucose tolerance test for ascertaining diabetes (LR+=1.97). LR- was consistently <0.5 throughout these study characteristics.
Conclusion: Using simple clinical information could be a modest help in considering whether a person will develop diabetes. However, there was not strong evidence for effectiveness in detecting persons at high risk of future diabetes without results of blood tests.

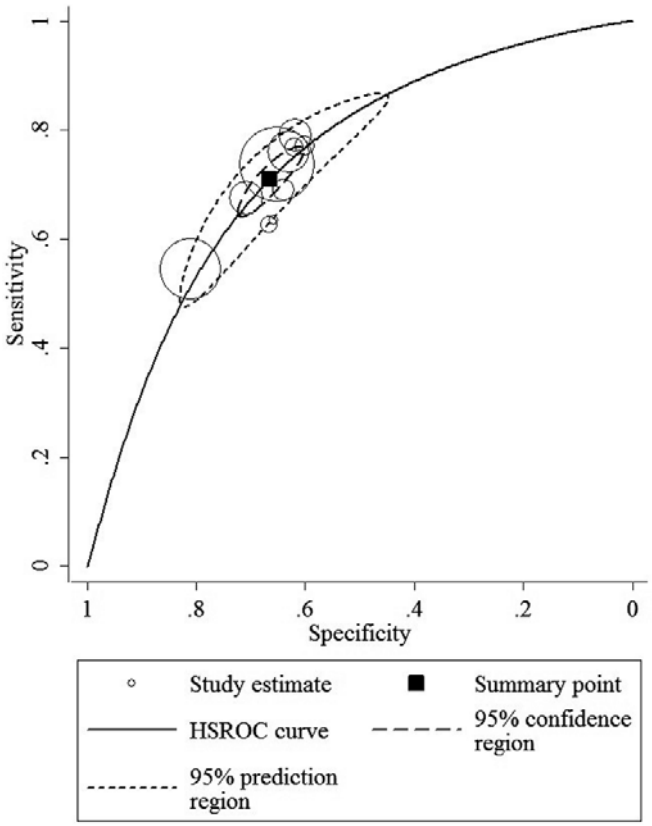

Figure Hierarchical summary receiver-operating characteristic curve with its accompanying 95% prediction region for predicting incident diabetes using simple clinical information without a blood test. Point estimate is indicated by a solid square. Each data point is indicated by a circle, the size of which depended on the study sample.

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Increased incidence of disorders of carbohydrate metabolism and metabolic syndrome in persons exposed to ionising radiation

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Background and aims: The most wide-scale man-made accident at the Chernobyl NPP (ChNPP) on April 26, 1986 was followed by the massive release of radioactive elements being tropic to many endocrine tissues. Fallout of the mentioned substances resulted in both external and internal irradiation of population. The hormone-producing pancreatic cells were exposed at that among other tissues leading to an increased risk of diabetes mellitus DM) and associated disease.
Materials and methods: Retrospective analysis (i.e. 20-25 years upon radiation impact) was applied among the ChNPP accident survivors (n=606) and general population of Ukraine (n=589) (control group) to the indices of: 1) DM incidence, 2) basal serum levels of insulin, C-peptide, leptin, thyroid-stimulating hormone and free thyroxin, 3) coefficients of tissue resistance (HOMA, QUICKI), 4) incidence of thyroid disease.
Results: Incidence of type 2 DM (21,4%) was higher in all subgroups of the ChNPP accident survivors (χ2Yates=25,64; p=0) vs. control group (10,5%). The supreme probability of type 2 DM was found in Chernobyl emergency workers engaged in work within so-called "non-iodine" period in 1986-1987, namely in 60% of persons (χ2Yates=89,72; p=0) who for the most long time had stayed in the 30-kilometer exclusion zone. High probability was found also in the emergency workers of the "iodine" period (April-August of 1986), namely in 23,5% of persons (χ2Yates=25,21; p=0).
Conclusion: Metabolic syndrome (MS) was more often diagnosed in the ChNPP accident survivors i.e. in 49,8% (χ2Yates=207,14; p=0) vs. control group (9,5%). Type 2 DM and MS were diagnosed mainly in people having an excess of body adipose tissue (body mass index >25 kg/m2). Cases of type 2 DM and MS were associated with increased indices of insulin resistance and leptin resistance.

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Social network and development of prediabetes and type 2 diabetes in middle-aged Swedish women and men

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Background and aims: In general, social ties are associated with health benefits. The aim of the present study was to explore if social network affects the development of prediabetes and type 2 diabetes (T2D).
Materials and methods: The study included 2924 women and 2039 men, aged 35-56 years and with normal glucose tolerance (NGT) at baseline. A follow-up was performed 8-10 years later and again the participants were investigated with an oral glucose tolerance test (OGTT). By then prediabetes (IFG and/or IGT) and type 2 diabetes (T2D, including also those who were diagnosed with T2D during the follow-up period) was detected in 168 and 50 women and in 236 and 93 men, respectively. We analyzed the associations of prediabetes and T2D with different measures of social network recorded by questionnaire at baseline; AVSI-index (availability of social integration i.e. quantity and quality of daily social contacts), marital status (married or living with partner) and social activity (being member and regularly taking part in meetings of association, club or congregation and/or taking part in study circles, sports or music playing together with other people). We calculated odds ratios (OR) and 95% confidence intervals (CI), age-adjusted and additionally adjusted for potential confounding variables (BMI, physical activity, smoking, socioeconomic status, psychological distress, decision latitude at work and sense of coherence), using multiple logistic regression analysis.
Results: Having AVSI scores in the highest tertile, i.e. better social integration, was associated with a decreased risk to develop T2D in women, age-adjusted OR=0.41 (95% CI: 0.19-0.88) although not significant after full adjustment, OR=0.51 (0.22-1.17). Contrary, in men AVSI was associated with an increased risk to develop T2D, especially in the middle tertile, OR=2.05 (1.20-3.49) and OR=2.48 (1.41-4.36) for age- and multi adjusted models, respectively. Taking part in social activities decreased the risk to develop prediabetes and T2D in women, age-adjusted OR=0.65 (0.46-0.91) and OR=0.43 (0.24-0.77) although less significant when adjusted for confounders, OR=0.77 (0.53-1.11) and 0.59 (0.31-1.13), respectively. Also in men a decreased risk was observed, but only for prediabetes, OR=0.60 (0.43-0.83) in the multi-adjusted model. Being married or living with partner was associated but only in men in whom a decreased risk for T2D was observed, OR=0.57 (0.33-0.97) and OR=0.60 (0.34-1.07) for age- and multi adjusted models, respectively.
Conclusion: Individuals having a social network in terms of family, friends, acquaintances and social activities seemed less likely to develop abnormal glucose regulation. When significance was decreased after multi-adjustments, this might be due to that the measures of social network and psychosocial confounders to some extent capture similar conditions. However, contradictory to an overall protective pattern of having a social network, AVSI showed in men an increased risk to develop T2D.
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PS 007 Basic science: environmental factors

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A chronic exposure to a mixture of low-dosed food contaminants predisposes to the risk of developing metabolic disorders

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Background and aims: Apart from genetic alterations and behaviors associated with excessive food intake and low physical activity, environmental pollutants have emerged as a new cause of metabolic diseases. Based on biodegradation, pollutants are classified as short-lived or Persistent Organic Pollutants (POPs). POPs are chemicals created intentionally (polychlorinated biphenyls, PCBs) or by-products (dioxins). Being lipophilic, POPs accumulate through the food chain. Bisphenol A (BPA) and phthalates are short-lived chemicals but because of their massive production (plastic goods), they largely contaminate food and beverages by leaching from containers and packaging. Consequently, non-occupational exposure is characterized by lifetime exposure to a whole host of chemical agents of which resulting effects could not be predicted from the effect of each individual pollutant, and food ingestion is a primarily route of exposure (especially fatty foods). Yet, human health risk assessment studies have focused primarily on single chemicals, setting up Tolerable Daily Intake (TDI) doses based on the No Observed Adverse Effect Levels (NOAELs) from animal studies. These findings prompted us to directly explore the hypothesis that a chronic exposure to a mixture of low-dosed food contaminants may predispose to the risk of developing metabolic disorders.
Materials and methods: Briefly, mice were challenged from preconception throughout life with a high-fat high-sucrose diet containing food pollutants (2,3,7,8-tetrachlorodibenzo-p-dioxin, PCB153, diethylhexyl-phthalate and BPA) added at doses 500-1000 lower than their NOAEL. Therefore, it grossly corresponded to the TDI for each pollutant. We measured several blood parameters, glucose and insulin tolerance, hepatic lipid accumulation and gene expression by RT-qPCR and Western blotting analysis in adult mice.
Results: Pollutant-exposed mice exhibited significant sex-dependent metabolic disorders (all with $p<0.05$) in absence of toxicity and weight gain. In males, there was no modification of either glucose tolerance or insulin sensitivity. However, we observed that pollutants increased the expression of hepatic genes (from 36 to 88% at the mRNA level) encoding proteins related to cholesterol biosynthesis, and decreased (40%) hepatic total cholesterol levels. In females, exposure to pollutants resulted in a marked deterioration of glucose tolerance. Analysis of gene expression in liver indicated reduced expression of estrogen receptor alpha by 25% (observed at mRNA and protein level) and of estrogen-target genes by >34% (mRNA level). Interestingly, a parallel two-fold induction of estrogen-sulfotransferase (EST) expression was observed indicative of enhanced estrogen metabolism and hence of reduced estrogen bioavaibility in liver. Estrogens protecting from metabolic disorders, it may well contribute to hepatic insulin resistance in exposed females.
Conclusion: Because of the very low doses of pollutants used in mixture, these findings may have strong implications in terms of understanding the potential role of environmental food contaminants in the development of metabolic diseases.
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Foodborne cereulide causes beta cell dysfunction and apoptosis

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Background and aims: The prevalence of both type 1 and type 2 diabetes mellitus is rising and the increase is believed to be related to environmental factors. Foodborne toxins that can directly induce beta cell dysfunction and apoptosis might partly explain the diabetes epidemic in this era of prepared and prepackaged meals. In our study, we investigate the beta cell toxicity of cereulide, a mitochondriotoxic peptide produced by certain strains of Bacillus cereus that is frequently found at low concentrations (approximately 4 ng/g of food) in rice, pasta and potatoes meals.
Materials and methods: Mouse and rat insulin producing beta cell lines, MIN6 and INS-1E respectively, as well as human hepatocellular HepG2 and monkey fibroblast-like COS cells were exposed to cereulide concentrations ranging from 0.05ng/ml to 5ng/ml for 24, 48 and 72h. Cell death was evaluated by a Hoechst/Propidium Iodide assay by two investigators blinded for exposure. Whole mouse islets, isolated from 2 week old C57Bl/6J mice, were exposed to similar cereulide concentrations for 1, 3 and 5 days and cell death was determined similarly. To evaluate the exact mechanisms leading to cell death, MIN6 cells were exposed to low concentrations of cereulide (0.15 - 0.5 ng/ml) for 24h and mechanisms of toxicity were evaluated by mRNA profiling and caspase 3/7 activation bioluminescence assay. Finally, beta cell function was assessed by analyzing glucose-stimulated insulin secretion of MIN6 cells, treated for 24 h with low concentrations (0.15 - 0.5 ng/ml) of cereulide.
Results: Cereulide exposure caused cell death in MIN6, INS-1E and pancreatic islets, but not in HepG2 or COS cells (see table 1). Caspase 3/7 activation confirmed the apoptotic cell death process (1.54 ± 0.07 fold induction by 0.25 ng/ml cereulide compared to control; $p < 0.01$). Interestingly, exposure to 0.25ng/ml cereulide induced markers of mitochondrial stress, including PUMA (p53 upregulated modulator of apoptosis; 180 ± 57 % of control; $p < 0.05$) but also markers of ER stress, such as CHOP (CCAAT/-enhancer-binding protein homologous protein; 294 ± 69 % of control; $p < 0.01$). Glucose-stimulated insulin secretion decreased from 10.48 ± 3.33 fold to 2.012 ± 0.51 ($p < 0.05$) after 24h exposure with 0.25 ng/ml cereulide.
Conclusion: Cereulide, a toxin frequently found in prepackaged or prepared starchy meals, increases levels of mitochondrial and ER stress markers in beta cells of rats and mice, even at low doses. In a dose dependent way, it also leads to impaired beta cell function and apoptosis. Cereulide might thus be involved in the current diabetes epidemic and in particular be responsible for observed clusters of sudden onset diabetes in Asia.

Table 1. Apoptosis induced after 24h exposure to cereulide (mean percentage ± SEM).

	MIN6 (n=5)	INS-1E (n=4)	HepG2 (n=3)	COS (n=3)	Islets (n=3)
Medium	7.3 ± 1.3	2.5 ± 0.3	5.8 ± 0.6	1.2 ± 0.6	3.1 ± 1.2
0.05 ng/ml cereulide	5.9 ± 1.0	3.2 ± 0.5	6.6 ± 2.1	1.6 ± 0.4	3.9 ± 1.5
0.25 ng/ml cereulide	31.6 ± 5.8 *	58.1 ± 11.4 *	6.9 ± 1.5	2.9 ± 0.7	8.6 ± 2.4
0.5 ng/ml cereulide	43.6 ± 6.1 *	100.0 ± 0.0 *	11.9 ± 2.5	2.6 ± 0.6	49.2 ± 9.0
5 ng/ml cereulide	100.0 ± 0.0 *	100.0 ± 0.0 *	7.7 ± 2.3	4.3 ± 0.9	96.4 ± 3.5*

* $p \leq 0.05$ vs control

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Pancreatic islet expression of enteroviral receptors and dsRNA sensors in human type 1 diabetes

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Background and aims: Type 1 diabetes mellitus (T1D) is a chronic autoimmune disease in which pancreatic beta cells are selectively destroyed by an autoimmune process. Among the environmental factors potentially involved in T1D etiology, viral infections have been suggested as key players, with several studies reporting evidence of enteroviral infection in pancreatic islets. Intriguingly, such infections are restricted to beta cells in recent-onset T1D patients. Susceptibility to viral infections is determined both by viral variants and by the nature of infection, while the specific tropism of viruses is modulated by local expression of cellular receptors, such as Coxsackie and Adenovirus receptor (hCAR). In addition, intracellular viral RNA sensors (e.g. RNA helicases RIG-I and MDA5) and other molecules (e.g. interferon -induced protein ISG-15) can trigger and/or modulate antiviral response. The aim of the present study was to characterize islet expression of molecules (hCAR, RIG-I, MDA5 and ISG-15) involved in virus-target cell interaction, in T1D and in non-diabetic pancreatic samples from multi-organ donors
Materials and methods: We analyzed pancreatic specimens obtained from 4 recent onset T1D, from 2 long standing T1D (disease duration 7 and 14 years respectively) and from 10 non-diabetic organ donors. Expression of

hCAR, RIG-I, MDA5 and ISG-15 was analyzed by immunohistochemistry. In order to establish which islet cell subset(s) express the molecules of interest (i.e. alpha-, beta- and delta cells) double immunofluorescence with confocal microscopy analysis was used. The extent of colocalization of hCAR, RIG-I, MDA5 and ISG-15 with insulin, glucagon or somatostatin was quantified by Leica Application Advance Fluorescence (LasAF) software.
Results: Viral receptor hCAR was expressed in islets from both T1D and non-diabetic donors almost exclusively within beta cells (average colocalization rate with insulin was 82.0±12.0% in T1D and 75.2±9.1% in non-diabetic donors; colocalization with glucagon was <15.0%), suggesting the existence of a differential distribution of enterovirus receptors among islet cell subsets. Of note, the few alpha cells expressing hCAR were single cells located outside the islets but scattered throughout the exocrine tissue. Expression of viral sensor RIG-I was observed mainly in delta cells both in T1D and in non-diabetic donors (colocalization rate with somatostatin 47.8±21.6% and 51.6±21.2% respectively; colocalization with insulin and with glucagon <5%). A similar delta-cell specific expression was observed for ISG-15 (colocalization rate with somatostatin 48.3±11.5% in T1D and 45.77±7.33% in non-diabetic donors). MDA5 was detected mainly within alpha cells (colocalization rate with glucagon 40.1±7.1%; colocalization with insulin <5%).
Conclusion: We show that the enteroviral receptor hCAR is expressed almost exclusively in beta cells, thereby providing a potential explanation for the beta cell specific tropism of certain enteroviruses. In addition, our discovery that expression of the viral sensors MDA-5, RIG-I and ISG-15 occurs mainly in non-beta cells, suggests that these cells are equipped with anti-viral response mechanisms that may limit their susceptibility to enterovirus infection.
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Therapies which lower advanced glycation end products influence experimental autoimmune diabetes in a time dependent manner
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Background and aims: The incidence of type 1 diabetes is increasing worldwide within lower risk genotypes most likely as a result of environmental risk factors. Increasing exposure to advanced glycation end products (AGE), attributable to altered food processing and dietary patterns, has been shown to influence islet secretory function. Thus we aimed to examine the effects of various interventions to reduce AGE exposure administered either as short term interventions prediabetes as compared with intervention for the remainder of life.
Materials and methods: Fifty day old female NODShiLt mice (n=10/group) were randomised to receive (i) no intervention (ii) a diet with 4-fold lower AGE content or (iii) the AGE lowering therapy, alagebrium chloride (ALT; 1mg/kg/day) from day 50 to day 100 or day 50 to day 200 of life. Two additional groups were infected at day 50 with an adeno-associated virus (AAV) encoding either the human endogenous soluble receptor for AGEs (esRAGE) or a GFP-labelled scrambled protein.
Results: Prediabetic NODShiLt mice treated with ALT from day 50 to day 100 of life, had a substantial reduction in autoimmune diabetes by day 200 as compared to control mice (80% vs 20%, p=0.005). Conversely, mice fed with a low AGE diet prediabetes from day 50 to day 100 of life or overexpressing esRAGE were not protected from autoimmune diabetes as compared to control (40% vs 20%, p=0.33) or control AAV mice (30% vs 60%, p=0.19), at day 200 of life. Specifically, glucose and insulin tolerance were each improved by ALT therapy while a low AGE diet only improved glucose tolerance prediabetes (day 80) despite a decline in insulin sensitivity compared to control mice. Both cohorts of mice treated with AAV exhibited an increase in insulin secretion as compared to control mice at day 80 of life, which only reached significance in the control AAV group (control AAV: p=0.01, esRAGE: p=0.06). However, long term administration of either a low AGE diet or ALT (day 50 to day 200) decreased circulating AGE concentrations and prevented the development of autoimmune diabetes compared to control mice (90% and 95% respectively vs 50%, p<0.05) as well as improving glucose tolerance and islet secretory function, although again the low AGE group had a demonstrable decline in insulin sensitivity prediabetes when compared to control mice (p<0.05).
Conclusion: Our results demonstrate that protection afforded by targeting advanced glycation in experimental autoimmune diabetes can vary depending on the type and length of therapy. A better understanding of the mechanisms responsible for the variability of this protection would be highly desirable in order to better assess the applicability of this type of approach in the prevention of type 1 diabetes in humans.
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Increased degradation of insulin granule proteins, caspase-like activity and MHC class I in Coxsackievirus infected insulin producing cells
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Background and aims: Type 1 Diabetes (T1D) is characterized by the progressive autoimmune destruction of pancreatic beta cells. Proteins of the insulin secretory granules (SGs) are among the dominant autoantigens of T1D. Human Enteroviruses (HEVs) such as Coxsackievirus B (CVB) are among the environmental factors thought to trigger and/or accelerate the development of the disease. However, the mechanistic relationship between viral infection and loss of tolerance toward beta cell antigens remains unclear. Here we investigated whether infection of HEVs alters the expression of SG autoantigens and induce changes that may favor their presentation to the immune system.
Materials and methods: Mouse MIN6, rat INS-1, and human 1.1E7 insulinoma cells were infected with CVB5 serotypes Faulkner (F) and MIN6 cell adapted (MCA). Infected cultures were compared to non-infected cultures 96h post-infection. Mouse, rat and human islets were harvested 72h post-infection with CVB5 strains. Proteasome activities were measured by luminescence assay and protein expression by immunoblotting.
Results: CVB5 infection of MIN6 cells inhibited the biosynthesis of total protein, but not the glucose-stimulated translation of SG proteins. In CVB5-infected cells, however, the expression of mature SG proteins and insulin release were dramatically reduced. CVB5 infection increased the caspase-like proteolytic activity associated with the proteasome, while trypsin-like and chymotrypsin-like proteolytic activities were not altered. Evidence for cleavage of caspase 3 and poly (ADP-ribose) polymerase (PARP) was also negative. The increased caspase-like activity was not associated with increased apoptosis, while necrosis was increased. Finally, the levels of Major Histocompatibility Complex class I molecules (MHC class I) did not change in CVB5 infected mouse MIN6, rat INS-1 and human 1.1E7 insulinoma cells, but were increased in CVB5 infected rodent and human pancreatic islets relative to control islets.
Conclusion: By reducing total protein biosynthesis while increasing i) the degradation of SG proteins, ii) the caspase-like activity of the proteasome, and iii) the expression of MHC class I, CVB infection may poise beta cells for preferential presentation of SG protein-derived peptides to the immune system, and thus directs autoimmunity towards them.
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Effect of nanopowder (TiO_2 and CO_3O_4) on circulating angiogenic cell viability, function and inflammatory response
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Background and aims: Recent evidences showed that nanoparticle (NP) exposure is associated to an increased cardiovascular (CV) disease risk. NPs size allows the passage through the pulmonary capillary barrier inducing systemic inflammation, accelerated atherosclerosis and altered cardiac autonomic function that have been claimed to account for NP-induced CV mortality. Among NPs, TiO_2 and Co_3O_4 are currently attracting enormous industrial interest due to their peculiar size- and shape-dependent properties and applications as pigments, catalysts, sensors for electrochemistry, magnetism, energy storage. NPs may contaminate living and working environments, thus representing a health hazard. The increased oxidative stress and inflammation associated to NP exposure, could exacerbate CV damage, especially in subjects at increased CV risk, such as diabetic patients. Studies on subjects exposed to NPs are not feasible, due to the difficulty to evaluate exposure. Thus,

hazard characterization and risk assessment ought to be based on NP toxicity to cell models derived from CV system, such as circulating angiogenic cells (CACs), considered key regulators of vascular homeostasis and repair. Aim of the study is the evaluation of possible effects of commercially available nanopowders on CAC viability, function and production of pro-inflammatory/oxidative stress species.

Materials and methods: CACs have been isolated from healthy donors' buffy coats after culturing lymphomonocytes on fibronectin-coated dishes in endothelial medium for 7 days. Different concentration of TiO_2 and Co_3O_4 (1-10-20-50-100 μg/ml) were added to the culture media to test nanopowder effects on EPC viability/proliferation, apoptosis, and function (adhesion assay). The effects of TiO_2 and Co_3O_4 on pro-inflammatory (IL1β, TNFα and monocyte chemoattractant protein-1 (MCP-1)) cytokine gene expression and oxidative stress (TBARS) were also tested.

Results: CAC viability was significantly reduced in the presence of 10, 20, 50 and 100 μg/ml Co_3O_4 following 24h and 48h exposure. Apoptosis assay (Caspase 3 activation) confirmed Co_3O_4 cytotoxic effect ($p<0.001$). The capacity of CACs to adhere to fibronectin resulted impaired when 50 and 100 μg/ml of Co_3O_4 were added to the culture media. On the contrary, no significant effect on CAC viability and function was observed in the presence of TiO_2. Oxidative stress induction was increased by 20% ($p<0.05$) in the presence of Co_3O_4 (20 μg/ml; 24h) and inflammatory cytokine (IL1β and TNFα) gene expression was significantly higher ($p<0.05$) following 6h exposure to Co_3O_4 and TiO_2 compared to controls.

Conclusion: Our results show that Co_3O_4 nanopowder induce cytotoxicity and an altered function in CACs accompanied by an increased inflammation and ROS production, suggesting potential adverse CV effects after occupational and/or accidental exposure to metal-containing nanopowder or NPs (Co_3O_4 in particular).

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DNA methylation analysis in adipose tissue after long term high-fat diet-induced obesity in mice

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Background and aims: Emerging evidence suggests that epigenetic factors, including DNA methylation, are involved in the development of obesity. Because of the importance of lifestyle factors in obesity development, DNA methylation can provide a putative mechanism by which specific environmental exposures convey risk for obesity. However, current understanding of the regulation and maintenance of the methylome, as well as its relationship with this disease, remains poorly characterised. In this study, we examined genome-wide DNA methylation status of white adipose tissue (WAT) of obese mice fed a high fat diet in order to identify epigenetic changes secondary to obesity.

Materials and methods: Five weeks old male C57BL/6J were fed either a standard (STD) or a high fat diet (HFD) for 5 months. Methylated DNA immunoprecipitation combined with high-throughput sequencing (MeDIP-Seq) on Illumina platform was performed on pooled DNA isolated from epididymal fat pads of STD fed mice and HFD fed mice (n=6/group). RT qPCR was performed in WAT and in skeletal muscle (SM) of these mice.

Results: The metabolic characteristics of the HFD fed mice are reported in Table 1. MeDIP-Seq analysis generated non-biased DNA methylation maps by covering - with sufficient depth and resolution - the entire mouse genome. Globally, methylated DNA regions showed a different distribution across the genome between the two mice groups. The analysis at gene level revealed a distinctive DNA methylation pattern in CpG islands and gene promoters of genes involved in inflammatory processes, gonadotropin releasing hormone receptor, Wnt and integrin signaling pathways, and in the regulation of transcription. Among the differentially methylated genes in the obese mice, Ankrd26 gene was identified, as well. We have recently demonstrated that Ankrd26 gene plays an important role in the development of both obesity and diabetes in vivo. Interestingly, Ankrd26 mRNA expression is decreased both in WAT (STD: 0.518 ± 0.005 vs. HFD: 0.478 ± 0.002; $p< 0.001$) and in SM (STD: 0.589 ± 0.009 vs. HFD: 0.548 ± 0.009; $p< 0.001$) from diet induced obese mice, indicating that Ankrd26 mRNA expression levels were consistent with its DNA methylation profile.

Conclusion: We used high-throughput sequencing for studying genome-wide DNA methylation in a well-characterised mouse model of diet-induced obesity. These studies indicate that, at least in this animal model, obesity is associated with DNA methylation changes in WAT on a genome-wide scale. Moreover, the differentially methylated regions are enriched in genes regulating transcription and in genes involved in the pathogenesis of obesity, such as Ankrd26, indicating the impact of nutrition on gene expression changes.

Table 1. Metabolic characteristics of the HFD fed mice.

	SD(n=6)	HFD(n=6)	p values
Body Weight (g)	28.51±0.99	39.11±1.30	P<0.001
AUC ITT (mg/dL*120min)	9343±424	21517±1812	P<0.001
AUC GTT (mg/dL*120min)	24525±1200	33909±1260	P<0.01
Glucose (mg/dl)	107±6.0	180±11	P<0.001
Insulin (ng/mL)	0.36±0.13	1.09±0.2	P<0.001

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Functional analyses of the type 2 diabetes associated 12-bp deletion in repin1

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Background and aims: *Repin1* maps within a quantitative trait locus for total cholesterol, body weight, serum triglycerides and serum insulin in the rat. Sequencing of human *repin1* revealed a 12-bp deletion within the coding region (rs3832490). We investigated the functional consequences of this variant.
Materials and methods: 1) *Population genetic studies:* The deletion in *repin1* was genotyped for subsequent association studies in two independent cohorts: the Leipzig cohort (N=1830) and the Sorbs (N=1046). In addition *repin1* mRNA levels were determined in paired human samples of visceral and subcutaneous adipose tissue (N=86). 2) *Functional studies:* 3T3-L1 preadipocytes were transfected with plasmids carrying either the *repin1-wildtype* or the *repin1-deletion*. We tested two different methods for transfection, lipofection (GeneJammer) and electroporation (NeonTransfectionSystem). The transfection efficiency was examined by using FACS and the overexpression of both variants was checked by Western Blot. After the differentiation we stained the cells with oil red O.
Results: 1) Compared with the non-carriers, subjects homozygous for the deletion variant seemed to be protected against type 2 diabetes (T2D) (P=0.011, after adjusting for age, sex and BMI). This variant was also associated with body fat mass and fasting plasma glucose. Compared with non-carriers, subjects with the deletion had a lower maximum adipocyte size in visceral and subcutaneous fat (P=0.065 and 0.014, respectively) and tended to have a lower mRNA *repin1* expression in the subcutaneous fat. 2) Substantially higher transfection efficiency was achieved by electroporation when compared with lipofection (84.2% vs. 15.2%). Using microscopy, there was no visual difference between the *repin1-deletion* and *repin1-wildtype* transfected cells. Compared with the untransfected 3T3-L1 adipocytes the *repin1*-transfected cells showed less and smaller lipid droplets.
Conclusion: The variant in *repin1* might reduce the risk for T2D most likely by influencing the adipocyte function and morphology. Furthermore, the study encourages further *in vitro* experiments in 3T3-L1 cells to pinpoint the role of the identified deletion in *repin1´s* biology.
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Regulation of alternative splicing in human obesity loci

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Background and aims: Genome wide association (GWA) analyses have significantly increased the number of confirmed candidate loci for obesity. We hypothesized that non-coding SNPs in these candidate loci could alter splicing of candidate genes. Aim of our study was to investigate alternative splicing events in known obesity loci.
Materials and methods: Genes in obesity loci (n=138) were selected based on recent GWA analyses. Alternative splicing in adipose tissue was investigated using PCR-capillary electrophoresis on subcutaneous adipose tissue samples extracted from 51 men (23 with type 2 diabetes, 28 with normal glucose tolerance) from the population-based Metabolic Syndrome in Men (METSIM) study. Furthermore, splicing pattern was determined in subcutaneous and visceral adipose tissue of 110 participants of Kuopio Obesity Surgery study (KOBS).
Results: Out of the 138 genes in known obesity loci 48 genes had known alternatively spliced isoforms based on UniProt and Alternative Splicing and Transcript Diversity (ASTD) databases. We found alternative splicing in adipose tissue for 11 of these genes. Altered splicing pattern between individuals with type 2 diabetes and non-diabetic was observed in 4 out of 11 genes: *BAT3* ($p= 2\times10^{-5}$), *SFRS10* ($p= 3\times10^{-5}$), *MSH5* (p= 0.002), and *LPIN1* (p= 0.003). Moreover, we found different distribution of *BAT3* ($p= 6\times10^{-12}$), *SFRS10* (p= 0.009) and *LPIN1* ($p= 5\times10^{-15}$) splice variants between visceral and subcutaneous fat. Finally, distribution of *MSH5* splice variants changed in response to weight loss (p= 0.002).
Conclusion: Alternative splicing was observed in genes located in the known obesity loci. The function of these splice isoforms needs to be investigated in experimental studies and their significance needs to be determined in larger population studies.
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Defective glucose homeostasis in mice inactivated selectively for *Tcf7l2* in the adult beta cell with an Ins1-controlled Cre

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Background and aims: Single nucleotide polymorphisms in the transcription factor T-cell factor 7 like-2 (TCF7L2) gene, including rs7903146, are associated with an elevated risk of type 2 diabetes in man. *TCF7L2* encodes a member of the TCF family of transcription factors involved in the control of cell growth and signalling downstream of wingless-type MMTV integration site family (Wnt) receptors. Mice inactivated for *Tcf7l2* throughout the pancreas using Pdx1.Cre-mediated recombination (Pdx1.Cre::Tcf7l2$^{fl/fl}$) display impaired glucose tolerance, beta cell expansion and insulin secretion, particularly in response to GLP-1. In this study we assess the impact of *Tcf7l2* deletion specifically in the adult pancreatic beta cell.
Materials and methods: To achieve deletion more specifically in the adult pancreatic beta cell, *Tcf7l2*-flox mice were crossed with mice in which Cre recombinase was knocked-in at the Ins1 locus to generate beta cell specific *Tcf7l2* knockout mice (Ins1.Cre::Tcf7L2$^{fl/fl}$). Intraperitoneal and oral glucose tolerance on mice maintained on a normal chow diet was monitored between 8-20 weeks.
Results: Compared to littermate controls Ins1.Cre::Tcf7L2$^{fl/fl}$ mice bred on a C57BL/6 background displayed impaired intraperitoneal glucose tolerance by 16 weeks (increase in AUC of 13.6 ± 2.8 %, n=6 mice per genotype), and impaired oral glucose tolerance from 8 weeks (increase in AUC of 10.6 ± 1.3 %, n=6), values similar to those previously observed in Pdx1.Cre::Tcf7L2$^{fl/fl}$ mice (where glucose intolerance was observed at 20 and 12 weeks when glucose was administered by the intraperitoneal or oral route, respectively).
Conclusion: These findings provide further evidence for a cell-autonomous role for *Tcf7L2* in the beta cell and suggest that lowered levels of active TCF7L2 in these cells may contribute to the effects of the risk allele. The impact of *Tcf7l2* deletion on isolated islet function, pancreatic beta cell mass, and the effect of maintenance on a high (60 %) fat diet are currently under investigation.
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Functional characterisation of the transcriptional machinery bound across the type 2 diabetes associated TCF7L2 intronic variant, rs7903146

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Background and aims: Resolving the underlying functional mechanism to a given genetic association has proven extremely challenging. However the key type 2 diabetes (T2D) associated locus, TCF7L2, presents an opportunity for translational analyses, as studies in multiple ethnicities and with Baysian modeling strongly suggest that SNP, rs7903146, is the causal variant within this gene.
Materials and methods: We hypothesized that protein factors bind to this intronic region to modulate TCF7L2 function. We carried out oligo pull-down combined with mass spectrophotometry (MS) to elucidate the transcriptional machinery across the SNP. Nuclear lysates from HCT116 cells, where TCF7L2 is abundantly expressed, were incubated with biotin-labeled, double-stranded 60bp oligonucleotides spanning rs7903146. The DNA-protein complexes were precipitated with streptavidin-agarose beads, and the bound proteins were isolated by denaturing SDS-PAGE. Following digestion with trypsin, the samples were analyzed by MS.

Results: We observed that poly (ADP-ribose) polymerase 1 (PARP1) is by far the most abundant binding factor. We also used stable isotope labeling to quantify binding affinity changes following insulin treatment. Although PARP1 binding was modestly increased by 20%, among the next most abundant binding proteins, ATP-dependent RNA helicase A and thyroid hormone receptor-associated protein 3 (THRAP3) showed markedly increased binding of 12 and 7 fold, respectively. Interestingly, all three proteins dimerize with TCF7L2 itself, supporting the notion of an expression feedback loop. In addition, we found evidence for an allelic difference for proteins with less abundant binding, namely X-ray repair cross-complementing 5 (XRCC5) and RPA/p70.
Conclusion: Our results point to a protein complex binding across rs7903146 within TCF7L2, that is both sensitive to insulin and allelic status, and reveal a possible mechanism by which this locus confers T2D risk.
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The type 2 diabetes-associated gene *ADCY5* modulates glucose-stimulated network connectivity and insulin secretion from human islets
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Background and aims: The coordinated behaviour of individual beta cells within the islet syncytium is thought to be important for the stimulation of insulin secretion. Whether, and how, this may be affected by known genetic risk factors for type 2 diabetes is unknown. Genome-wide association studies (GWAS) have recently identified a single nucleotide polymorphism (SNP), rs2877716, which increases the risk of elevated fasting blood glucose and type 2 diabetes. This SNP is located in the *ADCY5* gene, encoding the cAMP-generating enzyme adenylate cyclase 5. We sought here to determine how forced changes in *ADCY5* expression affect beta cell network coordination and insulin secretion from isolated human islets.
Materials and methods: Human islets were isolated from normoglycemic donors ($n \geq 3$) with appropriate local ethical permissions. Intracellular free Ca^{2+} ($[Ca^{2+}]_i$) was imaged in intact islets using confocal microscopy and cell-cell interactivity mapped by correlation analyses. Real-time insulin secretory dynamics were measured using the Zn^{2+}-probe ZIMIR. Gene-silencing was achieved by treatment for 72 h with lentiviral particles (MOI=100) harbouring shRNA against *ADCY5,* or scrambled RNA.
Results: Lentivirus treatment decreased *ADCY5* mRNA expression by ~90% in dissociated islets and ~30% in intact islets. *ADCY5* silencing dramatically (53.0±3.5%; P<0.01; n=11 islets) suppressed glucose- (11 *vs* 3 mM) stimulated rises in $[Ca^{2+}]_i$ and mildly (20.8±2.5%) reduced coordinated cell activity (P<0.01, n=11 islets). Apparent insulin release in the continued presence of 11 mM glucose was also lowered by *ADCY5* silencing (57.6±2.2%; P<0.01; n=7 recordings). These actions were not due to cAMP-independent effects on ATP-sensitive K^+- or voltage-gated Ca^{2+} channels, as silencing did not alter Ca^{2+} rises induced by the depolarising stimulus KCl. Paradoxically, *ADCY5* knockdown subtly but significantly enhanced the amplitude of GLP-1-evoked increases in $[Ca^{2+}]_i$ (P<0.01).
Conclusion: ADCY5 activity is required for normal glucose- but not GLP-1-stimulated insulin secretion from human islets. These data, which are consistent with clinical findings in carriers of rs2877716 risk alleles, suggest that distinct ADCY isoforms modulate cAMP increases in beta cells in response to nutrient *versus* incretin stimulation and exert differing effects on intercellular connectivity and insulin output.
Supported by: EFSD/MSD

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Effects of functional polymorphisms in GLIS3 and a putative target gene on insulin secretion
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Background and aims: Altered beta cell mass and/or dysfunction are central pathophysiological features in type 2 diabetes (T2D). Recent genetic findings have highlighted the transcription factor GLIS3 as a susceptibility gene for T2D, neonatal diabetes and congenital hyperthyroidism. GLIS3 maps to a locus associated with diabetes phenotypes in a genetic cross derived from the Goto-Kakizaki (GK) rat model of spontaneous T2D and Brown Norway (BN) controls. We identified DNA variants in Glis3 in the GK, including two non synonymous variants leading to potentially functionally important amino acid substitutions. We developed a congenic strain (CS1.a) which carries a short (1.7MB) GK genomic region containing Glis3 and surrounding genes (including Slc1a1), isolated on a BN resistant background, to test the relevance of these variants to diabetes, and carried out functional studies to identify GLIS3 targets
Materials and methods: We determined the genomic sequence across the entire GK haplotype in the CS1.a congenics. Glucose tolerance and insulin secretion tests were performed in CS1.a and BN rats. For transfection experiments, the promoter region of Slc1a1 was cloned into pGL3 basic vector upstream of the luciferase reporter gene, and co-transfected into INS-1 cells, together with the expression vector pcDNA3 containing the full cDNA for Glis3. A GLIS3 antibody (Abcam) was used for chromatin immunoprecipitation.
Results: Rats of the CS1-a strain exhibited hyperglycaemia and increased glucose-induced insulin secretion in vivo and in vitro. In INS1 cells, glucose induced insulin secretion was significantly altered when cells were transfected with a plasmid containing the GK allele of GLIS3. Sequence analysis of the GK congenic region identified polymorphisms in the promoter region of Slc1a1, close to a common binding motif for GLIS3. Luciferase assay of the cloned Slc1a1 promoter region showed that the GK variants are functional and are associated with decreased promoter activity. Results from chromatin immunoprecipitation experiments in INS1 cells showed an enrichment of SLC1A1 when GLIS3 was immunoprecipitated. Cotransfection experiments in INS1 cells demonstrated the interaction of GLIS3 with the promoter of Slc1a1, which could be further confirmed by ChIP-seq experiments.
Conclusion: We showed the impact of GK polymorphisms in GLIS3 on insulin secretion, which may at least partly account for the diabetes locus identified in the GKxBN cross. Our results demonstrate the functional impact of GK polymorphisms identified in Slc1a1 promoter and suggest that the gene encoding the glutamate transporter Slc1a1 is a direct target of GLIS3. These results provide advances in the characterization of the role of GLIS3 on insulin secretion and the molecular mechanisms involved.
Supported by: EFSD/MSD

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Functional analysis of MODY2 mutations in the nuclear export signal of glucokinase
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Background and aims: Glucokinase (GK) is a key enzyme in glucose homeostasis. GK acts as a glucose sensor in the pancreatic beta cell regulating insulin secretion and also plays a key role in glucose metabolism in the liver. Mutations in the GCK gene cause different monogenic glycaemic disorders, being the most common the familial, mild fasting hyperglycaemia or MODY2, caused by heterozygous inactivating mutations. The specific function of GK is based on the particular kinetic characteristics of this enzyme in addition to other regulatory mechanisms including covalent modifications, protein-protein interactions and subcellular localization of the enzyme. The aim of this work is to study the nuclear export mechanism of GK from the analysis of MODY2 mutations located in the nuclear export signal (NES) of the enzyme.
Materials and methods: MODY2 mutations located in the NES sequence of GK (residues 300-310) have been introduced by directed mutagenesis into

the human beta cell isoform of GCK. The biochemical effects of these mutations on the catalytic activity and thermostability of the enzyme were measured with bacterially expressed GST-GK fusion proteins. Physical interaction of GK with its regulatory protein GKRP was analyzed by enzymatic assays and/or the yeast two hybrid system. Glucokinase NES function was analyzed with a GFP fusion to rat GK residues 299-359 (pEGFP-N2-GK(299-359)). Confocal microscopy was used to study the nucleo-cytoplasmic localization of GFP constructs in transiently transfected Cos7 and Hek293T cells.
Results: Mutations V302E, L304P and L306R impaired GK enzymatic activity, reducing the activity index up to 3% of wild type level in the case of L306R. In addition, mutations V302E and L304P caused protein instability and mutations V302E, L306R and L309P altered the interaction of GK with GKRP. When transfected into Cos7 or Hek293T cells, the wild type GK(residues 299-359)-GFP fusion protein was homogenously localized in the nucleus and cytoplasm in more than 80% of transfected cells. This percentage decreased up to 65% in cells expressing GFP fusion proteins containing mutations L309P or L309H and up to 20% in cells expressing mutations V302E, R303W, L304P and R308W, where the fluorescence distribution is nuclear-enriched in more than 50% of transfected cells. Treatment with leptomycin b resulted in nuclear enrichment of fluorescence in more than 80% of cells transfected with the wild type fusion protein and did not affect the predominant nuclear distribution of mutants V302E, R303W, L304P and R308W. Moreover, overexpression of exportin-1 by cotransfection of cells with pCMV-HA-CRM1 and wild type GFP-fusion constructs resulted in an increase of cells with cytoplasmic enrichment of fluorescence, whilst cotransfection with the R308W mutant still resulted in a relative high percentage of cells with nuclear enrichment of fluorescence.
Conclusion: Our results indicate that GK nuclear export directed by the NES (residues 300-310) is mediated by the exportin-1 complex and MODY2 mutations in the NES sequence can affect this process, thus identifying an additional mechanism contributing to the disease.
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PS 009 Genetics: candidate gene association studies

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Usefulness of genetic risk score constructed from 49 loci for type 2 diabetes in Japanese individuals
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Background and aims: Genome wide association studies (GWAS) have identified over 60 susceptibility loci for type 2 diabetes (T2D). Although it has been shown that the ability of previous genetic information (~40 loci) to discriminate between susceptible and non-susceptible individuals is limited, effect of the updated genetic information for T2D has not been evaluated. Therefore, we intended to assess the clinical utility of GWAS-derived T2D susceptibility variants in a Japanese population.
Materials and methods: First, we examined up to 11,532 Japanese individuals for 55 GWAS-derived T2D susceptible single nucleotide polymorphisms (SNPs), and selected 49 SNPs, which showed consistent effect direction with the original reports [Odds Ratio (OR) > 1]. GRS was constructed in 2,613 T2D cases and 1,786 controls, whose genotype information for the 49 SNPs was complete, by counting the number of risk alleles of the 49 SNPs in each individual. We also constructed a GRS for β cell function (β-GRS) with 24 SNPs and a GRS for insulin resistant (R-GRS) with 7 SNPs based on the function of the genes encoded in these 49 loci. The association of the GRS with the disease or T2D-related traits was analyzed using a logistic regression model or linear regression analysis respectively.
Results: The GRS was significantly associated with T2D [OR/per risk allele = 1.12, 95% confidence interval (CI); 1.10 - 1.13, $p = 2.92 \times 10^{-44}$]. The association was similar even after adjustment for age, sex and log-transformed BMI (OR/per risk allele = 1.13, 95% CI; 1.11 - 1.15, $p = 8.75 \times 10^{-45}$) In receiver operating characteristic (ROC) analyses, area under the curve (AUC) for the GRS alone (model-1) and for age, sex, and BMI (model-2) were 0.624 and 0.743, respectively. Addition of the GRS to model-2 (model-3) resulted in a small, but significant increase in the AUC [AUC for model-3 = 0.773, p-value for model-2 vs. model-3 = 7.99×10^{-15}, ΔAUC (model 3- model 2) = 0.03]. The effect of the GRS was more conspicuous in non-obese individuals (BMI < 25, AUC = 0.640, ΔAUC = 0.029, $p = 2.31 \times 10^{-11}$) than in obese individuals (BMI ≥ 25, AUC = 0.601, ΔAUC = 0.021, p = 0.006). The GRS was associated with age at onset (β = -0.199, se = 0.073, p = 0.0069) in T2D patients (n = 1,591) and with fasting plasma glucose (β = 0.009, se = 0.004, p = 0.021) in non-diabetic controls (n = 804). The GRS was not associated with either Homeostasis Model of Assessment β-cell function (HOMA-β) or HOMA of insulin resistance (IR) in 574 control participants ($p \geq 0.05$), whereas β-GRS and R-GRS were significantly associated with HOMA-β (β-GRS: β = -0.017, se = 0.007, p = 0.011) and HOMA-IR (R-GRS: β = 0.031, se = 0.014, p = 0.028) respectively.
Conclusion: Currently available genetic information is still not sufficient for translation into clinical practice, but may have a greater impact on T2D susceptibility in non-obese individuals.
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A genetic risk score comprising common variants associated with fasting insulin is associated with OGTT- and clamp-based indices of whole body insulin sensitivity
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Background and aims: Recently, a genome-wide association study of glycaemic traits identified 19 variants associated with fasting insulin at genome-

wide levels of significance. These variants showed an association with the dyslipidaemic profile of higher triglycerides and lower HDL, consistent with an effect on insulin resistance. We sought to investigate the association of these variants with euglycaemic-hyperinsulinaemic clamp- and OGTT-based indices of whole body insulin sensitivity.
Materials and methods: We investigated the association of a genetic risk score comprising 18 variants associated with fasting insulin (after excluding *FTO*, which had an association with fasting insulin driven entirely by BMI) with clamp- and OGTT-based measures of insulin sensitivity in up to 3,409 non-diabetic individuals from the Ely (N=1,371), RISC (N=1,031) and ULSAM (N=907) studies using linear regression models adjusted for age and sex (and other covariates where specified). Per-fasting insulin-raising allele effects on log-transformed and standardised outcomes (including glucose and insulin at 0, 30, 60 and 120 min during the OGTT, anthropometric and lipid traits as well as clamp- and OGTT-based indices) were combined across studies using fixed effects meta-analysis.
Results: The genetic score was associated with clamp-based whole body insulin sensitivity (β-coefficient for M/I (95% CI): -0.02 (-0.04, -0.01); p=0.003) and with the OGTT-based Matsuda index (-0.03 (-0.04, -0.01); p<0.001); these associations remained significant after adjustment for triglyceride levels (p<0.01). Associations of the score with HDL and triglycerides were abolished after adjustment for M/I (both p>0.2). The score was not associated with BMI, waist circumference, or body fat percentage (all p>0.5), nor with glucose levels at 0, 30, 60, or 120 minutes during the OGTT (all p>0.15), although we did observe associations with higher insulin levels at all time points (all p<0.001), as well as with the insulinogenic index (0.03 (0.01, 0.04); p<0.001).
Conclusion: A genetic risk score comprising variants associated with fasting insulin is associated with clamp- and OGTT-based measures of insulin sensitivity independently of BMI or triglyceride levels. Confirmation that this score is associated with whole-body insulin sensitivity offers opportunities for understanding the role of insulin resistance in the aetiology of a range of disease processes.

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Type 2 diabetes genetic risk score is more strongly associated with basal insulin secretion and insulin requirement than family history in Japanese type 2 diabetic patients

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Background and aims: We have previously demonstrated that type 2 diabetes (T2DM) genetic risk score (GRS) was associated with the early age at diagnosis of diabetes (AOD), basal insulin secretion, and the future requirement of insulin injections. On the other hand, a family history of diabetes was well known to be a strong risk factor for the development of T2DM. However, no reports examined which genetic information was stronger associated with clinical information about T2DM. Therefore, in this study, we compared the clinical usefulness between GRS and family history.
Materials and methods: We analyzed 14 SNPs at HHEX, CDKAL1, CDKN2B, SLC30A8, KCNJ11, IGF2BP2, PPARG, TCF7L2, FTO, KCNQ1, IRS-1, GCKR, UBE2E2, and C2CD4A/B in 831 Japanese type 2 diabetic patients by using TaqMan PCR assay and the GRS was calculated according to the number of risk alleles by counting all 14 SNPs (T-GRS) as well as 11 SNPs related to β-cell function (β-GRS). We also examined self-reported family history of T2DM in first- and second-degree relatives and defined the number of parental history of T2DM as family history score (FHS). Furthermore, we obtained clinical information about T2DM and defined the subjects who were required to inject more than 10 units of insulin a day continuously as insulin requiring subjects.
Results: The FHS had stronger effects on AOD than both GRS (β for FHS=-4.448; $P = 3.7 \times 10^{-9}$, β for T-GRS=-0.663; $P = 8.0 \times 10^{-4}$, β for β-GRS=-0.608; $P = 2.9 \times 10^{-3}$) after adjustments for sex and the maximum BMI. However, both GRS had slightly stronger effects on F-CPR than the FHS (β for T-GRS=-0.032; $P = 0.025$, β for β-GRS=-0.036; $P = 0.014$, β for FHS=-0.115; $P = 0.035$) after adjustments for related co-variables. Moreover, both GRS were also significantly associated with insulin requirement, whereas FHS were not associated with insulin requirement (β for T-GRS=0.014; $P = 0.030$, β for β-GRS=0.014; $P = 0.041$, β for FHS=0.019; $P = 0.474$) after adjustments for related co-variables.
Conclusion: In this study, we have demonstrated that whereas family history of diabetes was stronger associated with onset of T2DM than GRS, GRS for T2DM was stronger associated with the basal insulin secretion and future insulin requirement than family history of diabetes.

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Loss of function mutation carriers (long QT type 1 patients) of the type 2 diabetes gene *KCNQ1* have reactive hyperinsulinaemia and postprandial hypoglycaemia

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Background and aims: Carriers of common intronic single nucleotide polymorphisms in *KCNQ1* have increased susceptibility of type 2 diabetes, presumably through a reduced beta cell function. *KCNQ1* expresses the voltage-gated K^+ channel in cardiomyocytes and pancreatic beta cells. Patients with type-1 long QT syndrome have loss of function mutations in *KCNQ1*. These mutations reduce the function of the potassium channel resulting in prolonged cardiac ventricular repolarization and thus lengthened QT-interval. The patients are thereby in increased risk of syncope, arrhythmia and cardiac arrest. Over-expression of *KCNQ1* in beta cells decreases insulin secretion due to premature repolarization of the action potential in beta cells, whereas inhibition of KCNQ1 in beta cells increases insulin secretion due to prolonged depolarization. The aim of this study was to investigate if carriers of loss of function mutations of *KCNQ1* have altered islet insulin secretion and glucose regulation compared to matched control subjects.
Materials and methods: 14 (5 men / 9 women) patients (age 44.4 ±2,5 years, BMI 28.5±1,7 kg/m2, QTcB 479 ± 9 ms) from six unrelated families, diagnosed with type 1 long QT syndrome were recruited. Each patient was BMI-, age- and gender-matched with two randomly chosen control subjects with normal electrocardiogram (28 (10 men /18 women), age 47.1±1,6 years, BMI 29.0±1,1 kg/m2, QTcB 425 ± 4 ms).
Results: KCNQ1 mutation carriers had significantly increased insulin secretion upon glucose stimulation compared to matched control participants, $p_{anova} < 10^{-6}$ (45,560 ± 6,300 vs. 26,040 ± 2,777 pmol /l insulin, $p_{AUCinsulin} < 10^{-5}$). Correspondingly, the patients had significantly increased C-peptide and proinsulin levels, $p_{anova} < 10^{-5}$. The glucose levels were lower for mutation carriers compared to controls with significant lower levels 3 hours after glucose ingestion (4.4 ± 0.3 vs. 5.4 ± 0.5 mmol/l glucose, $p_{180} = 0.03$). Furthermore, KCNQ1 mutation carriers had significantly increased insulinogenic index compared to matched control participants (41.5 ± 6.6 vs. 24.3 ± 3.7, $p < 10^{-3}$). The potassium levels were significantly lower in mutation carriers compared to control participants, $p_{anova} < 10^{-4}$. Follow-up studies on prolonged oral glucose tolerance test for 6 hours were made in four available patients and matching control subjects and showed that the patients developed hypoglycaemia in the time period 3.5 -5 hours after glucose ingestion in contrast to the matched control subjects that remained normoglycemic (plasma glucose range 1.4 - 3.6 vs. 4.1 -5.4 mmol/l glucose, $p < 10^{-2}$).
Conclusion: In this study we show that long QT type 1 patients, with known loss of function mutations in *KCNQ1,* have reactive hyperinsulinemia and postprandial hypoglycaemia. These findings confirm that the voltage-gated K^+ channel encoded by *KCNQ1* is a key player in insulin secretion and suggest that this mutation underlies some cases of "essential" postprandial reactive hypoglycaemia.
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PROX1 type 2 diabetes predisposing genetic variant is associated with visceral fat accumulation and lower carbohydrate oxidation

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Background and aims: Genome-wide association studies have shown that PROX1 gene, which encodes a co-repressor of hepatocyte nuclear factor 4α64 playing a crucial role in β-cell development - is associated with type 2 diabetes susceptibility. It has been recently suggested that reduced expression of PROX1 gene results in altered β-cell insulin secretion. We conducted genetic and phenotypic studies to better understand the role of PROX1 in type 2 diabetes.
Materials and methods: We genotyped rs340874 PROX1 SNP previously identified by GWAS as type 2 diabetes risk gene in 944 subjects (463 women,481 men). In all subjects body composition analysis: percent of body fat, visceral and subcutaneous abdominal adipose tissue by multi-frequency bioimpedance method have been performed. Total energy intake, energy density and content of total carbohydrates, fat, protein were estimated using 3 days nutritional report. Non-diabetic subjects underwent an oral glucose tolerance test (OGTT). Moreover, in a subgroup of 48 subjects carbohydrate and lipid oxidation was evaluated with indirect calorimetry during two consecutive: normal- carb and high-carb meal tests.
Results: In the studied group the PROX1 genotypes were significantly associated with visceral fat tissue accumulation (respectively for CC vs. CT vs.TT: 114.4 vs. 96.1 vs. 94.0 cm3, $p < 0.019$), but with lower calories ($p< 0.01$), and carbohydrates ($p< 0.001$) intake. Additionally, carriers of risk allel C showed higher glucose level during oral glucose tolerance test ($p<0.004$). Moreover, subjects with CC genotype presented lower carbohydrate oxidation rate after high-carb meal ($p = 0.033$) and had statistically higher glucose ($p =0.004$) and insulin levels ($p= 0.049$) after normal carb meal.
Conclusion: Our results suggest that analyzed PROX1 gene affects insulin secretion and is associated with accumulation of visceral fat tissue. We believe that our study may help to understand the mechanisms of type 2 diabetes risk development.
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The BDNF, NEGR1 and TMEM18 genetic variants are associated with body fat content and components of metabolic syndrome in humans

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Background and aims: It has been recently reported that genes encoding proteins involved in the regulation of brain function, including: BDNF (brain-derived neurotrophic factor), NEGR1 (neuronal growth regulator 1) and TMEM18 (transmembrane protein 18) are associated with BMI and risk of obesity. It is also generally accepted that risk of metabolic syndrome development depends on the amount of visceral fat rather than on total body weight or BMI. The aim of the study was to evaluate the association of common variants of NEGR1, BDNF and TMEM18 genes with the body fat distribution and occurrence of selected components of metabolic syndrome (including type 2 diabetes, dyslipidemia, hypertension).
Materials and methods: We genotyped BDNF (rs925946), NEGR1 (rs2568958, rs2815752, rs3101336) and TMEM18 (rs7561317) SNPs in 619 overweight/obese and 332 normal weight healthy subjects (484 men and 467 women), who underwent anthropometric (BMI, WHR) and body composition analysis: total body fat, visceral (VAT) and subcutaneous abdominal adipose tissue (SAT) content by multi-frequency bio-impedance method. Measured genotype analysis (MGA) was used to test association between SNPs and phenotypes.
Results: We have found a significant association of the studied SNPs with total fat mass: respectively for BDNF rs925946 ($p<0.05$) and for NEGR1: rs2568958 ($p=0.013$), rs2815752 ($p=0.008$), rs3101336 ($p=0.010$). On the other hand there was a preferential accumulation of visceral fat related to TMEM18 rs7561317 AA genotype ($p=0.022$). Moreover, different studied gene variants were related to different components of metabolic syndrome: NEGR1 SNPs were significantly associated with waist circumference ($p=0.0003$); BDNF variants with higher risk of type 2 diabetes ($p=0.002$) and hypertension development ($p=0.01$), while TMEM18 genotypes were associated with dyslipidemia ($p=0.001$).
Conclusion: Our results confirmed the association of genes encoding proteins involved in the regulation of brain function with obesity and fat accumulation, however we demonstrated that studied SNPs can have different effect on components of metabolic syndrome. We believe that our study may help to understand the mechanisms that control body fat mass deposition and development of metabolic syndrome.
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Association of mitochondrial respiratory chain polymorphisms with type 2 diabetes in the Spanish population

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Background and aims: Study the association between genes codifying for mitochondrial respiratory chain (MRC) subunits and type 2 diabetes mellitus (T2DM) in general population of Spain.
Materials and methods: Three thousand seven hundred and thirty-one subjects (age range 21 to 89) from three different population-based studies of Spain, were studied. Forty-eight single nucleotide polymorphisms (SNPs) of chromosomal genes which codify MRC proteins were selected and genotyped by the SNPlex method. Association between presence of T2DM and glycaemia levels and these SNPs were assessed.
Results: Significant associations were observed between polymorphisms rs1053517 (NDUFS1 gene), rs683943 (COX7A2 gene) and rs4806187 (COX6B1 gene) and glycaemia (p-value= 0.0054, 0.0096, 0.0059 and 0.0077, respectively). Moreover, rs4656993 and rs1136224 SNPs (NDUFS2 gene) were associated to T2DM (OR=0.74, with a p-value of 0.0098 and OR=2,2 with p-value=0.0038, respectively). Finally, polymorphisms rs2410718 (COX7C gene) and rs11205591 (NDUFS5 gene) were associated with both glycaemia (p-value of 0.04 and 0.029 respectively) and T2DM risk (OR=4.34 and p-value 0.0013 for the first SNP and OR=0.37 and p-value 0.0008 for the second SNP).
Conclusion: Polymorphisms of genes codifying MRC can be involved in glycaemia variation and might contribute to the risk of developing T2DM in the general population of Spain.
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Association of mitochondrial respiratory chain polymorphisms with the presence of obesity and their impact in type 2 diabetes mellitus development in the Spanish population

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Background and aims: Analyze a group of genes codifying for some important mitochondrial respiratory chain (MRC) subunits and their association with BMI and obesity in the general population of Spain, regarding the impact of these associations in the development of type 2 diabetes mellitus.
Materials and methods: Three thousand seven hundred and thirty one subjects from three different population based studies from different regions of Spain, with an age range 21 to 89 were studied. Forty eight single nucleotide polymorphisms (SNPs) of chromosomal genes which codify MRC proteins were selected and processed by SNPlex method.
Results: Significant associations were observed between rs4600063 (SDHC), rs11205591 (NDUFS5), rs10891319 (SDHD) SNPs and BMI (p-value= 0.04, 0.0011 and 0.0004, respectively) and obesity risk (p-value=0.0072, 0.039 and 0.0038, respectively). In addition, two of them, the rs11205591 and the rs10891319 polymorphisms, showed a significant epistatic interaction for BMI levels and obesity risk. Finally, the GG genotype of rs11205591 polymorphism significantly reduced the risk of being diabetic after including age and sex (OR= 0.32, 0.17-0.62; p-value =0.0001), and BMI (OR=0.37, 0.19-0.72; p-value =0.0008) as covariables.
Conclusion: Several polymorphisms of genes codifying MRC can be involved in BMI variation and can be related to the risk of being obese in Spanish general population. Furthermore, the rs11205591 (NDUFS5 gene) polymorphism might contribute to the risk to develop type 2 diabetes.
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PS 010 Genetics: methylation and post transcriptional modification

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Role of microRNAs in pancreatic beta cell dysfunction associated with ageing

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Background and aims: With age many different factors including increased insulin resistance and oxidative stress and decreased insulin secretion can result in the development of type 2 diabetes. MicroRNAs are short non-coding RNAs that are involved in a variety of metabolic diseases, including type 2 diabetes. In this study, we investigated the role of microRNAs in pancreatic beta cell dysfunction associated with aging.
Materials and methods: In order to identify changes in the global microRNA expression profile, associated with aging, RNA was isolated from pancreatic islets of 3 and 12 month old Wistar rats and analyzed by microarray. The results of the microarray for microRNAs of interest were confirmed by qPCR. To mimic changes observed in the aged rats, microRNA expression levels were specifically overexpressed or down-regulated by oligonucleotides or anti-miRs, respectively in MIN6 cells and dispersed human and rat islet cells. Apoptosis was triggered by exposure of MIN6 cells or primary islet cells to cytokine mix. Apoptotic cells displaying picnotic nuclei were counted using Hoechst staining Proliferation study was assessed by treating dissociated islet cells with Exendine-4 for 5 days. After that cells were stained for Ki-67 and analyzed in fluorescent microscope. Statistical differences were determined using a Student's t test or, for multiple comparisons, with one-way analysis of variance (ANOVA) of the means, followed by a post hoc Dunnett test.
Results: In order to validate our aging model we have measured GLUT-2 and pro-insulin expression in the pancreatic islets of aged rats and investigated their proliferative capacity. We observed that pancreatic beta cells of 12 month old rats display a significant decrease in pro-insulin and GLUT-2 mRNA levels and fail to proliferate after exposure to Exendin-4 ex vivo. From 312 micro-RNAs, detected by microarray 70 microRNAs were showing significant changes in their expression level in aged animals. We focused our investigations on miR-34a, miR-383 and miR-130b for which we confirmed by qPCR an up-regulation of 2 and 4 folds and down-regulation of 3 folds, respectively, in aged animals. We then investigated the impact on beta cell survival. We have previously shown that miR-34a overexpression in MIN6 cells increases apoptosis. Here we observed that miR-34a overexpression increases apoptosis also in dispersed rat and human islet cells. In contrast, miR-383 overexpression as well as down-regulation of miR-130b in MIN6 cells and dispersed rat and human islet cells was found to protect from cytokine-mediated apoptosis.
Conclusion: Pancreatic islets of 12 month old rats have a decrease of expression if pro-insulin and GLUT-2 and fail to proliferate after exposure to Exendine-4. These results correlate with aging phenotype. We have identified several changes in pancreatic islets in microRNA expression which are associated with aging. Among them miR-34a upregulation induces beta cell apoptosis, whereas miR-383 upregulation and miR-130b down-regulation promote beta cell survival. Ongoing experiments will determine the impact of these microRNAs on insulin secretion and beta cell proliferation.
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Genome-wide DNA methylation analysis identifies epigenetic alterations in pancreatic islets from patients with type 2 diabetes

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Background and aims: Impaired insulin secretion from pancreatic islets is a key characteristic of type 2 diabetes (T2D). Studying gene regulation in pancreatic islets from patients with T2D will help understanding the pathogenesis of the disease. Epigenetic modifications, including DNA methylation and histone modifications, control cell specific gene expression and may play a role in disease development. We have previously identified al-

tered DNA methylation of *PDX-1*, *INS* and *PPARGC1A* in parallel with decreased mRNA expression of respective gene and reduced insulin secretion in pancreatic islets from patients with T2D. To further examine if epigenetics play a role in the pathogenesis of T2D, we performed a genome-wide DNA methylation analysis in human pancreatic islets from donors with or without T2D.

Materials and methods: DNA methylation of ~480,000 sites in 21,231 RefSeq genes was analysed in human pancreatic islets from 15 patients with T2D and 34 non-diabetic donors using the Infinium HumanMethylation450 BeadChip from Illumina. The DNA methylation pattern was related to T2D status and the transcriptome in the human islets. Identified candidate genes were overexpressed in clonal beta cells and insulin secretion was analysed.

Results: Pancreatic islets from human donors with T2D cultured in vitro show decreased glucose-stimulated insulin secretion compared with islets from non-diabetic donors ($P < 0.05$). We identified 3,116 CpG sites with altered DNA methylation in T2D compared with non-diabetic pancreatic islets after correction for multiple testing using a false discovery rate analysis ($q < 0.05$). These include 1,649 sites in 853 unique genes with an absolute difference in DNA methylation ≥ 5%. The majority of these sites (97%) show decreased methylation in diabetic versus non-diabetic islets and several of the differentially methylated sites are located in genes with previously known functions in pancreatic beta cells e.g. calcium channels. We also identified 102 genes exhibiting both differential expression and DNA methylation in the diabetic versus the non-diabetic islets. Furthermore, reporter gene constructs were used to functionally test if DNA methylation affects gene expression and increased DNA methylation of gene promoters resulted in a decreased transcriptional activity in clonal beta cells in vitro. Finally, overexpression of some of the identified candidate genes in clonal beta cells resulted in decreased insulin secretion

Conclusion: Epigenetic modifications in human pancreatic islets seem to contribute to impaired insulin secretion in the pathogenesis of T2D.

Supported by: SRC, ALF, EFSD/Lilly, Söderberg, Påhlsson, Novo Nordisk, Exodiab/LUDC

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Identification of SNPs associated with DNA methylation in human pancreatic islets and its role in the pathogenesis of type 2 diabetes

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Background and aims: DNA methylation of cytosines in CpG dinucleotides has a regulatory role in multiple biological processes and altered DNA methylation patterns may contribute to the development of human diseases, e.g. type 2 diabetes (T2D). We have previously shown that ~50% of today known T2D associated single nucleotide polymorphisms (SNPs) introduce or remove a CpG site (CpG-SNPs). These loci are associated with differential DNA methylation of the CpG-SNP site in human pancreatic islets, representing the candidate genes TCF7L2, KCNQ1, PPARG, HHEX, CDKN2A, SLC30A8, DUSP9, CDKAL1, ADCY5, SRR, WFS1, IRS1, DUSP8, HMGA2, TSPAN8 and CHCHD9. Some of the T2D CpG-SNP sites that exhibit differential DNA methylation were further associated with gene expression, alternative splicing events and hormonal secretion in the human islets. The aim of this study was to further explore how genetic variation controls DNA methylation in human pancreatic islets by performing a global methylation quantitative trait locus (mQTL) analysis.

Materials and methods: Human pancreatic islets from 89 donors not diagnosed with diabetes mellitus were included in the study. SNP genotyping was performed using the HumanOmniExpress BeadChip (Illumina) and DNA methylation profiling in the islets was performed using the Infinium HumanMethylation 450K BeadChip (Illumina). Gene expression was quantified in the islets using the microarray GeneChip HumanGene 1.0 ST (Affymetrix).

Results: In total, 574,553 SNPs were analysed and related to DNA methylation levels of 468,787 CpG sites by using a linear regression model. Here 67,438 SNP-CpG pairs showed significant association in cis (≤ 500 kb between SNP and CpG site) and 2,562 in trans, with significance threshold < 0.05 after Bonferroni correction. Several of the identified SNPs in the mQTL analysis were further associated with differential gene expression in the human islets, including genes involved in diabetes related pathways.

Conclusion: Our results demonstrate how genetic and epigenetic factors can interact in human pancreatic islets and provide a molecular mechanism that may contribute to the pathogenesis of diabetes.

Supported by: SRC, ALF, EFSD/Lilly, Söderberg, Påhlsson, Novo Nordisk, Exodiab/LUDC

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Genome-wide analysis of DNA methylation variations caused by chronic glucolipotoxicity in beta cells

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Background and aims: There is a growing body of literature suggesting the role of interactions between genes and the environment in development of type 2 diabetes mellitus (T2DM). However, the interplay between environment and gene in developing and progressing T2MD are not fully understood. To determine the mechanism of glucolipotoxicity affecting beta cells, this study profiled the DNA methylation of Genome-wide genes in beta cells cultured with high-glucose-lipid medium.

Materials and methods: A high throughput NimbleGen RN34 CpG Island & Promoter Microarray was used to investigate the DNA methylation profile in RINm5f beta cell line cultured with high-glucose-lipid medium, and identified the genes which experienced aberrant DNA methylation with differential enrichment peaks (DEP) analysis. Methylated DNA immunoprecipitation(MeDIP) PCR was used for further validation of the microarray results. Growth viability of beta cells was evaluated with MTT, and RT-PCR and western blot were used for analysis of insulin expression to show glucolipotoxicity affecting beta cells.

Results: In MTT assay, the growth viability of beta cells was inhibited with one-month Glu+PA treatment compared with control cells (P<0.05), and insulin mRNA and protein expression in beta cells decreased (P<0.05). Compared to control, there were 6.44% genes abnormal hypermethylated and 8.14% genes abnormal demethylated in promoter region, 11.50% genes abnormal hypermethylated and 14.07% genes demethylated at CpG loci in beta cells cultured with high-glucose-lipid medium. Some of the genes have been confirmed to play important roles in regulating the function of beta cells, such as TCF7L2, ADCY5, HNF1B, DUSP9, WFS1, PRC1, and variants in these genes have been associated with T2DM in Genome-Wide Association Study (GWAS).The MeDIP PCR results were consistency to microarray,and the mRNA expression of these genes were consistent with the methylation status apart from TCF7L2 gene. TCF7L2 promoter region was hypermethylated after Glu+PA treatment while the mRNA expression increased (P<0.05) and protein expression decreased (P<0.05), which is similar to previous studies.

Conclusion: We conclude that chronic glucolipotoxicity could induce some genes to experience an aberrant DNA methylation and affect these genes expression in beta cells, which might contribute to beta cell function failure in T2DM and be helpful to explain, at least partially, the mechanism of glucolipotoxicity on beta cells deterioration.

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Implication of insulin-like growth factor binding protein 1 DNA methylation and protein variation for type 2 diabetes

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Background and aims: Low levels of circulating insulin-like growth factor binding protein-1 (IGFBP-1) are associated with type 2 diabetes (T2D). To evaluate whether epigenetic changes provide a link for translating environmental exposures into IGFBP-1 variation in T2D, we have investigated DNA methylation levels in the 5'-UTR of the IGFBP1 gene.

Materials and methods: IGFBP1 DNA methylation analyses were performed in 506 Swedish men, including normal glucose tolerance (NGT) subjects, newly diagnosed and anti-diabetic treated T2D patients with bisulfite pyrosequencing. Serum IGFBP-1 levels were measured with radio-immunoassay.

Results: DNA methylation levels of the IGFBP1 gene in T2D patients (newly diagnosed and anti-diabetic treated patients) were higher compared to NGT

subjects (19.8% and 18.9% vs. 16.9%, p<0.001). The IGFBP1 hyper-methylation in T2D was independent of body mass index (BMI). Furthermore, newly diagnosed T2D patients with a family history of diabetes (FHD) had higher IGFBP1 methylation levels than those without FHD (20.3% vs. 18.6%, p=0.014). Serum levels of IGFBP-1 in T2D were significantly lower compared to NGT (18 μg/l in newly diagnosed T2D and 17 μg/l in anti-diabetic treated T2D vs. 24 μg/l in NGT subjects, p=0.028, p=0.002). Unlike DNA methylation, serum IGFBP-1 protein variation was dependent of BMI.
Conclusion: Data from the present study provide the first evidence that DNA hyper-methylation in the IGFBP1 gene is associated with T2D in Swedish men, and suggest that increased IGFBP1 DNA methylation and decreased IGFBP-1 serum levels may implicate the risk for T2D.
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DNA methylation, cardio metabolic risk and type 2 diabetes in south Asians and Europeans
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Background and aims: People of South Asian origin have one of the highest rates of diabetes in the world. Genetic studies have not explained this excess risk. Epigenetic processes may play a role. This study aims to identify the association between DNA methylation, type 2 diabetes and related traits and to identify ethnic differences between individuals of South Asian and European origin living in the UK.
Materials and methods: Baseline samples (n = 192) from the extensively characterised population based SABRE (Southall And Brent Revisited) cohort were utilised. Men aged between 40-69 years at baseline were included, matched for ethnicity, age, smoking status and subsequent development of diabetes and/or CHD over 20 years of follow up. Genome-wide DNA methylation analysis was performed using the Illumina HumanMethylation450 array.
Results: Numbers of CpG sites associated with traits of interest were calculated with a family wise error rate (FWER) of 0.05. Effect sizes and standard errors are shown as the % change in methylation per unit change in metabolic measure described (mmol/L). Marked ethnic differences were observed in methylation sites throughout the genome (n = 2284). Methylation was also associated with metabolic traits including fasting insulin (n = 69 methylation sites including sites in ASXL2 (effect size = 0.20%, std error = 0.02%, t statistic = 8.72, p = 1.63E-15) and TET3 (effect size = -0.69%, std error = 0.08%, t statistic = -8.26, p = 3.65E-14)) and triglycerides(n = 585 sites in >45 loci including 14q32.31 (effect size = 3.10, std error = 0.33, t statistic = 9.35, p = 2.87e-17) and TXNDC12 (effect size = 0.42, std error = 0.05, t statistic = 8.95, p = 4.04E-16)). Association between fasting glucose and methylation was less marked (no methylation sites attained FWER cut-off, the strongest association observed was in RBM28 (effect size = -0. 92, std error = 0.18, t statistic = -5.14, p = 6.99E-07).
Conclusion: Wide-spread differences in methylation by ethnic group were observed. Associations between methylation and fasting insulin and triglycerides but not fasting glucose were also identified. Ongoing investigation of ethnic differences in these methylation sites and predictive power for subsequent cardio metabolic disease may provide valuable novel insights into the determinants of excess cardio metabolic disease risk in South Asians.
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Maternal transmission of variants in the THADA gene to offspring with type 2 diabetes
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Background and aims: Type 2 diabetes (T2D) is a complex heterogeneous disease resulting from a complex interplay between genetic and environmental factors. Several GWAS have established a genetic basis for T2D in the form of >65 genetic variants accounting for ~15% of the heritability. However, transmissions of risk alleles in families have been mostly unexplored and the function of most of these variants unknown. Our goal was to assess the transmission and parent-of-origin effects (POE) of SNPs in risk of T2D and related traits, including glucose metabolism and insulin secretion in families.
Materials and methods: The Botnia study comprising of 4189 individuals (1083 families) from Western Finland & Southern Sweden were genotyped on a custom-designed SNP panel containing 88 variants in risk of T2D and related traits, including glucose metabolism and insulin secretion using a Sequenom MassArray platform. Distorted transmission of T2D risk alleles to (1) T2D and (2) hyperglycemic (T2D+IFG+IGT) offspring were assessed in 180-280 independent trios using TDT (PLINK) and combined with parental discordant tests to increase power (parenTDT, PLINK). Transmissions within nuclear families with multiple offspring were assessed using FBAT. Parent-of-origin (POE) tests were performed using PLINK. 11 quantitative traits related to glucose metabolism and insulin secretion were assessed in non-diabetic individuals using qTDT and POE tests. OGTT values were adjusted for BMI.
Results: Distorted transmission was seen for 19 T2D risk SNPs to diabetic offspring, and for 20 to hyperglycemic offspring. The most significant results include excess-transmission of the risk T allele in rs7903146 in the *TCF7L2* gene applying the TDT (p=0.009), parenTDT (p= 1.432e-006) and FBAT (0.006) tests and rs163184 in the *KCNQ1* gene in the TDT (p=0.03) and FBAT (0.0028) tests to diabetic offspring. In support of previous findings, SNP rs163184 (KCNQ1) showed a trend towards maternal transmission (p= 0.035). The rs7578597 SNP in the THADA gene showed a consistent over-transmission of the maternal risk allele T to diabetic (p= 0.019) and hyperglycemic (p=0.006) offspring. Among 88 SNPs tested for distorted transmission to quantitative traits like glucose and insulin in offspring, the strongest effect was observed for rs10830963 (MNTR1B) for 30 and 60 min glucose, (p<0.01 and CIR (p=4,40e-03). SNP rs8042680 (PRC1) showed preferential maternal transmission for 30 min glucose (p=0,0002), rs7593730 (RBMS1/ITGB6 locus) (p=0,0038) for fasting insulin, rs7961581 (TSPAN8/LGR5) for ISI (p=0,0086) whereas rs6467136 (PAX4) (p=0,018) showed preferential paternal transmission for CIR.
Conclusion: Distorted parental transmission is not an uncommon feature of T2D risk SNPs with a very strong maternal transmission shown for rs7578597 in THADA. This is interesting in light of the view that variation in the *THADA* gene also has shown evolutionary selection from Neanderthals to modern humans.

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DOLCE: new cohort to study the effects of famine on the risk of diabetes and its complications
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Background and aims: We hypothesized that studies on long-term consequences of prolonged starvation during famine periods in Ukraine might shed light on key mechanisms associated with risk of or freedom from early-onset diabetes and its complications.
Materials and methods: DOLCE (Diagnostic Optimization and Treatment of diabetes and its complications in the Chernihiv region) is a population-based study of diabetic individuals and their healthy relatives in northern Ukraine. All participants underwent anthropometric and clinical measurements, blood samples were drawn for biochemical measurements and for DNA and RNA analyses, and all participants completed a questionnaire on family history, smoking, medication, and complication status for cardiovas-

cular diseases (CVD), nephropathy, retinopathy, angiopathy and amputation. We are currently investigating temporal effects on genetic risk associated with diabetes and its complications.
Results: Our current study population includes 3,670 individuals; 570 (54.9% male, age-at-onset/duration 25/11 years) were clinically diagnosed with type 1 diabetes (T1D) and 2,360 (32.7%, 54/5) with type 2 diabetes (T2D). 39.3% of T1D patients were GAD antibody positive (≥10 kE/l), possibly due to admixture with other diabetes types. Despite the differences in diabetes duration and age, prevalence of CVD was equally high in people born during the famine in the 1930's, World War II and the 5-year post-war period. Notably, while famine had a profound effect on risk of macrovascular complications of diabetes, this pattern was absent for prevalence of microvascular complications, which were similar across all birth year groups. Similarly to previous reports, the *TCF7L2* rs7903146 risk genotypes were significantly more frequent among T2D compared to T1D patients (p=0.03), as well as less frequent in T2D patients born before the 1950's (p<0.05), potentially due to survival selection. Additionally, preliminary genetic data suggest involvement of pleiotropic loci at *FADS1*, *HNF1A* and *DGKB* known to affect lipid metabolism in survival during starvation.
Conclusion: Famine is an important risk factor for development of macrovascular complications of diabetes, while other mechanisms seem to be operative for the progression of microvascular complications. Genetic markers could possibly help to pave the way to these pathways.
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PS 011 Genetics: monogenic forms

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Clinical widened diagnostic criteria and prediction models identify "MODY-X" patients who should benefit from new specific genetic screening
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Background and aims: Maturity Onset Diabetes of the Young (MODY) is underestimated because prevalence of obesity and type 2 diabetes in young patients are actually really increasing. Monogenic diabetes can be misdiagnosed as type2 diabetes. However, the correct molecular diagnosis has implication in patient treatment, prognosis and is important for patient's family.
Materials and methods: Direct sequencing of *GCK* and *HNF1A* genes were performed for 155 patients with a clinical suspicion of MODY. We compared the phenotype of all patients according to genetic results. We compared the probability to be MODY according to classical diagnostic criteria of MODY and according to the clinical prediction models proposed by Shields and all (2012) and by Béllanné-Chantelot and all (2011).
Results: A molecular abnormality of *GCK* was found in 15 patients ("MODY 2") and of *HNF1A* in 20 patients ("MODY 3"). 119 patients did not present any mutation of *GCK* or *HNF1A* ("No mutation "). Phenotype of the "MODY 2" and "MODY 3" patients help us to define "widened criteria " who are: age in the diagnosis < 40 years, BMI < 30 kg/m^2, ≥ 3 affected generations, among which at least 2 before age of 40ans. "No Mutation " are significantly older and have a higher BMI. MODY 2 and MODY 3 patients have more often ≥ 3 affected generations and a lower age at diagnosis. Algorithms find stronger probability of MODY for "MODY2" and "MODY3" than for "No Mutation". However, among "No Mutation" patients, 21 present the "widened criteria" of the MODY and 17 others have a probability of MODY X according to Shields > 25 %. 6 other patients present morphological renal anomalies suggestive of a genetic development abnormality.
Conclusion: In the "No mutation" group phenotype is variable. This group is probably constituted with MODY-X and with type 2 diabetes. Use of "widened diagnostic criteria" and on-line predictive probability calculators identifies, in this group, 44 patients who are likely to benefit from specific genetic explorations. This will be possible with the development of new methods for genetic screening.

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Reclassification of diabetes aetiology in a family with multiple diabetes phenotypes
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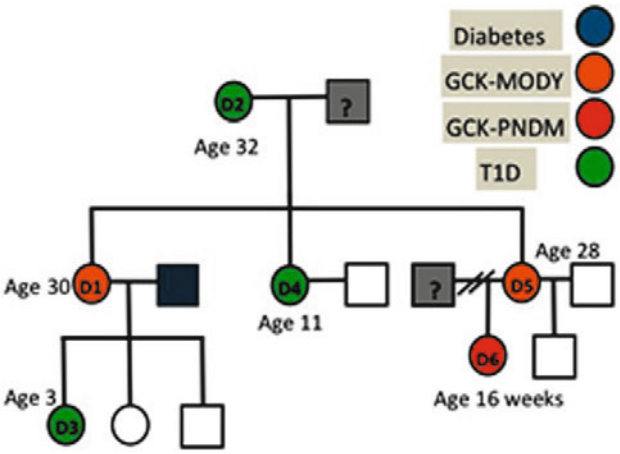
Background and aims: MODY is rare (1% of all diabetes) but the diagnosis has significant implications for probands and relatives by allowing personalised management and informing prognosis. The aim of this study was to show that appropriate use of genetic and non-genetic investigations leads to correct classification of diabetes aetiology.
Materials and methods: A Caucasian family had six members (D1-D6) with diabetes, with diverse phenotypes. Clinical history, HbA1c, C-peptide, pancreatic β-cell antibodies and sequencing for monogenic causes of diabetes were obtained.
Results: D1 had insulin-treated gestational diabetes (GDM), with normal delivery at 38 weeks. Insulin was discontinued post-delivery, but fasting hyperglycaemia persisted. She was β-cell antibody negative, with HbA1c 43 mmol/mol and C-peptide 0.79 nmol/l. Her sister (D2) also had a history of GDM. D1 and D2 were found to have the previously reported heterozygous GCK missense mutation T206M, confirming the diagnosis of GCK-MODY, consistent with the clinical scenarios.
Results: D3 (D2's daughter) had low birth weight, failed to thrive and presented with diabetic ketoacidosis (DKA) at 16 weeks. She was negative for

mutations in KCNJ11, ABCC8 and INS and was on insulin since diagnosis. Genetic screening showed that she carried the T206M and also the R43P missense mutation in GCK. Therefore, she has GCK- permanent neonatal diabetes (PNDM) due to compound heterozygous mutations. Both mutations have been functionally characterised in GCK-MODY and shown to be kinetically inactivating due to decreased affinities for glucose and decreased maximal specific activities compared to wild type GCK. The other three family members (D4: D1's mother, D5: D1's other sister and D6: D1's daughter) had a clinical diagnosis of type 1 diabetes (T1D). Both D4 and D5 had history of DKA, microvascular complications and investigations showed HbA1c >86 mmol/mol and undetectable C-peptide <0.02 nmol/l. D4 had positive GAD antibodies. D6 was diagnosed aged 3, had positive GAD and ICA at diagnosis and undetectable urinary C-peptide. These features were inconsistent with GCK-MODY and confirmed T1D in these three cases. We later showed D4 and D5 were negative for GCK mutations. We also deduced that the fathers of D1 and D3 carry undiagnosed GCK mutations.

Conclusion: Two family members, previously misclassified, were found to have GCK-MODY with important management consequences. Three family members were confirmed with T1D and along with the child with GCK-PNDM, should continue insulin therapy. This family demonstrated three different diabetes phenotypes and exemplifies the importance of careful phenotyping and investigation of relatives after discovering a genetic aetiology of diabetes.



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Detection of PAX4 mutations in Japanese patients with maturity-onset diabetes of the young

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Background and aims: Maturity-onset diabetes of the young (MODY) is the most common form of monogenic diabetes mellitus (DM), and mutations in twelve different disease-causing genes have been identified till date. However, in Japan, approximately 80% patients with a clinical diagnosis of MODY did not have pathogenic mutations in the screening with MODY1, 2, 3, and 5 genes. PAX4 is a transcription factor that plays an important role in the differentiation of islet β-cells. *PAX4* mutations were recently reported to be associated with MODY (also known as MODY9) in the Thailanders. Because there is no report regarding the association between *PAX4* and MODY in the Japanese, the aim of this study was to investigate the contribution of *PAX4* mutations to MODY in a subset of Japanese patients.

Materials and methods: Forty-three probands with MODY were studied. The inclusion criteria were as follows: (a) DM diagnosis before 25 years of age; (b) absence of obesity; (c) history of familial DM spanning either three or two generations, with one family member being diagnosed with DM before 25 years of age in the latter case; and (d) absence of disease-causing mutations in MODY1, 2, 3, and 5 genes. *PAX4* coding regions were examined in each subject by direct nucleotide sequencing. The frequency of each *PAX4* mutation was compared with that previously reported for Japanese patients with type 2 diabetes mellitus (T2DM) and non-DM. The χ^2 test was used for statistical analysis; $p < 0.05$ was considered significant.

Results: The mean age at DM diagnosis was 16.2 ± 4.6 years, and the mean maximum body mass index was 22.2 ± 2.2 kg/m^2. We identified five missense and one silent mutations: R31Q (minor allele frequency = 0.036, genotype frequency: homo/hetero/wild = 0/3/40), R121W (0.081, 0/7/36), Q173Q (0.012, 0/1/42), R192S (0.047, 0/4/39), R192H (0.151, 0/13/30), P321 (0.465, 14/12/17). These mutations were previously described in Japanese patients with T2DM and non-DM, indicating that these substitutions were not the genetic cause of MODY. However, we found the frequency of R192H mutation to be significantly higher in patients with MODY than in those with T2DM or non-DM ($\chi^2 = 11.45$, $p = 0.001$ and $\chi^2 = 7.939$, $p = 0.005$). Moreover, the prevalence of R121W mutation, which was previously reported to be associated with T2DM, was four times higher in our subset of patients ($\chi^2 = 12.49$, $p = 0.002$)

Conclusion: We identified five missense and one silent substitutions in *PAX4* in Japanese patients with MODY. The frequencies of two of these were significantly higher in patients with MODY than in those with T2DM, suggesting that R121W and R192H mutations may contribute to the early onset of DM.

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Phenotypic heterogeneity in Japanese patients with hepatocyte nuclear factor-1b mutations

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Background and aims: The aim of this study was to investigate clinical spectrum of hepatocyte nuclear factor-1b(*HNF1B*) mutation in Japanese patients with urogenital and /or pancreatic abnormality.

Materials and methods: Among 292 patients who were diagnosed with diabetes at age of 30 or younger, and gave consent for the study from 1992 to end of 2012, 185 patients were evaluated by abdominal ultrasonography. Kidney cysts and/or hydronephrosis, or pancreatic abnormality were detected in unrelated 46 patients among them, who were recruited in this study. *HNF1B* mutation was screened by direct sequencing. If the sample did not show any substitution, we next performed MLPA for screen genomic rearrangements. To confirm complete deletion in *HNF1B*, the array CGH experiment using the human genome CGH microarray (Agilent technologies, Santa Clara, CA, USA) was performed. Paired t-test and chi2 test were used.

Results: The age at diagnosis of diabetes and BMI in 46 patients (M/F=29/17) were 20.0±6.4 years old, and 25.8±6.6. Five heterozygous mutations (het) including R177X, W238R, Q275X, R276Q, and IVS 2nt+1G>A were identified. Seven patients were found to have hemizygous deletion mutation (del) spanning 1.4 Mb on 17q12 including whole *HNF1B*, and one case with aberration in exon 3 was identified. Detection rate of mutations in *HNF1B* was 13/46 (28.3%), and genomic rearrangements were found in 8/13 (61%). Since pathogenicity of exon aberration in this patient was unclear, we analyzed 12 patients for the further study. All patients with (het) had a family history; however, six patients were de novo in patients with (del) ($p < 0.05$). The age at diagnosis of diabetes and BMI were smaller in subjects with mutations compared to those without (Age 16.9±5.5 vs. 21.3±6.3, $p < 0.05$), (BMI 19.3±1.7 vs. 28.3±6.1, $p < 0.05$). Agenesis of pancreatic body and tail was observed in two with (del) and one with (het), hydronephrosis and/or ureterectasis were found in four with (del) and one with (het), kidney cysts were found in five with (del) and four with (het), though none of them were statistically significant. Three cases with (del) developed diabetes suddenly with DKA/ketosis, but none with (het).

Conclusion: Detection rate of mutation in *HNF1B* among patients with early onset and with malformations in kidney or pancreas was 28.3%, and genomic rearrangements was observed with 61%. Patients carrying mutations in *HNF1B* were characterized by lower BMI and younger onset. Deletion mutation was occurred with de novo except one. Those information will be useful for clinical diagnosis of MODY5.

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No attenuation of CRP response to acute inflammation is seen in HNF1A-MODY

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Background and aims: HNF1A is known to regulate hepatic C-reactive protein (CRP) production. We have shown that European subjects with *HNF1A* mutations (causing HNF1A-MODY) have low baseline levels of CRP. HNF1A

is part of the transcriptional complex involved in interleukin-6 stimulated CRP production during acute inflammation, so we hypothesised that they would also have an attenuated CRP rise during an acute inflammatory stimulus. This could lead to impaired complement activation and macrophage function in HNF1A-MODY. We used a standardised model of acute inflammation (endotoxin infusion) to investigate this.
Materials and methods: HNF1A-MODY subjects with diabetes (n=7) and normoglycaemic controls, matched for BMI and gender (n=9), were given intravenous endotoxin (E. coli lipopolysaccharide: 2ng/kg). Serum CRP, white cell count (WCC) and erythrocyte sedimentation rate (ESR) were measured at baseline and 2, 4, 6, 24 hours and 8 days after endotoxin administration.
Results: Apart from fasting glucose, baseline characteristics were well-matched between cases and controls.CRP and WCC rose as expected in response to endotoxin in both groups (Figure 1) while no consistent response was seen with ESR. There was no difference in either the CRP levels or the WCC at any time point during the acute inflammatory response in subjects with HNF1A-MODY compared to controls (p=0.42 and 0.15 respectively by repeated measure analysis of variance). Peak mean CRP (HNF1A-MODY vs. controls) was 28.1 vs. 31.7 mg/L at 24hrs (p=0.45) and peak mean WCC was 11.2 vs. 9.5 at 6 hours (p=0.31).
Conclusion: In this study we demonstrate that *HNF1A* haploinsufficiency does not appear to impair CRP production during the response to a moderate acute inflammatory stimulus. Whether HNF1A is present in adequate amounts or transcription occurs via an HNF1A-independant mechanism remains to be elucidated.

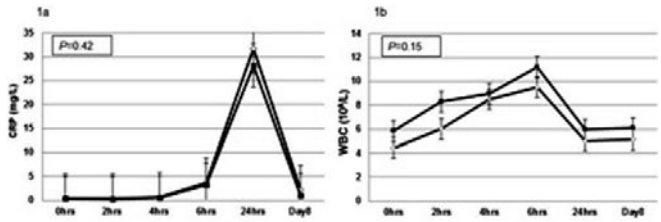

Figure 1 shows mean (±SEM) CRP (panel a) and WCC (panel b) for HNF1A-MODY (filled squares) and controls (open diamonds) for each time point. P values refer to differences between groups calculated by repeated measure analysis of variance.

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Cellular re-uptake of secreted carboxyl-ester lipase protein in CEL-MODY, a syndrome of diabetes and pancreatic exocrine dysfunction
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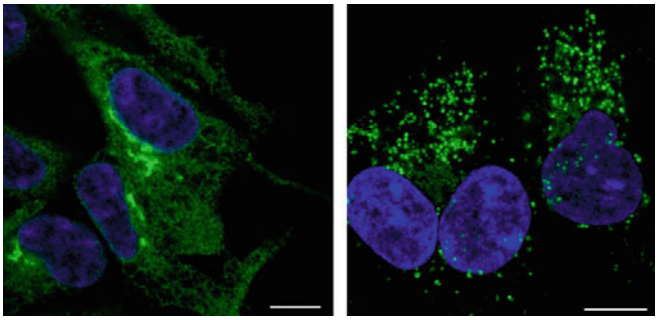
Background and aims: Single-base mutations in the carboxyl-ester lipase (CEL) gene have previously been identified in families with an autosomal dominantly inherited syndrome of diabetes and pancreatic exocrine dysfunction (CEL-MODY). The mutation c.1686delT is located in the last exon of the CEL gene and leads to a secreted protein with an altered C-terminus and a tendency to self-associate and aggregate. The aim of this study was to characterize the effect of the mutation in cellular model systems.
Materials and methods: Wild type (WT) and mutant (MUT) CEL proteins were expressed in stably transfected HEK293 cells and HeLa cells. We employed cell fractionation, immunoperoxidase electron microscopy and immunofluorescense confocal microscopy, and performed a comparison of the intracellular distribution of CEL-WT and CEL-MUT.
Results: In stably transfected HEK293 cells, we found that CEL-WT was predominantly detected in the endoplasmatic reticulum and Golgi compartments (left image), whereas CEL-MUT was present in a cytoplasmatic punctate pattern (right image). Electron microscopy demonstrated the presence of CEL-MUT aggregates at the plasma membrane and in the lumen of single-membrane vacuoles, which by immunofluorescense confocal microscopy was shown to express Lamp-1. Evidence for involvement of autophagy in CEL-MUT degradation was not found. However, when untransfected HEK293 or HeLa cells were grown in conditioned media from CEL-expressing HEK293 cells, we observed uptake and accumulation of the CEL-MUT protein variant in Lamp-1-positive organelles.
Conclusion: Our results suggest that the altered physicochemical properties of the C-terminal end of CEL-MUT causes this protein to be reabsorbed by endocytosis after its secretion, followed by degradation in the lysosomes. This finding may have implications for the understanding of how the acinar-specific CEL-MUT protein induces both exocrine and endocrine pancreatic disease.



Supported by: University of Bergen, INNOVEST, Helse Vest, K.G. Jebsen Foundation

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Preserved postprandial GLP-1 and GIP responses in patients with maturity onset diabetes of the young
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Background and aim: Maturity onset diabetes of the young (MODY) is a clinically and genetically heterogeneous subgroup of non-autoimmune diabetes, which constitutes 1-2% of all diabetes. We aimed to evaluate postprandial glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) responses in patients with MODY2 (glucokinase gene mutations) and MODY3 (hepatocyte nuclear factor (HNF) 1α gene mutations), respectively, and in healthy control subjects (CTRLs) matched for body mass index (BMI).
Material and methods: Ten MODY3 patients (age: 31±3 years (mean±SEM); BMI: 24±1 kg/m^2; fasting plasma glucose (FPG): 8.4±0.8 mmol/l; HbA_{1c}: 52±4 mmol/mol), 9 MODY2 patients (age: 43±5 years; BMI: 24±2 kg/m^2; FPG: 7.3±0.3 mmol/l: 49±2 mmol/mol) and 10 CTRLs (age: 40±5 years; BMI: 24±1 kg/m^2; FPG: 5.1±0.1 mmol/l; 34±1 mmol/mol) were examined with and 4h liquid test meal (525 kcal: 65 g carbohydrate, 20 g fat, 21 g protein).
Results: The 3 groups exhibited similar peak meal-stimulated plasma GLP-1 (27±5 (MODY3), 25±3 (MODY2), 27±2 pmol/l (CTRLs), p=0.89) and similar AUCs for plasma GLP-1 (3.7±0.4 (MODY3), 3.9±0.4 (MODY2), 4.3±0.3 nmol/l×min (CTRLs), p=0.50). Similar peak GIP (88±9 (MODY3), 114±16 (MODY2), 109±9 pmol/l (CTRLs), p=0.18) was also observed, but a higher AUC for GIP was observed for MODY2 patients (17.9±1.7 nmol/l×min) compared to MODY3 patients (13.9±1.3 nmol/l×min, p=0.022) but not compared to CTRLs (12.7±0.7 nmol/l×min, p=0.097). No difference in AUC between MODY3 patients and CTRLs was observed, p=0.76.
Conclusion: MODY3 and MODY2 patients exhibit preserved postprandial responses of the two incretin hormones GLP-1 and GIP.
Clinical Trial Registration Number: NCT01342939
Supported by: Novo Nordisk Foundation

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Differential glucagon responses to glucose in maturity onset diabetes of the young type 2 and 3
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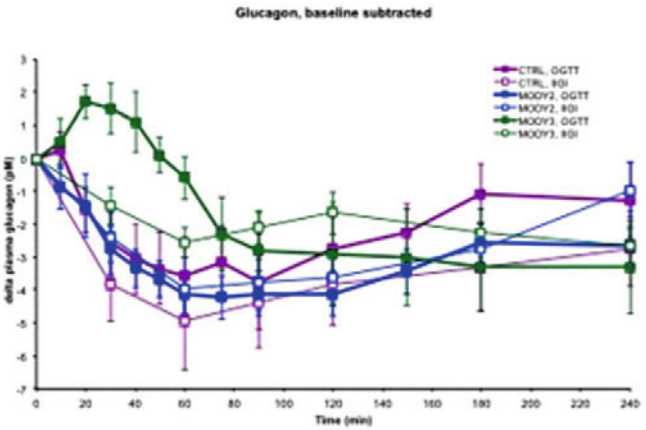
Background and aim: Maturity onset diabetes of the young (MODY) is a clinically and genetically heterogeneous subgroup of non-autoimmune diabetes, which constitutes 1-2% of all diabetes. Impaired suppression of gluca-

gon following oral ingestion of glucose is a characteristic feature of type 1 and type 2 diabetes. We aimed to study glucagon responses to oral and intravenous (iv) glucose, respectively, in patients with MODY2 (glucokinase gene mutations) and MODY3 (hepatocyte nuclear factor (HNF) 1α gene mutations) and in healthy control subjects (CTRLs) matched for body mass index (BMI).

Material and methods: Ten MODY3 patients (age: 31±3 years (mean±SEM); BMI: 24±1 kg/m^2; fasting plasma glucose (FPG): 8.4±0.8 mmol/l; HbA_{1c}: 52±4 mmol/mol), 9 MODY2 patients (age: 43±5 years; BMI: 24±2 kg/m^2; FPG: 7.3±0.3 mmol/l: HbA_{1c}: 49±2 mmol/mol) and 9 CTRLs (age: 41±5 years; BMI: 24±1 kg/m^2; FPG: 5.1±0.2 mmol/l; HbA_{1c}: 34±1 mmol/mol) were examined on 2 separate occasions: 4h 50g-oral glucose tolerance test (OGTT) and isoglycaemic iv glucose infusion (IIGI).

Results: A hyperglucagonaemic response was seen in MODY3 patients compared to MODY2 patients and CTRLs during OGTT, but not during IIGI ($\Delta AUC_{0\text{-}60min}$ (OGTT): 46±33 vs. −121±46 pmol/l×min, p=0.013, −142±36 pmol/l×min, p=0.006, respectively; $\Delta AUC_{0\text{-}60min}$ (IIGI): −83±20 pmol/l×min (MODY3) vs. −190±55 pmol/l×min (CTRLs) vs. −132±34 pmol/l×min (MODY2), p=0.15). No difference in glucagon response between OGTT and IIGI was seen in MODY2 patients, p=0.86 and CTRLs, p=0.34, but MODY3 patients showed a significantly (p=0.005) higher response during OGTT compared to IIGI.

Conclusion: MODY3 patients exhibit hyperglucagonaemia in responses to oral glucose, but are able to suppress glucagon normally following iv glucose. In contrast, MODY2 patients respond with normal glucagon suppression to both oral and iv glucose.



Clinical Trial Registration Number: NCT01342939
Supported by: The NovoNordisk Foundation

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A case of Transient Neonatal Diabetes (TND) in a young woman with Beckwith-Wiedemann Syndrome (BWS) and a molecular diagnosis of Hypomethylation of Multiple Loci (HIL)

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Background and aims: BWS is an overgrowth disorder associated with tumour predisposition. TND is a rare form of diabetes with neonatal presentation, remission in infancy, but subsequent relapse in ~50% of cases. TND and BWS have heterogeneous, partially-overlapping clinical features, and multiple genetic aetiologies causing deregulation of imprinting. Genomic imprinting is restriction of gene expression dependent on parent of origin, effected by changes not in DNA sequence but DNA expression. Notably, 50% of TND patients and 20% of BWS patients with imprinting errors have additional epigenetic mutations at multiple loci. We present a case with clear, separate, clinical features of both disorders.

Results: A 25 y old woman with BMI 25 and a background of BWS presented with recurrent genitourinary infections. She had glycosuria and an HbA_1C of 104mmol/mol (20-40mmol/mol). Fasting serum insulin was 8.3 mU/L (6-25) and C-peptide 506 pmol/L (364-1655) but islet antibodies were absent. Endocrine investigations for secondary diabetes were normal. She was born at 36 weeks gestation with birth weight 3.18kg (75th centile) and length 58cm (99th centile). Neonatal hyperglycaemia was treated with insulin for about 8 weeks. She also had a history of juvenile osteoporosis and fractures. After dietary advice and Metformin, she lost 6kg and her HbA_1C improved to 62mmol/mol after 4 months. Further molecular testing was carried out due to the history of neonatal diabetes and BWS. No mutations were discovered in the *KCNJ11*, *ABCC8* and *INS* genes. Imprinting analysis identified imprinting mutations at both the BWS and TND loci, as well as at other loci. These findings are consistent with her dual diagnosis of TND and BWS.

Conclusion: This is the first report where an individual has fully presented clinically and molecularly with TND and BWS in addition to epigenetic mutation of other imprinted loci. There is very little published literature on this subset of imprinting disorders. The cause may have been purely environmental or stochastic, or mutation of a regulatory factor involved in imprinting of all the affected loci; further genetic studies are ongoing.

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Diabetes mellitus and intestinal ulcers: it's a Wolfram Syndrome 2?

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Background and aims: Wolfram syndrome (WS) is an autosomal recessive disease with incomplete penetrance. WS is also known as DIDMOAD syndrome defined by the association of childhood-onset non-autoimmune diabetes mellitus (DM), optic atrophy (OA), diabetes insipidus (DI), and deafness (D). The majority of patients shows mutations in the WFS1 gene on chromosome 4p16.1, but a second WS gene on chromosome 4q22-24, named CISD2, is responsible of WS2. The mutation of CISD2 gene was identified only in few familial Jordanian consanguineous members. Patients affected by WS2 had additional features to those previously described in WS, such as defective platelets aggregation with collagen and upper intestinal peptic ulcers (UIU). We describe the first European girl affected by WS2.

Materials and methods: A 17 year old girl, first child of unrelated healthy parents, was referred to our hospital because of weight loss, polyuria and polydipsia. Laboratory investigations showed hyperglycaemia (glucose value 26.7mmol/L), HbA1c 13%, absence of chetonemia and acidosis, iron deficiency anemia (Hb: 10.7g/dl, MCV: 68.2fl, serum iron: 16μg/dl), presence of glycosuria, absence of chetonuria. Auto-antibodies for type 1DM were not detected. Diagnosis of both non-autoimmune diabetes and anemia were made, treated with insulin and martial therapy respectively. Her history was characterized by presence of UIU, from age of 5 years to 14 years, all diagnosed with esophagogastroduodenoscopy, and treated with pump proton inhibitors (PPI) therapy that has never been suspended during her entire life. On the age of 14 years, an abdominal laparoscopy, performed in the suspicion of annular pancreas, showed intussusception of the duodenum, that was treated with resection of a part of the duodenal and with a duodenal-jejunal anastomosis. At the age of 16 years an opthalmological evaluation, performed for visual impairment, revealed optic atrophy; the visual-evoked potentials executed, showed a pathological speed conduction, while both multifocal electroretinogram and Brain Magnetic Resonance were normal. The suspicion of Optic neuropathy Leber was placed without genetic testing. On the basis of clinical history and the presence of DM we suspected a WS. An audiologic evaluation was executed, showing sensorineural hearing loss at high frequencies. The presence of diabetes, optic atrophy, deafness and UIU, suggested the diagnosis of WS2. The platelets aggregation defect was explored, showing a defective platelet aggregation with collagen, while DI was absent. Genetic testing for WS2 was requested.

Results: The genetic testing showed a homozygous deletion of exon 2 and exon 3 of CISD2 gene in the patient, while her parents and brother were heterozygous for the same mutation.

Conclusion: The patient currently is 20 years old and presents: diabetes mellitus, treated with insulin therapy; visual impairment, because of optic atrophy, treated with lenses; tinnitus, due to sensorineural hearing loss at high frequencies; platelet aggregation defect; tendency to UIU treated with PPI therapy. This is the first case of a European girl with WS2 and reminds us that, although rare, the WS2 is a diagnosis to be suspected in presence of DM, OA, D and UIU.

PS 012 Genetics: gene-lifestyle interactions and pharmacogenetics

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Dietary polyunsaturated fatty acids and the Pro12Ala polymorphisms of PPARG regulate serum lipids through divergent pathways: a randomised crossover clinical trial

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Background and aims: Human and animal studies suggest an interaction between the Pro12Ala polymorphism of PPARG and dietary fat. To investigate if subjects with the Pro12Pro and Ala12Ala genotypes of PPARG respond differently to a diet supplemented with high saturated (SAFA) or polyunsaturated fat (PUFA).
Materials and methods: We recruited nondiabetic subjects from a population based METSIM study to obtain individuals with the Ala12Ala and the Pro12Pro genotypes. Seventeen men with the Pro12Pro genotype and 14 with the Ala12Ala genotype were randomized to both a PUFA diet and a SAFA diet for 8 weeks in a crossover setting. Adipose tissue biopsies for mRNA expression of PPARG2 variant were obtained during the diet intervention.
Results: At baseline subjects with the Ala12Ala genotype had higher levels of HDL cholesterol and lower levels of LDL cholesterol, total triglycerides and apolipoprotein B compared to those subjects with the Pro12Pro genotype ($p<0.05$, FDR <0.1). PPARG2 mRNA expression was higher in participants with the Ala12Ala genotype than in participants with the Pro12Pro genotype throughout the study (FDR <0.001), and PPARG2 mRNA expression correlated negatively with total triglycerides ($p<0.05$). PUFA diet resulted in lower levels of fasting glucose, total cholesterol, total triglycerides and apolipoprotein B ($p<0.05$, FDR <0.1) independent of the genotype and without affecting PPARG2 mRNA expression in adipose tissue. No effect of the genotype on serum glucose and insulin levels was observed.
Conclusion: Beneficial effects of dietary PUFA and the Ala12Ala genotype of PPARG on serum lipids are mediated through divergent mechanisms.
Clinical Trial Registration Number: NCT01274091
Supported by: Academy of Finland, Finnish Diabetes Research Foundation

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High heritability and genetic correlation of intravenous glucose- and tolbutamide-induced insulin secretion

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Background and aims: It has been established that the etiology of type 2 diabetes has a significant genetic contribution and that an altered beta cell function is central in the development of the disease. The aim of this study was to estimate the genetic influence on the beta cell response to a physiological (glucose) and a none-physiological (tolbutamide) stimulation.
Material and methods: 284 non-diabetic family-members of type 2 diabetic patients underwent a tolbutamide-modified frequently sampled IVGTT (t-FSIGT) during which glucose was injected at 0 min and tolbutamide at 20 min. Measurements of plasma glucose, serum insulin and serum C-peptide were taken at 33 time points from fasting to 180 min and insulin secretion rate (ISR), and acute insulin response (AIR) were calculated. Heritability estimates were performed using a polygenic variance component model calculating the additive effect and the unique environmental effect. The genetic correlations are presented as Pearsons genetic correlation coefficients (RhoG).
Results: We found high levels of heritability for measures of insulin secretion, especially after glucose stimulation, while plasma glucose was equally influenced by genes and environment. The highest level of heritability was found in the immediately stimulated state, with peak heritability for serum insulin and ISR within the first 7 min after glucose injection (90±14% and 69±14%, respectively) and within 4 min after tolbutamide injection (72±14% and 76±15%, respectively). A strong genetic correlation (correlations coefficients of 0.85-0.93) was found between measures of beta cell responsiveness after glucose and tolbutamide injections.
Conclusion: Our data suggest that the insulin secretion in response to both glucose and tolbutamide is under high genetic influence and that the response to both substances may be under control of the same genes.

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The CTRB1/CTRB2/BCAR1 locus is associated with GLP-1 stimulated insulin secretion and DPP-4 inhibitor treatment response

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Background and aims: The incretin hormone glucagon-like-peptide 1 (GLP-1) promotes glucose homeostasis and enhances beta cell function after a meal. GLP-1 receptor agonists (GLP-1 RA) and dipeptidyl peptidase-4 (DPP-4) inhibitors, which inhibit the physiological inactivation of endogenous GLP-1, are used for the treatment of type 2 diabetes. The goal of this study was to identify genetic loci influencing insulin secretion in response to stimulation with GLP-1. We also evaluated the effect of these loci on response to treatment with GLP-1 RA and DPP-4 inhibitors in type 2 diabetes patients.
Materials and methods: In the present study the Metabochip (200k SNPs) was used to identify genetic variants affecting GLP-1 stimulated insulin secretion (SIS) during hyperglycemic clamps in non-diabetic participants from the Netherlands and Germany. Subsequently their effect on response to GLP-1 RA and DPP-4 inhibitor treatment was analyzed in 527 type 2 diabetes patients from the Netherlands and UK. Data were analyzed using linear regression with adjustment for potential confounders. In addition we examined the effect of the genetic variants on mRNA expression and enzyme activity.
Results: We identified three novel genetic loci with large effects (32-46%) on GLP-1 stimulated insulin secretion during hyperglycemic clamps (*TMEM114; CHST3/SPOCK2/ASCC1* and *CTRB1/CTRB2/BCAR1*; n=232, all $p \leq 8.8*10^{-7}$). Furthermore the G allele of rs7202877 (MAF=0.11, +43% GLP-1 SIS) near *CTRB1/2* and *BCAR1* was associated with a lower decrease in HbA1c during DDP-4 inhibitor (0.51±0.16% (5.6±1.7 mmol/mol); $p=0.0015$) but not GLP-1 RA treatment ($p>0.5$). In addition we found that in human pancreas and islets rs7202877 enhances the expression of *CTRB1/2* but did not change the expression of *BCAR1*. *CTRB1/2* encode the enzyme chymotrypsin which cleaves proteins in food into smaller peptides suitable for further digestion and as expected we found that rs7202877 also enhanced enzyme activity in stool samples. Interestingly this locus is a known diabetes risk locus for both type 1 (G allele) and type 2 diabetes (T allele). Our data showing increased insulin secretion in G allele carriers thus provide a plausible mechanistic explanation for the protective effect of the G allele on type 2 diabetes susceptibility. We hypothesize that in the absence of a functional beta cell mass (i.e. in type 1 diabetics) this protective effect is lost thereby enhancing type 1 diabetes susceptibility via a yet unknown mechanism but possibly involving the gut which may also account for the observed difference in treatment response in DPP-4 inhibitor users.
Conclusion: In this study we have identified chymotrypsin in the regulation of the incretin pathway, type 1 and type 2 diabetes susceptibility and GLP-1 based treatment response in type 2 diabetes.
Supported by: BBMRI-NL, Dutch DDRF, Wellcome trust, UK MRC, German BMBF

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Polymorphisms associated with serum 25-hydroxyvitamin D concentration and their impact on subclinical atherosclerosis in patients with type 2 diabetes

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Background and aims: Vitamin D deficiency has been associated with type 2 diabetes (T2D) and myocardial infarction as well as multiple cardiovascular risk factors. Several single nucleotide polymorphisms (SNPs) associated with serum 25-hydroxyvitamin D (vitamin D) concentrations have been identified. The aim of this study was to investigate causality of vitamin D in cardiovascular disease using vitamin D-associated genetic variants and subclinical atherosclerosis in the IMPROVE study.
Materials and methods: IMPROVE is a European, multicentre, longitudinal cohort study, which enrolled individuals aged 54 to 80 years with at least three cardiovascular risk factors and no history or symptoms of cardiovascular disease from 7 centers in Finland, Sweden, the Netherlands, France, and Italy. Participants underwent carotid ultrasound examination at baseline, month 15 and month 30. Based on genome-wide association studies, 8 SNPs associated with serum vitamin D were identified for investigation here. Genotyping of SNPs was carried out using the Illumina Immunochip (6 SNPs) or Taqman (2 SNPs) platforms. In total 3430 subjects were included, of which 900 were T2D subjects.
Results: Genetic variants in the vitamin D binding protein *(GC or DBP*, rs2282679 and rs7041) and the 7-dehydrocholsterol reductase/NAD synthetase 1 gene (*NADSYN1*, rs12785878 and rs3829251) were negatively associated (bonferroni-corrected significance) with vitamin D levels after adjustment for established risk factors. Effect size and significance of associations between SNPs and vitamin D levels differed between subjects with and without T2D. SNPs in *GC* and *NADSYN1* were found to interact with T2D to influence vitamin D levels. In the multiple linear regression analyses including the interaction term, there were significant (bonferroni-corrected) positive associations between SNPs and segment-specific or composite carotid intima-media thickness (cIMT) measures (at baseline and after progression) independent of vitamin D levels and established risk factors.
Conclusion: SNPs in *DBP* and *NADSYN1* were associated with levels of vitamin D. Significant interactions between T2D and SNPs in *GC* and *NADSYN1* were independently related to cIMT and cIMT progression after adjustment of established risk factors and vitamin D levels. This suggests a role of vitamin D-related SNPs in early subclinical atherosclerosis, independent of vitamin D levels, but in a manner that depends on T2D status.
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TCF7L2 gene polymorphisms may influence decreased postprandial glucose oxidation - looking for personalised nutrition for obesity/type 2 diabetes mellitus prevention

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Background and aims: Genome-wide association studies have identified the number of genetic susceptibility loci for type 2 diabetes (T2DM). Single nucleotide polymorphisms (SNPs) in the transcription factor 7-like 2 gene (TCF7L2) seem to be one of the most predictive factors promoting T2DM development. Genetic variants of TCF7L2 have been implicated in the pathogenesis of T2DM primarily due to the influence pancreatic β-cell function, but the biological basis of these associations remains to be precisely determined. The aim of our study was to analyze whether SNPs of TCF7L2 gene influence postprandial insulin secretion and glucose utilization in nondiabetic men.
Materials and methods: We genotyped previously identified TCF7L2 SNPs: rs7901695 and rs7903146 in 943 subjects (330 normal-weight, 337 overweight, 276 obese; 463 women/480 men) who underwent anthropometric measurements, body composition analysis and OGTT. In randomly selected 48 subjects high-carb (HC), norm-carb (NC) and high-fat (HF) standardized meal tests were performed and postprandial carbohydrate utilization were evaluated with indirect calorimetry. Kruskal-Wallis non-parametric analysis of variance was used to evaluate difference between the studied parameters in subjects with the TCF7L2 genotypes.
Results: Subjects with CC genotype (rs7901695) presented 3 fold lower area under the curve (AUC) for glucose utilization (p=0.003) after high- carbohydrate meal intake in comparison to those with other genotypes. Similar results were found for TT homozygotes (rs7903146) for glucose utilization ($p<0.05$) and lower AUC for glucose oxidation (p=0.02) in the postprandial state. In non-diabetic subjects with the risk TCF7L2 genotypes glucose levels were significantly higher at 30, 60 and 120 min with 2.5 fold higher AUC for postprandial insulin levels (p=0.04) after standardized HC meals.
Conclusion: We believe that our study may help to understand the mechanisms that TCF7L2 gene influences the risk of T2DM. If our results are confirmed TCF7L2 gene-related personalized nutrition for obesity/type 2 diabetes mellitus prevention could be considered.
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Interaction between vitamin D receptor gene variants and dairy intake: effects on incident metabolic syndrome and incident IFG/type 2 diabetes in the D.E.S.I.R. study

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Background and aims: Epidemiological studies have suggested that vitamin D deficiency is associated with coronary heart disease. The vitamin D endocrine system is involved in the regulation of calcium metabolism. Actions of vitamin D are mediated by the specific cytosolic/nuclear vitamin D receptor (VDR). Several polymorphisms in the VDR gene have been reported to be associated with a variety of phenotypes, including insulin sensitivity and susceptibility to type 2 diabetes. In the D.E.S.I.R. cohort, we have shown that the consumption of dairy products is associated with the incidence of the metabolic syndrome and impaired fasting glycaemia/type 2 diabetes (IFG/T2D). Our aim is to study now the associations of VDR polymorphisms with the 9-year incidence of the metabolic syndrome and of IFG/T2D, testing for interactions with dairy product intake.
Materials and methods: Among 5212 subjects from the D.E.S.I.R. cohort, 4619 individuals born in mainland France were genotyped for three VDR gene polymorphisms (Fok1 G>A, Taq1 T>C, Bsm1 G>A) by the Kaspar method. Study participants completed a food frequency questionnaire. The metabolic syndrome was defined according to the International Diabetes Federation as waist circumference ≥ 94/80 cm for men/women plus two of the following factors: 1) elevated triglycerides: ≥ 1.70 mmol/l or specific treatment for this lipid abnormality; 2) reduced HDL cholesterol (HDL-C): ≤ 1.03 mmol/l for men and 1.29 mmol/l for women or specific treatment for this lipid abnormality; 3) elevated blood pressure: ≥ 130/85mmHg or treatment of previously diagnosed hypertension; 4) elevated fasting glycaemia ≥ 5.6 mmol/l or previously diagnosed type 2 diabetes (treated by glucose lowering drugs and/or fasting glycaemia ≥ 7.0 mmol/l). Associations were tested by logistic regression analysis with multiple adjustment (sex, age, alcohol, physical activity, smoking, BMI).
Results: No overall association was found between the VDR polymorphisms and the metabolic diseases; however the polymorphisms modulated associations with dairy product intake. A higher consumption of dairy products other than cheese was negatively associated with 9-year incident IFG/T2D in Bsm1 GG homozygotes (Odds Ratio [95%CI]: 0.67[0.47-0.95], P=0.025) but not in the other genotypes. A higher consumption of cheese was negatively associated with the incidence of the metabolic syndrome in Bsm1 G carriers (Odds Ratio [95%CI]: 0.72[0.59-0.87], P=0.001) and in Taq1 T carriers (Odds ratio [95%CI]: 0.73[0.60-0.88], P=0.001), but not in the other genotypes.
Conclusion: In conclusion, the associations between dairy product consumption and the risk of the metabolic syndrome and hyperglycaemia are modulated by genetic variation at the VDR locus.
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Several type 2 diabetes associated variants in genes involved in WNT-signalling interact with dietary fibre intake on type 2 diabetes incidence

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Background and aims: TCF7L2 is a principal transcription factor in the canonical WNT-signaling pathway. Short chain fatty acids, fermentation products of dietary fibers, are potent histone deacetylase inhibitors that can up-regulate WNT activity and *TCF7L2* rs7903146 was earlier reported by us to interact with dietary fiber intake on type 2 diabetes (T2D) incidence. Here we study whether other GWAS identified T2D associated single nucleotide polymorphisms (SNPs) in genes involved in the WNT pathway interact with dietary fiber on T2D incidence.
Materials and methods: We included 26,930 individuals without diabetes from the Malmö Diet and Cancer (MDC) population based cohort. Diet data was collected at baseline (1991-1996) using a modified diet history method (an extensive food frequency questionnaire, a 7-day food diary, and an interview). Altogether 51 gene loci (58 SNPs) were analyzed for links to WNT-signalling using annotations from Gene Ontology, Kioto Encyclopedia of Genes and Genomes, Biocarta, Panther pathways and Literature integrated within the DAVID-ABR. Over a mean follow up period of 14 years 2,860 cases of T2D were recorded. COX proportional hazards was used to analyze association with T2D (adjusted for age and sex) and interaction between genotypes and quintiles of fiber intake (adjusting for age, sex, BMI, total energy intake, season, diet method version, physical activity, smoking, alcohol intake, and education).
Results: Seven genes (9 SNPs) were annotated as involved in WNT-signalling (upstream of TCF/LEF) including *TCF7L2* (rs7903146 and rs12255372), *HHEX* (rs1111875), *HNF1A* (rs7957197), *NOTCH2* (rs10923931), *TLE4* (rs13292136), *ZBED3* (rs4457053) and *PPARG* (rs1801282 and rs13081389). SNPs in *TCF7L2*, *HHEX* and *HNF1A* loci predicted future T2D [HR 95% CI: 1.32 (1.24-1.39), 1.23 (1.16-1.30), 1.07 (1.01-1.12), and 1.14 (1.07-1.22) per risk allele]. SNPs in *TCF7L2*, *NOTCH2* and *ZBED3* loci interacted significantly with fiber intake on T2D incidence ($P_{interaction}$ = 0.04, 0.006, 0.01, and 0.003, respectively). Higher fiber intake associated with lower T2D risk only among homozygotes for the non-risk alleles of *TCF7L2* SNPs [N=13482 and 13522: HR 95% CI 0.95 (0.91-0.99) and 0.94 (0.90-0.98) per fiber intake quintile]. Higher fiber intake associated with lower T2D risk only among risk allele carriers of *NOTCH2* rs10923931 [N=4375 (GT) and 228 (TT): HR 95% CI: 0.90 (0.84-0.97) and 0.70 (0.50-0.99) per fiber intake quintile]. Higher fiber intake associated with lower T2D risk only among homozygotes for the risk allele of *ZBED3* rs4457053 [N=1643: HR 95% CI: 0.84 (0.75-0.94)]. *HHEX* rs1111875 had a tendency for interaction with fiber intake ($P_{interaction}$ = 0.11), but no interaction was observed for *HNF1A*, *TLE4*, and *PPARG* SNPs. Of the 44 loci not annotated to WNT signaling, none indicated interaction with fiber intake.
Conclusion: Our results indicate that several T2D susceptibility genes involved in WNT-signalling interact with dietary fiber intake on T2D incidence. The putative mechanisms by which fiber intake could modify the WNT signalling by the susceptibility variants in T2D pathogenesis include effects on beta cell survival, adipogenesis and adipocyte proliferation, and/or GLP-1 production in intestinal L-cells.
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Genetic variants within *AKR1B10* may influence human eating behaviour

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Background and aims: Preliminary data from a genome wide association study (GWAS; data unpublished) showed significant associations between Single Nucleotide Polymorphisms (SNPs) in the 5'UTR Region of the *Aldoketoreductase 1B10 gene* (*AKR1B10*) and human eating behavior. AKR1B10 is a monomeric enzyme that may play a role in detoxification processes and regulatory mechanisms like differentiation, proliferation and apoptosis. The aim of the present study was to investigate the effects of intragenic variants of *AKR1B10* on the eating behavior factors restraint, hunger and disinhibition.
Material and methods: The initial analysis included 548 Sorbs from Germany. This cohort is clinically well characterized for a variety of metabolic parameters including data on eating behavior (FEV). 4 tagging-SNPs (r^2=0.8, MAF<0.05) within the *AKR1B10* locus were genotyped in all subjects using the TaqMan SNP Genotyping assay (Applied Biosystems, Inc., Foster City, CA). Subsequently, genetic association analyses were calculated for restraint, hunger and disinhibition using linear regression models adjusted for age, sex and BMI. Replication analyses included another independent cohort from Leipzig (N=334).
Results: Among the Sorbs population the minor T-allele and the minor G-allele of the variants rs1834150 and rs782881 were significantly associated with increased disinhibition (additive mode of inheritance, *P*=0.006 and *P*=0.032). Further, we identified significant associations between the minor alleles and decreased waist circumference (rs1834150 and rs782881), increased alcohol consumption (rs782881) and higher coffee consumption (rs1834150) (all *P*<0.05). In our replication cohort we detected the same effect direction for the association with disinhibition (ß=0.152 for rs1834150 and ß=0.007 for rs782881). The meta-analysis for rs1834150 resulted in a combined *P* =0.0096 (Z-score=2.589). Moreover, in the replication cohort we further identified the variants rs1834150 and rs782881 significantly associated with increased restraint (respectively, *P*=0.008 and *P*=0.028). Albeit non-significant in the initial Sorbs population both SNP markers presented same effect directions for restraint (ß=0.439 for rs1834150 and ß=0.357 for rs782881). A meta-analysis for restraint resulted in combined *P*=0.0078 for rs1834150 (Z-score 2.659) and *P*=0.0178 for rs782881 (Z-score 2.371).
Conclusion: Our data suggest that genetic variants within the *AKR1B10* gene may play a role in the regulation of human eating behavior.
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The search for the variant explaining type 2 diabetes linkage on chromosome 9

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Background and aims: Previous studies have shown a suggestive linkage between type 2 diabetes (T2D) and a region on chromosome 9 in families from the Botnia region in Finland. One family with many affected individuals had a non-parametric LOD (NPL) score >15 to chromosome 9 p21-p23. Our aim in this study is to identify the variant(s) explaining the linkage using next generation sequencing and to explore the effect of these variants in T2D.
Materials and methods: The initial study was performed in 10 T2D and 3 non-diabetic family members using targeted sequencing of 3 Mb of chromosome 9 on the Illumina Genome Analyzer platform. Variants were selected for further genotyping in the extended family (10 T2D and 10 non-diabetics) based on four criteria; 1) novel, 2) segregation with diabetes, 3) location in or in the vicinity of a gene and 4) expression differences in peripheral blood leukocytes from the family members. Additional whole exome sequencing was performed in 20 members of the family (10 T2D and 10 non-diabetics). Replication genotyping was performed in 31 families from the same region traced back to the 17th century with 130 T2D patients and 213 non-diabetic subjects.
Results: After filtering of the variants identified in the targeted sequencing 52 variants remained. Fourteen of these have been additionally genotyped in the extended family, where a haplotype of three variants segregated with T2D (p=0.02) with an odds ratio of 21 (95% CI 1.78- 248.12). This haplotype was also seen in the replication cohort (p=0.02) although the frequency of the haplotype decreased from 20% in the family to 0.5% in the 31 families. The variants are located in the MPDZ gene encoding a G-protein interacting protein which is part of the signaling of both melatonin and serotonin. This gene seems to be differentially expressed in the peripheral blood leukocytes in risk genotype carriers (p=0.049) and has a nominally significant differential expression in the lymphocytes from diabetic patients compared to non-diabetic subjects in the family (p=0.07). In addition, exome sequencing also identified a novel, missense mutation in the ADAMTSL1 gene, segregating with T2D in the family (p=0.01).
Conclusion: Reported linkage of T2D to a region on chromosome 9 p21-p23 in this family may be caused by variants in the MPDZ and ADAMTSL1 genes.
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Novel gene-bases and pathway analysis GWAS methods for the identification of new candidate genes and pathways for type 2 diabetes

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Background and aims: Genome wide association studies (GWAS) have represented a promising tool to identify new genes involved in complex diseases, such as type 2 diabetes (T2D). However, despite large GWA meta-analyses efforts, less than 10% of the heritability can be explained by the to-date identified loci. A possible explanation for this limited success may be that several independent variants in a given gene or pathway, each of them with a small effect, contribute cooperatively to increase the susceptibility to the disease. To overcome this limitation, and to further exploit the available GWAS data, we have developed a gene-centred test able to take into account several SNPs in each gene and its pre-specified boundaries. We then applied the scores generated by this method to perform pathway analysis in T2D GWAS data.
Materials and methods: This test is based on a first step of SNP selection, followed by a multiple logistic regression fitting allowing correction for linkage disequilibrium. An empirical p-value for each gene is provided using adaptive permutation approaches. This method provides a single score for each gene, which can be applied to several pathway analysis techniques, including gene set enrichment analysis (GSEA) and Signalling Pathway Impact Analysis (SPIA), which also takes into account the network topology of the pathways.
Results: The application of this method to T2D GWAS data from the WTCCC, besides replicating previous loci, allowed the identification of several novel candidate genes for type 2 diabetes, including, one gene of the same family of the widely replicated KCNJ11 gene. Furthermore, pathway analyses based on the previously obtained gene scores ranked as the most associated pathway, the KEGG adipocytokine signalling pathway, which has been confirmed by other T2D meta-analyses, which suggest that reliable results can be obtained from applying this approach in modest GWAS datasets. The results applying this method on several extended replication datasets from the database of genotypes and phenotypes (dbGaP) and European Genome-phenome Archive (EGA) datasets involving six multi-ethnic cohorts involving 17.000 additional individuals will be presented.
Conclusion: We provide a novel gene-centred statistical method that allows the identification of novel T2D candidate genes, and provides a useful input for downstream systems biology analyses, such as pathway analyses methods. This study also proves the value of sharing individual-level GWAS data in public repositories, such as dbGaP or EGA, to apply novel statistical and systems biology methodologies to better understand the aetiology of complex diseases.

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Identification of new genetic associations to type 2 diabetes through accurate genotype imputation with *1000 Genomes* reference panel

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Background and aims: Despite the clear contribution of Genome-Wide Association Studies (GWAS) in the understanding of the genetics of Type 2 Diabetes (T2D), known associated loci published to date only explain a small fraction of the estimated heritability associated to T2D. Genotype imputation consists in predicting unobserved genotypes using a reference panel of haplotypes densely genotyped to increase the statistical power of the GWAS data. The *1000 Genomes Project* reference panel (1KG), based on low whole genome and exome sequencing data, constitutes a unique opportunity to perform a more accurate fine-mapping of known and replicated associated regions and to identify new genetic variants associated to T2D re-analysing published GWAS datasets stored in public repositories. The 1KG catalogue, including more than 37M SNPs, small insertions and deletions (INDELs) and structural variants, needs a thoroughly evaluation of the current post genotype imputation strategies in order to minimize false positive associations arising from inaccurate genotype imputation without compromising statistical power. The main goal of this study was applying this methodology to identify new candidate genes involved in the aetiology of T2D.
Materials and methods: Previous work of our group consisted in stating novel quality standards for genotype imputation adapted to the requirements of novel reference panels such as 1KG. For this purpose, we used the set of 1958 British Birth controls (~3.000 subjects) from the Wellcome Trust Case Control Consortium (WTCCC) genotyped by two independent platforms allowing us to select the appropriate quality filters to avoid inaccurate imputed variants that could result in false positive associations. We applied this strategy to the genotyped T2D data from the original WTCCC study involving ~2.000 individuals and ~3000 controls from the WTCCC2 study.
Results: Accurate imputed variants with minor allele frequency as low as 0.01 allowed the identification of 272 associated regions (p-value ≤ 5x10-8). The three loci found in the original WTCCC study have been identified and characterized with higher genomic resolution, enhancing functional annotation. Moreover, we detected 2 associated loci close to the ARAP1 and HNF1A genes identified in large meta-analysis (involving 34.412 cases and 59.925 controls) posterior to the WTCCC study from which we obtained the data of this study. These findings prove that imputation with 1KG allows the identification of new T2D loci without such large sample sizes and thus, less investment. In addition, 15 genes involved in the crosstalk between the insulin signalling pathway and the mitochondria function, recently reported by our group, are located in these novel T2D identified loci. The replication of these results in independent T2D case-control publicly available cohorts, encompassing more than 17.000 T2D individuals will be presented.

Conclusion: Genotype imputation using dense reference panels such as the 1KG allowed the identification of novel candidate genes as well as fine-mapping of already known associated regions using less sample sizes. These results, which we will confirm in independent populations, will lead to a better description of the genetic basis of T2D, allowing a better exploitation from the previously spent resources in large GWAS datasets.

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Large-scale genome-wide association meta-analysis of the 1000 genomes project imputed data identifies 19 novel lipid loci and new variants in previously known loci

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Background and aims: Hyperlipidemia is a frequent companion to T2D, reflecting shared insulin resistance, and contributes to diabetes-related complications. Genome-wide association (GWA) studies have been successful in detecting numerous associations with blood lipids (high and low density lipoprotein cholesterols (LDL/HDL), total cholesterol and triglycerides), through a substantial proportion of the genetic contribution to trait variances remains unexplained.
Materials and methods: In order to find more loci and to approach potential causal variants by fine-mapping in already known loci, we performed GWA meta-analysis of studies imputed from high density reference panel provided by the June 2011 release of 1000 Genomes project. This reference allows us to analyze markers with minor allele frequency as low as 0.5%. 17 population-based studies of European origin were used with total sample size up to 51,204 individuals.
Results: In a fixed-effects meta-analysis following imputation, we found 79 loci associated with at least one of the lipid measures with $P<5\times10^{-8}$, 19 of these were previously unreported (>2Mb from known loci) and lead variant at 7 of them had minor allele frequency less than 5%. We could replicate previously-reported associations at 3 missense SNPs (*APOE, ANGPTL4* and *PCSK9*, with MAFs 16.5%, 3.0% and 1.9% respectively) identified from candidate-gene resequencing efforts, and 3 others (*APOB, GCKR* and *HNF4A*, with MAFs 23.8%, 35.9% and 4.1%) found in GWA studies as the new lead SNPs. Initial fine-mapping analyses have identified at least two coding variants (*ABCA6/8* and *MOSC1,* with MAFs 1.9% and 28.0%) being lead SNPs at those loci and several other examples of low frequency lead SNPs.
Conclusion: Our results highlight the potential for the identification of novel associations using existing GWAS genotyping data, supplemented with imputation from high-density reference panel of 1000 Genomes Project without the need for costly re-sequencing experiments.
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Pleiotropic effects of obesity-susceptibility loci on metabolic traits: a meta-analysis of up to 37,874 individuals

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Background and aims: Obesity is a key risk factor for a number of metabolic diseases, including type 2 diabetes, dyslipedimia and cardiovascular disease. Although the exact biological mechanisms linking obesity to these co-morbidities are not fully understood, a set of common genetic factors with pleiotropic effects (i.e. affecting multiple traits) might in part explain the observed associations. We aimed to investigate whether the currently established obesity-susceptibility loci that were identified through genome-wide associations studies for body mass index (BMI) and waist-to-hip-ratio (WHR) are also associated with metabolic traits, independently of obesity-related traits.
Materials and methods: We systematically assessed associations of the 32 BMI and 14 WHR loci, individually and combined in two genetic predisposition scores (GPS-BMI, GPS-WHR), with glycaemic traits, blood lipids, and blood pressure (BP) by meta-analyzing data of up to 37,874 individuals of European ancestry from six population-based studies. We also examined whether these associations were influenced by (central) adiposity.
Results: The meta-analyses showed associations of both the individual obesity-susceptibility loci and the genetic predisposition scores with metabolic traits that were not driven by the obesity-related phenotypes. We observed significant associations of BMI-increasing alleles at five BMI-loci with lower levels of 2-hr glucose (RBJ, QPTCL: effect sizes -0.068 and -0.107 SD, respectively), HDL-cholesterol (SLC39A8: -0.065 SD, MTCH2: -0.039 SD), and diastolic BP (SLC39A8: -0.069 SD), and higher and lower levels of LDL- and total-cholesterol (QPTCL: 0.041 and 0.042 SD, respectively, FLJ35779: -0.042 and -0.041 SD, respectively) (all Ps<2.4x10-4), independently of BMI. The WHR-increasing alleles at two WHR-loci were significantly associated with higher proinsulin (GRB14: 0.069 SD) and lower fasting glucose levels (CPEB4: -0.049 SD), independently of BMI and WHR. A higher GPS-BMI was associated with lower systolic BP (-0.005 SD), diastolic BP (-0.006 SD) and 2-hr glucose (-0.013 SD), while a higher GPS-WHR was associated with lower HDL-cholesterol (-0.015 SD) and higher triglyceride levels (0.014 SD) (Ps<2.9x10-3), independently of BMI and/or WHR.
Conclusion: Our results provide evidence that obesity susceptibility loci have pleiotropic effects on metabolic traits, independently of adiposity. These findings bring a novel insight into mechanisms that link obesity with metabolic abnormalities and highlight that the genetic variants predisposing to obesity might also predispose to, or protect from, other metabolic disorders.
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Dissecting the pleiotropic effects of established type 2 diabetes and other cardiometabolic trait loci to define pathways and gene networks involved in type 2 diabetes pathogenesis

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Background and aims: Recent genome-wide association studies (GWAS) for human complex phenotypes have identified hundreds of genetic variants for cardio-metabolic traits and risk of disease. At many loci or specific variants associations are observed with multiple epidemiologically correlated traits. We formed the Cross-Consortia Pleiotropy Group to investigate the patterns of multi-cardio-metabolic trait associations across the genome. We aimed (a) to examine the associations of cardio-metabolic trait loci with epidemiologically correlated traits by grouping shared patterns of individual trait effects; (b) to define pathway and gene networks involved in the trait variability within the association pattern groups.
Materials and methods: We evaluated the genetic effects of 544 independent variants ($r^2<0.8$) from a total of 687 SNPs from published GWAS meta-analyses (thru Sep 2012) of 20 quantitative cardio-metabolic traits, including systolic/diastolic blood pressure, 8 glycaemic, 6 obesity/anthropometric, 4 lipid traits, and 2 diseases (Type 2 Diabetes (T2D), hypertension). We applied a complete hierarchical cluster analysis, which grouped variants according to their impact on the cardio-metabolic traits. We combined these data with annotated pathways, protein-protein interactions and semantic relationships from the published literature using GRAIL and DAPPLE software tools, which estimated the significance of connections between putative genes.
Results: We identified 33 groups of variants with shared patterns of associations with cardio-metabolic traits. Of these, 22 clusters contained groups of variants showing association with one or a group of highly correlated phe-

notypes. In the other 11 clusters, genetic variants were grouped according to their patterns of phenotypic effects. A group of 17 BMI/WC loci, such as *MC4R, TMEM18, BDNF, TFAB2B, NEGR1*, were related to BMI, lower HDL, higher triglycerides (TG), T2D risk, insulin resistance and obesity traits, the latter two being insignificant after BMI adjustment. 19 loci from another cluster were related to "healthy obesity/unhealthy leanness" by association with higher BMI and HDL, lower TG, glycaemic traits and risk of T2D, where three growth factors, *GRB14, PDGFC, VEGFA* showed significant connectivity ($p<0.001$). A cluster of 23 loci primarily associated with lower height or higher total cholesterol were also related to lower skeletal growth and higher HDL, accompanied by significant direct ($p=0.05$) and indirect ($p=0.001$) physical gene interactions. A cluster containing *CDKAL1, THADA, IGF2BP2, RREB1, DGKB, PROX1* and 6 other loci were related to beta cell function and glucose homeostasis traits and higher T2D risk.
Conclusion: This approach shows great promise for dissecting genetic effects on cardio-metabolic traits.

PS 014 Nutrition and mechanisms in diabetes: observational studies

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Anti-obesity and anti-diabetic effect of chronic coffee consumption in mice

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Background and aims: Chronic coffee consumption is correlated with a substantially lower incidence of type 2 diabetes. Apparently the association does not depend on race, gender or geographic distribution of the study populations. For the experimental exploration of the underlying mechanisms the demonstration of this effect in a mouse model of type 2 diabetes is needed.
Materials and methods: From week 10 on, male C57BL/6NCrl mice consumed regular coffee (i.e. coffee containing a natural content of caffeine) as filtered brew of 20 g ground coffee per litre (mean caffeine concentration 327 ± 30 mg/l, n = 29) or 40 g/ litre *ad libitum* or continued to drink tap water. The development of obesity and diabetes caused by a high fat diet (55% lipids, HFD) was observed for 35 weeks in comparison with mice feeding on normal chow (9% lipids, ND). In addition to parameters of metabolism, the morphology of the endocrine pancreas was quantified.
Results: The massive weight gain in HFD mice was dose-dependently delayed, the moderate weight gain in aging ND mice was abolished by coffee consumption. In both cases this was due to a lower feeding efficiency. Water or coffee consumption depended on the type of diet but not on the type of fluid. HFD mice had a 50% lower intake. At week 21 intraperitoneal glucose tolerance tests (IPGTT) showed a dose-dependent significantly faster decline of elevated glucose levels in coffee-consuming HFD mice, but not in ND mice. At week 39 the IPGTT showed diminished peak levels in coffee-consuming HFD mice, but also an improved glucose tolerance in all treatment groups vs. week 21. This spontaneous improvement was also visible as a decrease of the non-fasting glycaemia between week 21 and week 30, both in HFD and ND mice. Coffee-consuming ND mice and control ND mice had closely similar values for total cholesterol, triglycerides, HDL cholesterol and also blood glucose. The coffee-consuming HFD mice, in contrast, differed significantly from the corresponding ND mice in each single parameter. Irrespective of coffee consumption HFD mice were hyperinsulinaemic at week 21, grossly hyperinsulinaemic at week 39 and had significantly enlarged islets at week 45. Coffee consumption did not affect islet size or parameters of beta cell apoptosis, proliferation and insulin granule content.
Conclusion: The chronic consumption of regular coffee diminishes weight gain both in HFD and ND mice and favourably affects glucose tolerance in HFD mice. Thus, this model seems appropriate to explore which of the coffee constituents is responsible for the diabetes-preventive effect of coffee consumption in humans.
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Dietary intake of carbohydrates and risk of type 2 diabetes: European Prospective Investigation into Cancer in Norfolk study

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Background and aims: To better understand the role of dietary patterns in development of type 2 diabetes we investigated the association of dietary intake of carbohydrates and risk of type-2 diabetes in a large prospective study.
Materials and methods: A total of 25,639 men and women aged 40-79 were recruited in the European Prospective investigation into Cancer in Norfolk study. Incident cases of diabetes (N=749) were identified and compared with a randomly selected sub-cohort of 3496 participants. Seven-day food diary administered at baseline was used for dietary assessment. We performed modified Cox-proportional hazards regression analyses and compared results from the different methods of adjustment for total energy intake.
Results: Dietary intakes of sucrose, starch, lactose, maltose, or total carbohydrates were not significantly related to diabetes risk after adjustment for con-

founders. However, in the standard method for energy adjustment, intakes of fructose and glucose were inversely related to diabetes risk. The multivariable adjusted hazard ratio (95% CI) of diabetes comparing extreme quintiles of intake was 0.66 (0.48-0.91, P-trend 0.003) for glucose and 0.70 (0.52-0.95; P-trend 0.01) for fructose. In the nutrient density method, only fructose was inversely related with diabetes (HR 0.65, 95%CI 0.48-0.88). Exchanging 5% energy intake from fructose for saturated fatty acids was associated with 30% lower diabetes risk (HR 0.69, 95%CI 0.50-0.96). Results of the residual and energy partition method were similar to the standard method.
Conclusion: These prospective findings suggest that starch and sucrose are not associated, but that intakes of fructose and glucose are inversely associated with diabetes risk. Whether the inverse associations with fructose and glucose reflect the effect of substitution of these carbohydrate sub-types for other nutrients (i.e., saturated fatty acids), their net higher intake, or other nutrients associated with their intake, remains to be established through further investigation.
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The association between dietary flavonoid and lignan intakes and incident type 2 diabetes in European populations: the EPIC-InterAct study
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Background and aims: Epidemiological evidence on the relationship between flavonoid and lignan intake and type 2 diabetes risk is limited and inconclusive. Therefore, we aimed to study these associations prospectively.
Material and methods: The European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct case-cohort study included 12,403 incident type 2 diabetes cases and a stratified subcohort of 16,154 participants from among 340,234 participants with 3.99 million person-years of follow-up in 8 European countries. At baseline, country-specific validated dietary questionnaires were used. A flavonoid and lignan food composition database was developed from the Phenol-Explorer, the UK Food Standards Agency, and the US Department of Agriculture databases. Hazard ratios (HRs) from country-specific Prentice-weighted Cox regression models were pooled using random-effects meta-analysis.
Results: In multivariable models, a trend for an inverse association between total flavonoid intake and type 2 diabetes was observed (HR for the highest versus the lowest quintile 0.90; 95% confidence interval (CI): 0.77-1.04; *P*-trend 0.040), but not with lignans (HR 0.88; 95% CI: 0.72-1.07; *P*-trend 0.119). Among flavonoid subclasses, flavonols (HR 0.81; 95% CI: 0.69-0.95; *P*-trend 0.020), and flavanols (HR 0.82; 95% CI: 0.68-0.99; *P*-trend 0.012), including flavan-3-ol monomers (HR 0.73; 95% CI: 0.57-0.93; *P*-trend 0.029), were associated with a significantly reduced hazard of diabetes.
Conclusion: Prospective findings in this large and heterogeneous European cohort demonstrate inverse associations between flavonoids, particularly flavanols and flavonols, and incident type 2 diabetes. This suggests a potential protective role of eating a diet rich in flavonoids, a dietary pattern based on plant-based foods, on the prevention of type 2 diabetes.
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Glucosinolates for the prevention of type 2 diabetes? Reduction of glucose production and induction of protective principles
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Background and aims: Nasturtium (Tropaeolum majus) contain bioactive phytochemicals such as glucosinolates and isothiocyanates. We found that aglycons of glucosinolates modulate the intracellular localization of FOXO1. FoxO transcription factors can antagonize insulin effects and trigger a variety of cellular processes involved in tumor suppression, longevity, development and metabolism. Most of the glucosinolates have been evaluated in the cancer pathogenesis prevention, but only few have been evaluated in metabolic pathways. The current study evaluated the ability of Brassicales species to modulate the insulin-signaling pathway, the intracellular localization of FOXO1 and the expression of proteins involved in glucose metabolism, ROS detoxification, cell cycle arrest and DNA repair and their relation with the prevention of type 2 diabetes.
Materials and methods: FOXO1 translocation: Stably transfected cells expressing FOXO1-GFP (U-2OS human Osteosarcoma cells) were treated for 1h with benzyl-glucosinolate pretrated with myrosinase (formation of BITC) 1μM-100μM. Cells were stained with DAPI for the calculation of ratios of GFP-intensities in nuclear and cytoplasmic areas. Protein modification and gene expression: HepG2 cells (human hepatoma cells) were incubated with BITC for 30' followed by insulin 100nM for 15'. Proteins were analysed by Western immuno-blots using antibodies against AKT (pan AKT), p-AKT (phospho AKT), FOXO1, p-FOXO1/3a (Phospho FOXO1/3a), and GAPDH (housekeeping protein). For gene expression analyses HepG2 cells were incubated with 0.1μM-100μM of BITC for 24 hours followed by RNA-extraction. Semi quantitative real-time polymerase chain reaction assays were performed for: FOXO1, AKT, NRF2, SIRT1, and PEPCK (Phosphoenolpyruvate-carboxykinase), G6Pase (Glucose-6-Phosphatase) for gluconeogenesis; CAT (Catalase), MnSOD2 (Superoxid Dismutase) for antioxidative defense; CCNG2 (Cyclin G2), CDKN1A/1B (Cyclin Dependent kinase Inhibitor 1A p21 Cip1 /1B p27 Kip), GADD45 (Growth Arrest and DNA Damage induced Protein) for cell cycle arrest and DNA repair; SRXN1 (Sulfiredoxin 1), NQO1 (NAD(P)H dehydrogenase (quinone1)), and GPX2 (Glutathione peroxidase 2) for detoxification. RPL32 (Ribosomal Protein L32) was used as housekeeping gene. For analyses of involvement of specific factors HepG2 cells were transfected with siRNAs for FOXO1, AKT, NRF2, and SIRT1. Following 48h for knock down of these proteins, the gene expression for each factor and all targets was analyzed.
Results: BITC induced a dose-dependent nuclear translocation of FOXO1-GFP in U-2OS cells. In HepG2 cells an inhibition of AKT-phosphorylation and a reduction of FOXO1-phosphorylation were observed. BITC up regulated MnSOD2, CDKN1A, GADD45, SRXN1, NQO1, and GPX2 gene expression and induced a significant reduction of G6Pase and PEPCK-expression. The effect of BITC on the gene expression was not influenced by FOXO1, AKT, SIRT1, and NRF2 knock down except a less pronounced up regulation of SRXN1 under NRF2 knock down.
Conclusion: BITC potentially down regulates the hepatic glucose production, enhances the antioxidant response and promotes longevity. Despite knocking down the described factors the response to BITC was not altered indicating the involvement of additional molecular pathways.

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Effects of 12 weeks of trichosanthis kirilowii and manitolatodimolybdate in the treatment of Hispanic prediabetic patients
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Background and aims: Diabetes evolves through prediabetes, defined as having an impaired fasting plasma glucose (FPG) and HbA1c 5.7-6.4%. People with prediabetes and increased visceral fat (Vf) have higher risk of developing diabetes and cardiovascular disease compared to normoglycemic individuals. The prevalence of prediabetes in Hispanic population has increased appreciably. The aim of this study was to examine the effects of 12 weeks of treatment with trichosanthis kirilowii (Tk) and manitolatodimolybdate (Mm) as a novel combination insulin sensitizer on HbA1c, FPG, and Vf in adult Hispanic obese prediabetic patients.
Materials and methods: This prospective study enrolled subjects aged 18 or older, with HbA1c 5.7-6.4%, FPG 100-125 mg/dl, BMI ≥25 kg/m2, Vf ≥2.1 kg, without any medication, and with normal hepatic or renal function. The patients were instructed to take 2.0mg of Tk and 1.6mg of Mm in one tablet, twice a day during 12 weeks. HbA1c, FPG, and AST/ALT were measured initially and at week 12. Bioelectrical impedance analysis (BIA) was used to estimate visceral fat accumulation and total body water (Zeus 9.9 plus, Jawon medical). All data was obtained using data from labs and BIA. Registered Dietitians (RD) certified in diabetes management provided medial nutrition therapy (MNT) and exercise guidelines based on the American Diabetes Association´s clinical practice recommendations.
Results: 93 patients participated; 53.7% were female. The mean age was 42.1 (±9.4). After 12 weeks of treatment, significant reductions were observed in HbA1c from 6.2±0.26 to 5.6±0.2%, FPG (mean 114±8.60 to 102.9; p>0.05),

BMI (32.3±5.9 to 30.4±5.1; p<0.05), and Vf (4.64±1.2 to 3.8±1.0; p<0.05). 81% of the study sample reached HbA1c within normal ranges (<5.7%) after 12 weeks. No significant changes were seen in neither AST/ALT nor total body water levels.

Conclusion: The combined therapy with Tk and Mm significantly improved HbA1c and reduced both Vf and BMI in prediabetic patients. These findings highlight the potential to treat prediabetic state effectively while ensuring hepatic safety without edema. Further study is needed using a larger sample size but these results suggest significant comparisons useful for primary treatment.

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A short-term high-cholesterol diet deteriorates glucose tolerance to a different extent and through different mechanisms in controls and type 2 diabetes offspring

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Background and aims: In vitro and in experimental animals, extreme and prolonged exposure to lipid substrates induces insulin resistance and impairs insulin secretion. In addition, offspring of individuals with type 2 diabetes display a higher sensitivity to the detrimental effects of hyperlipidaemia. Whether and how changes in serum cholesterol concentrations affect glucose tolerance is still debated. While short-term LDL lowering with statins has no relevant effect, recent experimental and clinical studies have suggested that cholesterol rich lipoproteins can acutely influence both insulin secretion and insulin sensitivity. Whether more physiologic (diet-induced) short-term manipulations of serum cholesterol affect glucose metabolism and whether the genetic background influences this response is unknown.

Materials and methods: Twenty-one healthy young subjects (age: 26±1 yrs; BMI: 23±1 kg/m^2,12 males) of whom 10 with at least one parent with type 2 DM (T2Doff) were studied twice: after 14 days of an eucaloric low-cholesterol (100-150 mg/day) diet (LCD) and after a further 14 days of an eucaloric high-cholesterol (250-300 mg/day) diet (HCD). A 180-min OGTT was performed at the end of each dietary intervention. Insulin secretion was evaluated by C-peptide deconvolution and beta cell function modelling according to Mari et al (Best Pract Res Clin Endocrinol Metab 2003); insulin sensitivity was estimated by the OGIS method.

Results: In the whole study group, the HCD diet raised serum total- and HDL-cholesterol by 12% (p<0.04), while serum triacylglycerols and body weight were unaffected. On the OGTT, the glucose incremental area-under-curve (AUC) was 20% higher following the HCD (+68±33 mmol/L, p≤0.02) and beta cell glucose sensitivity was reduced by 20% (from 129±12 to 104±9 pmol min^{-1}m^{-2}nM^{-1}, p<0.04). When analysed separately, T2Doff showed a similar diet-induced change in serum lipids, but developed a more marked deterioration of glucose tolerance in response to HCD (AUC change: +104±34 *vs* +15±35 mmol/L, p<0.03). In this group, the HCD diet produced also a small decline in peripheral insulin sensitivity (from 442±18 to 414±20 ml min^{-1}m^{-2}, p<0.03), which was not observed in the 11 subjects without familial T2D.

Conclusion: A short-term increment of dietary cholesterol content, not affecting body weight or serum triacylglycerols, induces a worsening of glucose tolerance due to a decline in beta cell function; in T2D off, this change is associated with a reduction in peripheral insulin sensitivity.

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Lipopolysaccharide is negatively correlated with beta cell function

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Background and aims: Studies suggested that lipopolysaccharide (LPS) may induce insulin resistance via stimulating non-specific inflammatory response, which may lead to impaired glucose regulation. However, whether LPS is correlated with β-cell function, the crucial pathophysiology of type 2 diabetes, is still unclear. This study was designed to investigate the relationship of LPS and β-cell function in people with different glucose tolerances.

Materials and methods: Sixty-seven subjects were divided into normal glucose tolerance (NGT) group (n=23), impaired glucose tolerance (IGT) group (n=21), and type 2 diabetes (T2DM) group (n=23) based on OGTT, with sex, weight, BMI, blood pressure, lipid profiles matched. After 10h overnight fast, subjects were provided with a high-fat diet(486.6kcal, 47.7%fat). Blood samples were collected for measurement of glucose, insulin, C-peptide, LPS levels at 0-h, 0.5-h and 2-h after a high-fat diet and fasting expression of toll-like receptor 4(TLR4) on surfaces of plasma monocytes. β-cell function was evaluated by HOMA-β/HOMA-IR, $_{\Delta}$Ins30/$_{\Delta}$G30÷IR ($_{\Delta}$Insulin30/$_{\Delta}$Glucose30÷HOMA-IR), $AUC_{Ins120min}$/IR ($AUC_{Insulin120min}$/HOMA-IR), and $AUC_{Cp120min}$/ IR($AUC_{C\text{-}peptide120min}$/ HOMA- IR).

Results: LPS levels at all time points during a high fat diet and TLR4 expression increased from NGT to IGT and T2DM (Table 1). But LPS levels and TLR4 expression were not statistically different between T2DM and IGT (P>0.05). Fasting LPS, 0.5-hLPS, 2-hLPS were negatively correlated with β-cell function indices (HOMA-β/HOMA-IR: r= -0.304,r= -0.291, r= -0.297; $_{\Delta}$Ins30/$_{\Delta}$G30 ÷ IR: r = -0.467, r= -0.446, r= -0.451; $AUC_{Ins120min}$/IR: r= -0.445, r= -0.439, r= -0.447; $AUC_{Cp120min}$/IR: r= -0.519, r= -0.500, r= -0.506; P<0.05), respectively. TLR4 was negatively correlated with $_{\Delta}$Ins30/$_{\Delta}$G30÷IR, $AUC_{Ins120min}$/IR and $AUC_{Cp120min}$/IR(r= -0.274, r= -0.263, r= -0.344, P<0.05). Further multiple linear regression analysis showed that 0.5-h LPS was independently correlated with $_{\Delta}$Ins30/$_{\Delta}$G30÷IR, $AUC_{Ins120min}$ /IR, and $AUC_{Cp\text{-}120min}$, respectively.

Conclusion: Compared with NGT people, patients with IGT and T2DM have higher LPS levels and TLR4 expression. LPS may affect β-cell function.

LPS levels before and after a high-fat diet and fasting TLR4

		NGT (n=23)	IGT (n=21)	T2DM (n=23)
LPS (EU/ml)	0-h	0.62 (0.22, 0.64)	0.71 (0.39, 0.82) *	0.86 (0.45, 0.94) *
	0.5-h	0.73 (0.31, 0.76)	0.84 (0.50, 1.07) *	1.10 (0.55, 1.18) *
	2-h	0.96 (0.33, 0.99) #	1.08 (0.53, 1.22) *#	1.23 (0.62, 1.43) *#
TLR4 (MIF/10^5 cells)	0-h	15.66 (6.09, 9.76)	30.34 (15.00,45.18) *	36.96 (17.22, 55.19) *

* P<0.01 vs. NGT group, # P<0.01 vs. 0-h LPS

PS 015 Lifestyle factors as risk factors

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Smoking and risk of type 2 diabetes: the EPIC-InterAct study

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Background and aims: The aim is to investigate the association between smoking and incidence of type 2 diabetes (T2D) accounting for a large number of potential confounding factors and to explore potential effect modifiers and intermediate factors.
Materials and methods: The European Prospective Investigation into Cancer and Nutrition EPIC-InterAct case cohort study was conducted in 26 centres of the EPIC study in eight European countries and consists of 12,403 incident T2D cases and a centre-stratified subcohort of 16,154 individuals (including 778 T2D cases). Smoking was modelled by smoking status (never, former, current), with never smokers as the reference category. Prentice-weighted Cox-regression was used for the case-cohort analyses and random effects meta-analysis to estimate hazard ratios (HRs) for T2D.
Results: In men, the HRs (95% confidence interval (CI) of T2D were: 1.40 (1.26-1.55) for former smokers and 1.43 (1.27-1.61) for current smokers independent of age, education, centre, physical activity and consumption of alcohol, coffee and meat. In women, the associations were weaker (HRs (95%CI): 1.18 (1.07-1.30) and 1.13 (1.03-1.25) for former and current smokers, respectively. There was some evidence of effect-modification by BMI. The association tended to be slightly stronger in normal weight participants compared to those with overall or regional adiposity.
Conclusion: Former and current smoking was associated with a higher risk of incident T2D compared to never smoking in men and women, independent of educational level, physical activity, alcohol and diet. Smoking may be regarded as one of the modifiable risk factors for T2D and smoking cessation should be encouraged for diabetes prevention.
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Low birth weight is associated with alterations in dietary intake in later life independent of genetic factors

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Background and aims: Low birth weight is associated with an increased risk of type 2 diabetes and cardiovascular disease. These associations may, in part, be explained by alterations in dietary intake in later life. We examined whether low birth weight is associated with alterations in food intake, and whether this association is due to intrauterine environmental or genetic factors.
Materials and methods: Studies in dizygotic and monozygotic twin pairs offer a unique opportunity to investigate the influence of intrauterine environmental and genetic factors. Specifically, differences within dizygotic twin pairs are a function of both genetic and nongenetic factors, whereas differences within monozygotic (genetically identical) pairs can only be caused by nongenetic factors. We investigated data on birth weight and food intake in 42 dizygotic and 46 monozygotic adolescent same sex twin pairs. Birth weight was obtained from the mothers. Food intake was assessed by two-day food diaries and macronutrient content was calculated as proportions of total energy intake (E%). Fiber intake was calculated in grams/1000 kcal.
Results: In the overall sample of twins, birth weight was negatively associated with intake of saturated fat after adjustment for current weight (regression coefficient β: -0.9 E%/kg, p=0.02). Within pair analysis in dizygotic and monozygotic twin pairs combined demonstrated that twins with the lowest birth weight from each pair had a dietary intake of saturated fat that was 0.7% higher compared with their cotwins with the highest birth weight (14.8 ± 2.7 E% vs. 14.1 ± 3.0 E% p<0.05). This difference was similar in dizygotic and monozygotic twins (0.6% vs. 0.8% respectively). In a further within pair analysis, after adjustment for differences in current weight, intrapair differences in birth weight were negatively and significantly associated with differences in intake of saturated fat in dizygotic and monozygotic twins combined (β: -1.7 E%/kg, p<0.05). This intrapair association was also similar in dizygotic and monozygotic twins (β: -1.7 E%/kg and -2.0 E%/kg respectively). In the overall sample of twins, birth weight was not associated with intake of fibers after adjustment for current weight (β: + 0.1 g/1000kcal, p=0.8). However, in the intrapair analysis, a positive association was found between differences in birth weight and differences in intake of dietary fibers (β: 1.8 g/1000 kcal, p=0.04). Again, this association was similar in dizygotic and monozygotic twins.
Conclusion: In line with findings in singletons, we demonstrated that low birth weight is associated with higher intake of saturated fat and lower intake of dietary fibers within twin pairs. Our findings, indicating that these associations were similar in dizygotic and monozygotic twin pairs, suggest that intrauterine environmental rather than genetic factors explain the association between birth weight and alterations in dietary intake in later life. These data support the concept that improvement of the intrauterine environment may be beneficial for dietary habits in later life.
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Adherence to predefined dietary patterns and incident type 2 diabetes in European populations: EPIC-InterAct study

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Background and aims: Few studies investigated the relationship between predefined dietary patterns and incidence of type 2 diabetes; little is known about the generalizability of these associations. We aimed to assess the association between predefined dietary patterns and risk of type 2 diabetes in European populations.
Materials and methods: From among a case-cohort of 12,403 incident diabetes cases and 16,154 subcohort members nested within the European Prospective Investigation into Cancer and Nutrition study, we used data on 9,682 cases and 12,595 subcohort participants from seven countries. Habitual dietary intake was assessed at baseline with country-specific dietary questionnaires. Two widely-known diet-quality scores (Alternative Healthy Eating Index (aHEI), Dietary Approaches to Stop Hypertension (DASH) score) and three reduced rank regression (RRR)-derived dietary pattern scores were constructed. Country-specific HRs were calculated and combined using a random-effects meta-analysis.
Results: After multivariable adjustment including BMI and waist circumference, the aHEI and DASH dietary patterns were not significantly associated with diabetes, although there was a tendency towards an inverse association in countries with higher mean age. We observed inverse associations of the three RRR-derived dietary pattern scores with diabetes: HRs (95% CIs) for a 1-SD difference were 0.91 (0.86-0.96), 0.92 (0.84-1.01), and 0.87 (0.82-0.92), respectively. Random-effects meta-analyses revealed heterogeneity between countries that was explainable by differences in the age of participants or the distribution of dietary intake.
Conclusion: Adherence to specific RRR-derived dietary patterns, commonly characterized by high intake of fruits or vegetables and low intake of processed meat, sugar-sweetened beverages, and refined grains, may lower risk of type 2 diabetes.

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Fatty fish is associated with a reduced risk of latent autoimmune diabetes in adults (LADA) and type 2 diabetes

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Background and aims: It has been suggested that fish consumption, especially of fatty fish, may protect against type 2 diabetes (T2DM), possibly through

improvements in insulin sensitivity. Despite its autoimmune nature, latent autoimmune diabetes in adults (LADA) shares the T2DM characteristic of insulin resistance. Our aim was to investigate, for the first time, whether fish consumption is associated with the risk of LADA.
Materials and methods: The results were based on data from the ESTRID Study, an ongoing population-based case-control study including incident cases of LADA (n=143) and T2DM (n=295), and population-based controls (n=646). Information on diet was obtained by a validated 127-item questionnaire which also included questions on potential confounders including education, smoking, physical activity, and family history of diabetes. Cases of LADA had onset age ≥35 years and were distinguished from cases of T2DM by measures of GAD autoantibodies. Mean age was 60.5 years (LADA), 64.0 years (T2DM), and 58.8 years (controls). Odds ratios adjusted for age, sex, and other potential confounders were calculated using logistic regression.
Results: Weekly consumption of fatty fish (≥ 1 serving/week) was associated with a tendency for a reduced risk of LADA (OR=0.47, 95% CI=0.21-1.05). Similar results were seen for T2DM (OR=0.45, 95% CI=0.23-0.88), and there were no considerable differences between men and women. These associations remained after adjustments for BMI, physical activity, education, smoking, and family history of diabetes (OR=0.50, 95% CI=0.21-1.18 for LADA, and OR=0.37, 95% CI=0.16-0.87 for T2DM). Consumption exceeding two servings per week did not further reduce the risk (OR=0.44, 95% CI=0.15-1.25 for LADA, and OR=0.48, 95% CI=0.21-1.10 for T2DM). No reduced risk was observed for weekly consumption of lean fish neither for LADA (OR=1.12, 95% CI=0.49-2.57) nor T2DM (OR=1.24, 95% CI=0.64-2.43).
Conclusion: Our findings suggest that weekly consumption of fatty fish, but not lean fish, may be beneficial in the prevention of both LADA and T2DM, indicating a protective effect related to a shared disease characteristics. The lack of association for lean fish supports posed hypotheses that nutrients specifically prevalent in fatty fish, such as marine polyunsaturated n-3 fatty acids and/or vitamin D, may be mechanistically involved in a protective effect.
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The influence of the duration of exposure to westernised lifestyle and childhood environment on development of lifestyle-related diseases in Japanese-Americans
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Background and aims: Japanese-Americans, who are genetically identical to their Japanese progenitors, but have undergone rapid and intense westernization of their lifestyle. We reported that body mass index (BMI) was significantly higher and the prevalence of diabetes mellitus (DM) and metabolic syndrome (MS) were significantly higher, and atherosclerosis progressed more rapidly in Japanese-Americans by comparing with Japanese living in Hiroshima. The Japanese-Americans born of 2 Japanese parents were divided into 2 groups: first generation migrants (JA-1) who were born in Japan; and second or later generation migrants (JA-2) who were born and raised in the United States (USA). Therefore JA-1 and JA-2 have different backgrounds concerning the beginning and duration of their exposure to a westernized lifestyle. It is anticipated that the risk of obesity and DM differ between different generations of Japanese immigrants. On the other hand, it is well known that childhood lifestyle has a strong effect on the incidence of obesity and DM in the future. The second or later generation from the JA-2 group who are educated chiefly in Japan and return later to the USA are separated further into group Kibei. Due to this custom of returning to the USA later in life, Kibei often avoid exposure to westernized lifestyle in their childhood, and we speculated that Kibei would have a lower incidence of obesity and DM after maturity. The aim of this study was to determine whether the period of exposure to the westernized environment and a custom practiced by Kibei are associated with the incidence of lifestyle-related diseases.
Materials and methods: We investigated the prevalence of obesity, MS and DM among Japanese in Hiroshima in 2009 and Japanese-Americans in Hawaii in 2007 and Los-Angeles in 2010 (515 Japanese, 442 JA-1, and 375 JA-2). BMI cutoff for obesity was 25kg/m2. The diagnosis of MS was based on the International Diabetes Federation criteria. DM was diagnosed as a fasting glucose concentration >/= 126 mg/dl and/or at 2 hours post-load >/= 200 mg/dl or receiving medication for DM.
Results: The prevalence of obesity was 25.2, 28.5 and 42.1% for Japanese, JA-1 and JA-2, respectively. The prevalence of MS was 21.7, 20.8 and 32.8%, respectively. These two prevalence rates in JA-2 were significantly higher than in Japanese and in JA-1. The prevalence of DM was 8.7, 18.1 and 18.9%, respectively. This prevalence rate in JA-1 and in JA-2 was significantly higher than in Japanese. When accounting for Kibei status within the JA-2 group, we found the prevalence of obesity was 32.6 and 45.2%, the prevalence of MS was 21.7 and 36.4%, and the prevalence of DM was 16.3 and 19.8% for Kibei and Non-Kibei, respectively. The prevalence of obesity and MS in Non-Kibei were significantly higher than in Kibei, but the prevalence of DM was not significantly different.
Conclusion: We have demonstrated that the prevalence of obesity and MS in JA-2 were higher than in JA-1. It was also observed that the incidence rates for obesity and MS in Non-Kibei were higher than in Kibei within JA-2 subgroups. It was suggested that duration of exposure to westernized lifestyle and childhood environment were associated with an increase in lifestyle-related diseases.

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Chewing betel-quid and its association with metabolic disease, cardiovascular disease, and all-cause mortality: a meta-analysis of observational studies
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Background and aims: Asia already has 60% of the world's diabetic population and diabetes is increasing more rapidly in Asia than anywhere else. Metabolic diseases have a major influence on public health, since a modest increase in the risk of morbidity and mortality translates into a substantial social burden, so prevention of these diseases is extremely important. Betel nut (Areca nut) is the fruit of the Areca catechu tree, which grows in Asia. Approximately 600 million individuals regularly chew betel nut (or betel quid) worldwide, and betel quid chewing is common in South-East Asia and Western Pacific. Chewing betel quid is a known risk factor for oral cancer and esophageal cancer. It was recently proposed that an association may exist between inflammatory oral condition and systemic disorders. Numerous studies have shown that chewing betel quid is associated with the risk of systemic diseases as well as oral diseases. Thus, clarifying the relationship between chewing betel quid and metabolic disease may be important for the development of preventive strategies. Accordingly, we performed a meta-analysis to assess the influence of chewing betel quid on metabolic disease, cardiovascular disease, and all-cause mortality.
Materials and methods: We searched Medline, Cochrane Library, Web of Science, and Science Direct for pertinent articles (including the references) published between 1951 and 2013. The adjusted relative risk (RR) and 95% confidence interval were calculated using the random effect model. We employed the sex and the ethic group as an independent category for comparison within each study. The Newcastle-Ottawa Scale for assessing the quality of nonrandomized studies in meta-analysis was used to quantify the validity of each study.
Results: Of 580 potentially relevant studies, 17 studies from Asia (6 cohort studies and 11 case-control studies) covering 393,077 subjects (range: 1,049 to 97,244) were selected. Eight studies (N=126,622) showed significant dose-response relationships between betel quid consumption and the risk of events. According to pooled analysis, the adjusted RR of betel quid chewers vs. non-chewers was 1.47 (p<0.001) for obesity (N=30,623), 1.56 (p=0.001) for metabolic syndrome (N=28,328), 1.47 (p<0.001) for diabetes (N=51,412), 1.45 (p=0.06) for hypertension (N=89,051), 1.2 (p=0.02) for cardiovascular disease (N=201,488), and 1.21 (p=0.02) for all-cause mortality (N=179.582).
Conclusion: Betel quid chewing is associated with an increased risk of metabolic disease, cardiovascular disease, and all-cause mortality. Addition to reason preventing oral cancer, stopping betel quid use could be valuable for preventing metabolic diseases that are showing a rapid increase in South-East Asia and the Western Pacific.

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Association between urinary phthalate metabolites and diabetes mellitus: a pilot study

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Background and aims: Phtalates present widespread endocrine disrupting chemicals in many personal care and consumer products. A limited number of studies suggest that exposure to phtalates may be associated with metabolic disturbances of diabetes mellitus. The aim of this study was to examine the presence of urine phtalate metabolites in diabetic patients and their association with diabetes mellitus.
Materials and methods: We included 100 newly diagnosed patients with non insulin dependent diabetes mellitus who are without medical treatment, 50 males and 50 females, mean age 47,19±9,75 years. Body mass index (BMI), fasting glycaemia, fasting insulinemia and urinary phtalate metabolites (MEP - monoethyl phtalate, MEHP - mono -(2-ethylhexyl)-phtalate, MBP - monobuthyl phtatale) were mesured. Insulin resistance was calculated by HOMA IR. The same examinations were performed in 100 healthy persons as a control group.
Results: Patients with diabetes mellitus had significantly higher levels of BMI (29,75±7,22 vs 22,95±2,12 kg/m^2; p<0,0001), fasting blood glucose (9,06±4,06 vs 5,09±0,49 mmol/l; p<0,0001) and fasting blood insulin levels (13,64 +/- 13,29 mIU/l vs 7,74+/- 3,75 mIU/l, p<0,0001). HOMA IR was significantly higher in these patients than controls (3,41 +/- 2,05 vs 1,76+/- 0,95, p<0,0001). Urinary phtalate metabolites were non significantly lower in diabetic patients than controls (MEP 16,16 +/- 79,25 vs 35,57 +/- 133,91 ng/ml, p=0,22 ; MBP 9,59 +/- 59,53 vs 11,58+/- 82,63 ng/ml; p=0,33; MEHP 19,52+/- 59,28 vs 21,98+/-61,69 ng/ml; p=0,78). We found only positive correlation between MEHP and fasting glycaemia (r=0,23) in diabetic patients.
Conclusion: According to our data only exposure to diethylphtalate may have impact on glucose levels. Futher studies with more participants are needed to elucidate this association.

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Alcohol consumption is associated with a reduced risk of latent autoimmune diabetes in adults (LADA) and type 2 diabetes: the ESTRID case-control study

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Background and aims: Moderate alcohol consumption is associated with a reduced risk of type 2 diabetes. The protective effect is attributed primarily to improvement in insulin sensitivity. Our aim was to investigate whether alcohol consumption is associated with risk of LADA, an autoimmune form of diabetes with possible features of type 2 diabetes.
Materials and methods: We used data from an ongoing Swedish case-control study of LADA and type 2 diabetes-ESTRID (epidemiological study of risk factors for LADA and type 2 diabetes); where information on alcohol intake is collected by validated questionnaires. Logistic regression was used for carrying out analyses on data including 242 incident LADA and 445 incident type 2 diabetes cases and 689 controls; odds ratios (OR) were adjusted for age, sex, body mass index, family history of diabetes, smoking, and education.
Results: Moderate alcohol consumption (5-15g/day) was associated with a 37% reduced risk of LADA (OR; 0.63, 95% CI; 0.43-0.92) which persisted after adjustment for confounders; OR; 0.64, 95% CI; 0.44-0.94 (all); OR; 0.58, 95% CI; 0.33-1.02 (women), and OR; 0.67, 95% CI; 0.39-1.14 (men). The results were similar for type 2 diabetes; OR; 0.66, 95% CI; 0.47-0.93 (all); OR; 0.60, 95% CI; 0.35-1.03 (women); and OR; 0.72, 95% CI; 0.46-1.14 (men). A reduced risk was also suggested at higher levels of consumption (>25 g/day); OR; 0.55, 95% CI; 0.31-0.95 (LADA); and 0.71, 95% CI; 0.45-1.11 (type 2 diabetes). Beverage specific analysis suggest that consumption of liquor confers no protective effect; OR for per 5g/day; 1.04, 95% CI; 0.92-1.18 (Type 2 diabetes); and 1.08, 95% CI; 0.95-1.22 (Type 2 diabetes). The results suggest a protective effect of wine for LADA (OR for per 5g/day; 0.97, 95% CI; 0.91-1.04); and for type 2 diabetes the protective effect seemed linked to consumption of beer; OR for per 5g/day=0.88, 95% CI; 0.75-1.03.
Conclusion: Moderate alcohol consumption was associated with reduced risk of both LADA and type 2 diabetes, emphasizing that the risk of LADA alike type 2 diabetes can be reduced through lifestyle factors which improve insulin sensitivity.

PS 016 Lifestyle interventions

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Barriers to participation in a community-based lifestyle intervention programme to prevent type 2 diabetes following gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM) is growing and progression to type 2 diabetes is increased seven-fold following GDM.
Materials and methods: We have designed and recruited into a community-based randomised controlled trial (RCT) of an intensive lifestyle intervention compared to standard care for delaying diabetes onset following GDM. This paper compares the clinical, anthropometric and demographic characteristics of 89 consenters versus 156 non-consenters to the RCT using data available from post-partum clinic visits following GDM. It also examines the barriers to participation in women eligible for recruitment but who declined to participate.
Results: 410 women with prior GDM were invited to participate in the study; 89 consented, 156 refused, and the remainder were not contactable. Stated barriers to participation are available from the 156 non-consenters. The barriers cited by women can be grouped as follows: access/transport (distance, location, expense or lack of transportation) [n=75]; lifestyle (too busy, work schedule, caring for parents) [n=49]; parental commitments [n=48]; health-related (poor health, not concerned about health or diabetes risks, already taking action to improve health) [n=29]; research and intervention programme deterrents (research fatigue, test procedures, programme times/content) [n=26]; lack of social support [n=9].
Conclusion: To improve recruitment into similar programmes, researchers should consider offering home-based assessments; providing on-site child care; ensuring a variety of programme times; providing regular feedback, follow-up, and pragmatic advice for the target population. It is also important for health care providers to focus on updating and reinforcing knowledge about the future health implications of GDM. Finally, it would be valuable to integrate the use of cognitive behavioural strategies into such interventions in order to: (i) address underlying psychological barriers mitigating success in regard to behaviour change, and (ii) progress women who are still 'pre-contemplators' in regard to lifestyle modification to the stages of contemplation and action.
Clinical Trial Registration Number: ISRCTN41202110
Supported by: Irish HRB

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Prevention of type 2 diabetes; a systematic review and meta-analysis of different intervention strategies

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Different intervention strategies can prevent new cases of type 2 diabetes (T2DM). Aim of the present systematic review and meta-analysis was to evaluate the effectiveness of different strategies. Studies were grouped into 15 different strategies: 1: diet plus physical activity; 2: physical activity; 3-6: anti-diabetic drugs [glitazones, metformin, beta cell stimulating drugs (sulphanylureas, glinides), alfa-glucosidase inhibitors]; 7-8: cardiovascular drugs (ACE-inhibitors, ARB, calcium-antagonists); 9-14 [diets, lipid-affecting drugs (orlistat, bezafibrate), vitamins, micronutrients, estrogens, alcohol, coffee]; 15: bariatric surgery. Only controlled studies were included in the analysis, whether randomized or non-randomized studies. Appropriate methodology (PRISMA statement) was used. Seventy-one studies (490,813 subjects), published in english language as full papers, were analysed to identify predictors of new cases of T2DM, and were included in a meta-analysis (random-effects model) to study the effect of different strategies. Intervention effect (new cases of diabetes) was expressed as odds ratio (OR), with 95% confidence intervals (CIs). Body mass index was in the overweight range for 13 groups, obese or morbidly obese in lipid-affecting drugs and in bariatric surgery. Non-surgical strategies, except for beta cell stimulating drugs, estrogens, and vitamins, were able to prevent T2DM, with different effectiveness, from 0.37 (0.26,0.52) to 0.85 (0.77,0.93); the most effective strategy was bariatric surgery in morbidly obese subjects [0.16 (0.11,0.24)]. These data indicate that several, but not all strategies, prevent T2DM, making it possible to make a choice for the individual subject; for morbidly obese subjects bariatric surgery appears an adequate strategy.

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Effectiveness of an intervention in primary care to prevent type 2 diabetes in people with impaired fasting glucose, a cluster-randomised controlled trial

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Background and aims: Effective diabetes prevention strategies are needed. The Netherlands Diabetes Federation developed a protocol for coaching people with impaired fasting glucose (IFG) to a sustainable healthy lifestyle change: the 'Route planner diabetes prevention' (RP) within a primary health care setting. The protocol focuses on promotion of a healthy lifestyle among people with IFG within one year by providing 6 consultations and, if applicable, referrals to other health professionals (dieticians, physiotherapists or locally organised lifestyle activities). We evaluated the feasibility and effectiveness of care provided according to the RP.
Materials and methods: A cluster randomised clinical trial, including 22 general practices was performed. Patients with a newly diagnosed IFG were included in the trial. Participants in the intervention group received care according to the RP protocol (n=197). The control group received usual care (n=169). The outcome measure anthropometrics and biochemical parameters were assessed in the practices. Physical activity, food intake and their determinants were assessed by a validated questionnaire. All measurements were performed at baseline, and after one (post-intervention) and two years. A process evaluation among health professionals was performed by personal interviews.
Results: Multilevel analyses showed that after two years the number of participants adhering to the physical activity guideline increased more in the intervention (OR=1.97 95%CI 1.22, 3.20) than in the control group. Additionally risk perception (β=0.15; 95%CI 0.02,1.28) and the perceived knowledge about healthy lifestyle (β=0.17; 95%CI 0.10,0.33) were higher in the intervention group. Per protocol analyses showed an intervention effect on nutrition (β=-1.36; 95%CI -2.69.-0.03). Process evaluation revealed that participants in the intervention group received less consultations than described in the RP protocol while those in the control group received more consultations than expected. Post-hoc analyses showed that the number of consultations was associated with greater decrease in body mass index (β =-0.39; 95%CI -0.78;-0.01). Health professionals experienced the RP as time consuming; they found it hard to motivate unmotivated patients to participate in the RP.
Conclusion: Despite the small difference in consultations between the intervention and control group, the findings on physical activity suggest that promotion of a healthy lifestyle among people with IFG by providing the RP protocol are promising. Additionally, the study results showed that the intervention effect is bigger among motivated patients and those receiving care as prescribed by the RP. Adjustments must be made to make the RP protocol more applicable and (time-)efficient.
Clinical Trial Registration Number: METC 100218
Supported by: Ministry of Health, Wellbeing and Sports

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Sustained weight loss and risk reduction following lifestyle intervention in the community: results of the Rethinking Eating and ACTivity study (REACT)

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Background and aims: Understanding the long-term comparative effectiveness of multiple lifestyle intervention modalities in community settings is critical to assist consumers, clinicians, purchasers, and policy makers to make informed decisions. Evidence supports the effectiveness of lifestyle change, including weight loss and diabetes risk reduction, in the short-term; however mixed results are reported with follow-up greater than one year, particularly in underserved community settings. We therefore aimed to determined whether three Group Lifestyle Balance (GLB) intervention modalities that resulted in weight loss and diabetes and CVD risk reduction at 3 month follow-up were effective at achieving sustained improvements at 18 month follow-up.
Materials and methods: 555 individuals (86.1% female, 95.1% white, 55.8% obese) from 8 underserved, rural communities, in Pennsylvania were screened for BMI ≥ 25 kg/m2 and waist circumference ≥ 102 cm in men and 88 cm in women. Communities, along with their eligible participants (n=493; mean age: 51 yrs, 87.6% female, 94.1% Caucasian, 86.8% BMI ≥ 30 kg/m2) were assigned to 4 GLB intervention groups: Face to Face (FF) (n=119), DVD (n=113), internet (INT) (n=101), and self-selection (SS) (n=101). Participants in SS were empowered to select the modality of their choosing (60% chose FF, 40% INT, 0% DVD). Outcomes for these analyses included sustained improvement in the proportion of participants who met the 5% weight loss goal and the proportion of participants who decreased ≥ one diabetes and CVD risk factor at 18 months following the intervention. Diabetes and CVD risk factors included fasting glucose ≥100 mg/dL, waist circumference ≥ 102 cm in men and 88 cm in women, blood pressure ≥ 130/85 mmHg, triglycerides ≥ 150 mg/dL, and HDLc < 40 mg/dL in men and 50 mg/dL in women.
Results: Following the GLB intervention, 60.1% of participants lost at least 5% of their total body weight at the 3 month assessment (FF: 57.9%, DVD: 56.1%, INT: 62%, SS: 66.7%). All groups achieved maintenance of 5% weight loss in at least 50% of their participants at 18 months with the SS group having the largest number of participants who maintained weight loss (FF: 67.3%, DVD: 67.6%, INT: 67.7%, SS: 89.5%). A similar trend was observed in participants who reduced ≥ one diabetes and CVD risk factor at the 3 months. Nearly 75% of these participants maintained the reduction at 18 months. After adjustment for age, gender, baseline values, intervention group, and the clustering of communities within each intervention group, participants in SS were 2.3 times more likely to maintain 5% weight loss at 18 months compared to the other three groups (p=0.0007), but were not more likely to maintain reduction of diabetes and CVD risk factors (p=0.89).
Conclusion: These results demonstrate that despite the modality, the GLB intervention was effective at sustaining improvements in weight loss and risk reduction. Moreover, SS participants, who were empowered to choose their GLB modality, were twice as likely to sustain improvements compared to other groups. Indeed, the importance of patient-centered decision-making in healthcare is paramount.
Supported by: the Air Force

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Feasibility of a controlled intervention with physical activity in individuals with impaired glucose tolerance, recruited by FINDRISC

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Background and aims: Landmark studies have given proof of the positive effect of a life-style intervention with physical activity and diet in individuals with impaired glucose tolerance. The aim of this study was to explore the feasibility and effect of an intervention with isolated physical activity in individuals with impaired glucose tolerance (IGT) recruited by the FINDRISC questionnaire.
Materials and methods: The questionnaire was sent to a population of 9734 individuals, 35-75 years old, in a selected area in a middle-sized town in Sweden. Those with a risk-score >11 were encouraged to perform an oral glucose tolerance test and all with IGT were invited to participate in a randomized controlled trial with focus on physical activity. The participants were allocated to one of three arms; basic intervention with a recipe for physical activity, a step-counter and a personal nurse for support, an intensive care group with additional group sessions or to care as usual. A total of 52 individuals were examined for common risk factors for diabetes and CVD including anthropometric measurements, blood pressure, serum lipids and questionnaires about diet and lifestyle. A new examination was performed after one year.
Results: There was a high prevalence of co-morbidity in individuals recruited with the questionnaire. In these with IGT 17% were diagnosed with ischemic heart disease, 4% with chronic obstructive pulmonary disease and 6% had had a stroke. At base-line mean age was 64 (8.9) years, BMI 30 (4.2) kg/m^2, fasting P-glucose 6.0 (0.5) mmol/L, systolic blood pressure 150 (18) mmHg, diastolic blood pressure 83 (10.9) mmHg and mean cholesterol was 5.3 (1.2) mmol/L. During this first year six individuals developed diabetes, one died and another five dropped off because of disease or for social reasons (n=40). Differences between groups were assessed with general linear models adjusted for differences in age, sex and energy intake and in spite of the high co-morbidity the intervention was efficient. Body weight decreased significantly more Δ3.5kg, p=0.045 (CI, 0.076-6.881) in the intensive care group compared to the other groups, as did diastolic blood pressure Δ9.4 mmHg, p=0.013 (2.2-17.0), while the difference in decreased waist-circumference (p=0.091), and increased high density lipoproteins (HDL) p=0.071 both were non-significant. Furthermore, participants in the intensive care group had significant decreases in systolic blood pressure 12 mmHg (p=0.007), and waist-circumference 3cm (p=0.049) when assessed with paired samples T-test.
Conclusion: It is generally feasible to implement a life-style intervention with physical activity in screen-detected individuals with IGT, but high prevalence of co-morbidities needs to be considered. Though - despite these individual's burden of chronic diseases the intervention had effect on essential risk factors and may indicate a future prevention of type 2 diabetes.
Supported by: VR, GU, VGR, LU

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Characteristics of smoking behaviour in patients with diabetes attending a tertiary referral centre

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Background and aims: Cigarette smoking is a known cardiovascular risk factor. Smoking has been shown to have adverse effects on insulin resistance and body fat distribution. Studies have consistently highlighted an increased risk of morbidity and mortality in patients with diabetes who smoke, primarily from excess cardiovascular disease. There is little evidence that smoking is addressed consistently by healthcare providers, and smoking cessation trials rarely report separate results for diabetes patients.
Materials and methods: As a tertiary referral diabetes centre with 4000 active patients, we sought to better understand the burden of smoking within our patient cohort and its potential effects on glycemic control. 400 patients were surveyed regarding their smoking habits, their understanding of smoking with diabetes and potential barriers to quitting. We then matched for markers of glycemic control and other markers of cardiovascular risk.
Results: Of the 400 patients surveyed, mean age was 55.1 years (17-93.9 years), with 58% male. Current smokers accounted for 19.25%, 34.5% were ex smokers and 44% were never smokers. Mean age of current smokers was 49.2 years. Mean pack years in current smokers was 24.8 years, and 15.9% of never smokers were exposed to passive smoking at home. 87% of current smokers wanted to quit, and 70% thought having diabetes gave them an incentive to do so. The most significant concern about stopping smoking in 48% of patients was weight gain followed by increasing stress levels in 42.9%. Nicotine replacement had been used in the vast majority of smokers 97.4%. 49% typically relapsed within 1 month of a quit attempt. Patients typically attempted to quit smoking on 3 previous occasions. 56.7% of those current smokers who wanted to quit were accepting of help from a smoking cessation officer when offered this service. Current smokers had worse glycemic control (as measured by most recent HbA1c) compared to never smokers. Mean HbA1c of current smokers was 65.8 mmol/mol vs. 61.7 mmol/mol in the never smoking group (p= 0.043). Current smokers vs. ex smokers revealed

mean HbA1c of 65.8mmol/mol vs. 60.9 mmol/mol, but failed to reach statistical significance. (p=0.052). The number of anti-hypertensive medications prescribed for the patient was taken as a surrogate marker of blood pressure control. Significantly, current smokers were prescribed less antihypertensive medications (n=0.93) than ex smokers (n=1.65) (p=0.0001) or never smokers (n=1.29) (p=0.04). There was no statiscally significant difference in albumin creatinine ratio (ACR) or estimated glomerular filtration rate (eGFR) between the groups.
Conclusion: Smoking prevalence in patients with diabetes is less than that of the general population. Diabetes patients who smoke want to quit, yet they face similar barriers to smoking cessation as other smokers. Smokers with diabetes are keen to try pharmacological therapy and are accepting of smoking cessation support, have inferior glycemic control but are less troubled by systemic hypertension.

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Six month follow-up results from a registry observing Duodenal-Jejunal Bypass Liner treatment outcomes in subjects with type 2 diabetes and/or obesity

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Background and aims: The Duodenal-Jejunal Bypass Liner (DJBL) is an endoscopically placed and removed device to create a bypass of the proximal intestines mimicking the Roux-en-Y and Duodenal-Jejunal Bypass without the risks associated with surgery. The DJBL is indicated for the treatment of type 2 diabetes and/or obesity and remains implanted for up to 12 months. The aim of this registry is to report on the effectiveness and safety of the DJBL in the commercial setting.
Materials and methods: To date, 30 patients implanted with the DJBL in the commercial setting have been included in the registry study. Baseline mean BMI was 37.9 kg/m2 (29.7-48.1 kg/m2), weight 108.1kg (77-143.3kg) and 11 (36.7%) patients were reported to have had type 2 diabetes for a mean duration of 8.1 years (1-18 years), with HbA1c of 7.2% (5.0-10.2%). Data are collected at Baseline, Implant, 6 months post implant, 12 months post implant/removal, and 3, 6, 9 and 12 months post removal. Six month post implant weight, blood pressure, HbA1c, fasting plasma glucose, total cholesterol, LDL, and TG are presented here.
Results: At 6 months post implantation the mean BMI (34.9, - 3.0kg/m2,), weight (99.3, -8.8kg), HbA1c (6.7, -0.5%), fasting plasma glucose(6.8, -0.6 mmol/L), SBP (127.3, -5.8mmHg), DBP (82.1, -6.5mmHg), total cholesterol (4.84, -0.46mmol/L), LDL (2.76, -0.30 mmol/L) and TG (2.52, -0.30 mmol/L) were reduced from baseline/implant.
Conclusion: Use of the DJBL leads to improved glycaemic control, body weight, and associated biomarkers at 6 months compared to baseline in patients with type 2 diabetes and/or obesity in the real world setting.
Supported by: GI Dynamics, Inc.

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Duodenal-Jejunal Bypass Sleeve for obesity and type 2 diabetes: systematic review with meta-analysis of clinical studies

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Background and aims: The duodenal-jejunal bypass sleeve (DJBS) is designed to treat obesity and type 2 diabetes by mimicking the bypass effect obtained after gastric bypass surgery. DJBS is inserted and removed endoscopically, and thus, a fully reversible procedure. We aimed to evaluate clinical evidence of DJBS for the treatment of obesity and type 2 diabetes.
Materials and methods: We did a protocol-based systematic review with meta-analysis of clinical studies on the effects of DJBS vs. no intervention, sham-endoscopy and/or low-calorie diet in patients with obesity and/or type 2 diabetes. Randomized controlled trials were included in meta-analyses. Observational studies were included in sensitivity analyses to evaluate the risk of adverse events. The primary endpoint was change in body weight. Secondary endpoints included changes in glycated haemoglobin (HbA_{1c}). Meta-analyses were performed and results presented as mean differences (MD) with 95% confidence intervals (CI).
Results: Thirteen studies with a total of 273 patients were included. Meta-analysis showed that DJBS lead to a greater weight loss compared with controls (MD [95% CI]: -7.2 [-8.4,-5.9] kg; three trials). Furthermore, treatment with DJBS reduced HbA_{1c} compared with controls (-0.5 [-0.9,-0.2]%). Adverse events included transient abdominal pain, nausea and vomiting. Few cases of gastrointestinal bleeding and device obstruction were reported. No deaths occurred.
Conclusion: This systematic review found promising evidence to support additional trials on treatment with DJBS for obesity and/or type 2 diabetes.

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Bariatric surgery in France in 2011 and temporal trends since 2006

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Background and aims: To monitor practices and trends in bariatric surgery at a national level, including for people with diabetes.
Materials and methods: Hospital stays for bariatric surgery were extracted from the hospital discharge national database, and were linked with the national medical insurance data system (SNIIRAM) to collect patient characteristics from 2010 to 2011.
Results: In 2011, 30,529 patients underwent bariatric surgery (30,684 procedures), a rate of 47/100,000 inhabitants (men 16/100,000; women 75/100,000). Mean age was 39 years (n=114<age 18). The proportion of women (80%) and beneficiaries of the medical insurance for low income people (17%) were high. BMI was between 30 and 39 kg/m^2 for 29% of all patients, 40-49 for 60% and ≥50 kg/m^2 for 11%. Women underwent surgery at a younger age and a lower BMI compared to men. Treatments for obstructive sleep apnoea (23%), hypertension (24%), asthma/COPD (12%) and depression (17%) were more frequent compared to the general population after adjustment for age and sex. 3461 patients (11%) had diabetes. Sleeve gastrectomy was practiced in 44% of all cases, gastric by-pass in 31% and adjustable banding in 25%. Teenagers underwent more often adjustable banding (50%) than adults, and people with diabetes gastric bypass (39%) and sleeve gastrectomy (47%) than those without diabetes. A total of 426 different hospitals/clinics practiced bariatric surgery, with wide variation in numbers and types of procedure between hospitals/clinics. Major geographical variations in rates and types of procedures were also observed across France. The mean annual increase in all procedures was +16% from 2006 through 2011, with a major increase being recently observed for sleeve gastrectomy.
Conclusion: Our national administrative database provides a unique opportunity to contribute to monitoring medical practices at a national level as part of the national plan against obesity. This large national cohort of patients with and without diabetes will enable the analysis of consequences of bariatric surgery, with almost complete follow-up and a cost-effectiveness analysis.

PS 017 Body composition and diabetes aetiology in epidemiology

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Association between changes in body composition and risk of developing type 2 diabetes in Koreans

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Background and aims: Changes in body composition with aging are characterized by loss of muscle mass and increase of fat mass. Cross-sectional studies have shown that decreased muscle mass and increased fat mass are associated with insulin resistance and type 2 diabetes. However, there is a paucity of data that examined the effects of longitudinal changes in muscle or fat mass on the risk of type 2 diabetes. We assessed the association between changes in body composition and risk of developing type 2 diabetes in non-diabetic Korean individuals.
Materials and methods: We analyzed the clinical and laboratory data of 18,687 Korean adults (age 20-79 years, 11,510 men and 7,177 women) who underwent routine medical check-ups in 2007-08 (baseline) and again in 2011-12 (follow up) with a mean 4.3-year (range 3.0-5.7 years) interval. All non-pregnant adults who did not have diabetes at baseline examination were included for the analysis. Total fat mass, lean body mass, and soft lean mass were assessed using bioelectrical impedance. Odds ratios (ORs) for incident type 2 diabetes were estimated using multiple logistic regression analysis.
Results: Among the 18,687 participants who did not have diabetes at baseline, a total of 692 subjects (3.7%, 524 men and 168 women) developed type 2 diabetes during the 4.3-year follow-up period. Those who developed type 2 diabetes had significantly higher percentage of total body fat (23.5±5.5% vs. 21.6±5.4%, P <0.001) and lower percentage of lean body mass (76.6±5.6% vs. 78.5±5.5%, P <0.001) and soft lean mass (72.5±5.3% vs. 74.2±5.2%, P <0.001) at baseline compared with those who did not develop diabetes. After 4.3 years, those who developed diabetes showed greater increase of percent body fat (2.9±3.0 vs. 2.6±3.2 percentage points, P=0.043), decrease of percent lean body mass (-3.0±3.3 vs. -2.7±3.3 percentage points, P=0.008) and percent soft lean mass (-2.8±3.1 vs. -2.4± 3.1 percentage points, P=0.003) compared with those who did not develop diabetes. There was no significant difference in change of total body weight and BMI between the two groups. In multiple logistic regression analysis, increase of fat mass more than 10% was associated with increased OR for diabetes (1.32; 95% CI, 1.05-1.64), and decrease of fat mass was associated with lower OR (0.61; 0.47-0.81) after adjustments for age, sex, and baseline BMI, waist circumference, glucose level. Loss of lean body mass more than -5% was associated with increased OR for diabetes in age and sex-adjusted models (OR 1.32; 1.11-1.56), but this association became insignificant after further adjustments for baseline BMI, waist circumference, and glucose level (OR 1.09; 0.90-1.32). Increase of lean body mass was not significantly associated with lower OR for diabetes (0.84; 0.64-1.11).
Conclusion: These results showed that changes in total body fat mass are associated with development of type 2 diabetes independent of changes in BMI, while changes in lean body mass are not.

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Ethnicity-specific obesity cut-points in the development of incident diabetes: a prospective study including three ethnic groups in the United Kingdom

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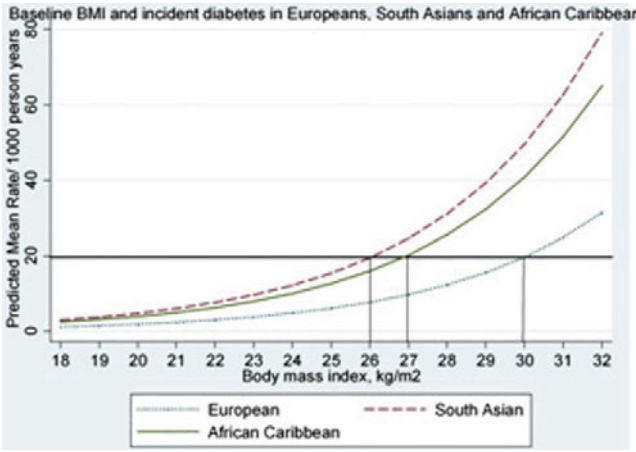
Background and aims: Conventional definitions of obesity, e.g. body mass index (BMI)> 30 kg/m^2 or waist circumference cut-points of 102cm (men) and 88cm (women), may underestimate metabolic risk in non-European populations. We compared incidence of diabetes in British Europeans, South Asians and African Caribbeans and identified equivalent sex- and ethnicity-specific BMI and waist circumference cut-points for estimation of diabetes risk.
Materials and methods: Population based cohort of 4202 non-diabetic Europeans, South Asians and African Caribbeans from London. Participants were aged 40-69 at baseline (1988-91), when they underwent anthropometry and fasting and post-load blood tests. Follow-up data to 19 years (median) were available for 2533 (60%). Incident diabetes was identified from primary care records, participant recall and/or follow-up biochemistry.
Results: Incidence rates of diabetes in men were greater in South Asians and African Caribbeans (respectively 20.8 (95%CI:18.4, 23.6) and 16.5 (12.7, 21.4)/1000 person years) compared with 7.4 (6.3, 8.7)/1000 person years in Europeans. Likewise, incidence rates in women were highest in South Asians (12.0 (8.3,17.2) and African Caribbeans 17.5 (13.0,23.7) compared with 7.2(5.3,9.8)/1000 person years in Europeans. For the equivalent incidence of diabetes at a BMI of 30 kg/m^2 in Europeans, age and sex adjusted BMI cut-points were: South Asian men and women: 26.0(24.6, 26.5) kg/m^2; African Caribbean men and women: 27.0 (26.7, 29.3) kg/m^2. For men of both ethnic minorities,age adjusted waist cut-points of 91.0 (87.4, 92.4) cm were equivalent in risk to 102cm in European men, and in women cut-points of 84.3 (76.5, 89.8) cm in South Asians and 81.6 (73.4, 85.5)cm in African Caribbeans were equivalent to 88cm in Europeans.
Conclusion: Middle-aged British South Asians and African Caribbeans had equivalent risk of developing diabetes at substantially lower cut-points of BMI or waist circumference than Europeans. Our findings suggest the potential benefit of public health measures for the prevention of metabolic risk in South Asian and African Caribbean populations long before the current conventional obesity cut-points are reached.



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Body fat distribution, glucose tolerance and metabolic profile in young adult twins: an exploration of sex differences

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Background and aims: We aimed to study a) whether gender differences in body composition account for differences in the metabolic profile among young adults; b) whether metabolic profile associates with body fat distribution and glucose tolerance independent of genetic factors; c) the extent to which genetic factors contribute to the covariance of central adiposity and metabolic profile.
Materials and methods: Metabolic profile of 46 metabolites (mainly lipids, lipid fractions and amino acids) was measured by nuclear magnetic resonance (NMR) in 1368 healthy young adult twins (age range: 22-36y, 531 monozygotic (MZ), 497 same sex dizygotic (SSDZ), 340 opposite sex dizygotic

(OSDZ) with BMI and waist measures. Oral glucose tolerance test (OGTT) was performed and body composition (android and gynoid fat by DEXA) was measured in a subsample of 128 MZ and SSDZ twin pairs. Metabolic profile was examined in men and women matched individually for BMI and android-to-gynoid (A/G) fat ratio and in OSDZ pairs partially matched for genes. Quantitative genetic modeling of twin similarity was used to assess genetic and environmental contributions to the covariation of metabolites and waist circumference (WC).
Results: Men had significantly higher ApoB, LDL-C, VLDL particle, and phenylalanine concentrations and lower levels of ApoA1, HDL-C and glutamine, tyrosine and branched chain amino acids (BCAA) than women. Sex-differences in ApoA1, HDL-C and amino acids remained significant even in males and females matched for BMI, A/G fat ratio and the genetic background (OSDZ). In individual twins, android fat and AUC insulin during the OGTT associated significantly with an unfavourable metabolic profile, and adiponectin and Matsuda index with a favourable profile in both males and females. Within MZ twin pairs, associations between AUC insulin and adiponectin and metabolic profile remained strong, but those with android fat were substantially weaker or non-significant indicating that genes may be important in explaining their association. Bivariate sex-limitation models utilizing the whole twin cohort confirmed that genes for abdominal obesity and metabolites partially overlap. For example, the genetic effects between BCAA's and WC correlated by r_g=0.37 (men) and 0.40 (women) and genes explained 66% and 94% of the covariance between BCAA and WC in men and women respectively.
Conclusion: Women have a more beneficial metabolic profile than men and this difference cannot be explained by differences in BMI or body fat distribution. Obesity and abdominal obesity in particular is associated with an adverse metabolic profile in young adults, which is largely explained by shared genes.
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Waist circumference and blood pressure are associated with microvascular vasomotion in a healthy population: the Maastricht study
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Background and aims: Microvascular vasomotion, i.e. rhythmic changes in vascular diameter, is thought to play an important role in ensuring optimal delivery of nutrients and oxygen to tissue. Impaired microvascular vasomotion has been demonstrated in states of obesity and type 2 diabetes mellitus (T2DM) (i.e. decreased vasomotion), and hypertension (i.e. enhanced vasomotion). It is unclear however, which factors influence microvascular vasomotion. Therefore, we investigated associations between traditional risk factors and microvascular vasomotion in a healthy population.
Materials and methods: We measured skin microvascular vasomotion in 241 healthy individuals (mean age 56 ± 8 years, 42% men, mean body mass index (BMI) 25.4 ± 3.6 kg/m^2) enrolled in the Maastricht Study. We selected a healthy population without T2DM, hypertension, prior cardiovascular disease, or use of cardiovascular medication. Skin blood flow was measured with a laser Doppler probe at the dorsal side of the left wrist. Fast-Fourier transform analysis was performed to determine the five frequency components to the variability of the laser Doppler signal (i.e., endothelial, 0.01-0.02 Hz; neurogenic, 0.02-0.06 Hz; myogenic, 0.06-0.15 Hz; respiratory, 0.15-0.40 Hz; and heart beat, 0.40-1.60 Hz). The associations of age, sex, waist circumference, total-to-HDL cholesterol, 24-h mean arterial pressure (MAP), fasting plasma glucose, and cigarette smoking with microvascular vasomotion was analyzed by use of multiple linear regression analysis.
Results: Higher waist circumference and 24-h MAP were associated with a lower and higher total microvascular vasomotion, respectively. Per one standard deviation (SD) higher waist circumference total microvascular vasomotion was -0.17 SD (95%CI: -0.32; -0.03) lower. One SD higher 24-h MAP was independently associated with a 0.18 SD (0.04; 0.32) higher total microvascular vasomotion. We found no associations of microvascular vasomotion with age, sex, total-to-HDL cholesterol, fasting plasma glucose, or smoking. Analysis of the five frequency bands revealed that these results were largely attributable to the contributions of the endothelial, neurogenic, and myogenic components. Sub-analysis with exclusion of the obese subjects (BMI>30 kg/m^2; n = 23) gave similar results.
Conclusion: In conclusion, in a healthy population waist circumference is inversely associated with microvascular vasomotion. In addition, blood pressure is directly associated with microvascular vasomotion. These data suggest that both elevations in waist circumference and blood pressure may affect microvascular vasomotion, and subsequently optimal nutrients delivery and tissue perfusion.

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The differences between serum fingerprints of healthy and pre-diabetic humans are dependent on BMI
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Background and aims: Development of type 2 diabetes mellitus (T2DM) is preceded by insulin resistance (IR). The IR may evolve to impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), which are considered as pre-diabetic states. The transition from pre-diabetic states to diabetes may take many years. However, it is estimated, that up to 70% of pre-diabates subjects eventually develop T2DM. The risk of T2DM development is increased in overweight (OW) and obese (OB) people; however lean (L) individuals also suffer from T2DM. The purpose of this study was to evaluate whether metabolic differences between healthy and pre-diabetes humans are dependent on BMI. Metabolomic approach by use of LC-QTOF-MS was used to fingerprint serum samples obtained from L, OW or OB humans whom were healthy (controls), IR or had IFG and/or IGT (pre-diabetes).
Materials and methods: 78 people (40 female, 38 male, mean age 45 years) took part in this study. Participants were classified according to health status (controls, IR or pre-diabetes) based on fasting glucose level, HOMA-IR, and 2-h 75-g OGTT. And as L, OW or OB based on BMI. Age and sex were matched between the groups. Fasting serum samples were fingerprinted by LC-QTOF-MS. Data were collected in ESI(+) mode (50-1,000 m/z). Chromatograms were aligned and quality assurance of obtained data was performed. LOESS function was used for signal correction. Statistical analysis was performed to compare controls with pre-diabetes in each BMI group. Significant metabolites were selected by univariate (t-test) and multivariate (s-plot) analyses. S-plot was obtained for OPLS-DA model. The same model was used to predict IR individuals in each BMI group. Identification of significant features was performed based on custom library with accurate mass and retention time of more than 100 metabolites.
Results: 21 metabolites discriminating controls from pre-diabetes were identified. In the L group fatty acid amides (FAA), lysophospholipids (LPL), cortisol, and sphingosine-1-phoshate were significantly higher in pre-diabetes as compared to controls. These metabolites were also increased in OW/OB controls in comparison to L controls. In the OW/OB groups LPL were main metabolites responsible for samples classification. Valine discriminated controls from pre-diabetes independently on BMI. Majority of the samples obtained from IR individuals, when predicted by OPLS-DA model, were classified between samples obtained from controls and pre-diabetes.
Conclusion: Metabolites discriminating controls from pre-diabetes are changing with BMI. As compared to L controls, serum fingerprint of pre-diabetes is characterized by increased amount of FAA, cortisol, and lipids. In OW/OB people elevation in mentioned metabolites is probably related to increased BMI. Mechanisms responsible for rising of these metabolites in L pre-diabetic humans require further investigations.
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A propensity-matched comparison of insulin secretion and resistance between subjects from 1990's and 2000's in a Korean population

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Background and aims: We aimed to evaluate the differences in insulin secretion and insulin sensitivity between individuals from 1990's and 2000's in Korean population.
Materials and methods: A total of 769 subjects during 1990's and 720 subjects during 2000's who underwent 75g-oral glucose tolerance test (OGTT) were recruited from Seoul St. Mary's Hospital in Korea. Insulinogenic index, Matsuda index and total-are-under-the-curve (insulin/glucose) (total AUC(I/G) were calculated. Individuals were categorized into 3 groups according to their glucose tolerance status; normal glucose tolerance (NGT), prediabetes (preDM) and diabetes (DM) group. Further subgroup analysis was performed according to body mass index (BMI) level. Propensity score model was constructed to adjust significant differences in the baseline characteristics of enrolled subjects in 1990's and 2000's.
Results: In NGT group, there was no significant differences in Matsuda index, Total AUC (I/G) and insulinogenic index between subjects from 1990's and 2000's. In preDM group, Matsuda index was lower in the subjects from 2000' scompared to those from 1990's, but total AUC(I/G) and insulinogenic index showed little differences. In DM group, Matsuda index was lower, and total AUC(I/G) and insulinogenic index was higher in subjects from 2000's compared to those from 1990's. There were no significant differences according to BMI levels across the glucose tolerance status. Hyperbolic relationship was observed between Matsuda index and total AUC(I/G) in subjects with NGT(ß=-0.67(-0.77, -0.57), preDM(ß=-0.80 (-0.91, -0.68) and DM (ß=-0.98 (-1.10, -0.88) in 1990's. Hyperbolic relationship was also confirmed in 2000's, in subjects with NGT (ß=-0.80 (-0.86, -0.73), preDM (ß=-0.90, (-1.01, -0.77) and DM (ß=-0.96, (-1.08, -0.84).
Conclusion: In conclusion, compared to subjects from 1990's, clinical phenotype of prediabetes and diabetes patients from 2000's showed increased insulin resistance regardless of their BMI levels, and the early-phase compensatory insulin secretion was only minimal in the prediabetes group. It is well known that type 2 diabetes in Asian population differ from those in Western countries. However, our study suggest that the clinical characteristics of prediabetes and diabetes have been westernised compared to those in the past decades.

Baseline Characteristics

		1990's (n=769)	2000's (n=720)	p-value†
Glucose tolerance	NGT PreDM DM	154 (20.0) 126 (16.4) 489 (63.6)	95 (13.2) 289 (40.1) 336 (46.7)	<0.0001
Age (years)*		48.6±12.0	51.1 ± 11.6	<0.0001
Male		346 (45.0%)	404 (56.1%)	<0.0001
Weight (kg) * Height (cm) *		63.9 ± 10.3 161.8 ± 8.5	67.9 ± 12.7 164.9 ± 8.9	<0.0001 <0.0001
BMI (kg/m2)	<23 23-<25 =25	223 (32.6%) 189 (27.6%) 272 (39.8%)	168 (27.5%) 158 (25.9%) 285 (46.6%)	0.035
Fasting plasma glucose (mg/dL)	NGT PreDM DM	86.01 ± 7.68 100.41 ± 13.82 152.58 ± 50.43	90.23 ± 6.34 109.64 ± 8.96 139.99 ± 47.28	<0.0001 <0.0001 <0.0001
Fasting insulin(μU/mL)	NGT PreDM DM	7.02 ± 5.81 7.73 ± 5.70 7.27 ± 6.15	7.90 ± 4.38 9.48 ± 5.88 10.99 ± 8.06	0.003 0.0002 <0.0001
Total Cholesterol (mg/dL) * Triglyceride (mg/dL) * LDL-Cholesterol (mg/dL) * HDL-Cholesterol (mg/dL) *		193.3 ± 42.7 175.7 ± 140.7 114.9 ± 38.1 45.4 ± 12.5	193.3 ± 42.7 175.7 ± 140.7 114.9 ± 38.1 45.4 ± 12.5	0.545 <0.0001 0.885 <0.0001

Baseline characteristics were assessed for normality and then compared between groups using the Fisher's exact test and Wilcoxon rank sum test* of independence. Data are means ± SD or n (%) unless otherwise indicated.

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Association of body fat mass and liver fat content with insulin resistance in euglycaemic Chinese middle-aged and elderly adults

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Background and aims: The risk of type 2 diabetes mellitus is heterogeneous among obese individuals. Recent studies indicated that a dysfunctional adiposity phenotype, characterized by the alteration of body fat distribution to visceral site, may contribute to the diabetes development and explain the heterogeneity. The aim of the current study is to investigate association of insulin resistance with whole body fat mass and ectopic liver fat content (LFC) in euglycemic Chinese middle-aged and elderly adults, and further determine the cutoff value for LFC to indicate insulin resistance in euglycemic Chinese people.
Materials and methods: The whole body fat mass were measured using dual-energy x-ray absorptiometry (Lunar iDXA, GE Healthcare) in 1445 euglycemic participants aged over 45 yr from Shanghai Changfeng community. Liver fat content was quantified via a newly-established computer-aided ultrasound quantitative method. Multivariate stepwise regression and general linear models were carried out to determine the independent association of insulin resistance with whole body fat mass as well as liver fat content.
Results: ① The body insulin resistance estimated by HOMA-IR is significantly correlated with fat mass (r=0.544, P<0.001) and LFC (r=0.316, P<0.001). ② Multivariate stepwise linear regression analysis shows that both body fat mass and LFC have positive independent associations with insulin resistance (Standardized β=0.376,P<0.001 and Standardized β=0.087, P<0.001, respectively). Besides, FBG, TG, HDL-c, SBP and gender are also independently associated with insulin resistance. ③ The subjects are divided into five groups according to LFC at 5%-increments and an increasing trend in HOMA-IR is found to accompany the increase in LFC when liver fat content exceeds 10% after adjustment for all potential confounders (P for trend<0.001) (Figure 1).
Conclusion: Both body fat mass and ectopic fat accumulation in liver are independently associated with insulin resistance level and liver fat content over 10% may indicate early insulin resistance state in euglycemic Chinese middle-aged and elderly adults.

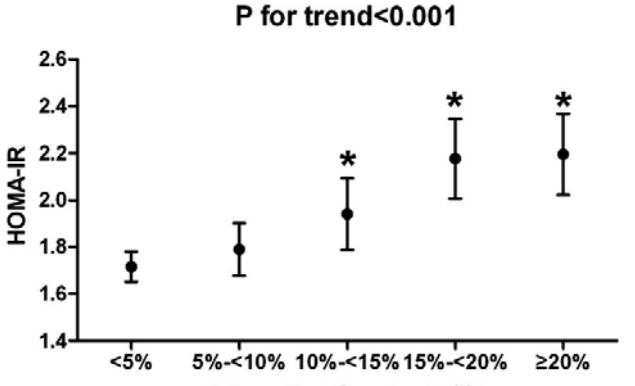

Figure 1 Comparison of HOMA-IR according to 5% increments of liver fat content after adjustment for age, BMI, gender, fat mass, components of metabolic syndrome, cigarette smoking, and alcohol drinking. P for trend<0.001.
*p<0.05, compared with subjects with liver fat content<5%.

Supported by: National Key Basic Research Program of China

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The impact of reproductive factors on glucose metabolism in postmenopausal women: Korean National Health and Nutrition Examination Survey 2008-2010

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Background and aims: Recent studies suggest that reproductive factors are related to metabolic syndrome in women. An earlier age at menarche has

been associated with an increased risk of cardiovascular disease. However, the impacts of reproductive factors on glucose metabolism has not been evaluated. The objective was to determine whether there is an association between the reproductive factors and glucose tolerance status in Korean postmenopausal women.
Materials and methods: The Korean National Health and Nutrition Survey (KNHANES) was a nationally representative survey with a stratified multistage sampling design. Datas were obtained from the KNHANES of 2008-2010. A total of 2,674 postmenopausal women were included in the analysis.
Results: The prevalences of impaired fasting glucose (IFG) and new diagnosed diabetes were 9.8% and 11.2%, respectively. Multivariate logistic regression analysis indicated that adolescent pregnancy was significantly associated with a decreased risk of IFG (odds ratio (OR), 0.87; 95% CI, 0.76-0.98) and diabetes (OR, 0.78; 95% CI, 0.65-0.85) after adjusting for risk factors associated with increasing glucose level. Other reproductive factors including age at menarche, age at menopause, parity, history of lactation, years since menopause, and oral contraceptives were not associated with a risk of IFG and diabetes.
Conclusion: Adolescent pregnancy is independently associated with a decreased risk of IFG and diabetes in Korean postmenopausal women.

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Sarcopenic obesity was associated with decreased renal function in elderly, diabetic Koreans: from KNHANES 2008-2010
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Background and aims: Sarcopenia, low skeletal muscle mass is an important age-related health implication for older people. Because muscle is the major organ contributing to insulin-mediated glucose disposal and glycogen storage, the loss of muscle mass may promote insulin resistance and risk of metabolic syndrome development. Important changes in body composition with aging are a progressive loss of muscle mass and increase of fat mass. This study aimed to examine the relationship of body composition with decrement of GFR among 2767 subjects (1176 men and 1591 women) aged 60 years or older from Korean National Health Examination and Nutrition Survey (KNHANES) 2008-2010.
Materials and methods: Cross-sectional analysis based on the data acquired in the second and third year (2008-2009) of KNHANES IV and first year (2010) of KNHANES V.1176 men and 1591 women aged 60 years and older had complete data on body composition. Data were collected by household interviews and direct standardized physical examinations. Sarcopenia was defined as an appendicular skeletal muscle mass (ASM) divided by weight (%) of <2 SD below the sex-specific mean for young adults. The ASM(kg) was defined as the sum of the lean tissue masses of the arms and legs. Obesity was defined as a body mass index (BMI) ≥ 25 kg/m^2. The renal function was measured by glomerular filtration rate(GFR) and chronic renal failure was defined as GFR <60 ml/min/1.73m^2.
Results: After adjusted age, BMI, smoking, alcohol, and exercise, we divided subjects into 4 groups ; normal, obesity, sarcopenia, and sarcopenic obesity group. In male (n=1176), odds ratio (OR) of chronic renal failure risk was 1.815 for obesity group(95% CI : 0.556-5.924), 2.353 for sarcopenia group (95% CI : 1.402-3.948), and 3.107 for sarcopenic obesity group (95% CI : 1.48-6.524) compared with normal male reference group aged 60 years and older In female (n=1591), odds ratio (OR) of chronic renal failure risk was 1.832 for obesity group (95% CI : 0.955-3.513), 1.119 for sarcopenia group (95% CI : 0.678-1.848), and 2.255 for sarcopenic obesity group (95% CI : 1.182-4.302) compared with normal male reference group aged 60 years and older.
Conclusion: In this study, Sarcopenic obesity was associated with decreased renal function in the elderly Korean diabetes. We consider that differentiation of body composition is closed related with the decreased renal function in the elderly diabetes.

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In the grey zone of type 2 diabetes prediction: Can we separate poorly discriminated incident cases and non-cases?
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Background and aims: Addition of many novel biomarkers to conventional diabetes risk scores has so far yielded only little incremental value in diabetes prediction. We aim to clarify possible reasons: (1) Measures of discrimination, particularly areas under the receiver operating curve (AROC), might be inappropriate to reflect potential gain in risk prediction, (2) (lifestyle) changes after baseline lead to changes in risk profiles not reflected by risk scores, (3) information from novel biomarkers is already captured by conventional risk factors.
Materials and methods: Analyses are based on the population-based KORA S4/F4 cohort study (873 participants without diabetes and aged 55-74 years at baseline). During 7-year follow-up, 91 incident cases of diabetes occurred. Using three different conventional risk scores, cases and non-cases were seen as well discriminated if the estimated risk was at least 10% larger in cases than in non-cases. Levels of biomarkers (e.g. adiponectin, insulin, hematocrit, white cell blood count) and changes in anthropometry were calculated separately for well and poorly discriminated cases and non-cases.
Results: Only under condition of good, but not of poor discrimination by conventional risk scores, are levels of biomarkers significantly more adverse in cases than in non-cases. Weight change after baseline was significantly larger in cases under condition of poor, but not of good discrimination. To give some examples (all analyses adjusted for age and sex): Under condition of good discrimination (with 58 cases and 458 non-cases), the median of the level of adiponectin was 5.7 μg/ml in cases and 10.2 μg/ml in non-cases (p<0.001); the median of insulin was 15.8 mikroU/ml in cases, and 8.3 mikroU/ml in non-cases (p<0.001); mean change in BMI was 0.48 kg/m2 in cases, and 0.39 kg/m2 in non-cases (p=0.49). Under condition of poor discrimination (with 33 cases and 324 non-cases), the median of the level of adiponectin was 8.3μg/ml in cases and 8.1μg/ml in non-cases (p=0.87); the median of insulin was 10.4 mikroU/ml in cases, and 11.7 mikroU/ml in non-cases (p=0.51); mean change in BMI was 0.97 kg/m2 in cases, and 0.15 kg/m2 in non-cases (p=0.01).
Conclusion: Separate analyses for good and poor discrimination by conventional risk factors give better insight into the discriminatory potential of novel markers than AROCs. Weight change after baseline, but not the selected biomarkers differ significantly between cases and non-cases which are poorly discriminated by conventional risk scores. In a more general view, there are various factors (changes in risk factors after baseline, correlations between novel markers and conventional risk factors, limited reproducibility of diabetes ascertainment) which make it questionable that discrimination between incident cases of diabetes and non-cases can steadily be improved once a level of good diabetes prediction (in terms of AROCs of about 0.85 to 0.90) has been obtained.
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Obesity paradox in diabetes mellitus: mortality and cardiovascular morbidity over 15 years of 12,025 patients
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Background and aims: Obesity is a key factor for the development of insulin resistance and Type-2 diabetes mellitus (T2DM). However, recent evidence suggests that obese patients with T2DM may have lower morbidity and mortality compared to patients of normal weight. These reports are limited by statistical power and confounders. In this analysis, the relationship between Body Mass Index (BMI), mortality and cardiovascular (CV) morbidity is investigated in a prospective cohort, with a long-term follow up and a large number of events.

Materials and methods: Between 1995 and 2011, the following data were collected in patients attending the diabetes service at Hull & East Yorkshire Hospitals NHS Trust; age, sex, height and weight (and therefore body mass index), blood pressure, biochemical and information on co-morbidities (cardiovascular (CV) disease, chronic renal failure (CRF), chronic obstructive lung disease (CPD) and cancer). Total mortality and hospital admissions for acute coronary syndrome (ACS), cerebrovascular accidents (CVA) and heart failure (HF) were gathered. Subjects were divided according to BMI quartiles and in age tertiles. ANOVA and Chi square were used to compare covariates among the BMI groups. Cox-Regression analysis was used to assess the prognostic impact of BMI and confounders on the above-defined events. Sensitivity analysis was performed accounting for T1DM and BMI<18.5.
Results: In total, 12025 patients with diabetes (54% men, mean age 60±15 years, 1761 (15%) T1DM) were enrolled and followed for a mean of 10±4 years during which ACS occurred in 1098 (9%), a CVA in 893 (7%) and a HF hospitalization occurred in 731 (6%) subjects and 4125 (34%) died. The risk of ACS was lowest in the normal BMI group and increased progressively with increasing quartiles of BMI, being greatest in obese subjects (BMI >30 HR 1.49; 95%CI 1.24-1.79; p30 HR 1.53 95% CI 1.53; 95% CI 1.24-1.88; p30) (HR 0.75 95% CI 0.69-0.82). Adjusting the analysis for comorbidity did not significantly affect the results but adjusting for age did. Therefore, we divided the results according to age tertiles. CV events showed a similar pattern in each age tertile as in the overall population. However, for all-cause mortality higher BMI was associated with a survival benefit in the eldest age tertile, whilst in the youngest age tertile, the relationship assumed a U shape, with the highest and lowest quartiles being associated with and increased risk of death. Sensitivity analysis was applied by excluding T1DM or underweight patients. However, this did not significantly affect the results.
Conclusion: In this study, in patients with T2DM, although being overweight was associated with an increased risk of CV events, higher BMIs were associated with a survival benefit, especially amongst older patients. DM induced by the metabolic stress of obesity may be a fundamentally different problem from DM that develops in the absence of the stress of obesity. Alternatively, obesity may provide a protective metabolic reserve in older diabetic patients.
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Evidence of reverse epidemiology between obesity and all-cause mortality in whites with type 2 diabetes from Italy

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Background and aims: While obesity is an established major risk factor for all-cause mortality in otherwise healthy individuals, its role in patients with type 2 diabetes (T2D) is controversial. Our aim was to address the relationship between obesity and all-cause mortality in a real-life set of White patients with T2D from central-southern Italy. We also used genetic data from GWAs-derived SNPs firmly associated with BMI, in order to address the biology underlying the association between obesity and reduced mortality rate we did observe (i.e. Mendelian randomization approach).
Materials and methods: Patients with T2D (n=764) are part of the Gargano Mortality Study, aimed at unraveling predictors of incident all-cause mortality. Time-to-death analyses were performed by Cox regression models. Of the 38 SNPs established associated to BMI in GWAs, we investigated those with a BMI β value ≥0.10 Kg/m2 and an allele frequency ≥10% (n=20). Genotyping of established BMI SNPs from GWAs, was performed by KBioscience (http://www.lgcgenomics.com/). Association between genotype risk score (GRS; number of risk allele carried by each individual) and obesity was tested by logistic regression analysis.
Results: During the 7.46±2.13 year follow-up 149 (19.5%) patients died. Annual incidence rates were 2.6% in the whole sample, 3.4% in non-obese and 1.9%, in obese individuals. As compared to non obese, obese patients had 45% decreased risk of all-cause mortality (HR=0.55, 95% CI=0.40-0.76; p=0.0004; age, sex, smoking habit, hypertension and dyslipidemia adjusted HR=0.64, 95% CI=0.45-0.90, p=0.010). Individuals with high "obesity genetic load" (i.e. 10 SNP-derived GRS being ≥ 9, the median value of whole sample) were more likely to be obese (OR=1.61, 95% CI = 1.21-2.12, p=0.001) as compared to individuals with low "obesity genetic load" (i.e. 10 SNP-derived GRS being <9). Annual incidence rates of all-cause mortality were 2.65% and 2.58% in individuals with high and low "obesity genetic load", respectively. As compared to individuals with low "obesity genetic load", the HR for all-cause mortality in those with high load was 0.97 (95%CI=0.70-1.33, p=0.877). Although the observed HR was not different from the expected one (HR=0.75, p=0.17), its approaching 1, strongly suggest that no causality underlies the association between obesity and reduced mortality risk.
Conclusion: Obesity is associated with reduced mortality risk in White patients from central-southern Italy. Based upon genetic studies, this association seems not to be causal. Further studies are needed to get deeper insights on the biological link between obesity and all-cause mortality in diabetic patients.
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Overweight is associated with LADA among women but not in men: results from ESTRID, a Swedish case-control study

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Background and aims: LADA (Latent Autoimmune Diabetes in Adults) may be the second most common form of diabetes. Data from the Nordic countries suggests that it accounts for ~10% of the total diabetes burden among adults. Still, risk factors are largely unknown - LADA is an autoimmune form of diabetes, but similarities with type 2 diabetes has also been shown, including insulin resistance. Our aim was to investigate the risk of LADA in relation to overweight and obesity, the strongest known risk factors for type 2 diabetes and an important source of insulin resistance.
Materials and methods: ESTRID (epidemiological study of risk factors for LADA and type 2 diabetes) is an ongoing population based case-control study - the largest of its kind. Incident cases of LADA and type 2, onset ≥ age 35, were recruited from the ANDIS registry (All New Diabetics in Skåne) together with a random sample of disease free individuals, matched over time (to date; LADA n=205, type 2 n=402, and controls n=670). Cases were classified as LADA if they had autoantibodies against the insulin producing beta cells (anti-GAD) at diagnosis or else, as type 2 diabetes. Mean age was 59 in LADA, 63 in type 2 and 59 in controls and the proportion of women was 44, 40 and 52%, respectively. Information on BMI (kg/m^2) was based on self-report. By means of logistic regression we calculated Odds Ratios (OR) with 95% Confidence Intervals (CI) adjusted for age, sex, family history, physical activity, education, alcohol and smoking. Population Attributable Risk (PAR) was used to estimate proportion of cases attributed to overweight/obesity.
Results: Excessive weight was associated with an increased risk of LADA, OR was estimated at 1.60 (CI 1.07-2.36) for overweight (BMI 25-29.9) and 3.03 (CI 1.90-4.84) for obesity (BMI ≥30). Stratified analysis revealed that this augmented risk primarily was seen in women, OR 4.32, CI 1.96-9.50 (obesity), while OR for obese men was 1.75, CI 0.89-3.43. We also found that women reporting being overweight at both age 20 and at onset, had a 5-fold increased risk of LADA, OR 5.48 (CI 1.27-23.74) with no corresponding increase in men, OR 1.26 (CI 0.55-2.90). The risk of type 2 diabetes increased dramatically with BMI; OR 31.66 (CI 17.10-58.61) for obesity and the excess risk was seen in men (OR 19.70, CI 8.45-45.94) as well as women (OR 47.97, CI 18.73-122.88). Estimation of PAR indicates that overweight accounts for 58% (32-76%) of all cases of LADA in women. Corresponding estimates for type 2 was 89% (77-95%) in men and 94% (87-98%) in women.
Conclusion: Our results indicate that at least for women, overweight and obesity are risk factors for LADA. Estimation of PAR suggests that a large proportion of LADA among women could be prevented through weight modification. The association was weaker than for type 2 which would fit with previous reports of LADA being characterized by insulin resistance, but to a much lesser extent than type 2. The sex inequality requires further investigation, but it is possible that the results might have been different with another indicator of excess weight such as WHR. These findings also suggest that early onset of overweight further augments the risk of LADA. Hence, it seems important to acknowledge excessive weight already during adolescence in order to prevent LADA.
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Comparison of abdominal obesity indicators and body mass index as a predictor of mortality among Europeans

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Background and aims: The body mass index (BMI) is the most commonly used surrogate marker for evaluating risk of cardiovascular (CVD) mortality in relation to general obesity, while abdominal obesity indicators have been proposed to be more informative in risk prediction. We aimed to compare obesity predictors including BMI, Waist circumference (WC), waist-hip ratio (WHR), waist-stature ratio (WSR), and A Body Shape Index (ABSI) in relation to mortality from CVD and all causes among four European populations enrolled in the Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe (DECODE) Study.
Materials and methods: Mortality from CVD and all causes in relation to baseline BMI, WC, WHR, WSR, and ABSI were investigated in a prospective cohort of 46651 Europeans aged 24-99. Hazard ratios were estimated by Cox proportional hazard models using age as time-scale.
Results: During a median follow-up of 7.9 years, 3435 participants died, 1409 from CVD. All obesity measures predicted CVD mortality but the prediction was mostly stronger with abdominal obesity indicators than with BMI ($P<0.05$ for all paired homogeneity tests except for ABSI in women), and independent of the BMI levels. The WSR appeared to be the strongest predictor among all the indicators, with a linear relationship with CVD and a quadratic relationship with all-cause mortality in both men and women.
Conclusion: Abdominal obesity indicators especially for WC, WHR and WSR, are stronger predictors for CVD mortality than BMI.
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Are we failing to capture the relationship between body mass index and cardiovascular disease and mortality in subjects with type 2 diabetes?

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Background and aims: There is considerable epidemiological evidence relating increasing body mass index (BMI) to an elevated risk of cardiovascular disease and mortality in subjects with type-2 diabetes mellitus (T2DM). Coronary heart disease (CHD) and mortality risk equations typically incorporate the effects of elevated BMI via the inter-relationship between modifiable cardiovascular risk factors (cholesterol and systolic blood pressure) and BMI; this approach may underestimate risk. Therefore, the objective of this study was to assess if the risk equations used in a well validated diabetes model accurately capture the relationship between BMI and the risk of CHD and total mortality.
Materials and methods: Using published observational data from the Swedish National Diabetes Register we compared the predicted incidence of fatal/nonfatal CHD (fatal/non-fatal myocardial infarction, ischaemic heart disease, unstable angina) and total mortality over a mean follow-up period of 5.6 years using the IMS CORE Diabetes Model (CDM). Hazard ratios (HR) from the model were compared with study HRs stratified by BMI after adjusting for baseline age, sex, duration of diabetes, BMI, smoking, HbA1c, blood pressure, use of antihypertensive and lipid-reducing drugs, and microalbuminuria.
Results: Comparing subjects with BMI<25 kg/m2 (mean age 60.4 years, 53.8% male; 20.7% current smokers; duration of diabetes 9.8 years; HbA1c 7.56%; SBP 141.4mmHg and BMI 23.0 kg/m^2) to those with BMI >30 kg/m2 (mean age 59.7 years, 49.0 % male; 14.7% current smokers; duration of diabetes 7.7 years; HbA1c 7.7.4%; SBP 148.0mmHg and BMI 34.1 kg/m^2) produced study HRs of 1.25 (1.09 - 1.44) and 1.47 (1.16-1.85) for CHD and total mortality respectively. HRs derived from the CDM were 1.062 (1.051-1.072) and 0.966 (0.956-0.977) for CHD and total mortality respectively.
Conclusion: This study demonstrates that despite using a diabetes model that has been shown to have good predictive validity to cardiovascular and mortality events reported across a large number of major T2DM outcomes studies the model significantly underestimates the relationship between increasing BMI and CHD and total mortality risk. There is a need for improved risk equations for use in diabetes models that adequately capture the deleterious effects of increasing body weight, particularly, if the true value of avoiding weight gain or weight loss is to be characterised.

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Trajectory of body mass index before the development of type 2 diabetes in Japanese men: Toranomon hospital health management centre study (TOPICS)

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Background and aims: To date longitudinal data on the trajectory of general adiposity with repeated clinical measurements before the actual onset of type 2 diabetes are sparse, especially in Asian individuals who are not predominantly overweight or obese. Evidence has shown rapidly increasing levels of glycemic concentrations could be observed before the diagnosis of diabetes. However, it remains unknown in which period of the natural history of diabetes an elevated BMI, increasing BMI or stable overweight/obesity could be observed among individuals who eventually develop type 2 diabetes. We aimed to investigate the trajectory of BMI and BMI histories before the development of type 2 diabetes in apparently healthy Japanese men.
Materials and methods: We reviewed data on 2068 non-diabetic Japanese men who underwent annual health examinations. Mean BMI and incident cases of diabetes (diabetes indicated by fasting plasma glucose level ≥7.0 mmol/L, self-reported clinician-diagnosed diabetes, or HbA1c ≥6.5%) were assessed on an annual basis over 8- to 10 years after the baseline examination. BMI trajectories before the development of diabetes were retrospectively evaluated from the baseline examination until the time of diagnosis of diabetes or to the end of the follow-up.
Results: Mean (SD) BMI at the time of diagnosis was 24.4 (3.2) kg/m^2 among diabetic individuals (n=241). An increasingly high BMI was associated with the 8- to 10-y pre-diagnosis period and diabetic individuals experienced a prolonged and stable elevated BMI of ≥24 kg/m^2 (range 24.1-24.4) over the 8 y prior to the diagnosis of diabetes. The mean BMI among the non-diabetic individuals did not exceed 23.2 kg/m^2 throughout the period. When we assessed the maximum BMI and the history of excessive obesity (BMI ≥27.5 kg/m^2), the mean maximum BMI was higher among diabetic individuals than non-diabetic individuals ($p<0.001$). The prevalence of individuals with a history of excessive obesity was also significantly higher among diabetic individuals (16.2% vs. 10.4%; $p=0.007$). Results of our assessment of the duration of overweight/obesity showed that diabetic individuals were more likely to have a history of stable overweight/obesity.
Conclusion: Japanese men who eventually developed diabetes during the 10-year observation period were not characterized as obese but had stable high-normal BMIs during the 8 y prior to onset of diabetes. Previous evidence indicated that values for glycemic markers rapidly increased before the development of diabetes; however, the present study showed a slight gain in BMI in the earlier stage of the natural history of diabetes followed by a prolonged period of overweight. Our findings would contribute to efforts to prevent the further deterioration of the glycemic state through a better understanding of the trajectory of general adiposity before the onset of diabetes.
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Thinness in childhood, weight gain over adolescence and risk of type 2 diabetes: a generational perspective

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Background and aims: Several growth trajectories in childhood have been associated with the risk of type 2 diabetes (T2D). We evaluated the link between adulthood T2D and the corpulence history between 8 and 18 years in a gender and generational perspective.
Materials and methods: Among the national sample of adult respondents to the ObEpi 2009 French survey, we included the subjects with available self-reported silhouettes at 8 and 18 years, diabetic status and maximum BMI in adulthood. We analyzed the relationship between T2D and silhouettes (thinnest: A to largest: G, regrouped in 4 main categories), as well as growth trajectories by logistic regression. We tested interactions with current age in three strata: 40-59 years, 60-79 years and 80 years or older.
Results: The inclusion criteria selected 23763 subjects (50.1±17.3 years, 52.8% women, 5.6% T2D). At age 8, leaner silhouettes (AB and C) were positively associated to T2D before and after adjustment on maximal adult BMI: adjusted ORs 1.54 (95%CI 1.30-1.82) and 1.35 (1.14-1.60) versus middle silhouette (D). The largest silhouettes at 18 years were significantly associated with T2D but the adjustment for maximum BMI reversed the association. The positive association T2D-large corpulence at 18 years persisted after adjustment only in the younger generation of men (40 to 59 years): adjusted OR 1.70 (1.04-2.79). For a similar maximum BMI in adulthood, subjects with the leanest silhouettes at 18 years had a higher risk of diabetes than the reference (D): OR 1.29 (1.09-1.52). In adjusted models, subjects of a D figure at age 8 had significantly protective ORs if growing thinner in adolescence, especially women: OR 0.54 (0.36-0.82), but change for a larger silhouette between 8 and 18 years increased the risk, especially in men: OR 1.51 (1.01-2.24).
Conclusion: Our results confirm, in a French context and for all age strata, previous data that childhood leanness is a risk factor for diabetes. They also show the detrimental effect of adolescent weight gain, independently of the obesity level reached in adulthood.
Supported by: The ObEpi surveys were funded by Institut Roche, France.

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Adiposity and all-cause mortality in individuals with type 1 diabetes

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Background and aims: The relationship of BMI with mortality in type 1 diabetes (T1D) has been suggested to start to resemble that of the general population, although with an exacerbated risk for mortality among the underweight. However, BMI may not capture the whole picture. We thus examined the relationship of mortality with different measures of adiposity: WHR, which specifically measures body fat distribution unrelated to BMI, Body Adiposity Index (BAI), which estimates the percent adiposity directly, as well as BMI in a large cohort of patients with T1D
Materials and methods: 4,426 patients with T1D (2322 M/2104 F, age 38.8±12.4 years, BMI 25.1±3.6 kg/m2) were followed until death (n=596 deaths) or for a mean of 11.2±3.4 years. HRs were calculated using Cox regression analysis for predefined WHO obesity categories (BMI) and for quartiles (BMI, BAI and WHR). Quartiles were created separately for men and women and thereafter combined for analysis. All analyses were adjusted for age, sex and duration of diabetes. Analyses were further stratified according to renal status into patients with and without end-stage renal disease (ESRD).
Results: Prevalence of overweight and obesity was 37.1% and 9.0%, respectively. The HRs for mortality are shown in Table. Underweight increased the risk for mortality even after stratification for ESRD (HR= 2.27 [1.21-4.25] with ESRD and HR=2.84 [1.26-6.43] without ESRD). Obesity was associated with mortality, without reaching statistical significance when all patients were included. However, obesity was a significant predictor of mortality among patients without ESRD (1.55 [1.15-2.09]). All quartiles of WHR increased the risk of mortality gradually with increasing quartile, even after stratification among patients without ESRD, but not in patients with ESRD. Quartiles of BAI did not predict mortality in this cohort.
Conclusion: The risk of mortality increased in parallel with higher quartile of WHR while there was a reversed J-shaped relationship between BMI and mortality, where the highest mortality risk was observed among underweight patients. BAI was not related to increased risk of mortality.

Risk of mortality in adiposity class (WHO) or quartile.

BMI WHO class	Underweight <18.5	Normal weight 18.5-24.9	Overweight 25.0-29.9	Obesity >30
N patients (%)	51 (1.2)	2329 (52.7)	1639 (37.1)	397 (9.0)
HR (95%CI)	4.04 (2.47-6.60)	1 (ref. category)	0.80 (0.67-0.95)	1.19 (0.91-1.54)
BMI quartiles	≤22.57	22.58-24.68	24.69-26.97	>26.98
N patients	1108	1108	1098	1102
HR (95%CI)	1.80 (1.43-2.27)	1 (ref. category)	0.87 (0.67-1.12)	1.28 (1.01-1.61)
WHR quartiles	≤0.87M ≤0.77F	0.88-0.91M 0.78-0.81F	0.91-0.96M 0.82-0.86F	>0.96M >0.87F
N patients	1169	1055	1076	977
HR (95%CI)	1 (ref. category)	1.43 (1.06-1.92)	1.91 (1.44-2.52)	2.86 (2.20-3.73)
BAI quartiles	≤21.67M ≤25.89F	21.68-23.76M 25.90-28.77F	23.77-26.02M 27.78-31.93F	>26.02M >31.93 F
N patients	1073	1073	1066	1064
HR (95%CI)	1.44 (0.96-1.60)	1 (ref. category)	1.02 (0.80-1.30)	1.15 (0.91-1.45)

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The development of age-related time-to-event epidemiological models: the importance of age of type 2 diabetes diagnosis by gender and BMI

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Background and aims: We have previously demonstrated the improved predictive ability of using age-related residual time-to-event modelling in the evaluation of disease risk, such as cancer-related outcomes. As an introduction to modelling body mass index (BMI) and cancer-related mortality in patients with type 2 diabetes, we explored the hypothesis that men develop type 2 diabetes at an earlier age compared with women across the range of body mass index (BMI) and waist circumference (WC) values.
Materials and methods: Data were analysed on 248,917 persons from the Salford Integrated Records healthcare electronic system, a stable predominantly Caucasian population, North West of England (1995 to 2010). Type 2 diabetes was defined based on age criteria (> 35 years), conservative text terms, and medication usage (n=14172). Data were available on BMI, WC, smoking and HbA1c (any measures recorded as a percentage using the DCCT method were converted into nmol/mol to conform to the IFCC standard) in 86%, 21%, 85%, and 63%, respectively. We modelled relationships between anthropometric measures and age, by gender, using linear regression.
Results: The mean ages at diagnosis per BMI category are shown in the Table - these decreased in men and women with increasing BMI category; mean ages were younger in men compared with women per BMI category. In regression models for age versus BMI sex is significant (in men compared to women the beta coefficient is -1.71 (-1.93- -1.49). In models separated by gender: the beta coefficient was -0.11 (95% CIs: -0.10 to - 0.12, p<0.001) in men; and -0.15 (-0.14 to -0.17, p<0.001) in women. The influence of smoking status on the model was significant for men and women (p<0.05). The mean ages at diagnosis per WC tertile are also shown in the Table - again, these decreased in men and women with increasing WC tertiles; mean ages were younger in men compared with women per WC tertile (significant difference between men and women p<0.001). For age versus WC, the beta coefficient was -0.16 (-0.09 to -0.23), p<0.001) in men; and significantly greater for women; -0.36 (-0.28 to -0.44, p< 0.001). There were no material effects on these relationships with the addition of HbA1c to the models.
Conclusion: A limitation of this study is high proportion of missing data for some of the variables examined. Nonetheless, with a population-based setting, the findings uphold the hypothesis that men develop type 2 diabetes at an earlier age compared with women across the range of body mass index (BMI) and waist circumference (WC) values. The baseline characterisations will inform more advanced modelling of BMI trajectory with cancer-related

outcomes in patients with type 2 diabetes, which in turn, will better inform clinical and health policy.

Mean age at diagnosis by BMI and waist circumfernce in Men and Women							
BMI (kg/m2) category	< 25	25-29.9	30-34.9	35-39.9	40-44.9	45-49.9	>= 50
MEN N	833	2367	1908	809	300	93	42
Mean age at diagnosis (SD)	58.1 (14.7)	58.8 (12.3)	56.0 (11.3)	53.6 (11.6)	50.4 (10.7)	48.5 (8.4)	49.3 (11.2)
WOMEN N	738	1417	1331	798	393	157	70
Mean age at diagnosis (SD)	64.1 (15.2)	62.3 (13.0)	59.4 (12.9)	57.0 (12.2)	54.1 (12.4)	52.1 (11.8)	51.9 (12.0)
Waist circumference (cm)	Tertiles	< 101.5	101.5-113.4	>= 113.5			
MEN N		573	587	541			
Mean age at diagnosis (SD)		55.7(12.5)	56.5 (10.9)	53.3 (10.4)			
Waist circumference (cm)	Tertiles	< 97	97 to 110.9	>= 111			
WOMEN N		401	436	363			
Mean age at diagnosis (SD)		60.8 (12.4)	56.6 (11.1)	53.4 (10.2)			

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PS 019 Risk markers for type 2 diabetes

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Age at menarche and type 2 diabetes risk: the EPIC-InterAct study

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Background and aims: Earlier age at menarche, a marker of pubertal timing in girls, is associated with higher risk of later type 2 diabetes. We aimed to confirm this association in a multi-centre European study and to examine whether it is explained by adiposity.
Materials and methods: The prospective EPIC-InterAct case-cohort study consists of 12403 incident type 2 diabetes cases and a stratified sub-cohort of 16154 individuals from 26 research centres across eight European countries. We tested the association between age at menarche and incident type 2 diabetes using Prentice-weighted Cox regression in 15168 women (including 5995 diabetes cases). Models were adjusted in a sequential manner for potential confounding and mediating factors, including adult body mass index (BMI).
Results: Mean menarcheal age ranged from 12.6 to 13.6 years across InterAct countries. Each year later of menarche was associated with 0.32 kg/m2 lower adult BMI. Women in the earliest menarche quintile (8-11 years, n=2418) had 70% higher incidence of type 2 diabetes compared with those in the middle quintile (13 years, n=3634), adjusting for age at recruitment, research centre and a range of lifestyle and reproductive factors (Hazard Ratio=1.70; 95% CI 1.49-1.94, P<0.001). Adjustment for BMI partially attenuated this association (HR=1.42; 95% CI 1.18-1.71, P<0.001). Later menarche beyond the median age was not protective against type 2 diabetes.
Conclusion: Women with history of early menarche have higher risk of type 2 diabetes in adulthood. Less than half of this association appears to be mediated by higher adult BMI, suggesting that early pubertal development may also directly increase type 2 diabetes risk.
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Type 2 diabetes and cardiovascular risks are present in adolescent girls with polycystic ovaries morphology

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Background and aims: The association between obesity, insulin sensitivity and metabolic risks is already evident in adolescence. Insulin has a central role in the regulation of pubertal development; furthermore insulin resistance and corresponding hyperinsulinemia are important factors reflecting early signs of PCOS. Menstrual disorders in adolescence are a marker of the development of PCOS in adulthood. Therefore identifying the subset of girls with polycystic ovary morphology may provide data regarding the mechanisms for the development of PCOS and also could explore the role of insulin resistance as an early factor for diabetes and cardiovascular diseases in later life.
Materials and methods: Consecutive 91 girls with menstrual irregularity and polycystic ovary morphology on ovarian USG (age 11-18 years) were divided into 2 groups: obese (OBS, n=38) and with normal BMI (NOR, n=53). In all girls we took a medical history, complete physical examination and detailed biochemical and endocrine profile. Then, we measured glycemic and insulinemic response (INS, mUI/ml) to 75g OGTT.
Results: Fasting plasma glucose was normal in all girls, in both study groups. However, 27% of the OBS participants met the criteria for impaired glucose tolerance diagnosis. All tested girls had various degree of hyperandrogenemia. Fasting and post challenge insulinemia, insulin resistance (IR-HOMA) were significantly higher in the obese PCO girls. (TABLE - All results are means ± SEM,*, NOR vs. OBS P<0.01)
Conclusion: Although the metabolic interrelationship between obesity and PCOS development have not yet to be fully understood, the co-occurrence of significant differences in adipokines, namely dramatically low levels of adiponectin and high levels of leptin, and severe peripheral insulin resistance in adolescent girls could have a significant impact on the development of diabetes and emerges as a risk factors for developing cardiovascular complications in later life.

Results in both study groups		
Group	NOR	OBS
BMI	22 ± 0.3	30 ± 0.5*
SBP (mm Hg)	106.5 ± 2.4	126.5 ± 1.5*
IGTolerance (%)	11%	27%*
Fasting INS	13 ± 0.7	22 ± 1.0*
OGTT-120 min INS	88 ± 4	194 ±14*
IR-HOMA	2.9 ± 0.2	4.9 ± 0.3*
Adiponectin (mg/ml)	11.4 ± 1.6	5.8 ± 1.3*
Leptin (ng/ml)	8.5 ± 2.3	24.1 ± 4.2*

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Cluster of insulin resistance and other metabolic risk factors predicts AMI morbidity in a Swedish population

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Backgrounds and aims: Risk factors for acute myocardial infarction (AMI) are known to cluster and insulin resistance is often included in such clusters; e.g. in the metabolic syndrome. The aim of the present study was to sex-specifically explore clusters of acknowledged risk factors for AMI, including metabolic factors such as insulin resistance, and to study whether such clusters are associated with AMI morbidity.
Materials and methods: In 2002-2005, 2816 subjects (30-74 years) were randomly selected from two municipalities in south-western Sweden (participation-rate 76%), and assessed with regard to cardiovascular risk factors. For the present study, 2328 subjects without known diabetes or hypertension were included.
Results: Factor analysis identified three identical factors in men and women. Homeostasis model assessment of insulin resistance (HOMA-ir), WHR, systolic blood pressure, and apolipoprotein B-to-A1 ratio loaded significantly on the principal "metabolic factor", leisure-time physical activity and self-rated health loaded significantly on the "vitality factor", and smoking and alcohol consumption loaded significantly on the "addiction factor". After a mean follow-up of 8 years, there were 29 first events of AMI in men and 7 in women. In a cox proportional hazard regression, adjusted for all factors, age, and educational level, only the metabolic factor was significantly associated with AMI morbidity in men, with a hazard ratio (HR) of 2.4 (95% CI: 1.6-3.5). There was a similar risk increase in women; however, due to the lower power in women the adjusted HR was only borderline-significant (HR: 1.9; CI: 0.96-3.9). When adding men and women together and instead adjusting the analysis also for sex, the HR was 2.3 (1.6-3.2).
Conclusion: Metabolic risk factors commonly known to constitute the metabolic syndrome clustered together in the present study and predicted AMI morbidity. As insulin resistance is believed to be one of the main factors that drive the metabolic syndrome and as it is also one of the main precursors to type 2 diabetes, these findings highlight the need of early preventive actions in insulin resistant men and women to decrease their risk of AMI.
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Predictors of normalisation of prediabetes and of persistence of normal glucose tolerance

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Background and aims: Reversion from prediabetes to normal glucose tolerance (NGT) without specific interventions has been rarely studied. We investigated in a population - based study which factors beyond blood glucose (lifestyle variable, clinical parameters) are associated with normalization of glucose tolerance. In addition, we investigated which factors contribute to long-term persistence of NGT.
Materials and methods: Population-based screening for glucose metabolism impairments (GMI) among 2508 adults in Moscow County was conducted in 2006. 661 subjects participated in the 3 - year follow - up, among which 110 had prediabetes and 464 had NGT at baseline. Subjects who were prediabetic at baseline and normoglycaemic at follow up were only classified as "return to NGT". GMI at baseline and at follow -up was diagnosed using standard OGTT according to WHO 1996/2006 criteria. OR and regression coefficient (B) was calculated using logistic regression analysis.
Results: 29 of 110 (26%) subjects who had been prediabetic at baseline returned to NGT after 3 year. In a logistic regression model with age, sex, BMI, change of BMI, glucose values, hypertension, diabetes family history and smoking as independent variables, age(p=0.002), fasting plasma glucose (FPG) (p=0.044), hypertension (p=0.005) and smoking (p=0.005) were significant predictors of normalization of prediabetes. After adjustment for all possible confounders, higher levels of FPG (B= - 1.608, p=0.011) and 2h glucose (B= - 0.340, p=0.023), higher levels of systolic hypertension (B= - 0.031, p=0.032) and less smoking (B= 0.706, p=0.006) significantly decreased the chance of returning to NGT. Among 464 subjects with NGT (baseline) 336 (72%) remained normoglycaemic in the 3 - year follow - up. In adjusted logistic regression model with age, sex, BMI, change of BMI, glucose values, hypertension, diabetes family history and smoking as independent variables, higher levels of FPG (B= - 1.041, p<0.001) and 2h glucose (B= - 0.214, p=0.011), higher BMI at baseline (B= - 0.069, p=0.001) decreased the likelihood of NGT persistence. For BMI change, decrease in BMI improves the likelihood of NGT persistence (OR, 95%CI: 2.8, 1.4 - 5.7 for BMI change <= - 1kg/m², compared to BMI change > 1kg/m²).
Conclusion: Reversion to NGT without specific interventions is not a rare event in prediabetic subjects. Systolic hypertension and smoking influence on normalization of prediabetes in 3 years. BMI and change of BMI are both related to persistence of NGT.

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Comparison between indexes of insulin sensitivity for risk prediction of cardiovascular diseases or development of type 2 diabetes

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Background and aims: The predictive effect of various insulin sensitivity indexes for risk of cardiovascular diseases (CVD) or type 2 diabetes (T2DM) is still unclear. We assessed two recent indexes, estimated at oral glucose tolerance test (OGTT), ISI-Ceder and ISI-Matsuda, with ISI-HOMA estimated using fasting glucose and insulin, and with M/I at the euglycemic insulin clamp as reference, in a large sample of subjects performing these tests.
Materials and methods: 71-years-old male participants (n=1049) from the Swedish ULSAM study, mean follow-up 9 years. All subjects performed the euglycemic insulin clamp with estimation of M/I [glucose disposal/mean plasma insulin], and a 75-g OGTT including insulin determinations with estimation of ISI-Ceder: metabolic clearance rate/mean plasma glucose/ log mean plasma insulin; ISI-Matsuda: 10,000/square root of [glucose0 (min) x insulin0 x glucose 120 x insulin120] and ISI-HOMA: 22.5 x 18 / [glucose0 x insulin0], respectively.
Results: Bland-Altman plots showed best agreement between M/I and ISI-Ceder with mean difference near zero, -0.07 (95% confidence intervalls: -1.52-1.66), while deviating from zero for M/I versus ISI-Matsuda-, -0.73 (-2.38-0.91) and M/I versus ISI-HOMA -0.85 (-2.89-1.19). Further, ISI-Ceder was a stronger predictor with best model fit at Cox regression for risk of non-fatal/fatal ischemic heart disease, CVD, and total mortality, when adjusted for BMI, systolic BP, smoking status, blood lipids, cystatin-C, microalbuminuria, Charlson-index and also the metabolic syndrome. Results for nonfatal or fatal CVDs combined are presented in table 1. Comparing highest versus lowest quartiles of each index, hazard ratios for these outcomes were strongly significant only using ISI-Ceder. Furthermore, ISI-Ceder was a stronger predictor for risk of development of T2DM in 1024 subjects free from T2DM at baseline, with best model fit at logistic regression, and also highest c-statistic, 0.84, when adjusting for BMI, systolic BP, HDL cholesterol, smoking, Charlson index as presented in table 1.
Conclusion: ISI-Ceder performed better as a predictor of risk for CVD/mortality and T2DM than ISI-Matsuda, ISI-HOMA and M/I, respectively.

Table 1. Type 2 diabetes and CVD prediction by indexes

Outcome		All patients						Q4 versus Q1	
T2DM (n=56)		OR [a]	Wald				C	OR [b]	P
CVDs (n=181)		(95% CI)	X^2	P	LR	AIC	stat	(95% CI)	value
T2DM	ISI-Ceder	0.12 (0.06-0.22)	43	<0.001	93	355	0.84	0.08 (0.02-0.26)	<0.001
	ISI-Matsuda	0.17 (0.08-0.38)	19	<0.001	57	391	0.78	0.11 (0.03-0.33)	<0.001
	M/I	0.59 (0.39-0.89)	6.3	0.01	34	414	0.71	0.48 (0.18-1.26)	0.1
	ISI-HOMA	0.35 (0.17-0.72)	8.1	0.004	38	410	0.72	0.37 (0.16-0.84)	0.02
CVDs	ISI-Ceder	0.76 (0.63-0.91)	9.0	0.003	51	2401	0.65	0.42 (0.24-0.71)	0.001
	ISI-Matsuda	0.73 (0.59-0.92)	7.4	0.006	51	2402	0.65	0.53 (0.31-0.93)	0.03
	M/I	0.80 (0.65-0.97)	5.2	0.02	47	2405	0.65	0.62 (0.34-1.13)	0.1
	ISI-HOMA	0.81 (0.65-1.02)	3.2	0.07	46	2409	0.65	0.86 (0.50-1.48)	0.6

Table 1. The ability of various indexes of insulin sensitivity to predict T2DM and CVDs during a follow-up for a mean of 9 years.

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Results from logistic regression in 1024 male participants free from T2DM at baseline and from cox regression in the total sample of 1049 male participants.

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T2DM - Adjustments for BMI, systolic BP, HDL-chdesterol, smoking and Charlson index.

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CVDs - Adjustments for BMI, systolic BP, smoker, LDL and HDL cholesterol, triglycerides, cystatin C, microalbuminuria, Charlson index, a history of CVD and of the metabolic syndrome.

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[a] Indexes with increase per 1 SD to allow direct comparison between odds ratios (OR) and between Hazard Ratios (HR), respectively.

[b] ORs and HRs, respectively, with patients within the highest quartile (Q4) compared to lowest (Q1) as reference.

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Wald X^2 statistic: a higher value indicates stronger association between a predictor and the outcome. Likelihood ratio (LR) X^2 statistics: a higher value indicates a better global model fit. AIC (Akaike Information Criteria): a lower value indicates a better model fit. C statistic: a higher value indicates a better discrimination. Q denotes quartile.

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Plasma fibrinogen level as a predictor of incident metabolic syndrome in a community-based prospective study in Hong Kong Chinese

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Background and aims: Metabolic syndrome (MS) comprises a constellation of metabolic abnormalities associated with a high risk of developing diabetes and cardiovascular diseases. Central obesity, with related insulin resistance and inflammation are considered the core of the underlying pathogenesis of MS. Fibrinogen, an acute-phase reactant in the coagulation cascade, has been shown to play pivotal role in determining the extent of local or systemic inflammation. In this study, we examined whether plasma fibrinogen was predictive of incident MS in a community-based Chinese cohort.
Materials and methods: Subjects were recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS) cohort. 2780 subjects were recruited in 1995-6 with baseline assessment performed. 1416 subjects without MS at baseline returned in 2005-8 for reassessment. MS was defined by ATP III criteria with waist circumference modified according to Asian cut-offs.
Results: Of the 2780 subjects at baseline, 560(20.1%) had MS. Fibrinogen level correlated positively with age, fasting glucose, waist circumference, systolic and diastolic BP, and triglycerides and inversely with HDL (all p<0.001). Fibrinogen level increased with the number of components (0 to 5) of MS (p<0.01) and was significantly higher in subjects with MS (p<0.001). Of the 1416 patients without MS at baseline, 286(20.2%) developed MS in 2005-8 reassessment. Subjects who had incident MS, when compared with those without, were older (45.7±11.2 vs 42.1±11.8, p<0.01) and had higher fibrinogen level (2.53[2.20-2.93] vs 2.40[2.10-2.79], p=0.001) at baseline. There was no difference in gender or smoking status. On multivariate analysis, baseline fibrinogen remained to be an independent predictor for incident MS with age-adjusted HR 1.92 (1.03-3.55, p=0.039) per log unit increase in fibrinogen.
Conclusion: Our findings confirmed plasma fibrinogen level, an indicator of inflammation, is an independent predictor of metabolic syndrome in this community-based Chinese cohort.
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Polymorphisms of the AGER gene are related to serum levels of soluble receptor for advanced glycation end products and autoantibody frequencies in children with type 1 diabetes

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Background and aims: The receptor for advanced glycation end products (RAGE) is a multiligand receptor involved in inflammatory and immune responses. RAGE and the encoding gene AGER are associated with the pathogenesis of type 1 diabetes (T1D) and the development of its complications. Strong linkage disequilibrium has been described earlier between AGER polymorphisms and HLA class II genes.
Materials and methods: The subjects (n=1444) were derived from the Finnish Pediatric Diabetes Register and Biobank. We analyzed three single nucleotide polymorphisms (SNPs) of the AGER gene associated with HLA-DR/DQ independent risk for T1D (rs2070600, rs9469089 and rs17493811) for possible associations with serum levels of soluble RAGE (sRAGE).
Results: T1D risk associated variants of two studied SNPs were found to correlate with lower levels of soluble RAGE. The mean level in subjects with the TT genotype of the rs2070600 (n=14) was 475 pg/ml compared to 932 pg/ml in CT (n= 309) and 1241 pg/ml in CC (N=1121) carriers [p<0.001 (ANOVA)], and the mean levels among subjects with various rs9469089 genotypes were 1140 pg/ml in GG homozygotes (N=980), 1214 pg/ml in CG heterozygotes (N=436) and 1401 pg/ml in CC homozygotes (N=28) [p<0.001 (ANOVA)]. Carrying the minor allele of the rs2070600 correlates with the frequency of IAA [54% vs. 43%, p<0.001(χ2)], whereas the protective minor allele of the rs9469089 is positively related to ZnT8A frequency [68% vs. 59%, p=0.009(χ2)] and inversely with IAA frequency [39% vs. 48%, p=0.001(χ2)]. Correlations between SNPs and autoantibody frequencies remained significant after controlling for the effect of age, sex and HLA genotype with logistic regression.
Conclusion: The association of two T1D risk affecting AGER gene SNPs with lower levels of soluble RAGE in children with newly diagnosed disease implies that polymorphisms of the gene influence the serum sRAGE levels. Studies in prediabetic children will be needed to assess the role of sRAGE levels in the development of T1D. The observed association between AGER gene polymorphisms and humoral autoimmunity suggests that the RAGE genotype might influence the autoimmune process leading to T1D.
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The serum activity of p53 in people with and without type 2 diabetes mellitus and its relation to the diabetes duration

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Background and aims: Growing evidence indicates that ageing is associated with cellular senescence that is manifested by irreversible cell growth arrest. Previously, cellular senescence was thought to be a results of shortening of the telomeres with each cell division. However, recently it has become clear that cellular senescence can be also induced by various stresses that damage DNA independently of cell replication. This complex process is controlled by tumor suppressor proteins, including p53 and pRb. T2DM patients reveal increased level of oxidative stress induced DNA damage. Since, the activity of p53 is associated with oxidative stress that accompanies T2DM we performed the analysis of serum activity of p53 in relation to the T2DM duration.

Materials and methods: The study population consisted of 241 unrelated Caucasians, recruited between 2008 and 2012, matched for sex and age enrolled into two groups: 165 T2DM patients and 76 control subjects with normal glucose homeostasis. All patients were not supplemented by any antioxidants. The study was approved by the Bioethical Committee of the Medical University of Lodz and met the tenets of the Declaration of Helsinki. Written consent was obtained from each patient. Assessment of anthropometric and biochemical parameters was performed. Plasma glucose, HbA1c and lipids were assayed by routine automated laboratory methods. The serum activity of p53 [U/mL] was determined in duplicate with competitive ELISA kits. All data were expressed as mean ± SD. Differences between mean values were tested using One-Way Anova test. P-value less than 0.05 were considered statistically significant.
Results: We noted that the serum activity of p53 calculated for the entire group of people with T2DM was significantly higher than in subjects without T2DM (2.49 ± 0.14 v 1.96 ± 0.13; p=0.019). However, the highest activity of p53 was found in patients with T2DM duration >10 years (3.32 ±2,54 vs 1.96±1.17; p≤0.001). Results and clinical characteristic of T2DM patients and control subjects are presented in Table 1.
Conclusion: In this study we show that the serum activity of p53 is significantly higher in people with than without T2DM. In addition, we found that the level of p53 activity appears to be associated with T2DM duration. Both of these findings may at least partially support hypothesis that p53 plays a role in cellular aging associated with diabetes and its duration.

Table 1. Anthropometric, biochemical and p53 results for study population.

	Control subjects n=76	T2DM duration 1 -10 years n=110	T2DM duration > 10 years n=55
Age (years)	67.75 ± 11.88	63.03 ± 13.47*P=0.015	67.82 ± 9.48†p=0.019
FPG (mg/dL)	92.04 ± 13.14	178.74 ± 75.82***	187.22 ± 92.98***
HBA1c (%)	6.01 ± 0.60	9.21 ± 2.54***	8.95 ± 1.65***
BMI (kg/m2)	28.57 ± 4.49	30.76 ± 6.05 *P=0.008	31.02 ± 5.05*p=0.004
WHR	0.95 ± 0.09	0.96 ± 0.11	0.98 ± 0.10
TC (mmol/L)	4.60 ± 1.51	4.77 ± 1.32	4.31 ± 0.97†p=0.023
LDL (mmol/L)	2.80 ± 1.38	2.89 ± 1.15	2.54 ± 0.84†p=0.047
HDL (mmol/L)	1.34 ± 0.49	1.12 ± 0.59	1.03 ± 0.35***
TG (mmol/L)	1.35 ± 0.72	2.09 ± 1.88***	2.05 ± 1.22***
p53 activity (U/mL)	1.96 ± 1.17	2.00 ± 1.21	3.32 ± 2.54***†††

****p≤0.001 vs control subjects*
††† p≤0.001 vs T2DM duration 1 -10 years

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Retrospective analysis of the Japanese diabetes risk score for incident diabetes and urinary albumin excretion in 2084 subjects and comparison with fatty liver markers

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Background and aims: The Finnish Diabetes Risk Score is a questionnaire used for detecting Europeans at high risk of developing type 2 diabetes. We modified it to create the Japanese Diabetes Risk Score (JPDRISC). Fatty liver and urinary albumin excretion (UAE) are risk factors for incident diabetes and cardiovascular disease, respectively. Variables of fatty liver are not included in the above scores. Moreover, the association between the above risk scores and UAE has yet to be sufficiently investigated. We retrospectively examined the odds ratio of JPDRISC for incident diabetes and compared it with fatty liver markers including serum cholinesterase (ChE). We also examined associations among JPDRISC, ChE and UAE.
Materials and methods: Study periods I and II were defined as January 2007 to May 2009 and June 2009 to December 2011, respectively. A total of 2084 subjects (1389 men, 695 women; mean age: 46 years) who underwent an annual medical check-up including blood sampling after an overnight fast in Periods I and II were assigned to JPDRISC analysis. They were accumulated as representatives of the regional population. In this study, diabetes was diagnosed by prescription of antidiabetic agents, fasting plasma glucose (FPG) ≥ 6.8 mmol/l, HbA1c (NGSP) ≥ 6.5% and/or a past history of diabetes in those not yet treated with antidiabetic agents.
Results: A total of 103 subjects were diagnosed with diabetes in Period I and excluded from analysis. The mean observation period was 2.23 years, with 42 subjects with newly developed diabetes from Periods I to II. The remaining 1939 subjects were nondiabetic. In logistic regression analysis, both JPDRISC (p=0.0003) and ChE (p=0.0169) in Period I were significantly associated with incident diabetes. In receiver operating characteristic analysis, the specific cut-off point in JPDRISC for detecting incident diabetes was 5.5 points (AUC 0.76, p<0.001, sensitivity 68%, specificity 0.71). In those with a JPDRISC ≥ 5.5 points (n=497), the odds ratio for incident diabetes was 5.2 (95%CI 2.5-10.1, $\chi 2$ p<0.0001) compared with others (n=1179). In those with a ChE ≥ 369 U/l (n=503), the odds ratio for incident diabetes was 4.1 (95%CI 2.2-7.9, $\chi 2$ p<0.0001) compared with others (n=1006). The UAE in Period II was significantly associated with fasting plasma glucose (FPG) and HbA1c in Period I (p<0.0001, r=0.3, both), but only very weakly with JPDRISC and systolic BP in Period I (p<0.05, r<0.1, both). The UAE in Period II was not significantly associated with ChE in Period I (p=0.2, r<0.1). In linear regression analysis, HbA1c (β=0.17, p<0.0005), systolic BP (β=0.20, p=0.02) and serum creatinine (Cr) (β=0.25, p<0.0001) in Period I were significantly associated with UAE in Period II.
Conclusion: The JPDRISC is more useful than ChE alone. UAE in period II was associated with HbA1c in Period I, but only very weakly with JPDRISC and not associated with ChE in Period I. Both JPDRISC and ChE affect incident diabetes, with elevated HbA1c being associated with increased UAE.
Clinical Trial Registration Number: 20-130 5420
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Fatty liver disease accompanies loss of glycaemic control on the 5 years follow-up of a previously non-diabetic population

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Background and aims: Diabetes and fatty liver disease are rising epidemics. PREVADIAB, the first Portuguese nationwide study on the prevalence of diabetes, showed that 11,7% of the population had diabetes; and nearly 25% presented at least one kind of defect of glycemic profile encompassed by IFG, IGT, or both ("prediabetes"). Five years after the initial study, we aimed to evaluate the evolution of glycemic control and the prevalence and impact of fatty liver on a follow-up study.
Materials and methods: People evaluated on the first PREVADIAB as non-diabetics (either designated "normal" or "prediabetes" based on both fasting glycaemia and 2h post-load challenge) were called for reassessment. Thus, subjects aged 23-83 years, from 33 Health Centers, were recruited 5 years after the original call. An OGTT was performed to evaluate glycemic control, and biochemical parameters were quantified. Fatty liver status, and peripheral and hepatic insulin sensitivity were estimated through surrogate indexes already validated on human studies (FLI, ISI, and HIR).
Results: The present cohort consisted of 519 people, representative of the overall distribution observed on the first study. The prevalence of diabetes, after only 5 years follow-up, was 9,6%. Additionally, IFG was 4,0%, IGT was 13,1%, and IFG+IGT was 3,3%, to a cumulative indication of "prediabetes" of 20,4%. In relation to the deterioration of glycemic control, 60% of individuals initially assessed as IFG+IGT have progressed to diabetes, while it happened in around 20% of IGT and IFG. Of normal individuals only 5% progressed to diabetes (T2D). Mean Fatty Liver Index (FLI) was found to be increasingly higher throughout disglycemic worsening, in comparison with normal glycemic control (FLI: 44.5±1.3 for normal subjects versus 56.4±3.1 for IGT, p<0.01; 62.5±4.5 for IFG, p<0.05; 64.3±5.6 for IFG+IGT, p<0.05; 70.2±3.4 for T2D, p<0.001). In terms of prevalence, fatty liver condition was identified on 66.0% of individuals with diabetes, and in almost half of people with "prediabetes" (47.0% of IFG+IGT, 47.6% of IFG, and 44.1% of IGT). Surprisingly, fatty liver was nonetheless identified on 28.9% of people with normal glycemic control. Hepatic insulin resistance was shown to correlate directly with FLI progression (r=0.631). Also, it showed a correlation with fasting hyperglycaemia (r=0,330), and, even stronger, with 2h post-OGTT hyperglycaemia (r=0.543), p<0.0001 for all. This seems consistent with the decrease in peripheral insulin sensitivity observed in all disglycemic groups in relation to normal individuals (from 271.6±6.1 for normal to 200.4±20.1 for IFG, p<0.05; 143.0±3.0 for IGT, p<0.001; 121.5±7.2 for IFG+IGT, p<0.001; and 122.5±5.3 for T2D, p<0.001).
Conclusion: The liver has a central role in energy homeostasis, and should be expected to influence not only hepatic glucose fluxes but also whole-body

glucose homeostasis. Here, we have shown that fatty liver is a prevalent condition in a previously non-diabetic population, and that it is strongly related to a worsening in glycemic control, both fasting and postprandial. This leads us to believe that one third of the normoglycemic population may be particularly prone to develop disglycaemia due to a present condition of fatty liver.
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PS 020 Circulating biomarkers in prediction of diabetes

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Metabolomic profiling identifies biomarkers associated with dysglycaemia and incident type 2 diabetes in the METSIM study
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Background and aims: Type 2 diabetes can be delayed or prevented with lifestyle or drug interventions in at risk subjects. There remains a need to better identify these at risk patients as they would be expected to benefit the most from early intervention. The aim of this work was to identify candidate biomarkers of dysglycaemia and for the prediction of type 2 diabetes.
Materials and methods: Age-matched case (N=220) and control (N=440) subjects (mean age: 60 years) were taken from the METSIM (Metabolic Syndrome in Men) Study including Finnish men. All subjects in this observational study were non-diabetic at baseline by both OGTT and A1C criteria. Case subjects were diagnosed with type 2 diabetes within the 5 year follow-up period and displayed this glycemic profile at baseline: 45% IFG, 7% IGT, and 38% combined IFG/IGT. Control subjects displayed normal fasting glucose and normal glucose tolerance at both baseline and at the 5 year follow-up. Non-targeted metabolomic profiling was carried out on fasted plasma samples using gas chromatography or ultrahigh performance liquid chromatography coupled with tandem mass spectrometry to identify metabolites whose levels differed in the case and control groups.
Results: A total of 404 named, identified metabolites were measured semi-quantitatively. Two-way ANOVA with contrasts and decision tree analysis was used to identify and stratify metabolites on their ability to separate cases from controls. All metabolites listed below have ANOVA p values $<5x10^{-6}$ (cases vs controls at baseline). Not surprisingly, given the high rate of dysglycaemia in the case group, glucose was the most significant metabolite found. The results replicated reports for several metabolites previously linked to incident type 2 diabetes: branched-chain amino acids (leucine, isoleucine, and valine), tyrosine, glycine, 2-hydroxybutyrate, and 1-linoleoylglycerophosphocholine (LGPC). A number of other metabolites associated with dysglycaemia and incident T2D were also identified. These include several compounds linked to branched-chain amino acid catabolism (3-methyl-2-oxovalerate, 4-methyl-2-oxopentanoate, 3-hydroxyisobutyrate, isovalerycarnitine, 2-methylbutyrylcarnitine). A number of fatty acids were also found: arachidonate, palmitate, stearidonate, oleate. Other compounds of interest include the Krebs cycle intermediate, alpha-ketoglutarate, the monosaccharide mannose, two compounds involved in energy metabolism: carnitine and creatine, and the purine xanthine. All of these metabolites were elevated in the cases with the exception of glycine and LGPC. Random Forest classification ranked the top 5 metabolites (after glucose) for distinguishing cases versus controls as mannose, alpha-ketoglutarate, 3-methyl-2-oxovalerate, 2-hydroxybutyrate, and 4-methyl-2-oxopentanoate.
Conclusion: A number of metabolites have been identified that associate with incident type 2 diabetes in Finnish men and which may also serve to distinguish normal glycemic control from IGT or IFG. These metabolites represent a variety of different metabolic pathways which may be perturbed prior to developing overt type 2 diabetes. In particular, dysregulation of branched-chain amino acid metabolism is replicated and highlighted by this work.
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Low serum total osteocalcin levels is associated with high risk of metabolic syndrome in Korean men
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Background and aims: Osteocalcin may play an important role in regulating insulin secretion and sensitivity, and fat metabolism. Evidence on the association of osteocalcin with metabolic syndrome(MS), however, is very limited. Therefore we investigated the relationship of serum osteocalcin levels and MS in Korean men.

Materials and methods: This study was a cross sectional study including 470 Korean adults who visited a health promotion center in a university hospital from January 2010 to May 2011. MS was defined according to the NCEP-ATP lll criteria. MS and its individual components were assessed as well as serum osteocalcin levels with multiple logistic regression analysis.
Results: The overall prevalence of the MS in participants of this study was 22.2%. Compared with the highest quantile serum osteocalcin level group (16.84-36.70 ng/dL), the odds ratio for MS in the lowest level group (13.73-16.80 ng/dL) was 4.62 (95% CI=2.24-9.51), in the lower level group (11.54-13.69 ng/dL) was 3.32 (95% CI=1.59-6.91), and in the intermediate level group (4.81-11.53 ng/dL) was 1.95 (95% CI=0.90-4.21). Among the components of metabolic syndrome, the odds ratios for abdominal obesity, impaired fasting glucose, elevated blood pressure, low HDL cholesterol level, and high triglyceride level in the lowest serum osteocalcin level group were 4.68 (1.82-12.03), 3.22 (1.74-5.95), 1.88 (1.11-3.20), 3.82 (1.83-7.96), and 1.46 (0.84-2.54), respectively.
Conclusion: We found that a low serum osteocalcin level is significantly associated with a high risk of MS and negative effect for some metabolic components, especially the abdominal obesity, impaired fasting glucose, elevated blood pressure, and low HDL cholesterol level in Korean male.

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Advanced glycation end products (AGEs) increases renal lipid accumulation: a pathogenic factor of diabetic nephropathy (DN)
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Background and aims: Advanced glycation end products (AGEs) is one of the pathogenic factors of diabetic nephropathy, and our previous work has suggested that AGEs may play a central role in the pathogenesis of glomerulosclerosis. The aim of this study is to further investigate the molecular mechanisms of glomerulosclerosis induced by AGEs.
Materials and methods: For the *in vitro* study, human renal mesangial cells (HMCs) were incubated with Nε-(carboxymethyl) lysine (CML; a member of the AGEs family) and low density lipoprotein (LDL) for 24h. On *in vivo* level, animal model with diabetic nephropathy was developed by high fat/sucrose diet feeding plus streptozotocin injection. Two weeks after STZ injection, rats were divided into three groups, namely, control, diabetic and diabetic treated with AminoguanidineHydrochloride (AG; an inhibitor of AGEs; 100 mg/kg/day) by gavage for 8 weeks. The effects of cholesterol accumulation were examined by Oil Red O staining and a quantitative intracellular cholesterol assay. Serum AGEs was determined by High performance liquid chromatography analyzer. 24-hour urine protein was measured by Coomassie brilliant blue protein assay. The gene and protein expression were analyzed by quantitative RT-PCR, western blot and immunohistochemistry .
Results: 1. CML increased Oil Red O staining in HMCs. 2. Intracellular cholesterol ester was increased in CML-treated cells. 3. CML upregulated both mRNA and protein expression of HMG-CoAR, LDLr, SREBP-2 and SCAP. 4. The level of serum AGEs was higher in diabetic rats than that in control rats, and AG treatment reduced serum AGEs. 5. 24-hour urine protein of the diabetic group was significantly increased compared with control group, and AG treatment improved this alteration. 6. There was very strong staining of Oil Red O in the glomerular of the diabetic group, less staining in the AG treated group, and almost no staining in the control group. 7. Immunohistochemistry showed the overexpression of LDLr, SREBP-2, and SCAP in the glomerular of the diabetic rats, which was suppressed by AG treatment. 8. RT-PCR and western blot showed the overexpression of HMG-CoAR, LDLr, SREBP-2, and SCAP in the renal of the diabetic rats was ameliorated after the AG treatment.
Conclusion: For the *in vitro* study, we demonstrated CML increased lipid accumulation in HMCs by disrupting the SCAP-mediated feedback regulation of HMG-CoAR and LDLr. On *in vivo* level, we also found this SCAP-mediated feedback regulation was disrupted in the renal of type 2 diabetic rats, but inhibiting AGEs producing improved the feedback regulation. These data demonstrated that AGEs (CML) caused glomerulosclerosis via disturbing the intracellular feedback regulation of cholesterol, and inhibiting AGEs-induced lipid accumulation have a potential renoprotective role in the progression of DN.

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Effect of fasting plasma glucose combined advanced glycation endproducts-peptide on screening for diabetes mellitus and pre-diabetes in Chinese high-risk population
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Background and aims: Diabetes mellitus (DM) has now become an epidemic disease worldwide which needs effective screening methods. This study evaluated the utility of combined use of fasting plasma glucose (FPG) and Advanced Glycation Endproducts -peptide (AGEsP) in DM screening among Chinese high-risk population.
Materials and methods: 1020 subjects with high-risk factors of DM were recruited from eight community health service centers for the measurements of FPG, haemoglobin A1c (HbA1c), AGEsP and 2h-plasma glucose (2h-PG) following 75g-oral glucose tolerance test (OGTT). 857 participants finished the screening procedure and completed all data collection. AGEs-specific fluorescence immunoassay (FIA) was used to measure total AGEsP content. Combined use of FPG and AGEsP was compared against either FPG alone, 75 g-OGTT alone or HbA1c for detection of DM. DM was diagnosed according to 2010 Chinese type 2 diabetes prevention guide. The appropriate cut-off points of AGEsP for DM was assessed. Sensitivity, Specificity, area under the curve (AUC) of receiver-operating characteristics (ROC) for DM detection were computed for FPG, 2h-PG, HbA1c and AGEsP.
Results: The incidence of both pre-DM and DM in the study population was 27.1% and 25.1% respectively. AGEsP was positively correlated with FPG, 2h-PG, and HbA1c (r = 0.602, 0.609, 0.751, $P = 0.000$ for each by Spearman Analysis). The AUC of the ROC curve for AGEsP predicting undiagnosed DM was similar to that of FPG, 2h-PG and the cut-off point of AGEsP 10.22 μg/ml was optimal for predicting DM, with a sensitivity of 84.2%, and a specificity of 88.5%. Compared to FPG alone, combined use of FPG and AGEsP had higher sensitivity for detecting DM (FPG ≥ 7.0 mmol/L and/or AGESP ≥10.22 μg/ml vs. FPG ≥ 7.0 mmol/L: 90.7% vs. 59.1%, $P < 0.001$). No significant difference exists between the AUC of AGEsP evaluated against FPG or 2h-PG alone in detecting DM. For pre-DM screening, combined FPG ≥ 6.1 mmol/L and/or AGEsP ≥ 5.18 μg/ml performed better than either FPG alone, OGTT alone or combined measurements of FPG and HbA1c (FPG ≥ 6.1 mmol/L and/or AGEsP ≥ 5.18 μg/ml vs. FPG ≥ 6.1 mmol/L vs. OGTT 2h-PG ≥ 7.8 mmol/L vs. FPG ≥ 6.1 mmol/L and/or HbA1c ≥ 5.9 mmol/L: 90.5% vs. 25% vs. 75% vs. 81.0%, $P < 0.001$).
Conclusion: This study identified that the utinity of AGEsP may be a practical and convenient tool for screening undiagnosed DM and pre-DM. The combined use of FPG ≥ 7.0 mmol/L and/or AGEsP ≥ 10.22 μg/ml, and FPG ≥ 6.1 mmol/L and/or AGEsP ≥ 5.18 μg/ml substantially improves the sensitivity of detecting DM and pre-DM relative to FPG alone, and may represent an efficient early detection of DM and pre-DM. This has implied a new efficient approach for current and future DM screening programmes, especially among high-risk population.
Clinical Trial Registration Number: BK 2010087

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Serum peroxiredoxin 4 as novel biomarker of oxidative stress and the risk of incident type 2 diabetes: the PREVEND study
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Background and aims: Oxidative stress plays a major role in the development of type 2 diabetes. It is unknown whether serum peroxiredoxin 4 (Prx4), as a potent antioxidant biomarker, predicts the risk of incident type2 diabetes (T2D).
Materials and methods: From the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, we included 3909 men and 4063 women (aged 28-75 years). Prx4 was measured by a novel immunoluminometric assay in baseline samples.
Results: Prx4 levels were significantly higher in men than in women (median [IQR], 0.71(0.45-1.16)U/l and 0.42(0.66-1.08)U/l resp.;P<0.001). During me-

dian (IQR) follow-up of 7.7 (7.4-8.0) years, 288 men and 208 women developed T2D. In a model adjusted for age, smoking, BMI and parental diabetes, OR for T2D risk per doubling of Prx4 levels was 1.31 (1.13-1.52) in men and 1.07 (0.90-1.27) in women (P<0.01 for sex-interaction). Further adjustment for glucose, HDL-cholesterol, triglyceride, hs-CRP, procalcitonin and urine albumin excretion did not materially change the association. The addition of Prx4 to the DESIR (data from the Epidemiological Study on the Insulin Resistance Syndrome) clinical model for prediction of diabetes improved discrimination (C-statistic change +0.010; P<0.001) and reclassification (integrated discrimination improvement = 0.004; P< 0.01) in men. We observed no improvement in women.
Conclusion: Elevated Prx4 levels are associated with a higher risk of incident T2D. This association was stronger in men than in women. Prx4 significantly improved prediction for risk of future T2D when added to a clinical model, but only in men. Further studies are warranted to confirm our findings and to investigate the underlying mechanisms for this gender disparity.
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Fasting plasma neurotensin and risk of type 2 diabetes according to dietary fat content
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Background and aims: Proneurotensin is a precursor of the regulatory peptide neurotensin involved in gut motility, gastric acid secretion and appetite regulation. Blood levels are elevated postprandially, and the secretion seems above all to be stimulated by fat intake, but may also depend on type of fat. High fasting plasma proneurotensin concentrations have been associated with development of type 2 diabetes (T2D) in individuals from the Malmö diet and cancer (MDC) cohort. We examined if dietary fat content modifies the association between fasting concentrations of proneurotensin and risk of T2D.
Materials and methods: This study included 3602 men and women, aged 46-68 years, from the population based MDC cohort with baseline examinations between 1991 and 1994. The included participants were without prevalent diabetes, and had measured fasting plasma proneurotensin concentrations and dietary data collected with a modified diet history method (combining a 7-day food record of cooked meals with a 168-item dietary questionnaire covering other meals). Cox proportional hazard models were used to calculate hazard ratios of incident T2D according to tertiles of fasting proneurotensin and energy adjusted intakes of total fat, saturated fat, monounsaturated fat and polyunsaturated fat. The mean time of follow-up was 13 years.
Results: We observed a significant statistical interaction between tertiles of proneurotensin and tertiles of dietary fat energy percent on incident T2D (P for interaction= 0.03) showing a significantly increased incidence of T2D with higher fasting proneurotensin among individuals in the lowest tertile of fat intake (P for trend <0.001), whereas no significant associations were seen among those in the higher tertiles of fat intake. Similar to our observations for total fat, we observed tendencies of stronger risk increasing associations among those with the lower intakes of saturated and monounsaturated fat than among those with higher intakes, but significant interactions were not detected (P=0.06 and P=0.17).
Conclusion: Our results suggest that dietary fat content could modify the association between fasting plasma proneurotensin and risk of T2D. High fasting proneurotensin may in particular be associated with increased risk among individuals with diets low in fat.
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Differential role of chronic inflammation in the development of glucose intolerance: relationship between HbA_{1c} and lower adiposity or insulin secretory function in women
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Background and aims: Local or systemic low-grade inflammation has been reported to increase the risk of the development of type 2 diabetes mellitus (DM). Helicobacter pylori (HP) can infect the mucosal lining of the stomach, induce chronic superficial gastritis, and decrease the serum pepsinogen 1(P1) level (ng/ml) and the ratio of pepsinogen 1 to pepsinogen 2 due to chronic inflammation. Some studies have shown an association between HP infection and DM. Our group conducted a cross-sectional study to clarify how chronic inflammation in gastric mucosa contributes to the development of glucose intolerance.
Materials and methods: The study subjects were 6,126 individuals (40-59 years) assessed for P1 and P2 levels, HbA1c (%, NGSP), IRI, and CRP in general health screenings performed at the Mitsui Memorial Hospital, in 2011. Gastritis was classified into four categories based on the serum level of P1 and the ratio of serum P1 to P2 (P1/2): Severe (P1 < 30 and P1/2 < 2.0), Moderate (P1 < 50 and P1/2 < 3.0), Mild (P1 < 70 and P1/2 < 3.0), and None(other values : P1≧70 orP1/2≧3.0). Gender difference was also examined.
Results: In females, the HbA1c levels were (n=2,640) 5.7±0.31 in the Severe group (n=67), 5.6±0.28 in Moderate (n=126), 5.6±0.33 in Mild (n=86), and 5.6±0.29 in None (n=847). The level in Severe was significantly higher than that in None (p=0.015). No differences in HbA1c were found between other categories in the females or between any categories in the males. Next an examination of the risk factors for diabetes on female revealed that BMI and body fat percentage (BFP; %) were significantly smaller (p<0.001) in Severe than in None: 19.7±2.8 and 22.5 ± 6.2 in Severe vs. 21.2±3.2 and 23.4±5.8 in None, respectively. There was no significant difference in insulin resistance, but the Severe group exhibited a significant impairment in insulin secretory function and a lower HOMA -β (47.3±15.5 compared to 58.4±29.4 in Neutral (p=0.007)). No significant differences were observed in the evaluation of the effects of CRP. In the univariate analysis, BMI was significantly correlated with HbA1c. The multivariate analysis of model 1 (P1/2, BMI, and age) and model 2 (P1/2, BMI, age, and HOMA -β) identified P1/2, BMI, age, and HOMA -β as independent risk factors for increment of HbA1c.In males, there was no significant difference in HbA1c between the Severe (n=87) and None (n=3,087) groups: 5.6±0.37 and 5.6±0.34, respectively. HOMA -β in the Severe group (55.6±33.7) was smaller than that in the N group (62.6±38.2), but the difference fell short of significance due to the large variability.
Conclusion: In the female subjects of this study, chronic inflammation with superficial gastritis represented by lower P1 and P1/2 induced a small but significant elevation in HbA1c without increasing either insulin resistance or adiposity, two common features of glucose intolerance caused by chronic inflammation in visceral adipose tissue. No such effect was observed in the male subjects of this study. Furthermore, subjects with severe gastritis were less adipose and had lower insulin secretory function. This suggests that chronic inflammation contributes differently to the development of DM in different tissues. The mechanism of gender difference should be further elucidated.

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Serum protein concentration predicts incident diabetes: the Insulin Resistance Atherosclerosis study
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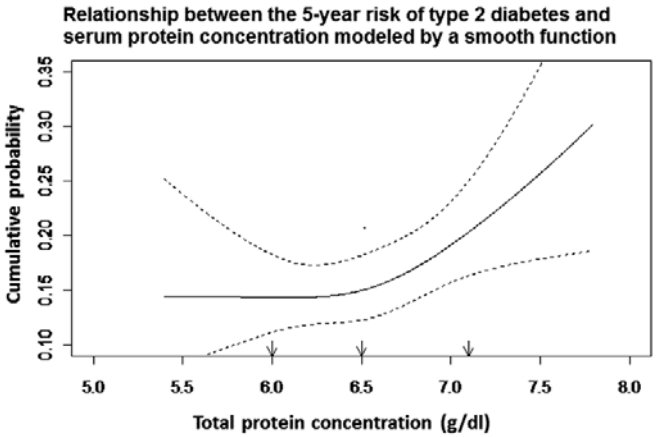
Background and aims: A number of acute phase reactants have been associated with insulin resistance syndrome and have shown to predict type 2 diabetes. Since albumin concentration tends to be stable in the absence of co-morbidities, we hypothesized that total protein concentration (TP) could predict future development of diabetes. Therefore, the aim of this study was to examine the relationship between TP and incident diabetes.

Materials and methods: Population data were obtained from the Insulin Resistance Atherosclerosis Study, which includes data from 864 participants who were non-diabetic at baseline. Using the 2003 American Diabetes Association criteria, incident diabetes was ascertained after a 5-year follow-up period. Insulin sensitivity index (S_I) and acute insulin response (AIR) were directly measured by the frequently sampled intravenous glucose tolerance test. Logistic regression analysis was used to assess the relationship between TP and incident diabetes.
Results: In cross-sectional analysis, TP had a significant relationships with BMI (r = 0.14, p <0.001), S_I (r = - 0.22, p <0.001), AIR (r = 0.17, p <0.001), C-reactive protein (r = 0.14, p <0.001), fibrinogen (r = 0.18, p <0.001), and albumin (r = 0.49, p <0.001). We performed a logistic regression to model incident diabetes with a restricted cubic polynomial spline for TP to estimate the varying effects of TP over its full range (Figure). This relationship was linear (Wald test, p >0.2) and statistically significant (p = 0.033). In contrast, serum albumin concentration modeled also by a smooth function was not associated with incident diabetes (p = 0.766). TP was an independent predictor of diabetes (OR x 1 SD, 1.32 [95% CI: 1.04 - 1.66]) after adjusting for age, sex, ethnicity, clinic, family history of diabetes, BMI, plasma glucose levels, S_I, AIR, C-reactive protein, alcohol intake, and smoking, and albumin excretion.
Conclusion: TP predicts the development of type 2 diabetes independently of multiple risk factors including plasma glucose, insulin sensitivity, and insulin secretion.



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Higher serum bilirubin level as a protective factor for the development of diabetes in healthy Korean men: a 4 year retrospective longitudinal study
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Background and aims: Bilirubin is a natural product of heme catabolism by heme oxygenase, one of key antioxidant enzymes. Recently, it has been recognized as a substance with potent antioxidant and cytoprotective properties. So far, there have been several studies which showed the significant negative relationship between serum bilirubin levels and the risk of metabolic disorders including cardiovascular diseases, type 2 diabetes, metabolic syndrome, arterial hypertension, and obesity. Regarding type 2 diabetes, however, most of the studies have been confined to the cross-sectional nature. In light of these findings, we designed this study to investigate the longitudinal effects of baseline serum bilirubin concentrations on the development of type 2 diabetes during a 4 year follow-up period in middle aged Korean Men.
Materials and methods: This 4 year retrospective longitudinal observational study was conducted at our medical center. The study population consisted of 5,960 men without type 2 diabetes who underwent routine health examination in 2007 (baseline) and 2011 (follow-up). Baseline serum bilirubin concentrations were determined by the vanadate oxidation method.
Results: During a 4 year period, 409 incident cases of diabetes (6.9 %) were identified. Incident type 2 diabetes decreased across the baseline bilirubin quartile categories (*P* for trend <0.001). In multivariate-adjusted model, the odds ratio for the development of type 2 diabetes was significantly lower in highest (i.e., 1.30-2.00 mg/dl) compared with the lowest bilirubin quartile category (i.e., ≤0.90 mg/dl), even after adjustment for confounding variables including homeostasis model assessment of insulin resistance (odds ratio=0.69, 95% confidence interval 0.48-0.99, *P* for trend=0.041).
Conclusion: The results indicate that serum total bilirubin level may provide additional information for predicting future development of type 2 diabetes, especially in subjects without chronic liver diseases.

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Circulating levels of selenoprotein P predict future hyperglycaemia in non-diabetic Japanese people
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Background and aims: We have recently identified selenoprotein P (SeP) as a hepatokine that induces insulin resistance and hyperglycaemia by using animal and cellular experiments. The aim of this study is to examine whether circulating levels of SeP predicts future hyperglycaemia in nondiabetic people.
Materials and methods: We followed 76 nondiabetic Japanese who went to a hospital for a complete physical examination for 4 years (age 52 ± 10; BMI 22.7 ± 3; A1c 5.2 ± 0.3%). Baseline levels of serum SeP were measured by a sol particle homogeneous immunoassay. 75 g oral glucose tolerance tests (OGTT) were performed before and after the follow-up period.
Results: (1) Baseline SeP levels correlated positively with baseline age and plasma glucose levels at 30 min and 60 min, and negatively with baseline insulinogenic index (r = 0.252, p = 0.028; r = 0.241, p = 0.036; r = 0.326, p = 0.004; r = -0.293, p = 0.010, respectively), but not with HOMA-IR. (2) Levels of plasma glucose at 0 min, 60 min, and 120 min significantly increased before and after the follow-up period (from 93 ± 8 to 97 ± 10, p < 0.0001; from 131 ± 41 to 144 ± 45, p = 0.004; from 109 ± 25 to 116 ± 26, p = 0.022, respectively). (3) Baseline SeP levels significantly correlated with plasma glucose at 0, 30, 60, and 120 min measured at the end of the follow-up period (r = 0.303, p = 0.008; r = 0.264, p = 0.021; r = 0.451, p < 0.0001; r = 0.230, p = 0.045, respectively). (4) A multiple regression analysis showed that baseline A1c and SeP predicted the fasting plasma glucose 4 years later independently of the other clinical markers such as age and insulinogenic index (β = 0.375, p = 0.001; β = 0.246, p = 0.024, respectively).
Conclusion: Circulating levels of SeP predicted the future hyperglycaemia independently of the other clinical parameters in nondiabetic healthy subjects. Our observation, combined with emerging functional and genetic evidence, support the concept that hepatokine SeP has a causal role in the development of type 2 diabetes.
Supported by: JST-A-STEP

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CARING: diabetes mellitus and risk of cancer - a systematic review and meta-analysis

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Background and aims: Patients suffering from diabetes mellitus (DM) may experience an increased risk of cancer, but results are conflicting. The aim is to examine the association between DM and cancer by a meta-analysis.
Materials and methods: A systematic review and meta-analysis was performed according to the PRISMA guidelines. The systematic literature search included Pubmed, Embase, Cinahl, Bibliotek.dk, Cochrane library, Web of Science and Svemed+ with the search terms: "Diabetes mellitus", "Neoplasms", "Prospective study" and "Risk of cancer". Odds ratios, relative risks, risk ratios, hazard ratios, prevalence ratios, incidence ratios and standardized incidence ratios were used in the pooled analysis. Statistical analysis was performed using random effects models.
Results: A total of 1,785 records were screened by title and abstract and 253 records were subsequently assessed by full text, of which 195 records fulfilled the criteria for the systematic review. The cancer types studied showed significant heterogeneity across studies by Chi-square test.Compared to non-diabetic controls, DM patients had an increased risk of any cancer (RR=1.14, 95 % CI [1.01-1.28], 26 records). Diabetics seemed especially at risk of liver (RR=2.27, [1.86-2.78], 47 records), pancreas (RR=2.21 [1.86-2.62], 47 records) and endometrial cancer (RR=1.9, [1.67-2.16], 24 records), although an increased risk was also seen for biliary tract, stomach, colon, rectum, kidney, bladder, breast and thyroid gland cancer. In contrast, DM patients appeared to experience a decreased risk for prostate cancer (RR= 0.87 [0.81-0.93], 44 records).
Conclusion: DM patients were at higher risk of cancer than non-diabetics, especially digestive tract cancers and hormone/related cancers (breast, endometrial). However, an apparent protective effect against prostate cancer was present. The observed effects could be due to confounding by factors not adequately adjusted for in the studies like obesity.
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Autonomic neuropathy, sexual dysfunction and urinary incontinence in women with type 1 diabetes: findings from the DCCT/EDIC

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Background and aims: Cardiovascular autonomic neuropathy (CAN) may play an important role in the pathophysiology of male diabetic erectile dysfunction but the impact of CAN on female sexual dysfunction (FSD) and urinary incontinence (UI) in women with T1DM remains unknown. We evaluated the association of cardiovascular autonomic neuropathy (CAN) and FSD and UI in women participating in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications Study (DCCT/EDIC).
Materials and methods: Measures of CAN (R-R response to deep breathing, Valsalva maneuver, change in postural blood pressure), FSD and UI were obtained in 580 DCCT/EDIC female subjects, 16-to-17 years after DCCT closeout. Mean age was 50.6±7.2 years, mean diabetes duration 29.6±5.1 years, and mean DCCT/EDIC HbA1c 8.0±0.9%. CAN was defined as 1) R-R variation<15 or 2) R-R variation between 15-19.9 and a Valsalva ratio≤1.5, or 3) postural drop of 10 mm Hg in diastolic blood pressure. UI was defined by the Sandvik Index categories of dry/mild (0-2) versus moderate/severe (3-12) and FSD was defined by the abbreviated Female Sexual Function Index (FSFI-R) score ≥22.75 defining presence of FSD at EDIC year 17. Multivariable logistic regression estimated the associations between CAN and FSD and UI, after adjusting for DCCT cohort, DCCT/EDIC HbA1c, DCCT/EDIC systolic blood pressure, age, smoking status, and drinking status at EDIC year 17.
Results: At EDIC year 17, FSD and UI were present in 26% and 30% of women, respectively. Subjects with FSD had significantly lower R-R variation (p=0.002) and Valsalva ratio (p=0.003) at EDIC year 16/17. (Table 1) Likewise, those with UI had similar findings. Forty seven percent of participants with FSD and 44% of participants with UI also had confirmed CAN at EDIC year 16/17 (p=0.02 and p=0.20, respectively). Participants with CAN had OR= 1.58, 95%CI (1.07-2.34) greater odds of FSD and 1.28 (0.88-1.85) greater odds of UI on univariate analysis. In adjusted analyses, participants with CAN had a 1.30 (0.83-2.02) greater odds of FSD and 1.02 (0.67-1.54) greater odds of UI. Although the trends remained consistent, they were no longer significant.

Table 1. Neuropathy measures at DCCT closeout and EDIC year 16/17 by FSD or UI status

	No FSD N=408	FSD N=146	p-value	No UI N=399	UI N=172	p-value
Neuropathy Measures¶	Mean±Std or N (%)					
At DCCT Closeout						
R-R Variation	41.50±21.01	40.04±22.63	0.4677	41.30±21.92	40.35±20.06	0.8031
Valsalva Ratio	2.05±0.40	1.98±0.38	0.1564	2.03±0.42	2.03±0.37	0.6931
Abnormal CAN	27 (7)	23 (17)	0.0014	36 (10)	15 (9)	0.8620
At EDIC Year 16/17						
R-R Variation	26.17±17.25	21.92±17.11	0.0021	25.48±17.24	22.58±16.45	0.0542
Valsalva Ratio	1.74±0.35	1.62±0.30	0.0028	1.72±0.34	1.64±0.32	0.0168
Abnormal CAN	138 (36)	65 (47)	0.0223	142 (38)	72 (44)	0.1986

Data are Mean±Std or N (%). P-values based the difference between those with and without FSD or UI using the Wilcoxon rank-sum test for quantitative variables or the Contingency chi-square for qualitative variables. Sample sizes may vary due to missing data.

Conclusion: The association between CAN and LUTS in the DCCT/EDIC cohort indicates that CAN may be a useful surrogate biomarker of more generalized autonomic neuropathy that plays a role in urologic complications in women with long-standing type 1 DM or vice versa. Further studies with longitudinal assessments to elucidate temporal sequence are warranted.
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The risk of acute pancreatitis with GLP-1 receptor agonists and DPP-4 inhibitors: a retrospective observational cohort study

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Background and aims: To evaluate the association between GLP-1 receptor agonists (GLP-1RA), DPP-4 inhibitors (DPP-4I) and acute pancreatitis compared to the association observed between this outcome and the use of other antidiabetic agents (OADA). In contrast to other published observational studies, this study includes both exenatide and liraglutide users and has a longer follow-up time.
Materials and methods: A retrospective cohort study was conducted in the Truven Health Marketscan® Commercial Claims and Encounters Database from 2005 to 2011. The Type 2 diabetes study population was classified as GLP-1RA user, DPP-4I user or OADA user based on the antidiabetic prescription received on the index date. Acute pancreatitis in the study groups was identified in the post-index follow up period by hospitalization claims with a primary discharge diagnosis of ICD-9 code 577.0 (acute pancreatitis). Cox Proportional Hazards model was used to compare the adjusted (refer to Table 1 for a list of adjusters) risk of acute pancreatitis among subjects treated with GLP-1RA and DPP-4I as compared to OADAs (reference group).
Results: We identified 124,925 GLP-1RA users, 208,683 DPP-4I users, and 1,187,502 OADA users 18-64 years old. The majority of subjects in these 3 cohorts were 55- 64 years old. The GLP-1RA cohort had a higher proportion of subjects who were obese (10.8%) compared to the OADA group (7.3%; p<0.001) and a higher proportion of females (57.6%) compared to the OADA

group (47.8%; p<0.001). We identified 5871 cases of acute pancreatitis in over 3 million person-years of follow-up. The unadjusted incidence rate of pancreatitis per 100,000 person-years was 1.92 (95% CI = 1.75 - 2.09) among the GLP-1RA cohort, 1.88 (95% CI = 1.74-2.04) among the DPP-4I cohort and 1.93 (95% CI = 1.88-1.99) among the OADA cohort. The adjusted hazard ratio for acute pancreatitis was 0.95 (95% CI = 0.86-1.04) for GLP-1RA and 0.92 for DPP-4I (95% CI = 0.85-1.00) compared to OADA (Table 1).

Conclusion: In this study neither the unadjusted incidence rates nor the adjusted hazards ratios demonstrated an increased risk of acute pancreatitis among patients prescribed GLP-1RA or DPP-4I compared to other antidiabetic agents. A follow-up analysis will be conducted when additional data from the last quarter of 2011 becomes available.

Table 1: Adjusted* risk of acute pancreatitis

Cohort Group	Adjusted HR (95% CI)	p-value
GLP-1RA (ref=OADA)	0.946 (0.860, 1.04)	0.251
DPP-4I (ref=OADA)	0.921 (0.847, 1.001)	0.0532

** Adjusted for age as of index date, Charlson comorbidity score, neuropathy, retinopathy, nephropathy, hypertension, myocardial infarction, congestive heart failure, ischemic heart disease, cerebrovascular disease, cardiac dysrhythmias, gall bladder disease, chronic liver disease, obesity, alcohol abuse, hyperlipidemia, hypercalcaemia, hyperparathyroidism, hypoglycemia, mental health disease, beta blockers, calcium channel blockers, anti-hypertensives, anti-thrombotics, steroids, diuretics, ACE inhibitors, angiotensin receptor blockers, fibrates, bile acid sequestrants, statins, anti-hyperlipidemics, pancreatitis level 1 drugs, pancreatitis level 2 drugs and history of pancreatic procedures ERCP.*

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Serum levels of pancreatic regenerating protein indicate the development of type 2 diabetes and diabetic chronic complications

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Background and aims: Type 2 diabetes mellitus (T2DM) is one of the most common non-communicable diseases globally, and its complications result in increasing disability, reduced life expectancy and enormous health costs. However, it is frequently not diagnosed until complications occur. Pancreatic regenerating protein (*reg*) is mitogenic to islet β-cells and associated with inflammation, while no data exist regarding the prognostic value of reg among T2DM patients. The aim of this pilot study was to evaluate the quantity of reg in different clinical stages of T2DM and its correlation with diabetic complications.

Materials and methods: We analysed serum reg and its correlation with clinical and biochemical parameters in 1004 subjects with T2DM at different clinical phases. A group of healthy control subjects was also analysed. reg values were measured by a newly developed ELISA.

Results: Reg was correlated with the duration of diabetes (spearman's rank correlation coefficient 0.319 p<0.001). Compared to healthy controls, reg levels were elevated in high-risk patients (18.7[15.0-26.4] vs 16.4[13.9-20.8], p=0.014), and patients with long-term diabetic mellitus (without complications: 26.4[17.4-38.2] vs 16.4[13.9-20.8] p<0.001; with complications: 32.1[22.1-55.5] vs 16.4[13.9-20.8] p<0.001). Interestingly, there is no statistically significant differences among the population of high-risk, IGR and incipient diabetic patients. The area under the curve (AUC) of reg for incidence of diabetes-onset and chronic complications were 0.640 and 0.754, separately. Two reg cut-offs potentially allow to identify individuals with a high risk to develop T2DM and its chronic complications. reg levels above 22 ng/ml in nondiabetes were associated with a high risk to develop T2DM in future, levels above 29 ng/ml among T2DM were the most significant parameter to predict the occurrence of diabetic chronic complications.

Conclusion: Reg might evolve as a promising new marker to predict the occurrence, development of T2DM and diabetic chronic complications.

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Haemorrheological approach for early detection of diabetic angiopathy

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Background and aims: Several key hemorheological parameters, such as erythrocyte deformability, erythrocyte aggregation are altered in patients with diabetes mellitus. Because erythrocytes remain in hyperglycemic environment throughout their life span and thus are subjected to series of compositional changes. These changes of erythrocyte in turn make whole blood more viscous and may play an important role on the pathogenesis of vascular complications of diabetes mellitus. So in this study, we intended to examine erythrocyte deformability and aggregation in patients with type 2 diabetes, and assess the differences of these parameters compared with healthy controls.

Materials and methods: 204 subjects were enrolled and their blood and urine samples were obtained. Also, they had a carotid IMT test and their eye's examination. The erythrocyte deformability was measured in terms of elongation index (EI) with microfluidic ektacytometer, RheoScan, which was very sensitive to detect changes in EI of erythrocytes due to hyperglycemic process. The aggregation index (AI) was measured with RheoScan, too. The hemorheological analyses including EI measurement were completed within 36 hours after blood collection. All subjects were divided by five groups according to their past history and test results as follows: Healthy control (n=32), prediabetes (pre-DM, n=38), diabetes without vascular complications (DM-no Cx, n=83), diabetes with microvascular complications (DM-microCx, n=24) and diabetes with macrovascular complications (DM-macroCx, n=27). The statistical analysis was performed using Kruskal Wallis test and Pearson's correlation coefficients with SPSS, version 20.

Results: A significant reduction of erythrocyte deformability was observed in DM-microCx group and DM-macroCx compared with healthy control (0.329 & 0.323 vs. 0.354 p<0.05). Also, EI was significantly decreased in the group of higher HbA1c level (a1c≥9%) compared with in the group of lower HbA1c level (a1c<7%, 0.322 vs. 0.337, p<0.05). The factors that had negative correlation with EI were DM duration, HbA1c, FPG, serum creatinine, triglyceride, HOMA-IR, calcium and results of PWV/ABI (no adjusted, p<0.05). Whereas, AI was observed significant decrease in DM-microCx and DM-macroCx group compared with healthy control (38.54 & 37.72 vs. 39.96, p<0.01) and these results implied that reduced cell deformability caused to decrease RBC aggregation even for increased fibrinogen concentration.

Conclusion: EI is a sensitive parameter to detect impairment of erythrocyte in diabetic process. The results in this study suggest that significant reduction in the EI may have correlation with diabetic vascular complications, and furthermore could be one of the indicators of these complications.

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Semi-automatic analyses of skin capillary density: proof of principle and validation

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Background: It is increasingly being recognized that microvascular dysfunction may be a key feature in the development of both obesity-related hypertension and insulin resistance. Skin capillaroscopy has been proven to be a valid tool to study microvascular function non-invasively in humans. Unfortunately, the analysis of capillary density from video-clips is very time-consuming, since this is done manually. This impedes the use of this technique in large-scale studies. We aimed to develop a (semi-)automated assessment of skin capillary density.

Methods: CapiAna (Capillary Analysis) is a newly developed semi-automatic image analysis application. The technique involves four steps: 1) movement correction, 2) positioning of the region of interest (ROI) and selection of the frame range, 3) automatic detection of capillaries, and 4) manual correction of detected capillaries. We assessed accuracy, reproducibility, and time-savings of the semi-automatic method as compared to the manual counting procedure (MCP). For this purpose, finger skin capillary density was measured in 10 healthy participants (6 women; mean age 55.7 (49 - 68) years). First, to investigate agreement between the semi-automatic image analysis application (CapiAna) and the classic MCP, we used weighted Deming regression and

Bland-Altman analysis. Second, intra- and interobserver reproducibility of the semi-automatic method was assessed by calculating coefficients of variation (CV). To this end, an experienced investigator counted the number of capillaries twice with a two week interval. In addition, a second experienced investigator counted the number of capillaries in exactly the same ROI. Finally, the difference in analysis-time between the semi-automatic and the manual method was assessed.
Results: We found a Pearson`s correlation coefficient of 0.88 ($P < 0.001$) between both methods. Weighted Deming regression analysis resulted in the equation $y = 0.87x + 16.16$. The comparison between CapiAna and the MCP by the Bland-Altman analysis showed a bias of -3.9 capillaries/mm^2 (<5%) and limits of agreement (± 1.96 SD) of +13.6 to -21.4 capillaries/mm^2 (approximately 17%). The intra- and interobserver CVs of CapiAna were 2.0% and 5.4%, respectively (as compared to MCP data from literature: 4.5% and 7.2%, respectively). Finally, the analysis time for CapiAna ranged between 25 - 35 minutes versus 80 - 95 minutes for the MCP.
Conclusion: We have developed a semi-automatic image analysis application for the assessment of skin capillary density. The results indicate that CapiAna is in good agreement with the MCP. In addition, CapiAna is more reproducible as compared to the MCP. Finally, analysis time was significantly reduced with CapiAna. As a result, skin capillaroscopy can be used in large-scale studies, which importantly extends the possibilities to perform microcirculation research in humans.

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Natural history of heart rate variability by glycaemic status in the Whitehall II cohort

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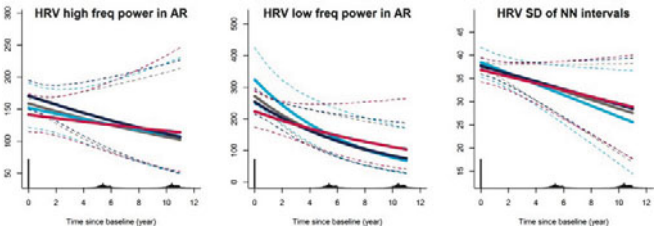
Background and aims: People with pre-diabetes and diabetes have an increased risk of cardiovascular disease (CVD). This increased morbidity and mortality is associated with adverse changes in cardiac autonomic tone which may be reflected in reduced heart rate variability (HRV) measures. Little is known about the natural history of HRV indices. We characterized changes in HRV indices in relation to baseline glycaemic state over ~12 years of follow-up.
Materials and methods: Data from 3,634 participants (7,290 person-examinations) without known diabetes at baseline (Phase 5) from the Whitehall II Cohort were used. 5-minute HRV recordings were collected at three phases approximately 5 years apart. HRV was analysed in the time domain (SD of intervals between R waves with normal-to-normal conduction (SDNN)) and in the frequency domain using an autoregressive method computing two power spectrum components: Low frequency power (LFP) and High frequency power (HFP). Individuals were divided into four subgroups based on glycaemic state at baseline: normal glucose tolerance (NGT)(n=3,110), isolated impaired fasting glucose (iIFG) (n=90), impaired glucose tolerance (IGT) (n=302), screen detected diabetes (SDM) (n=132). Trajectories of HRV recordings from baseline (time=0) and up to approximately 12 years later were estimated using multi-level longitudinal modelling adjusted for age, sex ethnicity, anti-hypertensive treatment and study phase. Participants with self-reported ischemic heart disease or use of nitrates were excluded.
Results: Preliminary results show a trend towards a decrease in all HRV indices over time in all subgroups (figure 1), with less decline in the SDM group. Baseline LFP measures are significantly lower (p=0.0244) in the SDM subgroup compared to baseline measures in the iIFG subgroup. No significant differences in HFP or SDNN values were found.
Conclusion: We show a decrease in SDNN, LFP and HFP over time in people with NGT, IFG, IGT and SDM which is in part related to ageing. Baseline values of LFP are lower in the SDM group compared to iIFG group suggesting an early imbalance in autonomic tone which could be the result of hyperglycaemia. The diminishing difference in HRV over time could be caused by convergence in ageing effects.



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The triglycerides-to-HDL-cholesterol ratio and cardiovascular disease risk in obese patients with type 2 diabetes: a report from the Swedish national diabetes register

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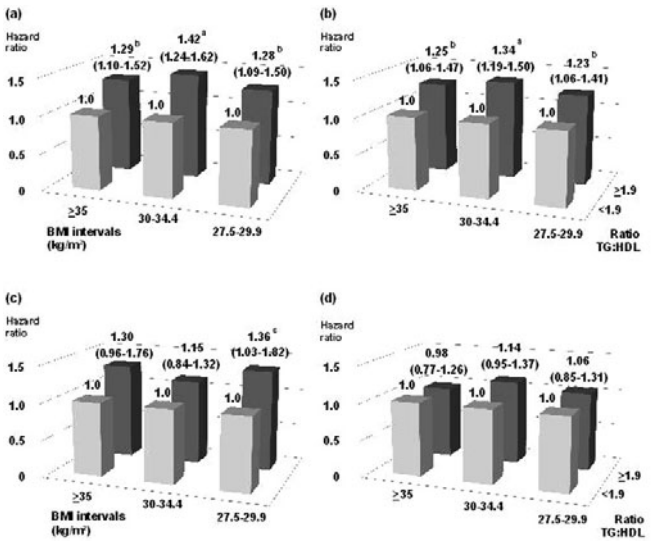
Background and aims: Studies in patients with type 2 diabetes (T2D) have shown both positive and negative results for the association between increasing BMI and cardiovascular disease (CVD) and mortality. We assessed the association between BMI and risk of coronary heart disease (CHD), CVD and mortality in T2D, also with regard to higher or lower levels of the ratio triglycerides-to-HDL-cholesterol (TG:HDL) as a marker for insulin resistance.
Materials and methods: 54,061 T2D patients, aged 30-74 years, with BMI ≥18.5 kg/m^2, mean age 61.5±8 years, diabetes duration 6.9±6 years, 14% with a history of CVD, from the Swedish National Diabetes Register, were included and followed for mean 4.8 years.
Results: Adjusting at Cox regression for characteristics not directly linked to BMI (age, sex, smoking, history of CVD), hazard ratios (HR) for fatal/nonfatal CHD and CVD were significantly ($p<0.001$) increased with baseline obesity (BMI 30-34.9 kg/m^2), 1.34 and 1.30, and prominent obesity (BMI ≥35 kg/m^2), 1.46 and 1.42, compared to normal weight (BMI 18.5-24.9 kg/m^2). With a second adjustment model including also BMI-linked covariates, as blood lipids, blood pressure, HbA1c, albuminuria, HR for these outcomes were significant only with prominent obesity, 1.19 ($p=0.01$) and 1.17 ($p=0.009$). Stratifying by 75th percentile of TG:HDL, with normal weight and TG:HDL <1.9 as reference and full adjustment, baseline obese and prominently obese with TG:HDL ≥1.9 had considerably increased HR around 1.7 for fatal/nonfatal CHD and 1.6 for CVD ($p<0.001$), while obese and prominently obese with TG:HDL <1.9 only had HR 1.2-1.3 for CHD and CVD ($p=0.003-<0.01$). A similar picture was found using updated mean BMI and TG:HDL. In each of the higher BMI categories, risks for CHD and CVD were increased by 25-40% with high TG:HDL compared to lower (Figure 1. Adjusted HR (95% CI) for outcomes with categories of baseline BMI and TG:HDL, a. fatal/nonfatal CHD; b. fatal/nonfatal CVD; c. fatal CVD; d. non-CVD mortality. Significance levels: a $p<0.001$, b<0.01, c<0.05). The risk for non-CVD mortality was not affected by lower or higher TG:HDL.
Conclusion: Obese T2D patients with high TG:HDL, associated with increased insulin resistance, had considerably increased risk of CHD and CVD. The TG:HDL ratio is clinically available and could add valuable information when assessing the individual patient risk.



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Short term mortality in people with diabetes: results from the national diabetes audit in England and Wales

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Background and aims: Research studies have provided a wealth of information on the associations between patient characteristics and mortality for those with diabetes but research populations often differ from clinical populations. The National Diabetes Audit (NDA) in England and Wales provides an opportunity to assess the independent association between patient demographic characteristics, treatment targets and diabetic complications on mortality in a 'real world' cohort.
Materials and methods: The NDA extracts data from primary care records on patient characteristics (age, sex, type of diabetes, ethnic group, social deprivation, body mass index - BMI) and treatment targets (HbA1c, blood pressure, cholesterol). The patients were matched to Hospital Episode Statistics and Patient Episode Data for Wales (databases containing a record of every hospital admission in England and Wales respectively) to identify hospital admissions for diabetic ketoacidosis, angina, heart failure, myocardial infarction, stroke, renal replacement therapy, minor amputation (below the ankle) and major amputation (above the ankle). They are also matched to nationally collated death records. A regression model was created to assess the risk of people aged 30 years and older with diabetes included in the 2009/10 audit dying in 2011.
Results: The risk of one year mortality increased with age (OR per additional year 1.082 95% CI 1.081-1.084) and male sex (OR 1.349 95% CI 1.316-1.382). People with Type 1 diabetes had a higher risk of dying than those with Type 2 diabetes (OR 1.222 95% CI 1.152-1.295). There was a statistically significant social deprivation gradient in the risk of one year mortality. People from South Asian and Black ethnic groups had lower mortality than those from white ethnic groups (OR 0.533 95% CI 0.504-0.563 and OR 0.529 95% CI 0.487-0.574 respectively). Having a very low BMI (<18.5) was associated with a significantly higher risk of dying (OR 2.372 95% CI 2.171 - 2.591 compared to BMI 18.5-24.9 (reference group). However, the lowest mortality were found in those with a BMI of 30-34.9 (OR 0.625 95% CI 0.604 - 0.648) and a BMI of 35-39.9 (OR 0.647 95% CI 0.618 - 0.677). This analysis found that there was a J shaped relationship between HbA1c and one year survival. Compared to those with a HbA1c of 42-53mmol/l (reference group) people with a HbA1c of <42mmol/l had an OR of 1.302 (95% CI 1.255 - 1.350). There was no significant difference in one year mortality for people with a HbA1c of 54-64mmol/l (OR 1.010 95% CI 0.980 - 1.041). Higher mortality was found in people with a HbA1c of 65-74mmol/l and 75+mmol/l (OR 1.145 95% CI 1.101 - 1.191 and OR 1.441 95% CI 1.385 - 1.498 respectively). A hospital admission for heart failure was associated with an OR of dying of 4.587 (95% CI 4.411 - 4.769) compared to 3.439 (3.232 - 3.660) for stroke and 1.991 (95% CI 1.853 - 2.138) for myocardial infarction. One or more minor amputations resulted in an OR of 1.709 (95% CI 1.415 - 2.065) and one or more major amputations was associated with an OR of 3.248 (95% CI 2.638 - 4.089). One or more hospital admissions for diabetic ketoacidosis were associated with an OR of 3.701 (95% CI 3.134 - 4.369).
Conclusion: This analysis has highlighted the risk factors for short term mortality in a real world cohort. Notable findings include the lower mortality risk in people from non white ethnic groups and in those with higher BMIs. It also identified the considerable additional risk of dying following a hospital admission for heart failure.
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Does the association of diabetes with mortality vary by sex? Results from a pooled cohort analysis

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Background and aims: Diabetes is associated with an increased risk of mortality. Whether this association varies by sex is unknown; findings from previous studies have been inconsistent and have mostly assessed prevalent diabetes without accounting for diabetes duration. The objective of this study was to investigate the association of incident diabetes with all-cause and CHD mortality and evaluate whether associations vary by sex.
Materials and methods: This pooled analysis included data from 35,665 participants, free of diabetes at baseline, from 5 epidemiologic cohort studies: Atherosclerosis Risk in Communities Study (1990-2006); Cardiovascular Health Study (1992-2008); Coronary Artery Risk Development in Young Adults Study (1985-2011); Framingham Offspring Study (1979-2007); and Multi-Ethnic Study of Atherosclerosis (2002-2011). Incident diabetes was defined as fasting glucose ≥ 7.0 mmol/l or use of diabetes medications. All-cause mortality and CHD mortality were determined from adjudicated event surveillance. Cox proportional hazards models with time-dependent exposure and covariates were used to assess the association of incident diabetes with mortality and determine whether this association varied by sex in models adjusted for socio-demographics (age, race, and education), cardiovascular disease (CVD) risk factors (BMI, smoking, hypertension, and hyperlipidemia), and prior CHD. Hazard ratios from cohort-specific analyses were pooled together using fixed-effects meta-analysis.
Results: Median age at baseline was 52 years. Over a median 17.9 years of follow-up, all-cause and CHD mortality rates were 173.5 and 35.5 per 1000 person-years, respectively. Diabetes was associated with an increased risk of all-cause and CHD mortality after adjustment for socio-demographics and a statistically significant interaction by sex was noted for all-cause mortality (Table). After additional adjustment for CVD risk factors and prior CHD, diabetes remained associated with all-cause and CHD mortality overall and within sex-groups, but the interaction between diabetes and sex was not statistically significant.
Conclusion: Incident diabetes was associated with an increased risk of all-cause and CHD mortality. The association of incident diabetes with all-cause mortality was greater among women compared with men, but this excess risk was explained by differences in CVD risk factors. Ongoing efforts to address modifiable CVD risk factors are needed to reduce the risk of all-cause and CHD mortality associated with incident diabetes.

	Overall	Men	Women	
		HR (95% CI)		p-value*
All-cause mortality				
Model 1	1.68 (1.55, 1.83)	1.58 (1.43, 1.76)	1.89 (1.65, 2.16)	0.03
Model 2	1.90 (1.67, 2.16)	2.06 (1.75, 2.42)	1.69 (1.36, 2.09)	0.30
CHD mortality				
Model 1	1.98 (1.66, 2.36)	1.82 (1.46, 2.28)	2.46 (1.83, 3.29)	0.06
Model 2	2.28 (1.76, 2.95)	2.20 (1.60, 3.04)	2.52 (1.66, 3.82)	0.35

Model 1 adjusted for age, race, and education.
Model 2 adjusted for age, race, education, BMI, smoking, hypertension, hyperlipidemia, and prior CHD.
*p- value for interaction of diabetes and sex

Table. Hazard ratios (HR) and 95% CI for association of incident diabetes with all-cause and CHD mortality, overall and by sex

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Total mortality by elevated transferrin saturation and haemochromatosis genotype in individuals with diabetes from two general population studies

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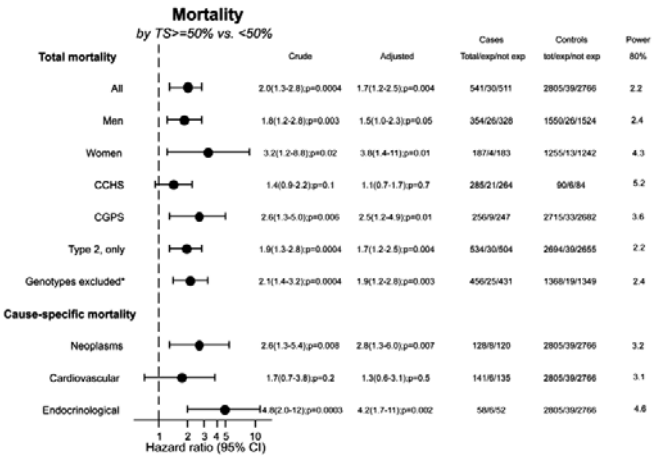
Background and aims: Iron overload and hemochromatosis genotype confer risk of diabetes. Mortality is increased in patients with clinically overt hereditary hemochromatosis, in individuals from the general population with increased transferrin saturation (TS) independent of hemochromatosis genotype, and also in patients with diabetes and increased TS or hemochromatosis genotype from a highly specialised diabetes clinic. Thus, we have recommended targeted screening for TS in specialised diabetes clinics. Whether mortality is also increased in patients ascertained from the general population with diabetes and increased TS or hemochromatosis genotype is unknown.
Materials and methods: In two Danish population-based follow-up studies, we examined mortality according to baseline TS(N=3346) or hemochromatosis genotype(N=1865) in individuals with diabetes (type 1 diabetes, N=118; type 2 diabetes, N=3228; total, N=3346). During a median of 4 years of follow-up, 541 patients with diabetes died.
Results: The cumulative survival was reduced in patients with diabetes with TS≥50% versus <50% (log-rank P<0.0001). Age-and sex adjusted hazard ratios for total mortality for TS≥50% vs <50% were 2.0 (95% CI 1.3-2.8; P =0.0004) overall, 1.8 (1.2- 2.8; P=0.003) in men, and 3.2 (1.2-8.8; P=0.02) in women; for neoplasms 2.6(1.3-5.4; P=0.008) and for endocrinological causes 4.8(2.0-12;P=0.0003); death due to cardiovascular causes was not different. Overall median survival time was 66 years (TS≥50%) and 79 years (TS<50%). Hazard ratio for total mortality in individuals with diabetes and hemochromatosis genotype C282Y/C282Y vs. wild type/wild type was 3.2(1.0-10;P=0.04) overall. Multifactorially adjusted hazard ratios and analyses for type 2 only or excluding hemochromatosis genotypes showed similar results. A stepwise increased risk of total mortality was observed for stepwise increasing levels of TS (log-rank P=0.0001), with the highest risk conferred by TS≥70% vs TS<20% with a hazard ratio of 4.8 (2.0-12; P =0.0006). Seven percent of premature death among diabetics in the general population can possibly be avoided by early screening for TS or hemochromatosis genotype.
Conclusion: Patients with diabetes, ascertained in the general population, with increased TS or hemochromatosis genotype have a 2-5-fold increased risk of premature death. Thus, we also recommend targeted screening with TS or hemochromatosis genotype in patients with diabetes in the general population.



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Prosomatostatin and mortality in patients with type 2 diabetes mellitus (ZODIAC-35)

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Background and aims: Prosomatostatin, the prohormone of somastatin, is suggested to be involved in glucose metabolism by inhibiting the secretion of glucagon and insulin. Prosomatostatin also influences the growth hormone-insulin-like growth factor-1 (GH-IGF-1) axis, by inhibiting the secretion of GH, the subsequent low IGF-1 concentrations are associated with an increased cardiovascular risk. Aim of this study is to investigate whether prosomatostatin is related to cardiovascular and all-cause mortality in patients with type 2 diabetes (T2DM).
Materials and methods: A total of 1687 primary care patients with T2DM participated in this prospective observational study, as part of the ZODIAC project. EDTA-plasma was stored at -80°C, until assessment of prosomatostatin by sandwich immunoassay. Cox proportional hazards models were used to investigate the relationship between the serum concentration prosomatostatin and mortality with adjustment for the selected confounders. Three models were chosen: model 1 included only prosomatostatin, model 2 included age and gender and model 3 included nine risk factors as additional potential confounders (diabetes duration, smoking, macrovascular complications, BMI, systolic blood pressure, HbA1c, serum creatinine, cholesterol-HDL ratio and albuminuria). Harrell's C statistic was used to investigate the capability of each model to predict mortality.
Results: Prosomatostatin was assessed in baseline serum samples of 1326 patients (78.6%) with a median (interquartile range) concentration of 591.5 (449.8-783.3) pmol/L. During median follow-up of 6.0 (3.2-10.0) years, 413 (31.1%) patients died, of which 176 (42.6%) were attributed to cardiovascular causes. As depicted in table 1, high prosomatostatin is significantly associated with cardiovascular and all-cause mortality, both in the crude analyses and after adjustment for age and gender. However, in model 3 the association of log prosomatostatin with mortality lost significance. There were no significant differences in Harrell's C statistics for the models with and without prosomatostatin.
Conclusion: High levels of prosomatostatin are significantly associated with increased cardiovascular and all-cause mortality in patients with T2DM, also after adjusting for age and gender. However, after adjustment for established cardiovascular risk factors this significant association is no longer observed.
Table 1. Results of the Cox proportional hazards models

	Log PSS HR (95% CI)	Harrell's C (95 % CI)	Harrell's C (95 % CI)*
All-cause mortality			
Model 1	2.80 (2.17-3.60)	0.62 (0.59-0.65)	-
Model 2	1.48 (1.14-1.93)	0.77 (0.75-0.80)	0.77 (0.75-0.80)
Model 3	1.09 (0.81-1.46)	0.79 (0.77-0.82)	0.79 (0.77-0.82)
Cardiovascular mortality			
Model 1	3.86 (2.64-5.62)	0.65 (0.60-0.70)	-
Model 2	2.21 (1.49-3.28)	0.76 (0.72-0.80)	0.75 (0.71-0.79)
Model 3	1.08 (0.69-1.68)	0.81 (0.77-0.84)	0.80 (0.77-0.84)

*Log prosomatostatin expressed in Hazard Ratio's and the capacity of the different models to predict mortality using the Harrell's C statistic. * Values of the Harrell's C statistic for the models without PSS. PSS: prosomatostatin, HR: Hazard ratio, 95% CI: 95% Confidence interval.*

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Predicting macro- and microvascular complications in type 2 diabetes: the Japan diabetes complications study / the Japanese elderly diabetes intervention trial risk engine

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Background and aims: To develop and validate a risk engine that calculates the risks of macro- and micro-vascular complications in type 2 diabetes.
Materials and methods: We analyzed pooled data from two clinical trials on 1,748 Japanese type 2 diabetic patients without diabetes complications other than mild diabetic retinopathy with a median follow-up of 7.2 years. Endpoints were coronary heart disease (CHD), stroke, non-cardiovascular mortality, overt nephropathy defined by persistent proteinuria, and progression of retinopathy. We fit a multi-state Cox regression model to derive an algorithm for prediction. The predictive accuracy of the calculated 5-year risks was cross-validated.
Results: Sex, age, HbA1C, years after diagnosis, BMI, systolic blood pressure, non-HDL cholesterol, albumin-to-creatinine ratio, atrial fibrillation, current smoker and leisure time physical activity were risk factors for macro- and micro-vascular complications and were incorporated into the risk engine. The observed-to-predicted ratios for each event were between 0.93 and 1.08 and Hosmer-Lemeshow tests showed no significant deviations between observed and predicted events. In contrast, the UKPDS risk engine overestimated CHD risk (observed-to-predicted ratios: 0.30 for CHD and 0.72 for stroke). C statistics in our Japanese patients were high for CHD, non-cardiovascular mortality, and overt nephropathy (0.696 to 0.767) but moderate for stroke and progression of retinopathy (0.636 and 0.614). By combining macro- and micro-vascular risks the classification of low- and high-risk patients was improved by a net reclassification improvement of 5.7% (p=0.02).
Conclusion: The risk engine accurately predicts macro- and micro-vascular complications and would provide helpful information in risk classification and health economic simulations.
Supported by: the Ministry of Health, Labour and Welfare

PS 022 Prediction of complications in type 1 and type 2 diabetes II

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Association between sodium and potassium urinary concentrations and cardiovascular and renal complications in a French cohort of type 2 diabetes patients

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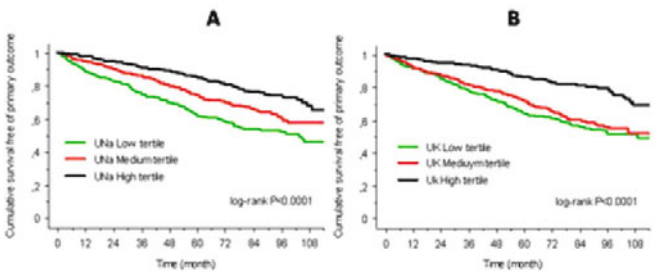
Background and aims: In type 1 diabetes and in the general population, epidemiological data suggest that sodium intake is associated with cardiovascular outcomes with a J-shape curve. However no data examined the association between urinary sodium and cardiovascular or renal complications in type 2 diabetes patients. We performed an observational evaluation in an inception cohort of 1467 type 2 diabetes patients focusing on sodium and potassium urinary concentrations (U_{Na} and U_K).
Materials and methods: Baseline urinary sodium and potassium concentrations were measured by indirect potentiometry on morning spot urine. Our primary outcome is a composite of cardiovascular death, non fatal acute myocardial infarction, cerebrovascular stroke, hospitalization for congestive heart failure or end-stage renal disease (dialysis or renal transplantation). We used Kaplan-Meier survival analysis, log-rank test and Cox proportional hazard model to estimate associations between predictors and complication outcome.
Results: Median follow-up was 56 months during which 386 primary outcomes and 193 cardiovascular deaths occurred. The mean value of U_{Na} and U_K were 88.5±41.3 mmol/day and 52.2±25.2 mmol/day respectively. Cumulative incidence of primary outcome increased significantly with decreasing tertiles of U_{Na} and U_K (Figure; all log-rank P <0.0001): lower tertile U_{Na} [7-68 mmol/l] : 9.67 %/pt-yr ; intermediate tertile U_{Na} tertile [69-103 mmol/l] : 6.41 %/pt-yr ; higher U_{Na} tertile [104-240 mmol/l]: 3.97 %/pt-yr. Lower tertile U_K [6-38 mmol/l] : 8.74 %/pt-yr ; intermediate U_K tertile [39-59 mmol/l] : 7.56 %/pt-yr higher U_K tertile [60-163 mmol/l]: 3.42 %/pt-yr. A similar relationship was found when considering cardiovascular death (all log-rank P <0.0001). This association remained significant after adjustment on age, sex, diabetes duration, estimated glomerular filtration rate, urinary albumin excretion, prior history of myocardial infarction, systolic blood pressure and use of diuretics (primary outcome : U_{Na}, P=0.0004:U_K, P <0.0001; cardiovascular death : U_{Na}, P =0.0041 and U_K, P <0.0036).
Conclusion: In type 2 diabetes patients, lower sodium and potassium concentrations are associated with higher cardiovascular and renal complications.

Kaplan-Meier survival curves of primary outcome-free survival according to urinary Sodium (A) and Potassium (B) concentration tertiles in a French type 2 diabetes cohort



Clinical Trial Registration Number: DGS2003/0530
Supported by: PHRC interrégional, AFD Recherche (2003) ALFEDIAM (2009) et GEMMS

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Albuminuria and renal function as predictors of cardiovascular events and mortality in type 2 diabetes: nationwide observational study

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Background and aims: Reduced renal function and albuminuria predict cardiovascular (CV) events and mortality in type 2 diabetes (T2D). We examined the role of these and several other cardiovascular risk factors including co-existing congestive heart failure (CHF) on cardiovascular (CV) events in a large observational population-based cohort of T2D patients.
Methods: We included 68 156 patients with T2D reported to the National Diabetes Register (NDR) in Sweden in 2003-06 with a mean follow-up of 5.6 years, censor date 31 December 2010. Data on outcomes were collected from the Cause of death and Hospital discharge registers.
Results: Ten per cent of patients experienced a CV event of which 3.7% were fatal. Increasing levels of albuminuria and renal impairment were independently associated with increasing risk of CV events and all-cause mortality, also when adjusting for CHF. In normoalbuminuric patients, a reduction in renal function was an important predictor of CV events and all-cause mortality. Glycemic control and smoking had important effects on risk for CV events in patients with albuminuria but not in patients with normoalbuminuric renal impairment.
Conclusion: Albuminuria and renal impairment are independent risk factors for cardiovascular outcomes and mortality in type 2 diabetes, albuminuria being the strongest risk factor and relevant at all levels of renal function. In normoalbuminuric patients, a reduction in renal function is an important predictor of CV events and all-cause mortality.

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Type 2 diabetes and risk of recurrent cardiovascular event following acute coronary syndrome (ACS): longitudinal analysis 2006-2011

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Background and aims: Cardiovascular disease (CVD) is the leading cause of mortality worldwide, resulting in extensive morbidity and mortality in patients with T2D. This study evaluated the effect of multiple risk factors on time to first subsequent CV event in patients with and without T2D following ACS.
Materials and methods: ACS patients hospitalised with an ICD-9 code for acute myocardial infarction (MI) (410.xx) or unstable angina (411.1x) were identified from the HealthCore Integrated Research Database (HIRD^{SM}) from 2006–2011. Patients with T2D were defined as ≥ 2 ICD-9 codes for T2D (250.x0, 250.x2) at least 30 days apart; or ≥ 1 claim for T2D and ≥ 1 claim for oral or injectable anti-diabetics; or ≥ 2 prescriptions of oral anti-diabetic (OAD) or glucagon-like peptide 1 medication. Patients without T2D were defined as not meeting the above criteria and having no type 1 diabetes diagnosis (250.x1, 205.x3). Multivariable Cox proportional hazards models were used to evaluate the effect of risk factors on time to first subsequent CV event (defined as stroke, MI or coronary heart disease-related mortality) in patients with and without T2D, adjusting for baseline demographic characteristics, comorbidities, treatment utilisation and index ACS characteristics.
Results: Of the 140,903 ACS patients identified, 38,553 (27.6%) had T2D at baseline. Mean (median) follow-up length was 1.82 (1.40) and 1.84 yrs (1.41) for patients with and without T2D, respectively. Patients with T2D were on average older (mean age 68.2 vs. 65.6 yrs) with more baseline comorbidities (Deyo-Charlson Index score 3.15 vs. 1.11) vs. patients without T2D. Mean (median) length of stay of the index ACS hospitalisation was longer in patients with T2D (7.62 [4.00] vs. 5.20 [3.00] days). Following discharge, 26.2% of patients with T2D had a subsequent CV event vs. 15.4% of patients without T2D during the follow-up period. After adjusting for baseline differences, patients with T2D had significantly increased hazard (all P-values < 0.05) of a subsequent CV event if older (adj HR = 1.30 in ≥ 65 vs. < 65 years), history of chronic renal dysfunction (adj HR = 1.36), heart failure (adj HR = 1.35), hypertension (adj HR = 1.10), baseline insulin use (adj HR = 1.19), or index STEMI and NSTEMI vs. UA (adj HR STEMI = 1.59, NSTEMI = 1.62). Patients with T2D had significantly lower adjusted hazards (all P-values <

0.05) with baseline statin (adj HR = 0.94), OAD agent use (adj HR = 0.95), or CABG during the index ACS event (adj HR = 0.83).
Conclusion: Following an ACS event, patients with T2D had increased hazard of a subsequent CV event if older, had more comorbidities or MI at index, or insulin use at baseline, while a lower hazard with statin, OAD use, or CABG during the index hospitalisation. These findings reinforce the need for aggressive management of known CV risk factors to reduce the risk of subsequent CV events in patients with T2D.

Table: Risk of Subsequent CV Event Following ACS Hospitalisation, by Diabetes Status

Parameter	Patients with T2D (n = 38,553)			Patients without T2D (n = 81,845)		
	Hazard Ratio	95% Hazard Ratio Confidence Limits		Hazard Ratio	95% Hazard Ratio Confidence Limits	
Baseline Characteristics						
>=65years (reference <65years)	1.30	1.25	1.35	1.60	1.55	1.65
Female (reference male)	1.01	0.98	1.04	0.97	0.95	1.00
Congestive heart failure	1.35	1.30	1.39	1.49	1.44	1.54
Peripheral artery/vascular disease	1.25	1.21	1.30	1.19	1.14	1.23
Hypertension	1.10	1.07	1.14	1.15	1.12	1.18
Dyslipidemia	0.99	0.96	1.02	0.95	0.92	0.97
Chronic renal dysfunction	1.36	1.31	1.41	1.37	1.32	1.43
Baseline Treatment						
Antiplatelets	1.05	1.01	1.09	1.12	1.08	1.16
Statins	0.94	0.91	0.97	0.96	0.93	0.99
ACE Inhibitor and/or ARB	0.95	0.92	0.98	1.00	0.98	1.04
Beta-blockers	1.07	1.03	1.11	1.06	1.03	1.09
OAD	0.96	0.92	0.99	n/a	n/a	n/a
Combination OAD	0.95	0.89	1.00	n/a	n/a	n/a
Insulin/insulin analog	1.19	1.15	1.24	n/a	n/a	n/a
Index ACS Characteristics						
STEMI (reference UA)	1.59	1.54	1.64	1.78	1.73	1.82
NSTEMI (reference UA)	1.62	1.56	1.69	1.73	1.67	1.79
CABG	0.83	0.79	0.87	0.93	0.89	0.97
PCI	0.97	0.94	1.00	1.08	1.05	1.11

PS 023 Receptor regulation of islet function

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The chemokine CCL5 is expressed by islets and regulates beta cell function through activation of GPR75

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Background and aims: Chemokine ligand 5 (CCL5) activates the chemokine receptors CCR1, CCR3, CCR5 and the atypical CCL5 receptor GPR75, which shares only 12-16% amino acid sequence homology with conventional chemokine receptors. It is known that GPR75 activation promotes inositol 1,4,5-trisphosphate (IP_3) formation, stimulates intracellular calcium ($[Ca^{2+}]_i$) and protects neurons against apoptosis. We have previously demonstrated that mouse and human islets express CCL5, that GPR75 is the most abundant receptor for this chemokine in mouse and human islets, and that CCL5 stimulates insulin secretion from mouse islets. The current study investigated the cellular localisation of CCL5 and GPR75 in mouse and human islets, the effects of exogenous CCL5 on insulin secretion and apoptosis in human islets and the requirement of GPR75 in mediating the effects of CCL5 in mouse β-cells.
Materials and methods: CCL5 and GPR75 localisation within mouse and human islets was detected by immunohistochemistry. Dynamic insulin secretion from human islets perifused in the absence or presence of exogenous CCL5 was quantified by radioimmunoassay. Apoptosis of human islets was induced by exposure to a cytokine cocktail (IFNγ:1000U/μl, TNF-α:1000U/μl and IL-1β:100U/μl) and detected by measuring caspase 3/7 activities using a luminescent assay. Changes in $[Ca^{2+}]_i$ were measured by single cell microfluorimetry of fura-2-loaded MIN6 β-cells transiently transfected with non-coding RNAs (ncRNAs) or with GPR75 siRNAs. The extent of GPR75 knockdown by siRNAs treatment was determined by quantification of GPR75 mRNA levels by qPCR.
Results: CCL5 was present in glucagon-secreting α-cells in mouse and human islets, and it was also expressed by β-cells of human islets, but not by mouse islet β-cells. CCL5 did not co-localise with somatostatin-secreting δ-cells of either species. The CCL5 receptor GPR75 was abundantly expressed by β-cells of mouse and human islets. Exogenous CCL5 (10nM) potentiated glucose-induced insulin secretion from human islets (peak stimulation above glucose plateau: 259±92%, n=4, P<0.05), and decreased cytokine-induced caspase-3/7 activities in human islets (luminescence units, control: 256,577±47,811; +1nM CCL5: 145,892±19,934, n=5-7, P<0.05). CCL5 stimulated $[Ca^{2+}]_i$ in MIN6 β-cells in a concentration-independent manner (% tolbutamide response, 0.25nM CCL5: 65.7±1.6%; 2.5nM CCL5: 50.5±1.4%; 25nM CCL5: 60.6±2.1%, n=34, P<0.01). Exposure of MIN6 β-cells to GPR75 siRNAs caused a significant reduction in GPR75 mRNA expression (non-coding RNAs, GPR75 mRNA expression: 0.48±0.07% of β-actin mRNA; siRNAs: 0.29±0.01%, n=3, P<0.05) and when GPR75 expression was down-regulated the $[Ca^{2+}]_i$ response to CCL5 was significantly diminished (0.25nM CCL5: 19.0±0.5%; 2.5nM CCL5: 28.4±0.9%; 25nM CCL5: 18.9±0.6% tolbutamide response, n=45, P<0.01 versus responses in ncRNA-treated cells).
Conclusion: GPR75 is expressed by mouse and human islet β-cells and it may be activated in a paracrine manner by islet cell-derived CCL5. Exogenous CCL5 stimulates insulin secretion from human islets and protects them from apoptosis, as we have previously found for mouse islets. Down-regulation of β-cell GPR75 diminishes $[Ca^{2+}]_i$ elevations in response to CCL5, suggesting that CCL5 may primarily exert its effects in β-cells via GPR75 activation.

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Fractalkine, a new factor protecting beta cell function and survival

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Background and aims: Fractalkine (CX3CL1) is a membrane bound chemokine with a soluble form generated by enzymatic shedding. Biological

actions of fractalkine are mediated through a specific G-protein coupled receptor, CX3CR1. We have previously shown the existence of a muscle-pancreas intercommunication axis in which fractalkine produced by skeletal muscle cells could be implicated. The aim of the study was to explore the impact of fractalkine on islets and sorted beta cells.
Materials and methods: Human islets and sorted beta and non-beta cells were used to measure, CX3CL1 and CX3CR1 mRNA expression. Glucose stimulated insulin secretion (GSIS: 1h 2.8 mmol/l followed by 1h 16.7 mmol/l glucose) was measured using human beta cells exposed to 1-50 ng/ml fractalkine for 24h or rat beta cells exposed to 1-100ng/ml fractalkine for 24h alone or with TNFα (20ng/ml) or cytomix (20 ng/ml each TNFα, IFNγ, IL1-β). Glucagon secretion (1h 16.7 mmol/l followed by 1h 2.8 mmol/l glucose) was measured on human islets exposed to 1-50 ng/ml fractalkine for 24h. Rat beta cell apoptosis (TUNEL assay), and proliferation (BrdU incorporation) were measured after 24h exposure to 1-100 ng/ml fractalkine alone, or to 20 ng/ml fractalkine for 48h with addition of cytomix for the last 24h. Data are mean +/- SE (5 independent experiments).
Results: CX3CL1 and CX3CR1 are both expressed in human islets. Within the islet, fractalkine seems to be principally expressed by non-beta cells while its receptor is more expressed in the beta cells. Treatment with fractalkine for 24h had no significant impact on GSIS or insulin content in human and rat sorted beta cells. In human islets, fractalkine treatment blocked stimulation of glucagon secretion at low glucose (e.g. 0.49+/-0.06% content/h fractalkine 25ng/ml vs. 2.23+/-1.09% control; $p<0.05$) without any effect on glucagon content. In rat beta cells, fractalkine treatment had no direct impact on proliferation or apoptosis. To test the hypothesis that fractalkine could protect against the negative effects of cytokines, rat sorted beta cells were treated with fractalkine in combination with TNFα or cytomix. Fractalkine completely blocked the adverse effect of TNFα on GSIS (1.46+/-0.11% content/h TNFα vs. 3.19+/-0.12% $p<0.05$ and 2.62+/-0.04% $p<0.05$ TNFα + 10 or 25 ng/ml fractalkine). Interestingly, fractalkine treatment was not able to block the effects of cytomix on GSIS, proliferation or apoptosis in rat sorted beta cells.
Conclusion: Taken together our results show that fractalkine could have a positive impact on beta cells via a paracrine and/or endocrine axis. Fractalkine can also directly regulate glucagon secretion. Moreover, fractalkine protects beta cells from the adverse effects of TNFα while offering no protection against cytomix treatment. This suggests a specific interaction between TNFα and fractalkine signalling pathways in beta cells.
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Epigenetic regulation of chemokine CXCL12 gene transcription influences its prosurvival effect on pancreatic beta cells

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Background and aims: The CXC chemokine ligand 12 (CXCL12/SDF-1) promotes the expression of the proliferative phenotype that improves the resistance of pancreatic beta cells (b-cells) to diabetogenic stimuli. Our aim was to demonstrate the positive impact of chemokine CXCL12 expression on increased survival of b-cells, to provide a novel insight into the epigenetic regulation of CXCL12 gene (*Cxcl12*) transcription and to initiate a screening study designed to test whether certain compounds present in the Epigenetic Compound Library (ECL-COST-TD0905) possess a DNMT1 inhibitory potential.
Materials and methods: The RIN-5F rat pancreatic b-cell line (wt), its counterpart that possesses a stably integrated human gene for CXCL12 (#1) and MIN6 cells were exposed to increasing concentrations of streptozotocin. The prosurvival potential of CXCL12 was assessed by the viability assay (MTT). For the DNA methylation studies, DNA was isolated from: rat RIN-5F wt and #1 cells, rat Langerhans islets and mouse MIN6, NIH 3T3 wt and PARP-1 knockout cells. DNA methylation of the rat and mouse *Cxcl12* was assessed using real-time methylation-specific PCR (MSP) with primers designed for each CpG island predicted within the promoter, the first exon and intron of *Cxcl12*. Each component from ECL-COST-TD0905 was used at 15 μM concentration for demethylation studies (5-aza-2'-deoxycytidine, was used as positive control).
Results: Our results confirmed that the ıncreased presence of CXCL12 improves pancreatic b-cell survival during oxidative stress induced by a diabetogenic stimulus. The CpG island analysis of the rat and mouse *Cxcl12* promoter, first exon and intron revealed the same number and very similar distribution of CpG islands in both species. MSP showed that the CpG-rich regions within the *Cxcl12* promoter, first exon and intron are semi-methylated in the rat Rin-5F cells. In the rat Langerhans islets, the core promoter is unmethylated, while the first exon exhibited methylation of both alleles. In mouse cells, large differences in methylation patterns of the core promoter were observed: wt cells possess a unmethylated and PARP-1 knockout cells a fully methylated core promoter. One of the eight analysed compounds from the ECL-COST-TD0905 possesses potential to inhibit DNMT1 *in vitro*.
Conclusion: We confirmed that CXCL12 exerts a prosurvival effect on pancreatic b-cells. The differences observed in the methylation status of the *Cxcl12* gene, points to decreased gene responsiveness to external stimuli. The clear differences in the methylation status of the promoter and the first exon in the rat insulinoma cell line and *ex vivo* isolated Langerhans islets have to be underlined. Furthermore, observed hypermethylation of mouse *Cxcl12* in PARP-1 knockout cells, points to the involvement of PARP-1 in the inhibition of the methylation *in vivo*.
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Role of GPR55 in the regulation of insulin secretion and islet survival

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Background and aims: We have recently shown that the novel cannabinoid receptor GPR55 is expressed by islets and that its pharmacological activation stimulates insulin secretion and protects islets from apoptosis. To further understand the role of GPR55 in islets, this study evaluated the effects of two GPR55 agonists (10μM O-1602 and 5μM LPI) on insulin secretion from islets isolated from GPR55 knockout (KO) mice and age-matched wildtype (WT) mice. The effects of the GPR55 antagonist cannabidiol (1μM CBD) on insulin secretion from isolated mouse and human islets, and on islet apoptosis, were also investigated.
Materials and methods: Islets were isolated from mouse and human pancreas by collagenase digestion and purified by density gradient centrifugation. Dynamic insulin secretion was quantified by radioimmunoassay following perifusion of islets from WT and GPR55 KO mice, and from human organ donors. Apoptosis of islets was induced by exposure to mixed cytokines (1U/μl IFNγ, 1U/μl TNFα, 0.05U/μl IL-1β) for 20 hours and caspase 3/7 activities were measured using a luminescent assay.
Results: 10μM O-1602 significantly stimulated insulin secretion from WT mouse islets at 2mM glucose (peak stimulation: 488±80%; n=3, $P<0.05$) and potentiated glucose-induced insulin stimulation (peak stimulation above 20mM glucose plateau: 232±53 %; n=3, $P<0.05$). The stimulatory effects of O-1602 on insulin secretion were abolished in islets isolated from GPR55 KO mice (n=4, $P<0.05$ versus response to O-1602 in WT islets). However, 10μM LPI significantly potentiated glucose-induced insulin secretion from islets isolated from both WT and GPR55 KO mice (peak stimulation above 20mM glucose plateau: WT: 142±11%; KO: 179±42%; n=4, $P<0.05$ versus 20mM glucose alone for WT and KO islets). Unexpectedly, the GPR55 antagonist CBD significantly stimulated insulin secretion from mouse islets at 2mM glucose (peak stimulation: 334±87%; n=4, $P<0.05$) and at 20mM glucose (peak stimulation above 20mM glucose plateau: 194±68%; n=4, $P<0.05$). Stimulatory effects in response to CBD were also observed in perifusion experiments using human islets, with increases over basal secretion at 2mM glucose (peak stimulation: 415±84%; n=4, $P<0.05$) and potentiation of glucose-stimulated insulin secretion (peak stimulation above 20mM glucose plateau: 255±50%; n=4, $P<0.05$). In addition, exposure of mouse islets to 1μM CBD for 20 hours protected against apoptosis (reduction in caspase 3/7 activities, basal: 39±6%; cytokine-induced: 45±2%; n=11, $P<0.01$).
Conclusion: These data indicate that the pharmacological GPR55 agonist O-1602, and the endogenous lipid LPI, stimulate insulin secretion from mouse islets, but only the effect of O-1602 is dependent on the presence of GPR55. In addition, the GPR55 antagonist CBD unexpectedly increased insulin secretion and promoted islet survival. Our results therefore suggest that GPR55 plays an important role in the regulation of islet physiology, but LPI and CBD exert their effects on islet function by a GPR55-independent pathway(s).
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Effect of in vitro and in vivo hyperglycaemia on muscarinic M3 receptor expression and activation in islets

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Background and aims: Islets are innervated by parasympathetic nerves which release acetylcholine (ACh) during feeding. In beta cells the effect of ACh is mediated primarily via muscarinic M3 receptors (M3R) leading to amplification of glucose-induced insulin secretion from the islets. Beta cell specific knock-out of M3R results in reduced insulin secretion and impaired glucose tolerance and we have previously shown that M3R expression and secretory sensitivity to muscarinic agonists are down-regulated in islets isolated from *ob/ob* mice. Here we investigate *in vitro* and *in vivo* whether this change is a consequence of the local hyperglycaemic environment of the islets.
Materials and methods: To provide an *in vivo* hyperglycaemic/diabetic environment lacking direct islet innervation, alginate-encapsulated mouse islets were transplanted intraperitoneally into streptozotocin-induced diabetic mice and retrieved after 7 days, and encapsulated islets were also transplanted into control, normoglycaemic mice. In the *in vitro* study, isolated mouse or human islets were maintained in the presence of 5.5 or 16 mmol/l glucose for 3 or 7 days. Blood glucose levels were assessed by commercial glucose meter, islet hormone secretion and content by RIA and M3R mRNA levels by qPCR.
Results: Blood glucose levels of diabetic mice remained high following minimal mass (200) transplantation of encapsulated islets (day -1: 28.6±1.3 mmol/l glucose, day 7: 22.5±3.5, n=5, P>0.2). Islets retrieved from both control and diabetic mice responded to glucose with release of insulin (control: 1 mmol/l glucose: 13.9±2.0 pg/islet/h, 20 mmol/l glucose: 79.2±20.8, P<0.05; diabetic: 1 mmol/l glucose: 18.4±1.8 pg/islet/h, 20 mmol/l glucose: 116.1±7.8, P<0.001, n=3-4), but enhancement of glucose-induced insulin secretion by the acetylcholine analogue, carbachol (CCh, 0.5 mmol/l), was reduced by 71±7% in islets from diabetic mice compared to controls (339±79% secretion at 20 mmol/l glucose vs 1156±346%, n=5-6, P<0.05). Maintenance of islets in the presence of 16 mmol/l glucose for 3 days significantly reduced the expression of M3R mRNA by 50±12% compared to islets maintained at 5.5 mmol/l glucose (n=8, P<0.001). Potentiation of glucose-induced insulin secretion by CCh was reduced by 33±7% (P<0.05) and 58±3% (P<0.001) following culture in 16 mmol/l glucose for 3 and 7 days, respectively, compared to 5.5 mmol/l glucose. In addition, CCh stimulated glucose-induced secretion from human islets that had been maintained at 5.5 mmol/l glucose for 7 days, but not those that had been exposed to 16 mmol/l glucose for 7 days (P<0.001 vs. 5.5 mmol/l glucose).
Conclusion: These results indicate that down-regulation of islet M3R and reduced insulin secretory sensitivity to muscarinic agonists is likely to be a consequence of the hyperglycaemic environment and suggests that M3 receptors could be potential targets in the treatment of type 2 diabetes.
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Receptor-mediated effects of melatonin on insulin secretion in INS-1 832/13 beta cells

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Background and aims: The vital role of the hormone melatonin on glucose homeostasis was highlighted by the discovery of the single nucleotide polymorphisms (SNPs) of the human Mtnr1b and more recently of Mtnr1a associated with impaired glucose metabolism. Despite several advances, the exact mechanism of melatonin actions on insulin release and its receptor involvement is still unclear. To unveil this issue, we examined the impact of melatonin receptor signaling on insulin secretion by using a knockdown approach in INS-1 832/13 β-cells.
Materials and methods: To generate knockdown of Mtnr1a and Mtnr1b receptors, the INS-1 832/13 β-cells were transfected with siRNA against these receptors. To examine the magnitude of receptor-mediated effects of melatonin on glucose-stimulated insulin secretion (GSIS), the transfected cells were stimulated with glucose in the absence or presence of melatonin (1μM). Moreover, the effect of silencing its receptors on cAMP-evoked insulin release was also studied considering the established cAMP-lowering effects of its receptors.
Results: Robust knockdown of both Mtnr1a and Mtnr1b receptors was achieved at the mRNA as well as at the protein level. The knockdown of either Mtnr1a or Mtnr1b did not affect GSIS in INS-1 832/13 β-cells (3.2±0.01 or 3.8±1.24 vs. 2.8±0.25 fold respectively). Melatonin decreased GSIS in control cells (2.8±0.25 vs. 1.8±0.11 fold respectively, P<0.05), while, in Mtnr1a knockdown cells no melatonin-induced reduction in GSIS was observed compared to negative control cells (2.7±0.09 vs. 2.8±0.25 fold respectively). Interestingly, melatonin only marginally lowered insulin secretion in Mtnr1b knockdown cells compared to Mtnr1a knockdown cells (2.2±0.12 vs. 2.7±0.09 fold respectively). However, fold changes in GSIS in melatonin-treated Mtnr1b knockdown cells were still significantly higher than the melatonin-treated negative control cells (2.2±0.12 vs. 1.8±0.11 fold respectively, P<0.05). This reflects that even though both receptors are involved in the insulin-lowering effect of melatonin, Mtnr1a exerted a stronger effect than Mtnr1b. No melatonin-mediated reduction in GSIS was observed in forskolin-stimulated Mtnr1a or Mtnr1b knockdown cells (5.9±0.62 vs. 6.1±1.00 or 6.6± 0.57 vs. 6.1±0.33 fold respectively). This supports previous reports of the involvement of cAMP signaling in melatonin receptor-mediated effects on insulin secretion.
Conclusion: Our results revealed that in the paucity of its receptors, melatonin failed to inhibit glucose- or cAMP-stimulated insulin release. This emphasizes the significant role of both of its receptors and cAMP signaling in this course.

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Inhibition of small-Maf function in pancreatic beta cells attenuates the effect of GLP-1 receptor agonist on improvement of glucose tolerance

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Background and aims: GLP-1 possesses protective effects on beta cells and increases both amount of insulin secretion on demand and beta cell proliferation. In addition, anti-oxidative and anti-inflammatory effects have been reported in some incretin-related agents. MafA, one of large-Maf factors, is a major regulator of insulin gene expression and beta cell function. On the other hand, small-Maf factors lack N-terminal transactivation domain and therefore act as a negative regulator of insulin expression. We previously reported that mRNA and protein levels of small-Maf factors were increased under oxdative or lipotoxic conditions, and concomitantly insulin gene expression was diminished in beta cell lines. Moreover, transgenic (Tg) mice with beta cell specific expression of dominant-negative small Maf factor (DN-MafK) on C57BL/6J background showed improvement of glucose tolerance under feeding high-fat diet (HFD), in spite of similar MafA expression pattern as compared with wild-type (Wt) mice. Here, to clarify the incretin effects on small-Maf factors under the lipotoxic condition, we evaluate the effects of liraglutide to HFD-fed Tg mice.
Materials and methods: Both Wt and Tg male mice were fed on HFD from 6 weeks of age and injected liraglutide (at a dose of 200μg/kg) or vehicle daily from 8 weeks. Body weight, food consumption and blood glucose were measured. At 16 weeks, insulin tolerance test and oral glucose tolerance test were performed, and then pancreatic sections were obtained for immunohistochemistry to evaluate beta cell mass using insulin staining. Proliferation of beta cell was evaluated by PCNA staining.
Results: Body weight and food intake were reduced in liraglutide-treated mice than in vehicle, but there were no significant differences between Wt and Tg mice. Treating with liraglutide resulted in significant lower levels of random blood glucose in Wt mice, not in Tg mice. Glucose tolerance test showed liraglutide-treated Wt mice and vehicle-treated Tg mice improved in blood glucose AUC as compared with vehicle-treated Wt mice (p<0.05, p<0.001 respectively) and, however, there was no significant difference between vehicle-treated Tg mice and liraglutide-treated Tg mice. In immunohistochemistry analyses, there were tendencies to increase beta cell mass and proliferation in liraglutide-treated Wt mice and Tg mice
Conclusion: The effect of GLP-1 in HFD-fed Tg mice seems to be focal. These data suggest a possibility that GLP-1 receptor signal in beta cells might be at least partially mediated by small-Maf factors.

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Cannabinoid CB1 receptors and mTORC1 signalling pathway interact to modulate glucose homeostasis

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Background and aims: The endocannabinoid system is an inter-cellular signalling mechanism that has been described to play a role in the islets of Langerhans in the modulation of insulin secretion as well as beta cell mass expansion. CB1 antagonism has been reported to ameliorate the metabolic profile, including glucose homeostasis, in diabetic patients. However, the downstream signalling proteins mediating these effects are poorly understood. The mammalian target of rapamycin complex 1 (mTORC1) is a key intra-cellular signalling pathway involved in energy homeostasis and has an important role in pancreatic islet's physiology. Recent reports have described a functional interaction between both systems in the central nervous system, but this aspect is still unexplored in the endocrine pancreas.
Materials and methods: We explored the possible interaction between the ECs and mTORC1 signalling by pharmacologically manipulating the CB1 or mTORC1 pathway in C57Bl/6 mice and used mice genetically lacking the CB1 receptor or the downstream target of mTORC1, p70S6K1 protein. In vitro static secretion experiments on islets, western blotting and in vivo glucose tolerance tests were performed.
Results: CB1 antagonism decreased glucose-stimulated insulin secretion (GSIS) and specific pharmacological blockade of mTORC1 by rapamycin, as well as genetic deletion of p70S6K1 protein, impaired the CB1-mediated decrease in GSIS. Phosphorylation of p70S6K1 protein within islets was increased after CB1 antagonism. In vivo experiments showed that CB1 antagonism decreased insulin levels and induced glucose intolerance; this effect was prevented by i.p. injection of low doses of rapamycin not increasing per se plasma insulin levels.
Conclusion: These findings suggest a functional molecular interaction between the ECS and the mTORC1 pathway within the endocrine pancreas and at the whole body level, which could have implications for the development of new therapeutic approaches for pancreatic beta cell diseases.
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Angiogenic factors regulate beta cell function

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Background and aims: Angiogenic factors play an important role in organ vasculature during development, quiescence as well as pathological conditions. The highly vascularised islets of Langerhans are in a close network with the islet endothelial cell (EC), which regulates blood supply as well as islet endocrine function. The Angiopoietin-Tie system is one such family of angiogenic factors consisting of Angiopoietin-1 (Ang-1)-the agonist, expressed by perivascular cells and β-cells, Angiopoietin-2 (Ang-2)-the antagonist, expressed by endothelial cells as well as β-cells and their common receptor Tie-2 expressed by endothelial cells. In this study we hypothesized that reduced islet vessel density under diabetogenic conditions occurs together with changes in the Angiopoietin-Tie system, which directly regulates β-cell function.
Materials and methods: Islet vessel density was quantified by immunostaining islets with CD31, and EC marker and insulin. The vessel density was expressed as a ratio of CD31:Insulin area. To investigate the effect of Ang-2 on islet function, human and mouse islets and INS-1E -cells were treated with 0.2 ug to 0.8 ug/ml of recombinant Ang-2. After treatment duration of 2-4 days the islets were subject to a glucose stimulated insulin secretion assay (GSIS).
Results: Endothelial cell area was reduced under diabetogenic conditions with 61% reduction ($p>0.05$) in mouse islets and a 46% reduction in human islets in response to glucotoxicity (22.2 mM glucose/palmitate for 4 days). Ang-1 and Tie-2 mRNA levels were decreased by 70% and 80% respectively whereas there was a 3-fold induction in Ang-2 mRNA in treated human islets, similar changes in Ang-1, Tie-2 and Ang-2 also occurred in mouse islets isolated from high fat diet fed mice. Such increased Ang-2 levels led to direct impairment in β-cell function. Glucose stimulated insulin secretion (GSIS) was depleted in human and mouse islets amd INS-1E cells upon exposure to recombinant-Ang-2.
Conclusion: The Ang/Tie angiogenic system plays an important role in the regulation of islet function, with Angiopoietin-2 causing its impairment under diabetogenic conditions.
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PS 024 Beta cell signal transaction

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FFAR1 is involved in both the acute and chronic effects of palmitate on insulin secretion

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Background and aims: Free fatty acids (FFAs) have pleiotropic effects on pancreatic beta cells. While acute exposure to FFAs stimulates glucose-stimulated insulin secretion (GSIS), prolonged exposure impairs GSIS and causes apoptosis. FFAs exert their effects via intracellular metabolism and interaction with the free fatty acid receptor 1 (FFAR1/GPR40). Here, we studied the role of FFAR1 in the acute and long-term effects of palmitate on GSIS and insulin content in isolated human islets.
Materials and methods: The role of FFAR1 in the acute effect of palmitate on GSIS was investigated by perifusing human islets with 20 mM glucose with and without palmitate (0. 5 mM, 0.5% BSA) in the presence or absence of the FFAR1 agonist TAK-875 (2μM), or the antagonist ANT203 (2μM). The role of FFAR1 in the chronic effect of palmitate was addressed by culturing human islets with and without palmitate (0.5 mM, 0.5 % BSA) in the presence or absence of the FFAR1 agonist TAK-875 (2μM), or the antagonist ANT203 (2μM) for 7 days. After culture GSIS and insulin content were measured.
Results: Acute palmitate exposure potentiated GSIS ~3-fold, whereas addition of FFAR1 antagonist decreased this potentiation to ~2-fold ($p<0.05$ n=4). In the absence of palmitate, FFAR1 agonist caused only a ~1.4-fold increase in GSIS ($p<0.05$, n=4). Treatment with palmitate for 7 days decreased GSIS to 70 % and insulin content to 25 % of control level ($p<0.05$, n=5). These negative effects of long-term exposure to palmitate were ameliorated by FFAR1 inhibition ($P<0.05$, n=5) and further aggravated by additional stimulation of the receptor ($P<0.05$, n=3). In the absence of extracellularly applied palmitate long-term treatment with the agonist caused a modest increase in GSIS.
Conclusion: We conclude that FFAR1 plays a substantial role not only in the acute potentiation of GSIS by palmitate, but also in the negative long-term effects of palmitate on GSIS and insulin content.
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Inhibition of the mitogen activated protein kinase pathway accentuates the pro-apoptotic effect of fatty acids but does not alter the fatty acid-induced insulin secretion

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Background and aims: The mitogen activated protein kinase (MAPK) pathway is involved in a variety of physiological processes such as proliferation, differentiation and, more intriguingly, apoptosis. MAPK is activated by growth factors including IGF-1 and insulin but also by metabolites such as glucose and fatty acids. The role of the MAPK pathway in insulin secreting cells is controversially discussed. Both, pro- and anti-apoptotic effects have been shown upon inhibition of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2). Also, reduced ERK1/2 activity affects insulin secretion. The stimulation of ERK1/2 primarily results in an induction of transcription factors. The activation of early genes, c-fos and myc, induces cell cycle proteins, such as cyclin D and cyclin dependent kinases, and promotes cell cycle progression. Furthermore, stimulation of ERK1/2 controls neuronal differentiation through paired box protein PAX6, a transcription factor also essential for differentiation of pancreatic endocrine cells. This study aims to understand the role of ERK1/2 during acute and chronic exposure of insulin secreting cells to fatty acids.
Materials and methods: In isolated islets from C57BL/6 and free fatty acid receptor 1 (FFAR1) knockout mice as well as in INS-1E cells, ERK1/2 was stimulated with palmitic acid (600 μmol/l in the presence of 0.6% albumine or 50 μmol/l in the presence of 0.05% albumine); with oleic acid (50 μmol/l in the presence of 0.05% albumine) and with a specific FFAR1 agonist (TUG-469, 10 μmol/l in the presence of 0.05% albumine); and inhibited by PD98059 (10 μmol/l). Insulin secretion was assessed after static incubation of the cells by radioimmunoassay. Apoptotic cell death was assessed using the TUNEL assay. Activation of ERK1/2 was evaluated on Western blot using phospho-specific antibodies.
Results: Palmitic and oleic acid transiently induced phosphorylation of ERK1/2 in INS-1E cells. This effect did not depend on FFAR1 as palmitic acid increased phoshorylation of ERK1/2 in wild type as well as FFAR1-deficient islets. Furthermore the FFAR1 agonist TUG-469 did not mimic the effect of fatty acids. Palmitic acid induced beta cell death, but oleic acid and TUG-469 did not. Although the ERK1/2 inhibitor PD98059 was neither apoptotic under control culture conditions, i.e. in the presence of 11 mmol/l glucose nor in the presence of TUG-469, it augmented palmitic acid-induced cell death. When insulin secretion was examined, inhibition of ERK1/2 by PD98059 did not affect basal secretion (i.e. at 2.8 mmol/l glucose) but significantly increased (by 50%) glucose-induced insulin secretion. PD98059 did not influence secretion in the presence of palmitic and oleic acid. Both fatty acids augmented 2-fold glucose (12 mmol/l)-induced secretion but had no effect at 2.8 mmol/l glucose.
Conclusion: These observations suggest that FFAR1 does not mediate fatty acid-dependent stimulation of ERK1/2. Inhibition of ERK1/2 augments fatty acid-induced cell death but does not affect fatty acid-induced insulin secretion, suggesting that ERK1/2 exerts primarily anti-apoptotic effects in beta cells.
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Prolonged exposure to palmitate deteriorates glucose-induced cAMP generation and pulsatile insulin secretion

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Background and aims: Type 2 diabetes is often associated with increased plasma concentrations of free fatty acids. Indeed, prolonged exposure of β-cells to the fatty acid palmitate induces lipotoxic effects with deterioration of glucose-stimulated insulin secretion, but the underlying mechanisms have not been fully clarified. Insulin secretion is triggered by a rise of the cytoplasmic Ca2+ concentration ([Ca2+]i) and is further amplified by elevation of cAMP. Glucose induces synchronous oscillations of [Ca2+]i and the subplasma membrane cAMP concentration ([cAMP]pm) in β-cells, which result in pulsatile insulin secretion. The aim of the present study was to investigate how [Ca2+]i and [cAMP]pm signalling in β-cells and insulin secretion are affected by long-term exposure to palmitate.
Materials and methods: Islets isolated from C57Bl mice were pre-incubated with 0.5 mM palmitate in 1% BSA for 48 hours. [Ca2+]i was measured with Fura-PE3 and epifluorescence microscopy. A translocation biosensor based on fluorescence-tagged subunits of protein kinase A was used to monitor [cAMP]pm in β-cells within intact islets. The time-course of insulin secretion was monitored using a translocation sensor for PtdIns(3,4,5)P3, a lipid formed in the β-cell plasma membrane following autocrine insulin receptor activation. Changes in localization of the reporters were detected with total internal reflection fluorescence microscopy. Insulin-secreting β-cells were identified by their large size and [cAMP]pm-lowering effect of adrenaline.
Results: In control β-cells, an increase of glucose from 3 to 20 mM induced a pronounced [cAMP]pm elevation, often with oscillations (frequency 0.09-0.27/min) whereas addition of 100 nM GLP-1 triggered stable [cAMP]pm increase. After 48 h exposure to palmitate, 91% of the β-cells failed to respond to glucose with rise of [cAMP]pm and the remaining cells showed only a modest and transient [cAMP]pm elevation. In contrast, the GLP-1-induced [cAMP]pm response was unaffected by palmitate treatment (amplitude 0.46±0.12 ratio units vs 0.38±0.04 in control). Basal [Ca2+]i in the presence of 3 mM glucose was slightly elevated after palmitate exposure (67±1 nM vs 48±0.8 nM in control; $P<0.05$), but the fatty acid did not influence the amplitude of the [Ca2+]i oscillations induced by 20 mM glucose (130±11 nM vs 132±10 nM in control) or the [Ca2+]i elevation triggered by 30 mM K+ (209±11 nM vs 220±22 nM in control). ¬¬¬¬Control islet cells responded to 20 mM glucose with pronounced PtdIns(3,4,5)P3 oscillations, reflecting pulsatile insulin secretion. This glucose-induced PtdIns(3,4,5)P3 response was lost in 93% of the palmitate-treated cells, and the remaining cells showed only a transient increase or minute stable elevation of PtdIns(3,4,5)P3.
Conclusion: Prolonged exposure of mouse islets to palmitate results in deterioration of glucose-stimulated insulin secretion with loss of pulsatility. While [Ca2+]i signalling is essentially unaffected, the secretory defect is associated with impaired generation of cAMP.
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Acute exposure of beta cells to low concentrations of IL-1beta improves insulin secretion through focal adhesion and actomyosin remodelling

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Background and aims: Defective insulin secretion in type 2 diabetes is associated with low-grade systemic and islet inflammation governed in part by IL-1beta. This cytokine is known to exert both negative and positive effects on beta cells. Numerous publications have documented the deleterious effects on beta cell function and survival of longer term (>24h) exposure to a high concentration (20ng/ml) of IL-1beta. While exposure to much lower concentrations of IL-1beta for >24h has been shown to improve beta cell function and survival, the acute effects of low concentrations of IL-1beta has been less well studied. We have recently demonstrated that focal adhesion (FA) and actomyosin II remodelling, and activation of the ERK1/2 signalling pathway occur rapidly in beta cells after glucose stimulation; this is crucial for insulin granule recruitment to the basal membrane. We hypothesized that IL-1beta might modulate these dynamic pathways leading to glucose-induced insulin secretion.
Materials and methods: All studies were performed using sorted rat primary beta cells cultured in monolayer on extracellular matrix. Beta cells were exposed to IL-1beta and insulin secretion (radioimmunoassay) measured during 1h at low glucose (2.8mmol/l), followed by 1h at high glucose (16.7mmol/l). Protein levels and phosphorylation were determined by western blot following 2h at low glucose and 10 min at high, in the continued presence of IL-1beta. Immunofluorescence was used to study dynamic changes in localization and appearance of FA proteins, focal adhesion kinase (FAK) and paxillin (PAX), and actin cytoskeleton. Data are mean±SE (n=3 unless mentioned otherwise).
Results: A low concentration of IL-1beta (0.1 ng/ml) for 2h increased glucose-stimulated insulin secretion (GSIS) by 42.6 ± 7.6% (p=0.028) whereas 20 ng/ml was without significant effect. Glucose stimulation led to remodelling of actin into filaments, with FAK and PAX phosphorylation and their localization in small newly formed FA. Presence of 0.1 ng/ml IL-1beta increased the length of FAs at the basal membrane measuring >2.5μm by 47.7 ± 16.6% (p=0.015) and glucose-induced phosphorylation of PAX and ERK1/2 by 39.8 ± 13.1% (p=0.031) and 37.06 ± 11.8% (p=0.024) respectively. By contrast, 20 ng/ml IL-1beta had no effect on glucose induced FA remodelling or protein phosphorylation. In order to determine whether 0.1 ng/ml IL-1beta also affects basal insulin secretion, beta cells were treated for 2h with the cytokine at 2.8mmol/l glucose. This led to a 19.8 ± 8.8% decrease in (basal) secretion (n=2) associated with actin restructuring into thick bundles.
Conclusion: Acute exposure of beta cells to 0.1 ng/ml IL-1beta improves beta cell function. The increase in GSIS was accompanied by changes in focal adhesion remodelling and intracellular signalling previously shown to be critical for insulin secretion. It is concluded that low-dose IL-1beta can act rapidly, directly and positively on insulin secretion through FA and actin remodelling.
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Relationship between islet angiogenesis and the positive effect of islet neogenesis-associated protein pentadecapeptide (INGAP-PP) upon beta cell function

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Background and aims: Islet neogenesis-associated protein pentadecapeptide (INGAP-PP) administration to normal rats increases their β-cell mass and function, but its mechanism of action remains unclear. On the other hand, embryonic β-cell differentiation needs the presence of endothelial cells, the vascular endothelial growth factor-A (VEGF-A) and integrin β. The aim of the present work was to study whether the effect of INGAP-PP upon β-cell mass and function is associated to a similar effect upon islet angiogenesis.
Materials and methods: Adult male Wistar rats were intraperitoneally injected for 10 days with saline solution (control group, C) or INGAP-PP (500 μg/day; intervention Group, I). At the end of the treatment period, glucose tolerance test (GTT) was performed in some animals from both groups while other rats were sacrificied and bled to measure plasma glucose, insulin and trilgliceryde (TG) concentration. Homeostatic model assessment-insulin resistance (HOMA-IR) and - β cell function (HOMA- β) were also calculated. Glucose-stimulated insulin secretion (GSIS), DNA content and levels of gene expression (qPCR and western blot) of Pancreatic and duodenal homeobox 1 (Pdx1), neurogenin 3, integrin β1, VEGF-A, VEGF-Receptor 2 (VEGF-R2), laminin β1 and insulin were measured on islets isolated from both experimental groups. Statistical data analysis was performed using the Students T-test and differences were considered significant when P value <0.05 (*).
Results: INGAP-PP did not affect significantly GTT (area under the curve: 2898 ± 649 vs. 2854 ± 525 mmol/L/120 min), plasma glucose (107 ± 2.5 vs. 113 ± 2.8 mg/dl), TG (122.6 ± 8.8 vs. 115.4 ± 7.9 mg/dl) and insulin (0.57 ± 0.08 vs. 0.63 ± 0.07 ng/ml) levels. Consequently, it did not affect HOMA-IR (4.01 ± 0.5 vs. 4.06 ± 0.6) and HOMA- β (52.4 ± 6.2 vs. 41.7 ± 7.1) values. However, I rats showed less islet DNA content than C animals (26 ± 1.3 vs. 18 ± 1.2* ng/islet) and higher GSIS at 16.7 mM glucose (10 ± 0.1 vs. 20 ± 0.1* ng ins/pg DNA). Their islets also showed increased mRNA levels of PDX1 (100 ± 1.82 vs. 221 ± 77* %), insulin (100 ± 0.09 vs. 134 ± 21* %), integrin β1 (100 ± 3.15 vs. 698 ± 29* %), laminin β1 (100 ± 3.05 vs. 201 ± 72* %) and VEGF-A (100 ± 0.22 vs. 149 ± 34* %) as well as protein concentration of integrin β1 (100 ± 28 vs. 191.9 ± 19.5* %) and VEGF-A (100 ± 16.78 vs. 154.79 ± 22.5* %).
Conclusion: These results suggest that the mechanism by which INGAP-PP exerts its effect upon β-cell mass and function in adult rats, mimics the normal process observed during the embryonic period where vascular neogenesis preceeds islet neogenesis.
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INGAP effect on pancreatic islet function: its potential molecular mechanism

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Background and aims: Islet neogenesis associated protein (INGAP-PP) increases insulin secretion and B-cell mass in normal rats but it is not clear the mechanism by which produces its effects. Thus, the aim of the present work is to study *in vitro* the effect of INGAP-PP on insulin secretion and on several enzymes and mediators involved in glucose metabolism and in PI3-K/Akt pathway.
Materials and methods: Islets isolated by collagenase digestion from adult normal rats were cultured for 4 days in RPMI with 2 g/L $NaHCO_3$, 5% FBS, 1% penicillin/streptomycin and 10 mM glucose (G), in the absence (controls) or presence of 10 μg/mL INGAP-PP, with/without addition of Wortmannin (150 and 300 nM), or LY294002 (10 and 25 mM) to the medium. Islets were preincubated in 3 mM G for 45 min and then incubated with different glucose concentrations to study: insulin secretion/content (radioimmunoassay), HK/GK activity (G-6-P production), glucose oxidation and utilization ($^{14}CO_2$ and ^3H_2O production, respectively), GK, IR, IRS-1/2 and PI3K quantification (Western blotting) and PI3K/IRS-1 association (immuneprecipitation and Western blotting). Results were statistically analyzed using ANOVA and Student´s t-test for independent samples.
Results: (C vs. INGAP-PP; *p<0.05): Insulin secretion (ng/islet/h): 3 mM G: 0.3 ± 0.03 vs. 0.3 ± 0.04; 8 mM G: 1.6 ± 0.2 vs. 2.4 ± 0.3*; 16 mM G: 2.4 ± 0.2 vs. 4.7 ± 0.3*; 16 mM +W 150 nM: 1.7 ± 0.3 vs. 1.8 ± 0.3; 16 mM G + W 300 nM: 0.9 ± 0.1 vs. 0.9 ± 0.3 (% inhibition respect to 16 mM G: C, 64 ± 6 and INGAP-PP, 81 ± 6*). Glucose oxidation $^{14}CO_2$ (pmol/ islet/ 120 min): 3 mM G: 0.3 ± 0.04 vs. 0.5 ± 0.07; 8 mM G: 0.4 ± 0.06 vs. 0.7 ± 0.1*; 16 mM G: 0.6 ± 0.1 vs. 1.4 ± 0.1*. Glucose utilization ^3H_2O (pmol/ islet/ 120 min): 3 mM G: 1.3 ± 0.2 vs. 1.8 ± 0.3; 8 mM G: 1.9 ± 0.3 vs. 3.2 ± 0.4*; 16 mM G: 3.3 ± 0.4 vs. 5.8 ± 0.6*. GK activity (pmol/islet/h): 2.4 ± 0.6 vs. 4.8 ± 0.3*. Protein levels (INGAP-PP % above control): GK 91.7 ± 3.8*; IR 75 ± 1.5*; IRS-2 170.8 ± 4.7*; PI3K 155 ± 15*; PI3K-IRS-1 complex 76 ± 5*; pIR 149 ± 5.6*; pIRS-1 138 ± 2.6*. Wortmannin and LY294002 significantly decreased the INGAP-PP-induced increase of GK protein concentration to levels similar to those measured in C.
Conclusion: Our data suggest that the positive modulatory effect of INGAP-PP on insulin release could be partly ascribed to its effect upon GK activity and glucose metabolism and it is mainly mediated by the PI3K/AKT pathway. All these INGAP-PP-mediated changes would elicit a more robust B-cell secretory function and result in a more sensitive B-cell to extracellular regulators such as insulin (increased protein IR level and phosphorylation). These results should be further investigated to prove the potential use of INGAP-PP to maintain/enhance the secretory capacity of cultured islets previous to

their transplant and also as an alternative therapy for diabetes prevention and treatment.
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Increased hexosamine biosynthetic pathway flux alters cell-cell adhesion in INS-1E cells and murine islets

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Background and aims: In type 2 Diabetes, beta cell failure is caused by loss of cell mass, mostly by apoptosis, but also by simple dysfunction (decline of glucose-stimulated insulin secretion, downregulation of specific gene expression). Apoptosis and dysfunction are caused, at least in part, by lipoglucotoxicity, in which increased flux of glucose in the hexosamine biosynthetic pathway (HBP) plays a role. In this study we sought to clarify whether increased HBP flux affects beta cell-beta cell adhesion and if this may participate in beta cell dysfunction and altered islet physiology.
Materials and methods: We used INS-1E cells and ex vivo murine islets. INS-1E cells and murine islets were subjected to glucosamine (GlcN) (5 and 7.5 mM) and high glucose (20 and 30 mM) treatments to increase the HBP flux. E-cadherin and beta-catenin expression was evaluated by WB, their intracellular localization by confocal immunofluorescence, Trypsin-Ca2+/Trypsin-EGTA digestions and sub-cellular fractionation. E-cadherin assembly into adherens junctions by TX-100 solubility assay. Cell-cell adhesion was evaluated by morphological examination of isolated and treated murine islets.
Results: We focused on cell-cell homotypic interactions. E-cadherin expression was not changed by increased HBP flux, however, there was a decrease of cell surface, and an increase in intracellular E-cadherin, detected by Trypsin-Ca2+/Trypsin-EGTA digestions (intracellular E-cadherin increased from 6±;1.8% to 18±;4% following a treatment of 7.5 mM GlcN for 48 hrs) and a change of intracellular E-cadherin distribution, from a prevalent Golgi to a prevalent endoplasmic reticulum distribution, detected by confocal immunofluorescence. Beta-catenin alterations were found to parallel those of E-cadherin, showing a dislocation from the plasmamembrane (where beta-catenin is bound to E-cadherin) to the cytosol, detected by confocal immunofluorescence and sub-cellular fractionation (cytosolic beta-catenin increased from undetectable levels to 16±;3.7% following a treatment of 7.5 mM GlcN for 48 hrs). Moreover, following increased HBP flux, there was a decrease in E-cadherin found in the Triton X (TX)-100-insoluble cell fractions, suggesting a defect of the assembly of E-cadherin into adherens junctions (a treatment of 7.5 mM GlcN for 48 hrs, in cells 50% confluent, decreased insoluble E-cadherin from 41±;6% to 26±;4% and, in cells 80% confluent, decreased insoluble E-cadherin from 76Ã‘±;8% to 28±;4%). Lastly, we measured cell-cell adhesion, by morphological examination of murine islets. Following increased HBP flux by both GlcN and high glucose treatments, there was a disaggregation of islet structure.
Conclusion: These results suggest that increased HBP flux changes the intracellular distribution of E-cadherin and beta-catenin and the ability of E-cadherin to assemble into adherens junctions. These changes were accompanied by decreased cell-cell adhesion, which may easily impact on beta cell function.
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Effects of the inhibitor of anoctamin 1, tannic acid, on insulin-producing cells

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Background and aims: We recently reported that anoctamin 1 mRNA and protein are expressed in human, rat and mouse insulin-producing cells, and proposed that anoctamin 1 acts as a volume-regulated anion channel in these cells. The present study concerns further investigations on the effects of the inhibitor of anoctamin 1, tannic acid, on insulin-producing cells.
Materials and methods: Cell volume was measured in BRIN-BD11 cells and dispersed rat islet cells. Insulin release, D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation were measured in rat pancreatic islets over 90 min incubation at 37°C. Voltage measurements were performed in zero-current nystatin-perforated patch-clamp experiments conducted in mice pancreatic islets.
Results: In both BRIN-BD11 and dispersed rat islet cells, tannic acid (100 μM) suppressed the regulatory volume decrease otherwise occurring during exposure to a hypotonic extracellular medium. Tannic acid (10 to 100 μM) inhibited insulin release evoked by 16.7 mM glucose, with a threshold value close to 3 μM, an ED_{50} close to 57 μM and a mean decrease of 43 ± 4% at a 100 μM concentration. However, in the presence of 8.3 mM glucose, tannic acid (100 μM) did not affect significantly insulin output. In islets exposed to 16.7 mM glucose, tannic acid (100 μM) decreased D-[5-^{3}H]glucose utilization by 45 ± 7%, whilst increasing D-[U-^{14}C]glucose oxidation by 40 ± 12%. Assuming that the difference between D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation corresponds to the production of lactic acid, the ATP generation attributable to the catabolism of glucose was virtually identical in the absence of tannic acid (995 ± 98 pmol/islet) and in its presence (998 ± 77 pmol/islet). As judged from the changes provoked by tannic acid (100 μM) in the duration, action potential height and action potential rate of the active bioelectrical phase in mice islets exposed to 16.7 mM glucose and assuming that the action potential height is proportional to the amount of Ca^{2+} entering into cells, it was calculated that the influx of Ca^{2+} only represented in the presence of tannic acid about 44% of the control value found in its absence.
Conclusion: These findings support the view that inhibition of anoctamin 1 by tannic acid decreases in islet cells both lactate output and Ca^{2+} influx independently of any change in the energy yield resulting from glucose catabolism, with a prevalent inhibition of insulin release at high concentrations of the hexose.
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Sphingosine kinase 1 interacting protein (SKIP) inhibits insulin secretion from beta cells through regulation of protein kinase A activity

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Background and aims: Compartmentalization of PKA (protein kinase A) is mediated by A-kinase anchoring proteins (AKAPs). The AKAP family consists of over 70 molecules and most AKAPs bind RII subunit of PKA. Several dual-function AKAPs can bind both RI and RII subunits; SKIP (sphingosine kinase 1 interacting protein, also called Sphkap) and MGC13057 are AKAPs that tether only PKA-RI subunit in human. PKA has been shown to be important in the insulin secretory process. Recent study on PKA-RI subunit-deleted mice reported a dramatic increase in glucose-stimulated and exendin-4-enhanced insulin secretion from beta cells. Because our preliminary data showed that SKIP is highly expressed in beta cells, we examined whether SKIP is involved in glucose-stimulated and exendin-4-enhnaced insulin secretion.
Materials and methods: Endogenous SKIP expression was detected by quantitative real time PCR, western blot analysis, and immunohistochemiscal study in several mouse and rat beta cell lines and islets isolated from Wister rats, C57BL/6 mice, and human. SKIP and PKA-RI interaction was confirmed by immunoprecipitation. Glucose-stimulated and exendin-4-enhanced insulin secretion was examined in SKIP-overexpressed and knock down INS-1D cells, a rat beta cell line, using SKIP expression vector or SKIP siRNAs, respectively. PKA activity was measured by PepTag assay for non-radioactive detection of PKA. Phosphorylation of CREB was detected by western blot analysis.
Results: Quantitative real time PCR showed that SKIP was highly expressed in beta cells, weakly expressed in heart and spinal cord, and was not detected in kidney, liver, gastrointestinal tract, testis, and skeletal muscle. Western blot showed that SKIP was strongly expressed in beta cells but not in spinal cord, heart or the other tissues. Immunohistochemical study revealed that SKIP was co-localized with insulin in INS-1D cells and was localized in pancreatic beta cells and not in alpha-cells of human, Wistar rat, and C57BL/6 mice. Immunoprecipitation showed that SKIP interacted with PKA-RI subunit but not with PKA-RII subunit in SKIP-overexpressed HEK293 and INS-1D cells. Overexpression of SKIP decreased PKA activity and CREB phosphorylation, while knock down of SKIP increased PKA activity and CREB phosphorylation in INS-1D cells. Overexpression of SKIP decreased glucose-stimulated insulin secretion by 60% ($P<0.05$) and exendin-4-enhanced insulin secretion

by 66% (p<0.05). In contrast, knock down of endogenous SKIP resulted in a 1.8-fold increase in glucose-stimulated (P<0.05) and a 1.3-fold increase in exendin-4-enhanced insulin secretion (P<0.05). However, insulin content did not differ between controls and SKIP-overexpressed or SKIP-knocked down INS-ID cells.
Conclusion: SKIP is specifically expressed in pancreatic beta cells. This molecule binds only PKA-RI subunit and modulates PKA activity. Overexpression of SKIP inhibited glucose-stimulated and exendine-4 enhanced insulin secretion, while knock down of SKIP increased glucose-stimulated and exendine-4-enhanced insulin secretion. These results demonstrate that SKIP inhibits insulin secretion through regulating PKA and CREB activity.
Supported by: MEXT

PS 025 Insulin secretion in vitro

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Effects of high extracellular KCl on insulin secretion beyond plasma membrane depolarisation
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Background and aims: The depolarization of the beta cell plasma membrane by a high extracellular K+ concentration stimulates insulin secretion without a preceding increase of the energy metabolism or changes in the KATP channel open probability. Also, the depolarizing strength can be predicted by the Goldman-Hodkin-Katz equation and, in contrast to depolarizing KATP channel blockers, the involvement of intracellular binding sites can be ruled out. Recently, however, the question arose as to whether the consequences of K+ depolarization differ from those of depolarization by KATP channel closure as occurs during glucose-induced insulin secretion.
Materials and methods: Membrane potentials and currents of isolated NMRI mouse beta cells were measured in the perforated patch mode of patch-clamp technique. The cytosolic Ca2+ concentration ([Ca2+]i) was measured using Fura-2/AM-loaded NMRI mouse islets. Insulin secretion was measured by batch perifusion of freshly isolated pancreatic islets.
Results: Using a voltage-clamp protocol, action currents were elicited in the presence of maximally effective concentrations of sulfonylurea and TEA to block all K+ conductance. These action currents were abolished by 10 μM nifedipine, demonstrating that the depolarization-induced action currents represent Ca2+ influx via L-type channels. Using the same protocol and raising the extracellular K+ concentration from physiological to 40 mM transformed the action current pattern into a continuous inward current after a short period of broadened action currents. This current could be abolished by 2.5 mM of the unspecific Ca2+ channel blocker CoCl2, partially reduced by 50 μM of the blocker of L-type Ca2+ channels, D600, but not by nifedipine. When the extracellular K+ concentration was only raised to 15 mM, the occurrence of significantly broadened action currents persisted. These currents could be completely antagonized by CoCl2 or nifedipine. To test the hypothesis that, in spite of the presence of a sulfonylurea and TEA, K+ currents are involved in the derangement of Ca2+ influx, the effects of 35 mM CsCl and 35 mM RbCl (each in addition to the physiological K+ concentration) were characterized. Rb+ is known to have practically the same permeability as K+, whereas that of Cs+ is only about 30%. In fact, Rb+ depolarized the beta cell like 40 mM K+, whereas the depolarization by Cs+ corresponded to that by 15 mM K+. Under voltage-clamp condition, Rb+ led to the same continuous inward current as 40 mM K+, whereas Cs+ produced broadened action current similar to 15 mM K+. Both, 40 mM K+ and 15 mM K+ were able to increase [Ca2+]i beyond the level established by a maximal sulfonylurea concentration. In both cases this increase correlated with a strong increase in secretion. The striking discrepancy between the near inability of 15 mM K+ to stimulate insulin secretion under basal condition and the ability to strongly augment the secretion by a maximal sulfonylurea concentration was also noted with 35 mM Cs+.
Conclusion: The presence of a high extracellular K+ concentration does not only depolarize the beta cell plasma membrane but also modifies the resultant activity of voltage-gated Ca2+ channels. Prolongation of the channel openings would explain the minimal insulinotropic property of 15 mM K+ under basal condition and the strong augmentation of secretion under depolarized condition.
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Differential role of NO in the control of insulin secretion in human pancreatic islets
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Background and aims: We have previously shown that rat pancreatic β-cells express an isoform of neuronal NO synthase (nNOS) that controls insulin

secretion through two catalytic activities: NO production and cytochrome c reduction. In humans, we also found the expression of nNOS in pancreatic β-cells. In the present study, we investigated whether nNOS, as observed in rats, is able to modulated insulin secretion in human pancreatic islets.
Materials and methods: Human islets were obtained from brain dead donors in the frame of the Gragil network (Geneva) and the IRB's isolation platform (Montpellier). Insulin secretion in response to glucose (2.8, 8.3, and 16.7 mM) was evaluated on pools of 10 islets during 90 minutes in the presence or not of pharmacological inhibitors of nNOS, Nω-nitro-L-arginine methyl ester (L-NAME) and 7-nitroindazole (7-NI), the NO donors, sodium nitroprusside (SNP) and hydroxylamine, and the scavenger of peroxynitrites, uric acid.
Results: Inhibition of nNOS by 10 mM L-NAME increased insulin secretion induced by 8.3 and 16.7 mM glucose (+35% et +33% respectively, P<0,001). In the presence of 100 μM 7-NI, a more selective inhibitor of nNOS, insulin secretion was stimulated by 20% in the presence of 8.3 mM glucose (P<0,01) and by 21% in the presence of 16.7 mM glucose (P<0,001). A substitutive treatment by the exogenous NO donor SNP (300 μM) was unable to normalize the amplificatory effect of L-NAME. At the opposite, SNP strongly stimulated insulin secretion induced by glucose + L-NAME (+76% with 8.3 mM and +70% with 16.7 mM glucose versus L-NAME alone, P<0,001), as well as that of glucose alone (+98% with 8.3 mM and +108% with 16.7 mM glucose, P<0,001). Moreover, hydroxylamine (300 μM), an endogenous NO donor, led to similar effects as SNP, as insulin secretion was increased in the presence of glucose + L-NAME (+43% with 8.3 mM and +57% with 16.7 mM glucose versus L-NAME alone, P<0,001) or glucose alone (+60% with 8.3 mM and +86% with 16.7 mM glucose, P<0,001). Conversely, we could demonstrate that uric acid (300 μM), a scavenger of peroxynitrites, was able to normalize the stimulating effect of SNP, with or without L-NAME, in the presence of 8.3 mM and partially in the presence of 16.7 mM glucose.
Conclusion: In human pancreatic islets, low amounts of NO modulate negatively insulin secretion, through S-nitrosylation of proteins involved in the metabolism, whereas, at higher concentrations, NO amplifies insulin secretion, probably via nitration of exocytotic proteins.
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Regulation of miR-335 expression contributes to orchestrate insulin secretion at the level of exocytosis in insulin secreting INS-1 832/13 cells
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Background and aims: MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression. Many miRNAs are implicated in insulin secretion including the well-established miR-375. We have previously identified a number of miRNAs with dysregulated expression in the pancreatic islets of non-obese type-2 diabetes (T2D) model Goto-Kakizaki (GK) rats as well as in human islets from glucose intolerant donors. Among the up-regulated miRNAs in the GK-rat was miR-335 which we showed to target Munc18-1. The regulation of miRNAs is still elusive, but there are indications that some miRNAs are glucose-regulated. Here we aim to investigate the glucose- and time-dependent regulation of miR-335 and miR-375, and the influence on exocytosis and insulin secretion by these miRNAs.
Materials and methods: INS-1 832/13 cells were cultured for 1, 2, 6, 12 and 24 h and at different glucose concentrations (2.8, 11 and 16.7 mM) or transfected with LNA (locked nucleic acid)-based anti-mir-335 or anti-mir-375 for 48 h. MicroRNA and mRNA expression was analyzed by qPCR. Glucose stimulated insulin secretion (GSIS) was measured by RIA and exocytosis was detected as changes in membrane capacitance using patch-clamp.
Results: In 2.8 mM glucose miR-335 expression was significantly higher when cultured for ≥ 2h relative to 1 h (n=3; p<0.05). At higher glucose concentrations this was valid for 12 and 24 h culture in 11 mM glucose (n=3; p<0.05) and for 6 and 12 h in 16.7 mM glucose (n=3; p<0.05). Interestingly, peak expression was reached earlier when cultured at 16.7 mM glucose (after 6 h) as compared to 2.8 mM glucose (after 12 h). This was accompanied with a reduced glucose stimulated insulin secretion after culture for 2 and 6 h in 2.8 mM glucose (n=3; p<0.01). Expression of the target gene S*txbp1* (coding for Munc 18-1) was reciprocally expressed except after culture for 24 h. On the other hand, expression of miR-375 was stable and did not vary with time at the different glucose concentrations. Furthermore, it was confirmed that culture in 16.7 mM glucose impairs GSIS. This effect was visible already after 1 h in culture. To further characterize the role of miR-335 and miR-375 in regulated exocytosis, we performed electrophysiological measurements on cells transfected with anti-miR-335 and anti-miR-375, respectively. Reduction of miR-335 levels in the knockdown experiments lead to Munc18-1 up-regulation. Exocytosis initiated by a train of ten 500-ms depolarizations from -70 to 0 mV was significantly increased by ~50% (n=8-10 cells; p<0.01) in the anti-miR-335 transfected cells, but there was no significant change in exocytotic response in the anti-miR-375 transfected cells. There were no changes in the voltage-dependent Ca^{2+} current after transfection with either of the anti-miRs.
Conclusion: We conclude that miR-335 influence exocytosis through the regulation of Munc18-1, and that low expression of this miRNA appears beneficial for enhanced exocytosis. The time shift in peak miR-335 expression suggests involvement of this miRNA in acceleration of impaired insulin secretion at hyperglycaemia.
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Involvement of heparan sulfate proteoglycan syndecan-4 in the mouse beta cell proliferation and insulin secretion
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Background and aims: Heparan sulfate proteoglycans (HSPGs) are composed of a core protein to which extracellular glycosaminoglycan chains are attached. Syndecans, one of the major heparin sulfate (HS)-containing core proteins, are distributed on the cell surface, where it interacts with various proteins, including growth factors, morphogens and extracellular matrix components to regulate their signalling. We recently found that HS is localized exclusively around beta cells in the islets of adult mice and is required for islet morphogenesis, beta cell proliferation and insulin secretion. Furthermore, we found that the 3-*O*-sulfate and 6-*O*-sulfate groups of HS are important for maintaining normal glucose-induced insulin secretion (GIIS) and beta cell proliferation, respectively. So far it is not known, however, which core proteins are crucial in this process and how they function to modulate beta cell function. The aim of this study is to clarify how the core protein including HS affects beta cell proliferation and insulin secretion.
Materials and methods: Subcloning of MIN6 cells, mouse pancreatic beta cell line, was performed by the limiting dilution method. The cells were then screened and selected by an index of GIIS, KCl-induced insulin secretion and HS expression. The expression of HS was analysed by western blot analysis. Semi-quantitative RT-PCR was used for analyzing mRNA expression of HS biosynthetic enzymes and HSPG core proteins. Expression of HSPG syndecan-4 was silenced, and cell proliferation and GIIS were examined in T3, insulin-secreting and HS-expressing cell line derived from the MIN6.
Results: MIN6 cells were subcloned, and 30 clones were obtained. Four sublines were selected for this study, designated T3, T5, T9 and T16. T3, T5 and T9 cells exhibit GIIS in a concentration-dependent manner, whereas T16 cells respond poorly to glucose. In the presence of KCl, T16 cells secreted almost the same amount of insulin as T3, T5 and T9. Next, we analysed the expression of HS in these subclones. HS was detected in T3, T5 and T9 cells. On the other hand, HS was not detected in T16 cells. The HS biosynthetic enzymes and HSPG core proteins such as Ext/Extl gene family, *N*-deacetylase/*N*-sulfotransferases, C5-epimerase, HS sulfotransferases, cell surface and secreted HSPG core proteins were almost expressed in all subclones, but syndecan-4, one of cell surface HSPG core proteins, was not expressed only in T16 cells. Silencing of a syndecan-4 by RNA interference reduced T3 cell proliferation to 85% of control treatment (p<0.005). Furthermore, GIIS was decreased in T3 cells with RNA interference of syndecan-4 to 52% of the control treatment (p<0.001).
Conclusion: Our data indicate that HSPG syndecan-4 plays important role(s) in the beta cell proliferation and insulin secretion.

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Flavanol metabolites produced by colonic microbiota improve glucose-stimulated insulin secretion and protect pancreatic beta cells against oxidative damage

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Background and aims: Oxidative stress is accepted as one of the causes of beta cell failure commonly observed in type 2 diabetes. Therefore, identification of natural antioxidant agents that enhance or preserve islet beta cell mass and function is considered an interesting strategy to prevent or treat diabetes. Dietary flavanols are natural occurring compounds abundant in fruits and vegetables. Oligomeric and polymeric flavanols are metabolised by the intestinal microbiota into various phenolic acids of low molecular weight, which are well absorbed in the colon. Recent evidences indicated that flavanol metabolites could possess biological properties and potential beneficial effects on various tissues. However, up to date, whether these compounds have a direct effect on pancreatic beta cells is unclear. The aim of this work was to establish the potential anti-diabetic properties of the microbial-derived flavanol metabolites 3,4-dihydroxyphenylacetic acid (DHPA), 2,3-dihydroxybenzoic acid (DHB) and 3-hydroxyphenylpropionic acid (HPP). To this end, we tested their ability to influence pancreatic beta cell function and to protect against oxidative stress-induced beta cell damage.
Material and methods: INS-1E beta cells and rat islets (isolated by collagenase digestion) were treated for 20h with physiological concentrations of DHPA, HPP and DHB (1-10 μM). Insulin content and insulin secretion in response to metabolites and metabolites plus glucose (4 and 10 mM) were quantified using an ELISA kit. Cellular signalling related to the effect of metabolites on glucose-stimulated insulin secretion (GSIS) was examined using specific inhibitors. Levels of the main protein kinases activated by metabolites were evaluated by Western Blot. In oxidative stress experiments, INS-1E cells were treated for 20h with the different phenolic compounds (5 μM) and further exposed to the pro-oxidant *tert*-butyl hydroperoxide (t-BOOH, 50 μM) for 2h. Generation of reactive oxygen species and carbonyl levels were evaluated by fluorimetric methods and cell viability by the crystal violet assay.
Results: We found that DHPA and HPP (but not DHB) amplified GSIS both in INS-1E beta cells and pancreatic islets without affecting insulin content. Experiments with pharmacological inhibitors suggest that enhancement of GSIS by DHPA and HPP is mediated by the activation of protein kinase C and ERK/MAPKs but is independent on the protein kinase A pathway. Furthermore, pre-treatment of cells with DHPA and HPP, and to a lesser extend DHB, was able to protect against beta cell dysfunction and death induced by the potent pro-oxidant t-BOOH.
Conclusion: These results suggest that the microbial-derived flavanol metabolites DHPA and HPP may have anti-diabetic potential by promoting survival and function of pancreatic beta cells. Further studies to evaluate their action *in vivo* are required.
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Insulin secretion and ZnT8 gene expression are decreased by ZnT3 overexpression

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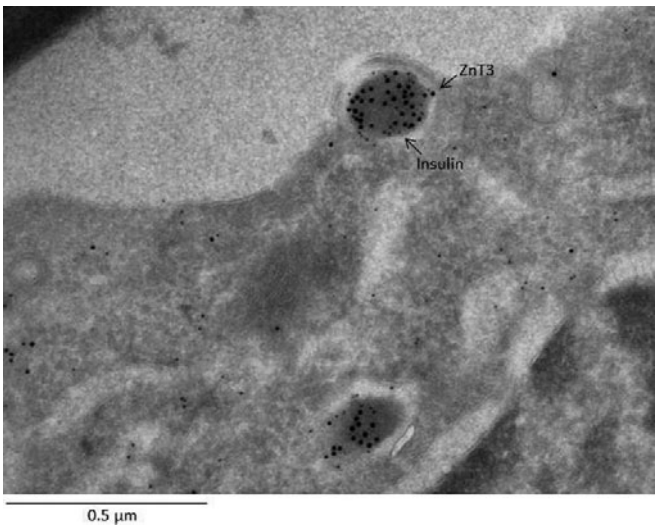
Background and aims: Insulin synthesis and processing rely on the presence of zinc. Zinc is abundantly present in pancreatic beta cells. The transport of zinc into beta cells is regulated by zinc transporting proteins (ZnTs). ZnT8 transport zinc into the insulin containing granules. Beta cell specific ZnT8 knockout mice show decreased insulin content, atypical insulin crystallization and reduced first phase insulin secretion. We have previously shown a transcriptional inverse correlation between ZnT8 and another zinc transporter protein, ZnT3. We found that ZnT3 knock down leads to a transcriptional up-regulation of ZnT8 and vice versa. Furthermore, ZnT3 knock down increases the apoptosis in beta cells and decreases the acute 2 hour-glucose stimulated insulin secretion. The aim of this present study was to investigate the effect of ZnT3 overexpression on beta cell functions including insulin secretion and beta cell survival.
Materials and methods: Insulin secreting INS-1E cells were stably transfected either with an empty pcDNA3.1 vector or with pcDNA3.1-hZnT3. Cells were treated with 5 mM, 11mM and 21 mM glucose for 24 hours prior to analysis. Gene expression analysis was performed by Q-PCR followed by normalization to three housekeeping genes (Beta-actin, Cltc, HSPcb). Glucose stimulated insulin secretion was analyzed by ELISA. Apoptosis was measured by DNA fragmentation analysis by Nucleo Counter NC3000. Immuno-gold EM analysis was performed on cryosections of cell pellets on a FEI Morgani microscope. Goat anti-rabbit IgG conjugated to 10-nm and 5-nm colloidal gold particles were used for visualization of ZnT3 and insulin, respectively.
Results: The gene expression of ZnT8 was significantly decreased by overexpression of ZnT3 at all tested glucose concentrations (5 mM, 11 mM and 21 mM). ZnT3 overexpression decreased the insulin transcription, insulin content and insulin secretion compared to the vector transfected cells. ZnT3 overexpression did not affect the level of DNA fragmentation in INS-1E cells after 24 hours of glucose treatment (5mM, 11 mM and 21 mM). EM analysis showed that ZnT3 was localized to some insulin-empty compartments in the cytosol. Interestingly, ZnT3 was primarily localized to insulin containing granules close to the plasma membrane.
Conclusion: We show that ZnT3 and ZnT8 expression levels are inversely correlated. ZnT3 overexpression reduces insulin synthesis and secretion. We show that ZnT3 is present in insulin-empty compartments in the cytosol but primarily located in insulin containing granules close to the plasma membrane.



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Zinc buffering by exogenous Zn7-metallothionein-2A affects insulin production, insulin secretion and intracellular zinc regulation in beta cells

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Background and aims: Preservation of beta cell mass and beta cell insulin secretion ability appears to be related to a balanced cellular zinc homeostasis as pancreatic beta cells depend on zinc for their primary function; insulin biosynthesis, insulin storage and insulin secretion. Metallothioneins are ubiquitously expressed proteins well-known for their metal-binding capacity and secures sufficient zinc for the cellular needs. The aim of the study was to examine how important zinc binding proteins are regulated at normal and high glucose concentrations and to investigate if increasing extracellular metallothionein levels by means of exogenous Zn7-MT-2A is beneficial for beta cell function and survival by contributing to an optimal zinc supply and regulation in the pancreas.
Materials and methods: INS-1E beta cells were pre-conditioned with +/- Zn7-MT-2A for 6 hours at standard 11 mM glucose and then stimulated with 6 or 21 mM glucose respectively for 24 hours. A concentration of 1 μg/mL (152 nM) Zn7-Metallothionein-2A (Zn7-MT-2A) was maintained throughout the stimulation period. Following stimulation cells were harvested for

gene expression analysis of Bax, Bcl-2, ZnT-8, ZnT-5 ZnT-3, MT-1A or MT-3 determined by RT-PCR and for measurements of insulin content and insulin secretion by ELISA.
Results: Zn7-MT-2A treatment induced a 2-fold increase in the level of insulin secretion and elevated the insulin content (2-fold) at 6.6 mM glucose (P=0.0001 and P=0.0325). Zn7-MT-2A exposure reduced the transcriptional regulation of the Bax/Bcl-2 ratio at both 6.6 and 21 mM glucose (P=0.0343 and P=0.0472). Furthermore, ZnT-5 and ZnT-8 gene expression was down-regulated (1.6 and 1.8 fold) by 21 mM glucose (P<0.0001 and P=0.0025) and by treatment with Zn7-MT-2A (1.2 fold) at the basal glucose concentration. Exposing INS-1E cells to 21 mM glucose lead to statistically significant (2-fold) down-regulation of MT-1A transcription (P=0.0037) and a further down-regulation of MT-1A was observed after exposure Zn7-MT-2A. The MT-3 gene expression was up-regulated (2-fold) at 21 mM glucose (P<0.0001) and at the basal glucose level MT-3 gene expression was down-regulated (1.15 fold, P=0.0199) when beta cells were exposed to Zn7-MT-2A. Unpaired T-test was used to determine statistical significance.
Conclusion: This study shows that Zn7-MT-2A can regulate important beta cell functions in INS-1E cells. The study suggests that there is a therapeutic potential of metallothionein administration for regulating beta cell insulin processing, cell death and zinc turnover. Exogenous metallothionein administration not only protects the INS-1E beta cells during glucose stress but also optimize the core function of the cells under normal glucose conditions.

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Modulation of incretin actions in pancreatic beta cells by metformin
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Background and aim: Metformin improves blood glucose concentration through activation of LKB1 and AMPK in liver and skeletal muscle. On the other hand, incretins, GLP-1 and GIP, directly raise intracellular Ca2+ and cAMP concentration in pancreatic β-cells, resulting in insulin secretion. We examined the effect of metformin on pancreatic β-cells and especially on the mechanisms of insulin secretion by incretins. We focused these Ca2+ response and cAMP production related with the mechanisms of insulin secretion.
Materials and methods: Amount of insulin secretion was measured by ELISA. For cAMP assay, pGloSensor™20F cAMP Plasmid was transfected into MIN6 cells by Neon™ Transfection System and cAMP production was measured by luminescence spectroscopy using GloSensorTM cAMP assay (Promega, USA) reagent. Intracellular Ca2+ concentration was measured by the Fura-2 fluorescence-imaging analytic method using Meta Fulora video image analyzer. LKB1 and phosphorylated LKB1 were detected by Western blotting.
Results: Ca2+ response by 10 nM GLP-1 was better than that by the same dose of GIP due to their receptors' mRNA expression in MIN6 cells and the peak response was dependent on glucose concentration because of significantly lower response in 0 mM glucose condition. 1 mM metformin pretreatment reduced to 78% of Ca2+ response by GLP-1 but concomitant metformin treatment with GLP-1 showed only 31% reduction. Moreover, inhibition of Ca2+ response by metformin occurred under 5.5 mM glucose as a physiological concentration but not 16.7 mM glucose condition. This inhibitory effect was time-dependent, 30 min (13 %), 60 min (30 %), 120 min (74 %) and 180 min (61 %). It showed that maximum inhibitory effect of Ca2+ response needed the pretreatment of metformin for 120 min. Metformin phosphorylated LKB1 and reached to maximum activation at 1hr under both glucose conditions. And also metformin modulated cAMP production in MIN6 cells by incretins under 5.5 mM glucose condition but not 16.7 mM glucose. Metformin affected in the similar way on insulin secretion by incretins. However, under high glucose concentration (16.7mM), metformin didn't show any effect on incretin functions (Ca2+ response and cAMP production).
Conclusion: Metformin reduced Ca2+ response by incretins under physiological glucose concentration and modulated on cAMP production by them. The peak of LKB1 activation by metformin did not correspond the time of maximum inhibitory effect against Ca2+ response. Glucose requirement was also different in these activities. Analysis of these mechanisms have still been investigating. As a result, it is suggested that metformin control the reaction of pancreatic β-cells to incretins under physiological glucose condition.

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The DPP-IV inhibitor, sitgliptin enhances glucose-stimulated insulin secretion from mouse isolated islets
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Background and aims: The action of DPP-IV inibitors are considered to prevent degradation of glucagon-like peptide-1 (GLP-1) in the circulation thus enhancing the effects of GLP-1 on glucose-stimulated insulin secretion (GSIS). DPP-IV activity has been identified in several tissues but its presence and role in pancreatic islets have not been determined. The aims were to determine the direct effects of DPP-IV inhibitor, sitagliptin on GSIS in isolated mouse islets and identify DPP-IV in islet cells.
Materials and methods: Mouse or rat islets, isolated by collagenase digestion were incubated with glucose, 1 or 12mM (60min) with and without sitagliptin (5nM). Long term effects were tested by overnight culture of islets with the DPP-IV inhibitor (5nM) followed by measurements of GSIS (1 or 8mM glucose). Insulin secretion was measured by radioimmunoassay and GLP-1 production by ELISA. DPP-IV and GLP-1 receptors were identified in islets cells by immunohistochemistry.
Results: GSIS (1-12mM glucose) was enhanced by 30% compared to GSIS without sitagliptin, from 0.12±0.01 to 0.16±0.01% of insulin content/h (n=6, p<0.05). Overnight pretreatment of islets with sitagliptin (5nM) resulted in a doubling of GSIS, from 0.05±0.008%/h to 0.10%±0.01 (n=3, p<0.05). Immunolabelling demonstrated DPP-IV to be present in mouse jejunum and mouse islet alpha and beta cells. GLP-1 receptor was detected in mouse beta cells but absent from alpha cells. In rat islets, GLP-1 was co-released with glucagon at molar ratios 1:4.
Conclusion: The presence of DPP-IV in pancreatic islets and the direct enhancing effects of sitagliptin on GSIS suggest that DPP-IV inhibitors can act directly on islets raising the possibility that GLP-1 could be released and act locally in islets.
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Anti-diabetic effect of oral borapetol B, isolated from the plant tinospora crispa, by stimulating insulin release in type 2 diabetic Goto-Kakizaki rats
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Background and aims: *Tinospora crispa* (*T.crispa*) has been used in traditional medicine for the treatment of diabetes mellitus but the exact mechanism of its anti-diabetic effect is unknown. From *T.crispa* extract, we have isolated a biologically active small compound, Borapetol B (C1). The current study was undertaken to evaluate the anti-diabetic properties of C1 compound.
Materials and methods: The effect of C1 on the blood glucose and serum insulin was assessed by an oral glucose tolerance test in normoglycaemic control Wistar (W) and spontaneously type 2 diabetic Goto-Kakizaki (GK) rats. For *in vitro* studies, the stimulation of insulin secretion was assessed by performing batch incubation and perifusion experiments using isolated W and GK pancreatic islets.
Results: C1 (10 μg/100 g) which was administrated orally by gavage 30 minutes prior to an oral glucose challenge significantly improved blood glucose levels in treated compared to placebo group with areas under the glucose curves 0-120 min being 72 ± 17 vs 344 ± 10 mmol/L (P<0.001) and 492 ± 63 vs 862 ± 55 mmol/L (P<0.01) in W and GK rats respectively. Plasma insulin levels were significantly enhanced by 2-fold in treated W and GK rats at 30 min compared to the placebo group with mean values of 27 ± 4 vs 61 ± 9 μU/mL (P<0.05) and 30 ± 4 vs 63 ± 8 μU/mL (P<0.05) respectively. For the *in vitro* study, C1 dose-dependently increased insulin secretion from W and GK isolated islets in both low (3.3 mM) and high glucose (16.7 mM). In W, incubation of islets at 0.1, 1 and 10 μg/ml of C1 significantly stimulated insulin release by 6.2-fold (15.1 ± 2.2; P<0.01), 8.1-fold (19.5 ± 4; P<0.05) and 9.2-fold (21.9 ± 2; P<0.001) in low glucose and 1.5-fold (48.8 ± 6; P<0.05), 1.9-fold (63.1 ± 8.9; P<0.05) and 5.0-fold (164.5 ± 11.3; P<0.001) in high glucose respectively compared to the control group. In GK, islets treated with C1 at 0.1, 1 and 10 μg/ml significantly stimulated insulin secretion by 3.9-fold (4.7

± 0.3), 6.3-fold (7.5 ± 0.9) and 8.8-fold (10.5 ± 1.8) (all P<0.05) respectively in low glucose and by 1.5-fold (20.6 ± 1.1), 2.3-fold (30.9 ± 3.8) and 4.2-fold (57.3 ± 6.3) (all P<0.05) respectively compared to the control group. The perifusions of W and GK rat islets with 10 μg/ml of C1 increased insulin secretion in low (0-16 min) and high (16-30 min) glucose. In W, insulin secretion increased by 5.5-fold (1.1 ± 0.16 μU/islet/min)(12 min) and 7.5-fold (1.5 ± 0.16 μU/islet/min)(22 min). There was a significant difference observed from 2 min to 32 min (P<0.001). In GK rat, insulin secretion increased by 2.5-fold (0.43 ± 0.05 μU/islet/min)(2 min) (P<0.001) and 4.7-fold (0.8 ± 0.03 μU/islet/min) (20 min) (P<0.001). There was a significant difference observed between (2-4 min) and (20-30 min) (all P<0.001). The insulin secretion returned to basal level in both W and GK when C1 was omitted from the perifusate indicating that the compound did not cause leakage of insulin by damaging islet beta cells.

Conclusion: This study provides evidence that Borapetol B (C1) isolated from *Tinospora crispa* has anti-diabetic properties mainly due to its stimulation of insulin release. Further studies are needed to understand the mechanisms involved by which C1 induce insulin release from pancreatic islets.

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PS 026 Beta cell metabolism and mitochondrial function

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Nutrient regulation of thiols redox state in the mitochondrial matrix of rat pancreatic islet cells: dynamic measurements using redox-sensitive GFP2 fused to GRX-1

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Background and aims: Both reactive oxygen species and NADPH production are putative metabolic coupling factors in pancreatic β cells. Through a network of oxidation-reduction reactions, they exert opposite effects on protein thiols redox state that may play a role in the control of β cell function and survival. So far, little is known about nutrient-induced changes in β cell thiols redox state, and even less about their changes in subcellular compartments. Here, we used the redox-sensitive GFP2 fused with glutaredoxin (GRX) 1 to investigate the acute nutrient regulation of glutathione redox potential, a good indicator of thiols redox state, in the cytosol and mitochondrial matrix of rat β cells.

Methods: Male Wistar rat islet cell clusters were cultured on glass coverslips in RPMI medium containing 10mM glucose (G10) and 10% FBS. They were infected with an adenovirus encoding cytosolic GRX1-roGFP2 or mitochondrial mt-GRX1- roGFP2. One to two days later, the fluorescence ratio of the probe (exc 400/480nm; em 535nm) was measured every 30s during perifusion with a Krebs solution containing various glucose concentrations and test substances. The results (mean±SE for 8-11 clusters from at least 3 islet isolations) were normalized to the fluorescence ratio measured in the presence of 10mM dithiothreitol (set to 0%) and that measured in the presence of 1mM H_2O_2 (set to 100%). The significance of differences between groups was assessed by one-way ANOVA and a post-test of Newman-Keuls.

Results: Coinfection with Ad-RIP-DsRed showed that 90% of mt-GRX1-roGFP2 positive cells were β cells. The correct expression of the probes in the cytosol and mitochondria was confirmed by confocal microscopy using Mitotracker Red. In the presence of G10, the fluorescence ratio of cytosolic GRX1-roGFP2 was low (1.5±0.3% of min-max difference) and unaffected by stepwise changes in glucose concentration between G0 and G30. In comparison, the fluorescence ratio of mitochondrial mt-GRX1-roGFP2 was higher and significantly affected by the glucose concentration, ranging from 31±1% in G0 to 27±1% in G2, 21±1% in G5, 17±1% in G10, and 10±1% in G30. The fluorescence ratio of mt-GRX1-roGFP2 was also significantly reduced by addition of various metabolic substrates (α-ketoisocaproic acid, succinate methylester, Leu with Gln) to G5. These nutrient effects, which were opposite to previously reported nutrient-induced changes in NAD(P)H autofluorescence, were not due to changes in pH, as addition of 30 mM NH_4Cl or sodium acetate to G10 only slightly affected mt-GRX1-roGFP2 fluorescence ratio. They were also independent from changes in Ca^{2+} influx, as mt-GRX1-roGFP2 fluorescence ratio was not affected by addition of 500μM tolbutamide to G5 or 250μM diazoxide to G10. The glucose reduction of mt-GRX1-roGFP2 fluorescence ratio was similar when β cells were depolarized with 30 mM extracellular K^+ in the presence of 250μM diazoxide than under control conditions.

Conclusion: Acute nutrient stimulation rapidly reduces thiols (mainly glutathione) redox state in the mitochondrial matrix but not the cytosol of rat islet cells. These changes could play a role in the nutrient stimulation of insulin secretion (triggering and metabolic amplifying pathways), or in the long-term effects of glucose on the functional β cell mass.

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Influence of K_{ATP} channel inhibition on ROS production in pancreatic beta cells

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Background and aims: In pancreatic beta cells glucose metabolism represents a major source for reactive oxygen species (ROS) and it is well known

that oxidative stress induces beta cell failure. Sulfonylureas (SUs) have been used in the treatment of diabetes mellitus type 2 for decades and their effects on stimulus-secretion coupling are well documented. In previous studies we showed that SUs upregulate antioxidative enzymes and protect beta cells against oxidative stress. Our current aim was to investigate short and long-term effects of SUs on mitochondrial metabolism and ROS levels in beta cells.
Materials and methods: Cytosolic Ca^{2+} concentration $[Ca^{2+}]_c$ was measured by fura-2, mitochondrial membrane potential (ΔΨ) was determined by Rh-123, FAD and NAD(P)H concentrations were analyzed by changes in autofluorescence and intracellular ROS were captured with 2',7'-dichlorofluorescein.
Results: In glucose-stimulated beta cells elevation of Ca^{2+} influx by a SU partly depolarised the mitochondrial membrane potential (ΔΨ) (15 mM glucose: 418±19 a.u., + 100 μM tolbutamide: 428±20 a.u., n=31, p≤0.05). The L-type Ca^{2+} channel blocker nifedipine completely prevented this effect (15 mM glucose + 5 μM nifedipine: 414±16 a.u., + 100 μM tolbutamide: 406±16 a.u., n=22, p≤0.05). To investigate the influence of SUs on the concentration of reduction equivalents, changes in NAD(P)H and FAD were determined. As expected NAD(P)H concentration was increased by elevating glucose (0.5 mM glucose: 479±14 a.u., 15 mM glucose: 504±14 a.u., n=16, p≤0.001). SU application reduced NAD(P)H autofluorescence by 5±1 a.u. and the L-type Ca^{2+} channel blocker gallopamil reversed this effect (n=16). FAD autofluorescence that could be increased by the specific complex II inhibitor 3-nitropropionic acid (3-NPA) (15 mM glucose: 422±7 a.u., + 1 mM 3-NPA: 440±7 a.u., n=40, p≤0.001) was unaffected by SU (4 mM glucose: 687±39 a.u., + 100 μM tolbutamide: 682±41 a.u., n=8, n.s.). 1 h incubation of beta cells with 15 mM glucose led to ROS accumulation (0.5 mM glucose: 315±19 a.u., 15 mM glucose: 789±46 a.u., n=56-63, p≤0.001). At 15 mM but not at 0.5 mM glucose SUs increased ROS. This effect could be reversed by gallopamil (1 h: 15 mM glucose + 100 μM tolbutamide: 996±56 a.u., + 50 μM gallopamil: 649±34 a.u., n=47-72, p≤0.001). 3-NPA did not elevate ROS indicating that the effect of SUs is independent of complex II (1 h: 15 mM glucose: 434±31 a.u., + 1 mM 3-NPA: 248±26 a.u., n=26-37, p≤0.001). Interestingly, oxidative stress induced by long-term incubation of islet cells in a glucolipotoxic milieu (2 days: 25 mM glucose, 100 μM palmitate) was not increased but drastically ameliorated by co-administration of a SU (25 mM glucose + 100 μM palmitate: 1192±298 a.u., + 10 μM gliclazide: 783±216 a.u., n=250-251 of 4 independent preparations, p≤0.05).
Conclusion: Beside their insulinotropic effect SUs are able to modulate ROS accumulation in metabolically active beta cells. Acute stimulation of Ca^{2+} influx by SUs accelerates respiratory chains and favours ROS formation possibly by interacting with mitochondrial structures like complex I. In agreement with upregulated antioxidative defense our data show that prolonged exposure to SUs reverses this situation and protects beta cells against metabolic stress by reducing intracellular ROS formation.
Supported by DFG

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The role of the reactive oxygen species producing NADPH oxidase 5 (NOX5) as a regulator of islet function
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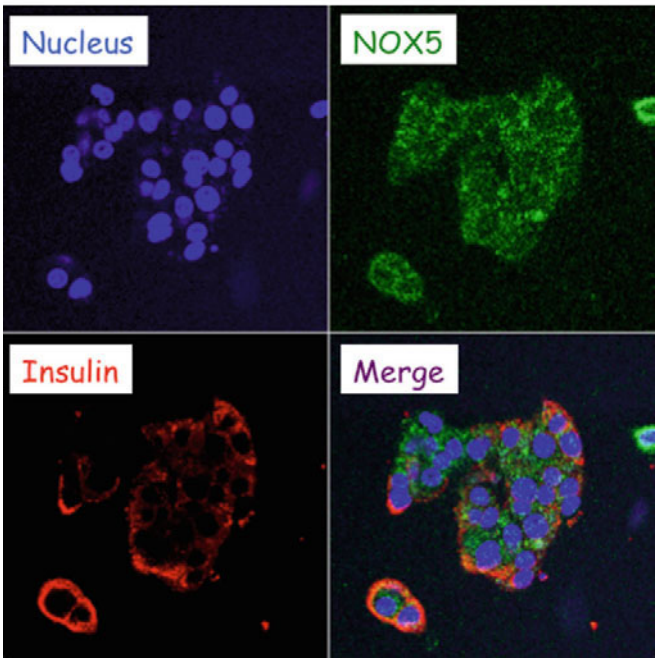
Background and aims: Production of reactive oxygen species (ROS) is a well-known modulator of islet beta cell insulin secretion and apoptosis. Different isoforms of the family of NADPH oxidase enzymes (NOX-es) are major intracellular sources of ROS. The goal of our study was to characterize NOX isoform expression in human islets and to investigate their role in islet insulin secretion and survival in a systematic way.
Materials and methods: We analyzed isolated human islets by real-time PCR, Western blot analysis and immunofluorescens labeling.
Results: We detected NOX2, NOX4 and NOX5 mRNA expression in whole islets and in isolated beta cells using an RT-PCR approach. In the following part we focused our attention on NOX5 as this isoform has not yet been described in islets. NOX5 is expressed in humans but has no rodent homologue. NOX5 produces reactive oxygen species (ROS) in a calcium-dependent manner and regulates cell proliferation in diverse cell types. When isolated islets were stimulated with high (16.7mM) glucose concentration, NOX5 protein displayed a two-fold increase compared to basal (5.6mM) glucose concentrations. Immunofluorescens co-localization experiments in islets demonstrated an intriguing pattern for NOX5 expression. In basal condition, we detected few, but highly NOX5-positive cells which were also labeled for somatostatin. By contrast, in glucose-stimulated islets NOX5 labeling was detected in insulin positive cells with lower intensity and a punctuated expression pattern. NOX5 did not directly co-localize with insulin-containing vesicles. To extend our investigations we analyzed DNAchip data and found increased NOX5 expression in islets of Type 2 diabetic subjects.
Conclusion: Taken together, our data imply NOX5 as a novel modulator of islet beta cell function and a potential regulator of intra-islet crosstalk. Therapeutic alteration of different NOX isoforms is considered a novel and viable approach in different pathologies. Our data suggest that modulation of NOX5 activity might be envisioned in the context of islet function and survival.

Immunofluorescens co-localization of NOX5 and insulin
in isolated human islets after 12 hours of 16.7M glucose incubation



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Oxidative stress and activation of the intrinsic pathway of apoptosis cause beta cell loss in Friedreich's ataxia
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Background and aims: Friedreich's ataxia (FRDA) is a neurodegenerative disease caused by reduced expression of the mitochondrial protein frataxin (Fx). In addition to the neurological manifestations, FRDA patients have a high prevalence of impaired glucose tolerance and diabetes as a consequence of β-cell demise. We have previously shown that Fx deficiency sensitizes β-cells to apoptosis induced by metabolic or endoplasmic reticulum stress. Moreover, we found that cAMP induction by incretin analogs is β-cell protective and may have therapeutic potential in FRDA. The activation of the intrinsic or mitochondrial pathway of apoptosis is controlled by the balance between anti- and pro-apoptotic proteins of the BCL-2 family. The aim of this study was to elucidate the molecular mechanisms of β-cell demise in FRDA.
Materials and methods: Fx was silenced by RNA interference (siFx) in stable clones of INS-1E cells expressing the HyPerCyto or HyPerMito vectors, to monitor H2O2 production in the cytosol or mitochondria, respectively, and in INS-1E cells overexpressing catalase in the mitochondria. RNA interference was used to silence BAD, DP5, Puma or Bim (≥ 50% knockdown) together with siFx. 48h after transfection cells were exposed for 24h to oleate (0.5 mM) or the endoplasmic reticulum stressor brefeldin-A (0.1 μg/ml), with or without the cAMP inducer forskolin (20 μM) or the reactive oxygen species scavenger MnTMPyP (25 μM). β-cell apoptosis was examined by Hoechst 33342/propidium iodide staining, caspase-3 activation and immunofluorescence for cytochrome c release. Protein and gene expression was analyzed by Western blot and real-time PCR.

Results: Fx silencing increased SOD2 expression and mitochondrial H2O2 production in β-cells. MnTMPyP protected Fx-deficient β-cells from oleate (40±4% apoptosis for siFx oleate vs 21±2% for siFx oleate + MnTMPyP, n=3, p<0.05) and brefeldin (47±3% for siFx brefeldin vs 37±2% for siFx brefeldin + MnTPyP, n=4, p<0.05). Mitochondrial catalase overexpression also protected Fx-deficient β-cells from brefeldin (n=3, p<0.05). Fx deficiency activated the intrinsic pathway of apoptosis in β-cells, as indicated by mitochondrial cytochrome c release, induced the pro-apoptotic proteins DP5, Puma and Bim (around 60% increase in mRNA expression, n=9-14, p<0.05), and reduced BAD phosphorylation. Silencing of DP5, BAD and Bim but not Puma protected Fx-deficient β-cells in control condition and after oleate and brefeldin exposure (n=4, p<0.05). Forskolin protected Fx-deficient β-cells by restoring BAD phosphorylation and reducing Bim expression (n=4, p<0.05).
Conclusion: β-Cell death in FRDA is mediated by enhanced mitochondrial oxidative stress and activation of the intrinsic pathway of apoptosis. The pro-apoptotic BCL-2 proteins BAD, DP5 and Bim are key mediators of β-cell death. cAMP inducers protect Fx-deficient β-cells via BAD phosphorylation and reduced Bim expression. Selective modulation of pro-apoptotic BCL-2 proteins by incretin analogs may have therapeutic potential to prevent β-cell loss in FRDA.
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Glucose causes a pronounced activation of ATP-synthase dependent respiration in pancreatic beta cells
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Background and aims: Mitochondria are essential for metabolism-secretion coupling in pancreatic beta cells. Glucose stimulation accelerates mitochondrial oxidative metabolism and respiration to increase the cytosolic ATP/ADP ratio, which initiates plasma membrane electrical activity and thereby insulin granule exocytosis. Cytosolic calcium signals occurring as a result of plasma membrane depolarization are sensed by mitochondria and serve as a potentiating signal during second phase insulin secretion. The proposed main target of mitochondrial calcium signals are pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and isocitrate dehydrogenase. Their activation will increase TCA cycle activity and the formation of NADH. Here the effect of beta cell calcium signaling on the regulation of glucose-induced NAD(P)H formation and ATP-synthase dependent respiration were assessed.
Materials and methods: Glucose-induced respiration was studied in INS-1E cells or groups of intact human islets. Mitochondrial respiration was calculated as the difference before and after addition of rotenone (complex I inhibitor) in combination with antimycinA (complex III inhibitor). ATP-synthase dependent respiration was determined similarly using oligomycin. Oxidative metabolism in human islets was followed by measuring NAD(P)H autofluorescence on a two-photon confocal microscope. Cytosolic calcium signals were analyzed at the single cell level in INS-1E and primary beta cells using the calcium sensitive probe YC3.6.
Results: Glucose stimulates mitochondrial respiration in INS-1E and human beta cells. We find that in insulin secreting cells a surprisingly large fraction of total respiration under resting conditions is ATP-synthase independent. Following glucose stimulation this fraction of the total respiratory rate remains constant while ATP-synthase dependent respiration is strongly induced. This activation of ATP-synthase is almost completely suppresses when calcium signaling is prevented. Under similar conditions, NAD(P)H levels rapidly increase in response to glucose. At this point the NAD(P)H/NAD(P) ratio is in a new steady-state determined by on the one side enhanced oxidative metabolism and on the other re-oxidation by the respiratory chain. Consistent with this view, when glucose is removed the NAD(P)H signal rapidly decreases while inhibition of respiration further augments the NAD(P)H/NAD(P) ratio. Interestingly, calcium signaling had no impact on the NAD(P)H steady-state signal.
Conclusion: Glucose-induced calcium signaling is required to stimulate ATP-synthase dependent respiration in pancreatic beta cells. In contrast calcium signaling does not influence steady-state NAD(P)H levels, which are determined mainly by the glucose concentration. The data are consistent with the hypothesis that calcium accelerates oxidative metabolism (NADH formation) and respiration (NADH oxidation) about equally.

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MICU2 modulates nutrient-sensing and insulin secretion in INS-1 832/13 cells
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Background and aims: The Mitochondrial Calcium Uniporter (MCU) is a low affinity Ca^{2+} channel-complex. It is responsible for mitochondrial Ca^{2+} (Ca^{2+}_m) influx during metabolic activation. Ca^{2+}_m influx potentiates mitochondrial metabolism by the activation of dehydrogenases in the TCA-cycle, which increases metabolic flux and hence NAD(P)H availability. The molecular composition of the MCU-complex was recently resolved. Thus, the proteins MCU and MICU1 (mitochondrial calcium uptake 1) are potential regulators of nutrient-stimulated ATP generation and insulin secretion in beta cells. Recently, MICU2 (EFHA1) was reported to be part of the MCU complex. Here, we investigated the role of MICU2 in glucose-stimulated insulin secretion (GSIS) and mitochondrial function in the clonal beta cell line INS-1 832/13.
Materials and methods: *MICU2* mRNA expression in human islets was determined by microarray analysis in islets from 85 donors. GSIS (at 2.8 or 16.7 mM glucose) was determined 72 h after siRNA-mediated knock-down (KD) of *Micu2* in INS-1 832/13 cells. ATP content was determined by luciferase-dependent luminescence. The Seahorse XF24 instrument was used to measure respiration.
Results: *MICU2* mRNA expression in human islets was negatively correlated with fold change in insulin secretion (p=0.0160). KD of *Micu2* in INS-1 832/13 cells for 72 h decreased basal insulin secretion by 33 ± 4.0% (p = 0.0003). There was a trend towards decreased GSIS. Both basal and glucose-stimulated respiration were decreased in *Micu2* KD cells by 44 ± 9% (P=0.0257) and 33 ± 7% (p=0.0364), respectively, compared to control cells. While KD of *Micu2* did not affect basal nor glucose-stimulated total ATP levels, respiration-driven mitochondrial ATP generation (oligomycin-sensitive respiration) and proton leak (oligomycin-insensitive respiration) were decreased by 27 ± 7% (p=0.0229) and 57 ± 11% (p=0.0156), respectively. Interestingly, KD of *Micu2* abolished spare respiratory capacity (FCCP-induced uncoupling) by decreasing the maximal respiration rate by 43 ± 6% (p=0.0047).
Conclusion: MICU2 seems to modulate nutrient-sensing and insulin secretion in INS-1 832/13 cells. This modulation awaits full elucidation but likely involves Ca^{2+}_m influx leading to activation of dehydrogenases in the TCA-cycle and production of NAD(P)H. This is supported by our findings of decreased metabolic flux and the abolishment of spare respiratory capacity by *Micu2* KD. It may limit availability of electrons, causing the mitochondria to run out of reducing equivalents when respiration is uncoupled from ATP-generation.
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The mitochondrial fission proteins Fis1 and Drp1 are important for proper glucose-induced insulin secretion in INS1 832/13 cells
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Background and aims: Mitochondria in living cells exist as a dynamic network that continuously cycle through fusion-fission events. Dysfunctional parts of the network were sorted out by mitophagy to support mitochondrial vitality. Mitofusin 1 and 2 (Mfn1 and Mfn2) and the optic atrophy 1 (Opa1) are essential for mitochondrial fusion, whereas the fission protein 1 (Fis1) and the dynamin related protein 1 (Drp1) control fission. It is currently being discussed if both fission proteins, Fis1 and Drp1, are essential to maintain mitochondrial function and glucose-induced insulin secretion of pancreatic beta cells. Thus, the aim of this study was to investigate changes in mitochondrial dynamics and cellular function after knockdown of Fis1 and Drp1 in glucose-responsive INS1 832/13 cells.
Materials and methods: Down-regulation of Fis1 and Drp1 were achieved using the GIPZ Lentiviral short hairpin RNAmir expression system. INS1 832/13 cells were infected with lentiviral particles containing shRNA for Fis1 or Drp1, or a non-silencing shRNA as control. Gene and protein expression were analysed by Real-Time PCR, western blot, and immunofluorescence analyses, respectively. The ATP content was measured using the ATPlite assay. Glucose-induced insulin secretion was determined by ELISA. Mitochondrial morphology and membrane potential were determined by MitoTracker and TMRE staining.

Results: Knockdown of Fis1 and Drp1 in INS1 823/13 cells resulted in a significantly reduced gene expression compared to control cells. Immunofluorescence and western blot analyses showed that Fis1 and Drp1 protein expression was likewise decreased. The mitochondrial membrane potential was significantly reduced in shFis1 and shDrp1 cells compared to control cells by 40 and 20 %, respectively. We observed a homogenous mitochondrial network structure in INS1 823/13 control cells. Knockdown of Fis1 and Drp1 resulted in a significantly higher mean mitochondrial area compared to control cells. However, whereas the Fis1 knockdown especially increased the value of elongated mitochondria in INS1 823/13 cells, after Drp1 knockdown also a number of clumped mitochondria were detectable. The ATP content and glucose-induced insulin secretion was significantly reduced, both in shFis1 and shDrp1 cells compared to control INS1 823/13 cells.
Conclusion: Our results suggest that both proteins, Fis1 and Drp1 are important for the stimulus secretion coupling in INS1 823/13 cells. Because the reduced expression of Drp1 and Fis1 evoked a different mitochondrial morphology, we propose for both proteins independent mechanisms on mitochondrial dynamics and cellular function in pancreatic beta cells.
Supported by: DDS

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Activation of glutamate dehydrogenase stimulates insulin secretion and implicates a metabolic pathway in beta cell stimulus-secretion coupling
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Background and aims: Leucine is an amino acid that stimulates insulin release under physiological conditions through the activation of glutamate dehydrogenase (GDH). GDH catalyzes the oxidative deamination of endogenous glutamate. Previous studies have shown that leucine and 2-aminobicyclo [2.2.1]heptane-2-carboxylic acid (BCH), a nonmetabolizable leucine analog, are activators of GDH and largely mimic the effects of glucose on insulin release from pancreatic islets. To better understand stimulus-secretion coupling in pancreatic β-cells, the functional, secretory and metabolic responses to glucose and BCH are compared in INS-1 832/13 clonal β-cells, as well as in human islets.
Materials and methods: INS-1 832/13 clonal β-cells and human islets were stimulated with 16.7 mM glucose or 20 mM BCH. Glucose- and BCH-stimulated insulin secretion was measured, as well as mitochondrial and plasma membrane potentials. Metabolite profiles were determined by gas chromatography-mass spectrometry and compared at 2.8 and 16.7mM glucose as well as 20 mM BCH, using multivariate statistics.
Results: Similar to 16.7 mM glucose, BCH provoked insulin release in INS-1 832/13 β-cells in a biphasic manner. This was associated with a depolarization of plasma membrane and an increase in the cytosolic Ca^{2+} level. Conversely, metabolite profiles in INS-1 832/13 β-cells kept in 2.8 and 16.7 mM glucose or 20 mM BCH were perfectly separated by multivariate statistical analysis. Notably, glucose or BCH increased α-ketoglutarate levels while glutamate levels decreased in response to BCH stimulation in either INS-1 832/13 β-cells or human islets.
Conclusion: BCH through the activation of GDH induces the same effects as high glucose on insulin secretion and plasma membrane potential in INS-1 832/13 β-cells. Notwithstanding this activation of GDH by BCH involves other metabolic pathways, mainly anaplerotic, in β-cell stimulus-secretion coupling.
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PS 027 Functional analysis of beta cells

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DNA methylation of the glucagon-like peptide-1 receptor (GLP1R) in human pancreatic islets
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Background and aims: Glucagon-like peptide-1 (GLP-1) is an incretin hormone which exerts its effects via the glucagon-like peptide-1 receptor (GLP1R) located on several different cell types and organs including pancreatic beta cells. Binding of GLP-1 to its receptor on beta cells will enhance the exocytosis of insulin. GLP1R protein expression is reportedly reduced in pancreatic islets from patients with type 2 diabetes. However, whether epigenetic mechanisms e.g. DNA methylation affect *GLP1R* in human islets remains unknown. Here, we examined if DNA methylation of the *GLP1R* gene is associated with *GLP1R* mRNA expression in pancreatic islets from human donors. We further examined if pancreatic islets from patients with type 2 diabetes show differential DNA methylation of the *GLP1R* gene compared with non-diabetic donors.
Materials and methods: DNA methylation of 12 CpG sites located both upstream and downstream of the transcription start site of *GLP1R* were analysed in human pancreatic islets of 55 non-diabetic donors and 10 donors with type 2 diabetes. DNA methylation of the *GLP1R* promoter was also analysed in FACS sorted alpha and beta cells from pancreatic islets of three human donors.
Results: DNA methylation of one CpG site of the *GLP1R* promoter correlated negatively with islet gene expression (rho=-0.34, p=0.008) and positively with BMI (rho=0.30, p=0.02) and HbA_{1c} (rho=0.30, p=0.03). This specific CpG site is positioned in an area previously shown to have SP1 and SP3 transcription factor binding sites, suggesting that methylation of this specific site could influence the binding of these factors and thereby the transcription of the gene. We also found significantly higher levels of DNA methylation of this CpG site in human alpha cells compared with beta cells (p=0.009). Furthermore, one CpG site showed nominally increased DNA methylation in pancreatic islets from patients with type 2 diabetes compared with non-diabetic controls (p=0.022).
Conclusion: Our results suggest a mechanism where obesity and/or hyperglycaemia influence DNA methylation and eventually gene expression of *GLP1R* in human pancreatic islets.
Supported by: VR, ALF, LUDC/Exodiab and Söderberg, Påhlsson and Novo Nordisk Fondations

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Targeting pancreatic alpha and beta cells with adeno-associated viruses (AAVs)
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Background and aims: Adeno-associated viruses (AAVs) are useful gene therapy vectors and are being tested clinically in various diseases. We have designed AAV8s containing the rat glucagon promoter (700 bp) or the rat insulin I promoter (410 bp) to specifically target pancreatic alpha- and beta cells, respectively. These novel vectors may allow for the acute expression or knockout of a desired gene and for the development of gene therapies targeted to these cell types.
Materials and methods: Constructs with the glucagon promoter driving enhanced green fluorescent protein (GCG-EGFP) or with the insulin promoter driving Cre recombinase (RIP-Cre) were used to produce the vectors. AAV8s were delivered by intraperitoneal or intraductal injection to wild type and mT/mG reporter mice. Transgene expression and function of the targeted

cells were analyzed at different time points post-injection. Additionally, body weight and several metabolic parameters were examined.
Results: AAV RIP-Cre delivery resulted in specific beta cell Cre expression within the islets. Cre activity was confirmed by delivery of AAV RIP-Cre to reporter mT/mG mice, in which EGFP was expressed upon Cre-mediated recombination. Administration of AAV RIP-Cre up to the dose of 1x10^12 viral genomes did not produce alterations in body weight, blood glucose, glucose tolerance, glucose-stimulated insulin secretion or plasma hormone levels. AAV GCG-EGFP delivery allowed for exclusive EGFP expression in alpha-cells, which exhibited normal [Ca2+]i when stimulated with low and high glucose and adrenaline, and did not produce alterations in body weight, blood glucose, arginine and glucose tolerance, or plasma hormone levels. Moreover, preliminary data showed that AAV GCG-EGFP could be used to successfully sort alpha-cells from a pancreatic cell population.
Conclusion: We conclude that AAV8s may be suitable vectors to specifically and acutely target gene expression in alpha- and beta cells.
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Microelectrode array (MEA) technology allows functional analysis of human and mouse pancreatic islets
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Background and aims: Electrical activity of beta cells in intact mouse islets consists of membrane potential oscillations that couple blood glucose levels to insulin release. We have shown before that the oscillations measured with MEAs are an excellent marker of beta cell activity and metabolic integrity. We aimed 1) to apply the MEA technology to human islets in order to elucidate whether human islets exhibit oscillatory activity similar to that described in mice, and 2) to demonstrate in mice that the MEA is suitable for the long-term assessment of islet functionality in vitro.
Materials and methods: Oscillations of V_m were recorded from whole islets as field potentials using MEA technique. They were quantified by calculating the fraction of plateau phase (FOPP = percentage of time with burst activity). Mouse islets were cultured on MEAs. Electrical activity from human islets, obtained from biopsies during pancreatic surgery, was recorded on MEA electrodes in tissue slices or individual islets.
Results: We can show for the first time that the membrane potential of human islets is able to oscillate in a similar manner as observed in mouse islets (n=1). The islet within a tissue slice covered two electrodes (200 µm distance) of a MEA. The oscillations appeared at a glucose concentration of 10 mM and could only be followed for 2'30 min because of a fast decay of signal amplitude. The apparent FOPP was 48 % which closely resembles the EC_{50} value for glucose stimulation of mouse islets which is at 12±1 mM glucose (n=5-10). In another human islet electrical activity occurred in 10 mM glucose after 4 d in culture. The signal was too noisy to resolve oscillations properly, but responded to diazoxide and tolbutamide. In a further approach, mouse islets were kept up to 34 days in culture (DIC) and five to ten islets were usually recorded in parallel with MEA electrodes. The FOPP was summarized for the experiments performed at DIC 6 and 7, and compared to those at DIC 33 and 34. Similar to acute recordings obtained previously* islets were silent at 3 mM glucose and the FOPP amounted to 32±3 % (n=8) and 36±2 % (n=7) at 10 mM glucose and to 70±6 % (n=8) and 72±5 % (n=7) in 15 mM glucose at DIC 6/7 and DIC 33/34, respectively. This long-term experiment shows for the first time that beta cells in culture keep unchanged oscillatory activity and responsiveness to glucose over weeks.
Conclusion: This study presents for the first time that human islets show membrane potential oscillations comparable to those found in mice. It discloses the proof-of-principle that MEA technology can be used to evaluate the functionality of human islets. Further investigations will elucidate whether this technique is suited for quality control of islet transplants. Moreover, we show for the first time, that MEA technology is suitable to assess the functional properties of individual islets over weeks. As the glucose-dependent FOPP did not change with time, this opens numerous possibilities for complete new concepts and designs of in vitro studies on islets of Langerhans which may contribute to reduce the number of laboratory animals, too.

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Syndapin I is a key regulator of beta cell endocytosis and plasma membrane protein homeostasis
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Background and aims: Syndapin was originally identified as a synaptic dynamin-associated protein in the brain. Later, this neuron-specific isoform was named syndapin I since other isoforms, syndapin II and syndapin III, were detected in other tissues. All these three isoforms participate in the regulation of endocytosis. The best understood one is syndapin I, which plays a crucial role in dynamin-mediated endocytosis in neuronal tissue. Like other secretory cells, the highly specialized secretory pancreatic β cell undergoes profound exocytosis in both constitutive and regulated fashions. Such profound exocytosis is inevitably followed by effective endocytosis to preserve β cell integrity and plasma membrane homeostasis in particular. Significant efforts have been devoted to the understanding of insulin exocytosis, the central function of the β cell. However, β cell endocytois is much less understood. The molecular details of the endocytic machinery in the β cell have not been well clarified. The present work dissects mechanistically the role of syndapin I in constitutive and regulated endocytosis as well as plasma membrane protein homeostasis in the pancreatic β cell.
Materials and methods: We combined patch-clamp techniques, confocal microscopy, FM1-43 staining, siRNA transfection, immunoblot analysis, immunocytochemistry and cell culture for characterizing the role of syndapin I in endocytosis and plasma membrane protein homeostasis in the mouse islet β cells and insulin-secreting RINm5F cells.
Results: Immunoblot analysis shows that syndapin I siRNA transfection significantly decreased syndapin I expression at the protein level. Confocal microcopy reveals that syndapin I knockdown effectively abrogated internalization of the fluorescent endocytosis marker dye FM 1-43 into β cells under basal conditions, exposed to D-glyceraldehyde/D-glucose or stimulated with KCl. In addition, intracellular application of inositol hexakisphosphate ($InsP_6$) evoked a significant reduction in β cell capacitance and activated β cell CK2 resulting in the elevation of syndapin I phosphorylation in β cells. Furthermore, CK2 inhibition not only reduced the $InsP_6$-induced phosphorylation, but also decreased $InsP_6$-induced capacitance reduction in β cells. Importantly, syndapin I knockdown appreciably increased L-type Ca^{2+} channel density in the β cell plasma membrane.
Conclusion: The results reveal that syndapin I serves as a key regulator of both constitutive and regulated endocytosis in β cells. This key endocytic protein also mediates $InsP_6$-induced β cell endocytosis via its CK2-dependent phosphorylation. Importantly, it plays a crucial role in the maintenance of plasma membrane protein homeostasis, as exemplified by the L-type Ca^{2+} channel. Overall, syndapin I is a key regulator of β cell endocytosis and plasma membrane protein homeostasis. These findings provide a better understanding of β cell endocytosis and its physiological importance.
Supported by: EFSD/Novartis, SSF, VR

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Stimulus secretion coupling in a human beta cell line - EndoC-betaH1
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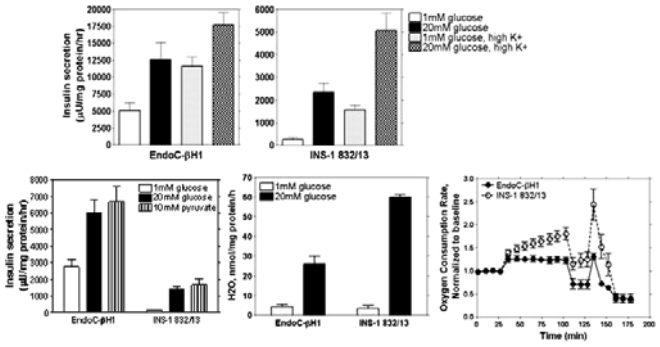
Background and aims: Clonal cell lines have been extensively used in investigations of ß-cell metabolism as they are accessible at large quantities, and as opposed to the islet, contain only one cell-type. Thus far, most research has been conducted in rodent ß-cell lines due to the lack of stable human ß-cell lines. However, findings from rodent models cannot generally be directly translated into humans. Here we examine and compare stimulus-secretion coupling in a recently developed human ß-cell line (EndoC-ßH1) with a widely used rodent ß-cell line (INS-1 832/13).
Materials and methods: EndoC-ßH1 and INS-1 832/13 clonal ß-cells were challenged with various secretagogues after which insulin secretion, insulin content, metabolite profiles, glucose utilization, ATP, oxygen consumption rate, plasma membrane potential and cytoplasmic free calcium were analyzed.

Results: Overall, the patterns of responses in the measured parameters were similar in the two cell-lines when stimulated with glucose, pyruvate and KCl. Basal insulin secretion, however, was higher in the human than in the rat cells. Conversely, the fold difference in the response to secretagogues and the plasma membrane potential was lower in the human cells. Metabolite profiling revealed clear similarities, but also differences in e.g. metabolites associated with mitochondrial shuttling.
Conclusion: To this end, the response in metabolism to glucose stimulation was found to be largely similar in human EndoC-ßH1 cells and rat INS-1 832/13 ß-cells. Hence, previous knowledge generated from studies on stimulus-secretion coupling in the INS-1 832/13 ß-cell line may to a large extent translate into human ß-cell biology.



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Interaction of incretin and sulfonylurea through Epac2A signalling in insulin secretion
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Background and aims: Incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) potentiate glucose-induced insulin secretion by increasing the intracellular cAMP level. This potentiation is mediated by both protein kinase A (PKA)-dependent and PKA-independent pathways, the latter involving Epac2A, a protein possessing guanine nucleotide exchange factor activity towards the small G-protein Rap. Recently, we found that Epac2A is directly activated by sulfonylurea (SU) and is important in SU-induced insulin secretion. Thus, Epac2A is a target of both cAMP and SU. To investigate the interaction of incretin and SU through Epac2A in insulin secretion, we examined the effects of combination of SU with incretin or cAMP analog on insulin secretion and activation of Epac2A/Rap1 signaling.
Materials and methods: We examined the effects of combination of SUs with GLP-1 on insulin secretion from isolated mouse pancreatic islets. We also examined dynamics of insulin secretion from perfused pancreases stimulated with combination of GLP-1 and SUs using wild-type and Epac2A-deficient mice. Activation of Epac2A by SUs and 8-pCPT-2'-*O*-Me-cAMP (8-pCPT), an Epac-selective cAMP analog, in insulin-secreting MIN6 cells was examined by fluorescent resonance energy transfer (FRET) system using Epac2A FRET sensor. Activation of Rap1 by SUs and 8-pCPT in MIN6 cells was examined by GTP-Rap1 pull-down assay. The effect of Rap1 activation on insulin secretion was examined using MIN6 cells infected with adenovirus carrying constitutive-active Epac2A mutant (CA-Epac2A), which lacks both cAMP-binding domains.
Results: In MIN6 cells, Epac2A was activated by glibenclamide (GLB) or glimepiride (GLM) but not gliclazide (GLC). In pancreatic islets, combination of GLP-1 with GLB or GLM augmented insulin secretion at both 4.4 and 8.8 mM glucose. Combination of GLP-1 with GLC augmented insulin secretion but the extent is smaller than that by combination of GLP-1 with GLB or GLM. In perfusion experiment, GLB and GLM induced insulin secretion in a biphasic manner: a transient increase immediately after stimulation (early phase), followed by sustained release (late phase). In the presence of GLP-1, GLB-induced insulin secretion was markedly potentiated in both early and late phases in wild-type mice. In contrast, potentiation of both phasic GLB-induced insulin secretion by GLP-1 was markedly reduced in Epac2A-deficient mice. On the other hand, there was no significant difference in the potentiation of GLC-induced insulin secretion by GLP-1, which was observed predominantly in late phase, between wild-type and Epac2A-deficient mice. In MIN6 cells, Epac2A was synergistically activated by combination of GLB and 8-pCPT. Activation of Rap1 was enhanced by combination of 8-pCPT with GLB or GLM but not GLC. We next examined the association of enhancement of Rap1 activation and insulin secretion, using CA-Epac2A. Introduction of CA-Epac2A into MIN6 cells induced strong activation of Rap1 in the absence of cAMP and significant potentiation of GLB- and glucose-induced insulin secretion.
Conclusion: Incretin and SU synergistically augment insulin secretion through Epac2A/Rap1 signaling. This synergistic effect depends upon SU structure.

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Central role of osteopontin in pancreatic islets: regulation by glucose and incretin hormones
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Background and aims: In diabetes, metabolic memory refers to a situation where the body can remember glycemic control years later, emphasizing the need for initial optimal metabolic control to prevent late complications. Elevated glucose levels can turn on genes with deleterious effects, among which we in this project focused on osteopontin (OPN). In patients with type 2 diabetes, circulating concentrations of OPN are elevated, and over expression of OPN has been associated with inflammation and accelerated diabetic complications. Previous studies have revealed that OPN is up-regulated in the serum of type 1 diabetic patients as well as in diabetic vascular walls and kidneys. Recent publication from our own group showed that in insulin-secreting cells, OPN could stimulate cell proliferation and prevent cytokine-induced apoptosis. These data suggest that OPN plays an important role in pancreatic islets. We therefore aimed at determining the function of OPN in pancreatic islet and how hyperglycaemia and incretins regulate the expression and function of OPN under normal and diabetic conditions.
Materials and methods: We conducted our study on diabetic mouse models and human pancreatic islets, involving methods as islet hormone secretion assay, immunohistochemistry, quantitative PCR and ELISA.
Results: Our data showed that in isolated single mouse islets, high glucose significantly increased OPN secretion about threefold ($p<0.001$) and elevated expression level by fivefold ($p<0.05$). GLP-1 upregulated OPN secretion by 1.5 fold ($p<0.001$) when islets are exposed to high glucose (16.7 mM) but has no effect at low glucose (1 mM), while GIP stimulated twofold OPN secretion at low glucose ($p<0.001$), but not at high glucose. We also observed that OPN exerts direct inhibitory effect on glucose-stimulated insulin secretion (GSIS) in both mouse (~50%, $p<0.05$) and human islets (~50%, $p<0.01$). Interestingly, in diabetic human islets, GSIS was elevated about 1.5 fold ($p<0.01$) by OPN. In addition, we explored the role of OPN in diabetic SUR1-E1506K mutant mice, in which an excessive secretion of OPN was found in islets. We observed that OPN is present in both pancreatic beta- and alpha-cells by confocal microscopy, and high glucose is able to upregulate OPN expression and secretion in islets from wild type C57Bl (1.5 fold, $p<0.05$), but not in SUR1-E1506K mutant mice. However, OPN secretion is significantly upregulated in SUR1-E1506K mice islets compared to wt when challenged by high glucose ($p<0.001$).
Conclusion: Our study shows that OPN inhibits GSIS in both human and mouse islets, but stimulated GSIS in diabetic human islets. The secretion and expression of OPN is regulated by glucose and incretins. When exposed to high glucose, diabetic mouse islets secreted more OPN compare to its wildtype.
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Effects of glucose and potassium depolarisation on the granule mobility in the submembrane space of insulin-secreting MIN6 cells
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Background and aims: Physiologically, insulin secretion is initiated by an increase of the glucose concentration. This involves a depolarization-induced

Ca^{2+} influx, which is experimentally often mimicked by raising the K^+ concentration in the medium. It has come into doubt, however, whether the stimulatory effect of a strong K^+ depolarization, which produces a first phase-like insulin secretion, is based on the same mechanisms as the first phase of glucose-induced secretion.

Materials and methods: Granules in the immediate vicinity of the plasma membrane were visualized by transient transfection of insulin-secreting MIN6 cells with an insulin-EGFP fusion protein and imaged by TIRF microscopy. The cells were continuously perifused with HEPES-buffered Krebs-Ringer medium (saturated with 95% O_2 and 5% CO_2) at 37.0 °C. The TIRF field had a calculated decay constant of 84 nm. The image files (1 sequence = 200 images = mean duration of 25 seconds) were evaluated by an in-house written program (MATLAB 7.6.0) to achieve an observer-independent quantification. The insulin secretion of MIN6 pseudoislets was measured by perifusion with ELISA of the fractionated efflux.

Results: Insulin release from perifused MIN6 pseudoislets was elicited by 30 mM glucose followed by 40 mM K^+ after an interval of 10 min with basal glucose. These stimuli were also applied in the reversed sequence. Under each condition 40 mM K^+ produced a robust increase of secretion whereas glucose was markedly less effective when following the K^+ depolarization. The same experimental protocol was used to measure the mobility of insulin granules in the submembrane space of perifused single MIN6 cells. Depending on the duration of their presence in the submembrane space, insulin granules were classified as either short-term (presence < 1 s) or long-term residents (presence for the entire image sequence ≥ 25 s). In a typical sequence ca. 80 % of all the granules that were identified were short-term residents and 30 to 40 % of the granules identified in the first image were still visible in the last image and were classified as long-term residents. Additionally, arrivals at and departures from the submembrane space were quantified, which represent the bidirectional mobility in the z-direction, i.e. orthogonal to the plasma membrane. Independently of the sequence of exposure 30 mM glucose and 40 mM K^+ led to an increase of arrivals and departures within 2 min. During the next 7 min there was a further slight increase with high glucose but not with 40 mM K^+. The increase of arrivals and departures was reversible upon wash-out of the stimuli. The increased rates of arrivals and departures led to a transient increase of the total number of granules in the submembrane space and a transient decrease of the number of long-term resident granules. None of these effects was seen with a constant basal glucose concentration.

Conclusion: High glucose and K^+ depolarization exert similar effects on the mobility of submembrane insulin granules in MIN6 cells, suggesting a role for depolarization-induced Ca^{2+} influx. However, increased mobility and secretion coincided only with K^+ depolarization, but were separable with glucose stimulation, suggesting the control of exocytosis by additional regulatory elements further downstream.

Supported by: DDG, DFG, DDS

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Autocrine signals mediate plasma membrane translocation of protein kinase C in insulin-secreting cells

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Background and aims: Activation of protein kinase C (PKC) typcially involves Ca^{2+}- and/or diacylglycerol (DAG)-dependent translocation of the kinase from the cytoplasm to the plasma membrane. In insulin-secreting β-cells, PKCs have been implicated in the regulation of metabolism, cell death, proliferation and secretion. In the present study we investigated PKC translocation to the plasma membrane in response to transient increases in plasma membrane DAG after autocrine activation of $P2Y_1$-purinoceptors by ATP co-released with insulin.

Materials and methods: MIN6 β-cells were transfected with different fluorescent-protein-tagged PKC isoforms and a fluorescent translocation biosensor for DAG. The cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with the fluorescent indicator Fluo-4. Changes of fluorescence in the sub-plasma membrane space were recorded with total internal reflection microscopy.

Results: Glucose stimulation of MIN6 β-cells triggered brief (<10 s), repetitive DAG elevations ("spiking"), which were perfectly mirrored by rapid recurring translocation of the novel PKC isoforms PKCδ, -ε, and -η. The conventional PKCβI and βII isoforms showed a more complex response with both the rapid and a slower translocation pattern. Depolarization with K^+ also induced parallel DAG spiking and PKC translocation. Whereas PKCε dissociated from the membrane between the K^+-induced DAG spikes, PKCβI dissociation was incomplete and the sustained component due to $[Ca^{2+}]_i$ elevation. Inhibition of $P2Y_1$-purinoceptors interrupted both DAG spiking and the intermittent translocation of the PKCs, but did not affect $[Ca^{2+}]_i$ or the associated sustained PKCβI translocation.

Conclusion: Exocytosis from β-cells induces transient increases of DAG, which translate into recurring translocation of conventional and novel PKCs to the plasma membrane, indicating that PKC signalling is involved in the autocrine regulation of β-cell function.

PS 028 Islet gene regulation

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The clock gene Rev-erb alpha regulates glucagon secretion in mouse pancreatic alpha cells

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Background and aims: Disruption of pancreatic clock genes impairs pancreatic beta cell function, leading to the onset of diabetes. Specifically, the clock gene Rev-erbalpha was shown to regulate insulin secretion and proliferation. Despite the importance of alpha-cells in the regulation of glucose homeostasis and diabetes pathophysiology, nothing is known about the role of Rev-erbalpha in these cells. Therefore, the aim of the present work was to study the role of the clock gene Rev-erb alpha in the regulation of glucagon secretion.
Materials and methods: Pancreatic mouse alpha TC1-9 and mouse primary alpha cells were used. Gene expression and protein levels were measured by RT-PCR and western blot respectively. Gene silencing was done by siRNA technique. Intracellular calcium levels were measured by fluorescence microscopy. Purification of primary mouse alpha cells were done by FACS and glucagon secretion was measured by ELISA.
Results: Rev-erb alpha gene and protein down-regulation by siRNA in alphaTC1-9 cells inhibited glucagon secretion ($p<0.01$). The Rev-erb alpha agonist GSK4112 increased glucagon secretion (1.6 fold) and intracellular calcium signals in alphaTC1-9 cells and mouse primary alpha-cells, whereas the Rev-erb alpha antagonist SR8278 produced the opposite effect. AlphaTC1-9 cells exhibited intrinsic circadian Rev-erb alpha expression oscillations at 0.5mM glucose that were inhibited by 11mM glucose. In mouse alpha-cells, glucose induced similar effects ($p<0.001$). High glucose inhibited key genes controlled by AMPK such as Nampt, Sirt1 and PGC-1alpha ($p<0.01$). AMPK activation by metformin completely reversed the inhibitory effect of glucose on Nampt-Sirt1-PGC-1alpha and Rev-erb alpha genes. The Nampt inhibitior FK866 decreased Sirt1, PGC-1alpha and Rev-erb alpha mRNA expression and inhibited glucagon release ($p<0.01$).
Conclusion: Rev-erb alpha is as a new intracellular regulator of glucagon secretion via AMPK/Nampt/Sirt1 pathway. Strategies to target the Nampt-Sirt1-Rev-erb alpha in pancreatic alpha-cells can be useful for the treatment of hyperglucagonemia present in diabetes.
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Roles of the type 2 diabetes-associated gene products Arap1 and StarD10 in the control of insulin secretion

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Background and aims: Investigation of the function of genes identified by genome-wide association studies for Type 2 diabetes may bring a better understanding of disease aetiology and ultimately provide new therapeutic targets. Recently-identified common genetic variants in the ARAP1 locus affect fasting proinsulin levels (rs11603334) and glucose-induced insulin secretion (rs1552224) in man. Two genes are implicated at this locus: ARAP1 and STARD10. To determine which of these might confer altered disease risk, and to explore the molecular mechanisms involved, we have studied the effect on insulin secretion of manipulating Arap1 and StarD10 levels in mouse islets and rodent beta cell lines.
Materials and methods: Arap1 and StarD10 expression was silenced by siRNA and proteins overexpressed via a coding plasmid transfected by Lipofectamine 2000 (cell lines) or adenoviral infection (mouse islets). Insulin secretion was measured by radioimmunoassay.
Results: Western (immuno-) blot analysis revealed that both Arap1 and StarD10 are expressed in mouse and human islets and in beta cell lines. Confocal immunocytochemistry demonstrated chiefly extranuclear localisation of both proteins. Forced over-expression of StarD10 cDNA in MIN6 cells decreased insulin secretion provoked by 30 (versus 3.0) mM glucose (control: 2.54 ± 0.21-fold; StarD10: 1.55 ± 0.10-fold; $p<0.01$) or 30mM KCl (control: 5.20 ± 0.35-fold, StarD10: 2.63 ± 0.14-fold; $p<0.0001$). Over-expression of the full-lengh variant of Arap1 (variant 3) exerted no apparent effect on insulin secretion in response to either 30mM glucose (control: 2.31 ± 0.28-fold; Arap1-V3: 2.22 ± 0.25-fold; NS) or 30mM KCl (control: 4.24 ± 0.48; Arap1-V3: 4.13 ± 0.72; NS). Although overexpression of a shorter form of Arap1 (variant 1), lacking an N-terminal inhibitory domain and thus supposed to be more active, had no significant effect on insulin secretion, we observed a strong tendency toward an inhibition of insulin secretion provoked by 30 mM glucose (control: 4.01 ± 0.63-fold; Arap1-V1: 2.84 ± 0.53-fold; NS) or 30mM KCl (control: 3.68 ± 0.28; Arap1-V1: 2.90 ± 0.32; $p=0.083$). siRNA-mediated silencing of either gene exerted no significant effect on stimulated insulin secretion
Conclusion: These data suggest that StarD10, implicated in phospholipid transfer between intracellular organelles, may act downstream of glucose metabolism to modulate insulin granule trafficking and/or exocytosis. Increased levels of StarD10 in beta cells from risk allele carriers may thus lead to diminished insulin secretion.
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PS 028 Islet gene regulation

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Identification of mitochondrial targets in human islets exposed to high glucose or fatty acids

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Background and aims: Exposure of pancreatic beta cells to metabolic stresses, such as chronic high glucose, fatty acids and oxidative stress, alters mitochondria and interrupts the transduction of signals normally coupling glucose metabolism to insulin secretion. However, it remains unclear what are the respective contributions of different diabetes-associated stresses on human beta cell dysfunction and death. Here, we aimed at identifying putative mitochondrial targets of beta cell injuries secondary to metabolic stresses.
Materials and methods: Freshly isolated human islets from three different donors, who had provided written informed consent, were cultured for 3 days in different conditions: 1) in control 5.6mM glucose 2) at high 25-30mM glucose 3) with 0.4mM palmitate 4) with 0.4mM oleate and 5) at 5.6mM glucose after a transient oxidative stress (200μM H2O2 for 10 min at day 0). Effects of the different metabolic stresses on expression profiles were repeatedly investigated in the three independent islet preparations. The NanoString-based molecular screening allowed absolute quantification of mRNAs of 57 mitochondrion-associated genes. The expression profile was compared to non-endocrine human HeLa cells. Molecular targets of interest were further evaluated at the protein level by immunoblotting.
Results: We first established the transcriptome of 30 mitochondrial inner membrane proteins, including the recently identified pyruvate carriers MPC1 and MPC2. Then, we observed that the different stresses induced specific responses in terms of expression profiles. Chronic exposure of human islets to high glucose impaired glucose-stimulated insulin secretion, decreased insulin content, promoted caspase-3 cleavage and cell death. High glucose specifically reduced transcript levels of the energy sensor SIRT1, of key transcription factors (HNF4α, PPARα, TFAM), and of mtDNA-encoded respiratory chain subunits (MT-ND3, MT-ATP6). Conversely, transcript levels of the uncoupling protein UCP2 and of the dicarboxylate transporter DIC and the aspartate/glutamate carrier AGC2 were increased by high glucose, a profile compatible with important mitochondrial anaplerotic/cataplerotic activities and NADPH-generating shuttles. All tested metabolic stresses down-regulated IPF1, MAFA, and the pyruvate carboxylase PC. Three days after acute oxidative stress, no significant alteration or apoptosis were detected in human islets. Immunoblotting revealed complex regulation of protein expression, not necessarily correlating with mRNA levels. Interestingly, absolute transcripts levels of PC, TFAM and UCP2 were lower in human islets compared to HeLa cells.
Conclusion: NanoString data provided first insights into absolute transcript levels in human islets, both in safe and metabolically stressful conditions. Expression profile of mitochondrion-associated genes was selectively modified by glucose in human islets, delineating a glucotoxic-specific signature.
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TCF7L2* is the master regulator or proinsulin expression through *ISL1, MAFA* and *NEUROD1

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Background and aims: Type 2 Diabetes is characterized by elevated glycaemia as a consequence of reduced insulin secretion along with compromised insulin sensitivity. Genetic variants in the*TCF7L2*, shows the strongest association with type 2 diabetes (T2D) by affecting various aspects of beta cell function, including reduced insulin secretion.
Materials and methods: The screening of downstream pathways of TCF7L2 was performed using transcriptome RNA-seq in *Tcf7l2* depleted INS-1 832/13 cells. Pathway analysis was performed using Ingenuity IPA. Downstream targets were verified using Real-Time PCR, Western Blot, immuno histo chemistry imaging, and measurement of glucose stimulated insulin secretion.
Results: Knockdown of *TCF7L2* using siRNA reduced the expression of proinsulin gene expressionas well as of *Isl-1*, *MafA*, and *NeuroD1* expression in INS1 831/13 cells, and consequently glucose stimulated insulin secretion. *Isl1* was identified as a direct target of TCF7L2 and ISL1 is a known enhancer of proinsulin gene expression. In human pancreatic islets from cadaver donors, siRNA mediated *TCF7L2* depletion caused down regulation of *Isl1*, *MAFA* and *NEUROD1* mRNA expression. In addition, expression of *ISL1, MAFA* and *NEUROD1* correlate significantly with the *INS-IGF2* mRNA in human islets. Both NEUROD1 and MAFA were bound to the proinsulin promoter.
Conclusion: We report a novel pathway by which TCF7L2 can influence insulin synthesis (proinsulin) and secretion, by regulating the expression of ISL1, NEUROD1 and MAFA in adult pancreatic islets.
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cAMP mediated regulation of miR-212/132 expression in insulin secreting cells acts through CREB-regulated transcription co-activator 1 (CRTC1)

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Background and aims: MicroRNAs (miRNAs) are small non-coding RNAs controlling gene expression. We have previously demonstrated miR-212 and miR-132 to be regulated by glucose in Wistar rat islets. Others have demonstrated that the transcription factor CREB binds to CRE-sites on the miR-212/132 promotor. Here we aim to investigate mechanisms involved in cAMP-mediated transcriptional regulation of the miR-212/132 cluster in insulin secreting cells.
Materials and methods: INS-1 832/13 cells were cultured for 2, 6 and 24 h in 2.8, 5.0 and 16.7 mM glucose in the absence or presence of 1 μM forskolin (FSK) and 100 μM IBMX. To investigate the role of co-activators of CREB, INS-1 832/13 cells were transfected with siRNA against CRTC1, 2 and 3. Glucose stimulated insulin secretion (GSIS) was determined as the fold-increase in insulin released during 1 h incubation at 16.7 as compared to 2.8 mM glucose. The release at the different conditions was measured using RIA. Relative expression of miRNAs and mRNAs was determined by qPCR. Western Blot was performed to verify phosphorylated CREB.
Results: Expression of miR-212 and miR-132 was significantly increased after 2h culture in 16.7 mM as compared to 2.8 mM glucose ($p<0.05$; n=3 for each miRNA). A more pronounced increase in expression of miR-212 and miR-132 was observed after incubation in FSK/IBMX (3-and 4-fold, respectively; $p<0.01$) as compared to glucose alone and this increased to 5- and 7-fold and 6- and 8-fold after 6 and 24 h, respectively. GSIS was 3-fold ($p<0.01$; n=3) after culture in 2.8 mM glucose for 2h, which was significantly reduced (to 2-fold increase; $p<0.05$) after culture in the simultaneous presence of FSK/IBMX. The reduction in GSIS was more pronounced after culture for 6 an 24 h. Increased level of phosphorylated CREB was observed at 16.7 mM glucose, which was further enhanced in the presence of FSK. Transfection of INS-1 832/13 cells with siRNA against the three CRTCs separately reduced each of them significantly (>50%). Expression of miR-212 and miR-132 ($p<0.05$; n=3) and GSIS were significantly decreased ($p<0.01$; n=3) in cells transfected with siRNA against CRTC1, whereas expression of miR-212 and miR-132 was unaffected after silencing of CRTC2 and 3.
Conclusion: We confirm our previous observation that expression of miR-211 and miR-132 is glucose dependent in insulin secreting cells. Moreover, our data suggests that the miR-212/132 cluster is strongly regulated by cAMP in insulin secreting cells and that this is, at least in part, mediated through CREB and its co-activator CRTC1.
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GLP-1 rescued dysregulated miRNA by hyperglycaemia in diet-induced obese mice

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Background and aims: GLP-1 has shown direct beneficial effects on beta cells, such as anti-apoptotic effect, increasing beta cell mass, and improvement of beta cell function. However, its mechanism is still not fully understood. MicroRNAs could be involved in insulin secretion, pancreatic development, and

beta cell differentiation. This study investigated the role of microRNA in beta cell development with GLP-1 in obesity-associated diabetes.
Materials and methods: We administered GLP-1 to diet-induced obese mice fed high fat diet(HFD) for 2 weeks. Body weight, food intake were monitored regularly throughout the treatment. beta cell mass, islet size, plasma glucose, and plasma insulin were measured after 2 weeks of dosing. We have selected 6 microRNAs(miR-15a, 29c, 34a, 124a, 146a, and 375), as candidate according to literature data and to a computational analysis and analysed the microRNA expression by real-time quantitative PCR in serum and pancreas.
Results: The HFD Mice receiving GLP-1 had a body weight gain similar to that of the HFD mice. The HFD Mice receiving GLP-1 were significantly increased beta cell mass relative to the HFD mice. The HFD Mice receiving GLP-1 were significantly decreased both plasma glucose and insulin levels relative to the HFD mice. The HFD mice were significantly increased miR-15a, 29c, and 375 levels in pancreas relative to the low fat diet mice. Expression of miR-15a, 29c, 146a, and 375 was significantly decreased by administration of GLP-1 in pancreas of the HFD mice. The HFD mice ware significantly decreased serum miR-15a, 29c, 34a, 124a, 146a, and 375 levels relative to the low fat diet mice. Expression of serum miR-15a, 29c, 34a, 124a, 146a, and 375 was significantly increased by administration of GLP-1 in the HFD mice.
Conclusion: Our results provide experimental evidence that dysregulated miRNA contributes to development of obesity-induced insulin resistance. miR-15a, 29c, 34a, 124a, 146a, and 375 play an important role for beta cell development with GLP-1 in obesity-associated diabetes and are a potential target for the treatment of obesity-associated diabetes.
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Differences in microRNA expression between embryonic stem cells and induced pluripotent stem cells during differentiation to a pancreatic lineage

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Background and aims: Type 1 diabetes mellitus (T1DM) results from autoimmune destruction of pancreatic ß cells. Islet transplantation has shown proof of principle for cell replacement therapy to treat T1DM. Insulin-expressing cells have been produced in vitro from embryonic stem cells (ESCs), however problems associated with ESCs, primarily ethical concerns & immunogenicity, mean an alternative cell source is needed. Induced pluripotent stem cells (iPSCs) are an alternative source of pluripotent stem cells which can be derived in a patient-specific manner. iPSCs have been shown to differentiate into insulin-expressing cells in vitro but it is unknown whether iPSCs are equivalent to ESCs, since important differences have been shown to exist between ESCs & iPSCs which may affect the ability of iPSCs to give rise to cells of a pancreatic lineage & thus limit their usefulness for treatment of T1DM. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. They are differentially expressed depending on cell type & stage of differentiation. A unique miRNA signature characterises pancreas development at the definitive endoderm (DE) stage. Given the recognised differences in miRNA expression between iPSCs & ESCs, we have investigated the profiles of miRNA expression at the DE stage, a critical step in the in vitro differentiation of these cells toward an insulin-expressing phenotype.
Results: Multiple iPSC & ESC lines were differentiated into DE using an Activin A-based protocol. Individual cell lines showed different propensities to form DE. As a result, microarray analysis was carried out on undifferentiated & differentiated iPSCs & ESCs in order to compare miRNA expression. Microarray analysis allowed identification of miRNAs that are up- or down-regulated upon differentiation to DE, & which may therefore play a role in this process. The 10 miRNAs that are most upregulated in DE are miR-375, miR-708-5p, miR-744-5p, miR-4792, miR-4530, miR-26b-5p, miR-4472, miR-27b-3p, miR-4289 & miR-30b-5p. The 10 miRNAs that are most downregulated in DE are: miR-5002-5p, miR-378a-3p, miR-3941, miR-4451, miR-516b-5p, miR-4436b-5p, miR-4732-3p, miR-32-3p, miR-124-5p & miR-4285. Several of these miRNAs have been previously implicated in pancreas development. Comparison of undifferentiated iPSCs & ESCs showed that they share a miRNA signature, with no miRNAs differentially expressed ($p<0.05$). However, upon differentiation, 91 miRNAs were differentially expressed in iPSCs vs. ESCs. This suggests that miRNA expression may play an important role in the ability of iPSCs & ESCs to differentiate into DE. Further elucidation of the role of these miRNAs may be useful for improving the efficiency of differentiation into a pancreatic lineage. Microarray results have subsequently been validated by qRT-PCR. We are now investigating the role of miRNAs that are involved in DE formation & that are differentially expressed between ESCs & iPSCs through the identification of putative targets & the manipulation of miRNA expression in differentiating cells.
Conclusion: iPSCs are a promising alternative to ESCs for the treatment of T1DM. However, differences in miRNA expression between these two cell types may affect their ability to differentiate into the pancreatic lineage, which may be important for any future clinical application of these cells.

PS 029 Quantification of beta cell mass

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Dual modal MR/fluorescent zinc sensing probes for in vivo beta cell imaging

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Background and aims: Changes in pancreatic beta cell mass contribute to the development of both Type 1 and Type 2 diabetes. At present, however, robust approaches to following these changes prospectively *in vivo* are lacking. Compared with other modalities, Magnetic Resonance (MR) imaging has excellent anatomical resolution but suffers, at the molecular scale, with low intrinsic low sensitivity. To produce a detectable change in water signal intensity, a relatively high concentration of contrast agent (0.01 - 0.1 mM) is required. This creates problems when imaging at the molecular level, as the most interesting targets are present at much lower concentrations, typically in the nano-molar range. In order to overcome the inherent sensitivity problem of the MRI, dual modal contrast agents show great promise for use both in experimental models and potentially in the clinic. Given the high concentration of zinc ions in insulin granules, and relative scarcity of these ions throughout the rest of the pancreas, we hypothesised that MRI-active agents capable of binding zinc may be used to quantify beta cell mass in vivo. Specifically, we have modified DOTA to allow both zinc binding and the incorporation of fluorescent sensors. Coordination with gadolinium yields novel dual-modal, MR/fluorescent zinc sensing probes.
Materials and methods: DOTA was modified with various organic fluorophores, AQA (Gd.1) and Dansyl (Gd.2), and zinc sensing motifs, to create zinc sensing agents. Probes were tested using both pancreatic beta cell-derived (MIN6, INS1(832/13) and non-beta (HEK293, HeLa) cell lines and in vivo after injection into CD1 mice. Fluorescence was monitored by confocal or multiphoton microscopy and MRI using a 9.4T magnet.
Results: Of compounds synthesised, Gd.1 showed ratiometric fluorescence changes upon zinc binding with a Stokes shift, from λem = 410 nm to λem = 500 nm upon zinc chelation, and a Kd = 22 μM. Gd.1 has an r_1 = 4.2 $mM^{-1}s^{-1}$, which increased to 6.2 $mM^{-1}s^{-1}$ (9.4T) upon zinc binding. Gd.2 shows an increase in fluorescence intensity at λem = 520 nm, with a Kd = 44 μM, Gd.2 has an r_1 = 3.7 $mM^{-1}s^{-1}$, which decreases to 3.4 $mM^{-1}s^{-1}$ (9.4T) upon zinc chelation. Examined in beta cell lines, both agents also showed localisation to secretory granules identified by co-expression of granule-resident protein phogrin, and relatively poor uptake into non beta cells. Injection of Gd.1 into mice also revealed uptake into islets with modest staining of other tissues.
Conclusion: We describe dual modal imaging agents that sense zinc, with excellent fluorescent and MRI properties, which may be used to monitor beta cell mass in vivo.
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^{111}In-exendin for non-invasive quantification of beta cell mass in patients with type 1 diabetes and healthy controls

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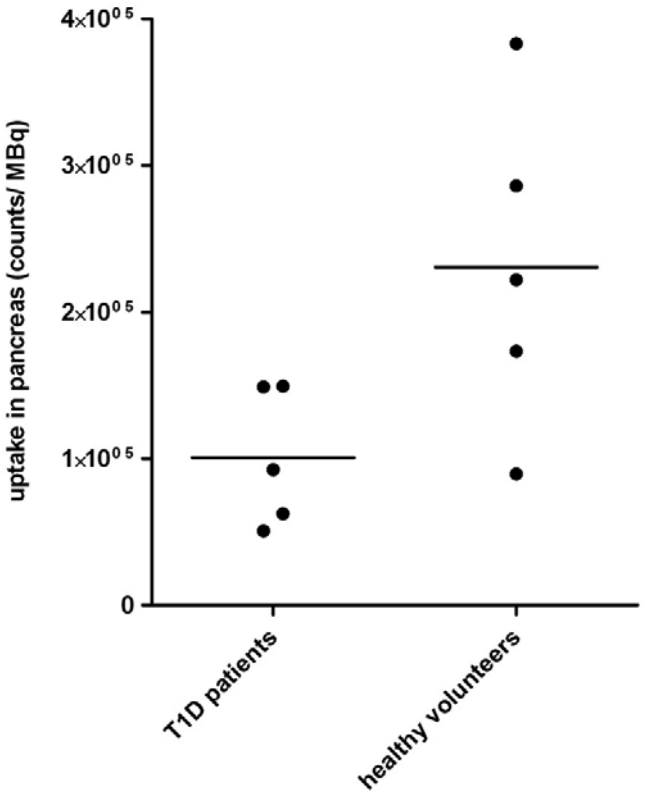
Background and aims: To date, many questions remain concerning the role of beta cell mass (BCM) in the pathophysiology and course of type 1 and type 2 diabetes (T1D and T2D). Various tests exist to quantify beta cell (BC) function (insulin secretion), but do not necessarily provide information on BCM, while BCM seems the most important predictor of future BC failure. A method that reliably can quantify BCM would be a tremendous asset to better elucidate the pathophysiology of T1D and T2D, and to determine the effects of therapeutic interventions. We developed a non-invasive imaging technology based on targeting of ^{111}In-labelled Exendin to the glucagon-like peptide-1 (GLP-1) receptor, which is specifically expressed on beta cells. In previous studies in animal models we showed that the pancreatic uptake of this tracer correlates with BCM. Here, we report the first results of an ongoing clinical trial using single photon emission computed tomography (SPECT) with ^{111}In-Exendin for BCM quantification in patients with T1D and in healthy controls.
Materials and methods: Patients with long standing T1D (no measurable C-peptide after glucagon stimulation, BMI below 27, age 21-60 yr) and healthy controls (normal glucose tolerance, matched for age, gender and BMI) are eligible for the study. 24 hours after i.v. injection of 150 MBq ^{111}In-Exendin-4, SPECT/CT scans were acquired for quantitative assessment of pancreatic BC targeting. A volume of interest (VOI) of the pancreas was delineated on SPECT/CT images and counts within this VOI were corrected for administered activity and time after injection.
Results: So far, 5 T1D patients and 5 matched healthy volunteers were studied. The pancreatic uptake of ^{111}In-Exendin (see figure) varied widely among these subjects. In healthy volunteers, it ranged from 0.9x10^5 to 3.8x10^5 counts/MBq (mean 2.3x10^5 counts/MBq). The uptake was decreased in T1D patients, but interestingly not completely absent in all, ranging from 0.5x10^5 to 1.5x10^5 counts/MBq (mean 1.0x10^5 counts/MBq). Our main observations are: a) pancreatic uptake in T1D patients is up to 8-fold lower than in healthy volunteers, b) pancreatic uptake varied by a factor of 3-4 amongst individuals within one group, and c) in some T1D patients, the radiotracer uptake suggests a substantial residual BCM.
Conclusion: Our results suggest that quantification of BCM with ^{111}In-Exendin SPECT is feasible. The data show large differences between T1D patients and healthy controls, as well as a high inter-individual variability in pancreatic uptake. Our *in vivo* imaging data are in line with autopsy data from the literature, suggesting that T1D patients may have a relevant remaining BCM, and that large inter-individual differences in BCM exist. ^{111}In-Exendin SPECT seems a valuable tool for non-invasive determination of BCM.



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Towards clinical imaging with ^{18}F-labelled exendin-4, synthesised via click chemistry

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Background and aims: The glucagon like peptide-1 receptor (GLP-1R) is abundantly expressed in pancreatic beta cells and is a candidate molecular

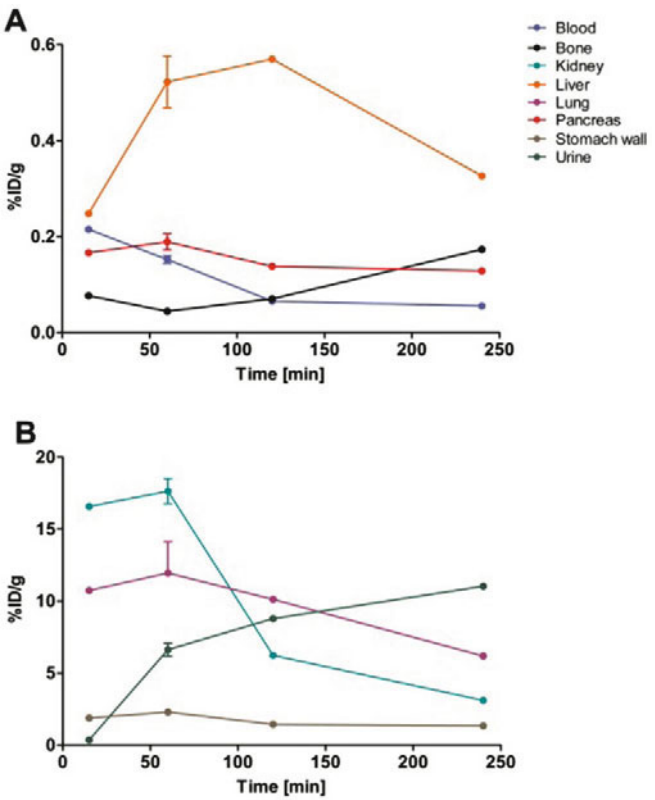
target for beta cell imaging. Click chemistry (Cu(I)-catalysed alkyne/azide cycloaddition) was explored for the ^{18}F-labelling of GLP-1R specific exendin-4 peptide. We aim to develop [^{18}F]exendin-4 tracer for clinical imaging of beta cells with PET.

Materials and methods: Synthesis of [^{18}F]exendin-4 was started by reacting alkyne tosylate precursor with ^{18}F-fluoride-Kryptofix complex in DMSO. Subsequently, exendin-4 azide was added to react with [^{18}F]alkyne in the presence of $CuSO_4$/Na-ascorbate. Radiochemical analysis and isolation of [^{18}F]exendin-4 was performed using HPLC. *Ex vivo* biodistribution of [^{18}F]exendin-4 was evaluated in male Sprague-Dawley rats (N = 1-3 per time point). Rats (250-300 g) were injected intravenously with 6 ± 1 MBq (mean ± SD) [^{18}F]exendin-4 (mass 0.10 ± 0.04 nmol, specific activity 66 ± 24 MBq/nmol) and sacrificed at 15 min, 1 h, 2 h or 4 h after injection. The organ-specific radioactivity was reported as a percentage of the injected dose per gram of tissue (%ID/g). Intrapancreatic distribution of radioactivity was assessed with autoradiography and islet-to-exocrine tissue ratios were analyzed. For PET scans, one rat was imaged twice (dynamic 0-1 h, static 3.5-4 h) using Inveon Multimodality PET/CT.

Results: The radiochemical yield of the Click reaction was 40-45 %, and the specific activity of [^{18}F]exendin-4 was 80-140 MBq/nmol after synthesis. Most of the radioactivity was observed in the kidneys (Fig. 1B). Renal clearance was high (3 %ID/g 4 h p.i.), while radioactivity was nearly constant in the pancreas and stomach wall over the course of the study (Fig. 1A and B). The islet-to-exocrine tissue ratio in pancreas was 10 in one study using [^{18}F]exendin-4 with a specific activity of < 1 MBq/nmol, and increased to a maximum of 130 (*modus:* 60-70) with a specific activity of 140 MBq/nmol. Radioactivity in bone was low, indicating low defluorination. In PET images, the highest tracer uptake was found in kidney, lung and stomach wall.

Conclusion: To be clinically applicable for beta cell imaging, a sensitive radiotracer with high specific activity (> 1 GBq/nmol) is required. We have established a synthetic route towards ^{18}F-labelled exendin-4 based on Click chemistry. Our preliminary results of [^{18}F]exendin-4 showed specific and sustained uptake in pancreatic islets with an islet-to-exocrine tissue ratio of up to 130. Although far from clinically viable, these indications are promising for the development of an ^{18}F-labelled exendin-4 analogue with a specific activity that would render clinical imaging of beta cells.

Figure 1. *Ex vivo* biodistribution of [^{18}F]exendin-4 in rat.



Supported by: CoE, BetaImage

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^{19}F-mannoheptuloses as contrast agents for the non-invasive imaging of GLUT2-expressing cells: in vivo and ex vivo MRI studies

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Background and aims: Suitable analogs of D-mannoheptulose are currently considered as possible tools for the non-invasive imaging of pancreatic islet insulin-producing cells. In the present studies, we examined whether ^{19}F-heptuloses could be used for non-invasive imaging of GLUT2-expressing cells. It was previously shown that the GLUT-2 transporter is expressed in both β-cells and hepatocytes and that mannoheptulose has high uptake specificity for the GLUT-2 transporter .

Materials and methods: *In vivo* ^{19}F MRI was used to confirm an extracellular concentration of 10 mM of either 1-^{19}F or 3-^{19}F fluoro-deoxy-mannoheptulose (FMH) after injection. Male BL6 mice were anesthetized using isoflurane according to the ethical committee's recommendations. For the *in vivo* ^{19}F MR imaging experiments, a Bruker Biospec 9.4T small animal MR scanner was used in combination with an in-house build, single loop, saddle-shape surface coil (42 mm x 66 mm). The animals were sacrificed immediately after the final imaging session (2 to 5 hours after the ^{19}FMH injection). The pixelwise ^{19}F signal was considered significant if larger than 2*SD of background noise ($p<0.025$) at post-processing stage. Hereby, ^{19}F NMR spectra of homogenized tissue samples were acquired by using an NMR spectrometer (Bruker Avance II, 9.4T) after addition of a known concentration of 5-fluorocytosine as an external concentration and chemical shift reference. The effect of fluorinated heptoses, D-mannoheptulose and 2-fluoro-2-deoxy-glucose administrated as indicated above upon blood glucose and insulin concentrations was examined at 5, 15, 30, 60, and 120 min after intraperitoneal glucose injection.

Results: Using an in-house build ^{19}F MRI coil resulted in reproducible, sensitive and quantifiable 19F MR images with a detectability limit of approximately 10^{15} atoms per pixel. *In vivo* images show clear signals of 1-FMH and 3-FMH from the bladder, kidney, liver and potentially from the pancreas. Repeated MRI scans indicate rapid clearance of 1-^{19}FMH and 3-^{19}FMH from the circulation (kidney, bladder) and only low retention in the liver and pancreas. ^{19}F NMR spectroscopy of excited tissue confirms the presence of ^{19}F FMH in these organs (1 to 5 mM). Both *in vivo* MRI and *ex vivo* NMR show almost complete clearance of ^{19}F FMHs 24 hours after injection. The effect of the ^{19}FMH on the IPGTT was comparable to that of unlabeled D-mannoheptulose. Indeed, 30 min after injection of high concentration of D-glucose (1mg/g of mice) and coinjection of these heptoses, the glycaemia remained higher than in the control experiments.

Conclusion: *In vivo* experiments using the different FMH derivatives indicate rapid clearance of the compound. While the FMH signal remaining in the liver and pancreas is potentially sufficient for in vivo β-cell and hepatocyte imaging, the sensitivity has to be improved for applications in models of diabetes. The IPGTT experiments demonstrate that the fluorinated mannoheptuloses exert the same effect as the unlabeled heptose. These results support the hypothesis that these fluorinated mannoheptuloses could be suitable candidates in the perspective of their use as non-invasive imaging tools.

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Are we overestimating the loss of beta cells in type 2 diabetes?

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Background and aims: Decreased beta cell mass (-30/40% on average) is a consistent finding in human type 2 diabetes (T2D), and increased apoptosis is considered the major driving mechanism. However, recent work has shown that beta cell dedifferentiation, could also lead to beta cell failure in T2D. In addition, clinical findings such as the rapid recovery of insulin secretion after bariatric surgery suggests the presence of a relevant beta cell mass even in long-standing T2D patients. We therefore hypothesized that beta cell loss, as assessed so far, may be overestimated in T2D subjects, and performed morphological, morphometric, ultrastructural and functional studies with

pancreatic samples and isolated islets from non-diabetic (ND) and T2D individuals.
Materials and methods: We studied pancreas tissue samples and/or isolated islets from 6 ND (age: 69±3.5 years; gender: 3 males and 3 females; body mass index, BMI: 26.5±1.5 kg/m^2) and 7 T2D (age: 63±8 years; gender: 3 males and 4 females; BMI: 26.2±2.2 kg/m^2; known duration of diabetes: from 3 to 15 years) subjects. Immunocytochemical staining for insulin, glucagon and chromogranin, and electron microscopy analyses were performed, together with insulin secretion evaluations.
Results: By light microscopy, islet insulin-positive area was approximately 25% lower (p<0.001) in T2D (55.7±5.6%) than ND (74.3±8.7%). However, when islet beta cells were counted by electron microscopy, the 13% decrease in T2D (61.0±8.7% vs 70.3±6.4%) was barely significant (p=0.054). Beta cell amount as measured by light and electron microscopy differed significantly in T2D (p=0.036), but not in ND (p=0.41), and insulin granule density volume was lower (p<0.01) in T2D cases. Chromogranin expression was similarly detected in ND and T2D islets, including in cells negative for insulin. Glucagon containing cells, as quantified by both light and electron microscopy, did not differ between ND and T2D islets. When ND isolated islets were studied after 24h exposure to 22.2 mmol/l glucose, a marked reduction of insulin positive area by light microscopy assessment (non-treated: 74±8%; treated: 49±11%, p=0.03), but not upon electron microscopy (67±5% vs 71±7%, p=0.5) was found, together with marked insulin degranulation (p<0.05). Again, chromogranin staining detected cells negative for insulin. Acute glucose and glibenclamide-stimulated insulin release was 50% lower (both p=0.01) from T2D isolated islets.
Conclusion: These results show that the loss of beta cells in T2D islets may be currently overestimated due to insulin degranulation, and support the concept that insulin secretion defects play a key role. If so, rescuing beta cell function in T2D could be more realistic than pursuing regeneration.
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Effects of altered insulin sensitivity on islet morphology in humans
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Background and aims: Type 2 diabetes develops when insulin secretion fails to cope with worsening insulin resistance. It is becoming evident that insulin resistance can also impact non-classical tissues including the pancreatic β-cells. For example, obese insulin resistant patients exhibit an enhanced β-cell mass as an adaptive response although the underlying mechanism(s) is not fully understood.
Materials and methods: To explore the alterations that occur in islet morphology, as part of the adaptive mechanism, we performed hyperinsulinemic euglycemic clamps in 18 Caucasian nondiabetic patients, dividing them into insulin resistant (IR, n=9) or insulin sensitive (IS, n=9) (10 F/8 M, 51±15 yrs., BMI 27.9±5.3 kg/m2) groups according to glucose uptake (GU), incretins were evaluated during a Mixed Meal Test and insulin secretion were evaluated during Hyperglicemic Clamp, before and then after the surgery. Subsequently, all patients underwent duodeno-pancreatectomy, and pancreases were collected for immunohistochemistry for glucagon, insulin and somatostatin+ cells to assess islet morphology. Apoptosis was evaluated by TUNEL, proliferation by Ki67, and ductal cells by CK19 immunostaining.
Results: Assessment of the entire group revealed a direct correlation between GU and islet size (r= -0.74; p<0.001), and between GU and % glucagon area expressed as a fraction of the total pancreas (%GLUCA, r=-0.65; p=0.003). IR group displayed a significant reduction in β/α ratio and increased islet size (2456±332.2 vs 5156±944.8 μm2, p<0.01). While no differences were evident in proliferation, apoptosis or β cell area, the IR group displayed increased insulin+CK19+ cells (p<0.001),scattered islets (<8 cells)(p=0.04), insulin+glucagon+ double cells (p<0.01) and a larger β-cell nuclear area (p=0.03). Further, GLP1 levels correlated with %GLUA (r=0.63, p=0.04) and we detected GLP-1 immunoreactivity in α-cells. After the 50% pancreatectomy, We observed that the IRs experience an higher reduction of insulin secretion capacity compared to ISs (IS, 924.2± 900.3 vs. IR 6952±1951 ΔAUC insulin secretion tot, p=0.04). Moreover, greater increase in the glucagon secretion after pancreatic mass reduction have been described in insulin resistance status (IS 112.5±101.2 vesus IR 1812±326.3, p=0.02).
Conclusion: Our data suggest that neogenesis from duct cells and potential transdifferentiation of α cells towards the β-cell lineage could contribute towards a compensatory increase in β cells production in insulin resistance. This may be, in part, driven by the presence of GLP-1 immunoreactivity in the α cells. Together these data indicate that a failure of compensation in patients with insulin resistance leads to the development of overt diabetes following partial pancreatectomy.

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Islet number rather than islet size may be a major determinant of beta cell mass in non-diabetic humans
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Objective: Both type 1 and type 2 diabetes are characterized by a deficit of β-cell mass(BCM). Although enhancing endogenous β-cell regeneration is considered to be a potential therapeutic strategy to cure diabetes, little is known about factors determining BCM in humans. Both islet number and islet size(IS) contribute to BCM, however, the relative contribution of both factors in humans is unclear. Therefore in this study we examined associations between islet density(ID), mean IS and BCM in human pancreas.
Methods: We obtained autopsy pancreas from 72 Japanese adults aged from 20 to 70 years(age 47 ± 12 (mean ± SD), BMI 24.1 ± 5.0). To be included, cases were required to have 1) had a full autopsy within 24 h of death and 2) pancreatic tissue stored that was of adequate size and quality, 3) no history of diabetes, pancreatitis or pancreatic surgery, 4) no use of glucocorticoids. Cases were excluded if pancreas tissue showed autolysis or any abnormal change such as pancreatitis. Pancreas tissues were stained for insulin or glucagon, and fractional β-cell area(%BCA) and α-cell area(%ACA) to the total pancreas area were measured, respectively. Since pancreas weight was not available in the most cases, to determine BCM or α-cell mass(ACM), the pancreas weight in each case was estimated by use of equations based on the population data. Then estimated BCM or ACM were calculated as a product of %BCA or %ACA and reference values of pancreas parenchymal mass. ID, IS and β-cell turnover(i.e., replication, neogenesis and apoptosis) were quantified in randomly selected areas of pancreas that contained more than 100 islets in each case. Islet was defined as a cluster of four or more β-cells and a total of 7,778 islets were assessed. Islet number per section was calculated as a product of ID and total pancreas area. Replication and apoptosis were quantified by immunohistochemistry for insulin and Ki-67 or single-stranded DNA staining, respectively. Neogenesis was evaluated by the frequencies of scattered β-cells within the acinar tissue and insulin(+) duct cells.
Results: Islet morphometric analysis revealed that there was a considerable variation in ID(5.28 ± 2.73 /mm^2) and IS(7,342 ± 2,729 μm^2) among individuals. There was a negative correlation between ID and IS($r = -0.25$, $p = 0.03$). %BCA was not correlated with IS($p = 0.6$), while %BCA was positively correlated with ID($r = 0.63$, $p < 0.01$). These results did not change even when BCM was used in the analysis and there was a positive correlation between BCM and ID($r = 0.63$, $p < 0.01$), but not IS($p = 0.5$). Islet number per section was also associated with %BCA and BCM($r = 0.63$ and 0.64, both $p < 0.01$). The frequency of scattered β-cells was positively correlated with ID($r = 0.68$, $p < 0.01$) and negatively correlated with IS($r = -0.36$, $p < 0.01$). There was no correlation between the frequency of insulin(+) duct cells and ID or IS($p > 0.05$). The frequency of replication was correlated with IS ($r = 0.26$, $p = 0.03$), whereas there was no correlation between replication and ID($p = 0.2$). Apoptosis was not detectable in all cases. %ACA and ACM were also correlated with ID($r = 0.39$ and $r = 0.38$, both $p < 0.01$), whereas there was no correlation between IS and %ACA or ACM($p > 0.05$).
Conclusion: We report that there was a considerable variance in islet morphology in non-diabetic human pancreas and ID, but not IS was significantly correlated with BCM in humans. Our findings imply that islet number rather than IS is a major determinant of BCM in human.
Supported by: NMTREF, DSHF, JDF

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Slow and minimal decline of beta cell mass associated with ageing in non-diabetic Japanese and lack of compensatory islet hyperplasia in obesity

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Background and aims: Reduced beta cell mass is established to be a major pathological finding in type 2 diabetic subjects. Since the population of diabetes increases in the aged, it may be hypothesized that enhanced aging process may account for the reduced beta cell mass. It remains unclear, however, how beta cell mass is influenced by aging or obesity in Japanese subjects who are prone to type 2 diabetes in the environment of westernization. In this study, we therefore examined islet and beta cell masses in different ages in Japanese non-diabetic subjects and their relationship to body mass index (BMI) and cell proliferation activity.
Materials and methods: Pancreata obtained from 115 autopsied cases (male 69, female 49) were subjected to morphometric analyses of masses of islet and beta cells after immunostainings of chromogranin A, and insulin. To explore underlying mechanism of the islet changes, immunohistochemical expressions of Ki67, pancreatic duodenal homeobox (PDX)-1, cell cycle inhibitor P16, and oxidative stress marker γH2AX were also examined. The values of immunopositive reactions were estimated at decile age levels.
Results: The largest islet volume density (Vi)(%) was evident in a group of age below 10 years old (y.o.)(4.33±1.0, mean±SE). Thereafter, Vi was sharply reduced until 20 y.o. and consistent throughout the rest of life. In contrast to Vi, beta cell volume density (Vb) was gradually reduced with aging without any peak, yielding a significant difference between groups of below 10 y.o. (2.26±0.45) and 70ties (1.93±0.23). BMI did not influence on the changes of Vb ($p=0.07$). During maturation until 10 y.o., beta cell proliferation activity was gradually decreased ($p<0.05$) and reached a low plateau (<0.5%), which was independent of BMI. PDX-1 expressions were robust in the young, but equivocal after maturation, while expressions of P16 and γH2AX in the adults or seniles were more intensitied compared to those in the young.
Conclusion: We found that age-associated decline of beta cell mass was marginal after maturation and the reduction of beta cell mass encountered in type 2 diabetes may be a specific process. In Japanese, there was no evidence that increased BMI was associated with beta cell hyperplasia.
Supported by: JMESCS, JMC, JDF

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Beta and alpha cell trajectories during development and after high fat programming in neonate, weanling and adolescent Wistar rats

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Background and aims: Islet cell populations fluctuate over the early life course and are influenced by nutritional programming. Previous studies revealed that exposure to high fat diets during fetal and early postnatal life induced immediate, transient and durable changes in islet cell structure and function in progeny throughout life. This study assessed beta and alpha cell trajectories in neonate, weanling and adolescent Wistar rats during development (physiology) and after exposure to a high fat diet.
Materials and methods: Pancreata from neonatal (one-day-old), weanling (three-week-old) and adolescent (three-month-old) Wistar rats were double immunolabeled with insulin and glucagon in control (10% fat as energy) progeny (physiology) and progeny maintained on a high fat (40% of mainly saturated fat as energy) diet during fetal, lactational and/or postnatal life (high fat programming). Beta and alpha cell number, size and volume, beta cell:alpha cell and alpha cell:beta cell ratios were assessed with data presented as means ± SEM and significance established at $p < 0.05$.
Results: Beta cell hyperplasia presented in adolescent rats (659 ± 37.80, n = 8) compared to neonate rats (115 ± 41.83, n = 7) and after postnatal high fat maintenance at weaning (640 ± 112.80, n = 7) that was preserved at adolescence (1189 ± 61.30, n = 7). Postnatal high fat maintenance induced beta cell hypertrophy at adolescence (182 ± 11.72 μm^2, n = 7 compared to 115 ± 15.47 μm^2, n = 5 in neonates) and was mirrored in adolescent rats maintained on a high fat diet during fetal life (116 ± 7.66 μm^2, n = 6 compared to 85 ± 10.03 μm^2, n = 12 in neonates). Physiologically and more markedly after postnatal high fat maintenance, alpha cell numbers increased with age, emerging at weaning and prevailing in adolescence. Further, alpha cell hyperplasia was maintained after fetal and postnatal high maintenance at weaning (224 ± 32.68, n = 6) and adolescence (226 ± 29.76, n = 5; both compared to 102 ± 20.26, n = 10 in neonates). Physiologically, alpha cell hypertrophy presented in adolescence (125 ± 11.82 μm^2, n = 8 relative to neonates 55 ± 4.43 μm^2, n = 5). However, after postnatal high fat maintenance, alpha cell hypertrophy emerged earlier at weaning (92 ± 7.35 μm^2, n = 6) and persisted in adolescence (137 ± 7.29 μm^2, n = 7). Physiologically, after fetal, lactational or fetal and lactational high fat maintenance, alpha cell volume was enhanced at weaning.
Conclusion: Beta and alpha cell populations vary in number, size and volume throughout development and are altered in response to high fat programming. Preserving beta and alpha cell populations (islet cell numbers that constitute islet cell volumes) will maintain islet cell function to regulate glucose homeostasis.

PS 030 Beta cell mass expansion and survival

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Molecular mechanism of pancreatic islet proliferation during the neonatal development in mice

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Background and aims: Pancreatic islet cells undergo a transient burst of proliferation immediately after birth, followed by substantial remodeling of cell mass in the neonatal period, with a progressive decrease in cell replication. The different rates of cell proliferation are thought to be due to differences in the percentage of cells recruited for entry into the cell cycle. To understand this adaptation at the molecular level, we undertook a time course analysis of β/α cell mass, cell proliferation and cell cycle genes expression in mice.
Materials and methods: Mice islet β/α cell mass and the expression of pocket protein family (Rb, p107, p130), E2F1, cdk1 and cyclinB1 at 1-, 3- to 8-week after birth were detected by immunohistochemistry. Pancreatic islets were isolated by collagenase. Cell cycle was detected by flow cytometry. The expression of cell cycle genes were detected by real-time quantitative PCR and Western blotting.
Results: 1) Compared with 1w, islet β/α cell mass significantly increased at 3w and 8w ($P<0.05$); cell size increased 1.7 times at 8w ($P<0.01$); cell number increased nearly 3.5/3.7 times at 3w ($P<0.01$). Rb, p107, p130, E2F1, Cdk1 and cyclinB1 were dynamically expressed in nucleus and cytoplasm of islet cells. 2) Compared with 8w, there were less islet cells in the G0/G1 phase and more cells in the G2/M phase at 1w and 3w ($P<0.05$), and the proliferation index were significantly higher at 1w and 3w ($P<0.05$). 3) The mRNA level of Rb, p107, E2F1/2, cyclin D1/E1/A1/A2/B1/B2, cdk1/2/6, cdc25b/c and Wee1 at 8w were much lower than that at 1w ($P<0.05$), whereas the level of p130 and cdc25a were much higher than that at 1w ($P<0.05$). The level of cdk4 expressed more at 3w than that at 1w and 8w ($P<0.05$). 4) Compared with 1w, the protein level of Rb, p107, E2F1, cyclinB1 and cdk1 were much lower, but the level of p130 was significantly increased at 8w ($P<0.05$).
Conclusion: Islet β/α cell mass increased significantly during neonatal development in mice, primarily in cell numbers and proliferation. Cell cycle genes were dynamically expressed. The higher level of Rb, p107, E2F1/2, cyclin D1/E1/A1/A2/B1/B2, cdk1/2/6, cdc25b/c and Wee1 might be involved in the regulation of islet cell proliferation.
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Modulation of C/EBP beta expression via AMPK regulates pancreatic beta cell mass

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Background and aims: The endoplasmic reticulum (ER) is often compared to a quality control plant. ER homeostasis is maintained by the unfolded response induced by environmental conditions, which leads to instability of the ER, known as ER stress. During the development of type 2 diabetes, ER stress can lead to not only insulin resistance but also pancreatic β-cell failure. We have recently reported that accumulation of the transcription factor C/EBPβ in β-cells causes ER stress and reduced β-cell mass, leading to β-cell failure. We established β cell-specific C/EBPβ transgenic (TG) mice, which had a non-fasting glucose level of approximately 200 mg/dL, and showed reduced β-cell mass. Therefore, we considered TG mice to be mild diabetes model mice. Next, we examined whether the DPP-4 inhibitor vildagliptin (Vilda) could preserve pancreatic β-cell mass in the TG mice owing to reduced C/EBPβ expression, which was concomitant with reduced ER stress and amelioratied insulin signaling. We investigated the mechanism through which Vilda suppresses C/EBPβ expression.
Materials and methods: MIN6 cells, pancreatic β cell lines, were treated in vitro with tunicamycin, Exendin-4, Metformin (Met) and AICAR. We evaluated the relationship between AMPK and C/EBPβ in an in vitro assay; TG and wild-type (WT) mice were orally administered Vilda with or without Met, starting at 4 weeks of age.
Results: Incubation of MIN6 cells with Exendin-4 or Met resulted in the activation of AMPK, which suppresses C/EBPβ expression. Furthermore, co-expression of dominant-negative AMPK increased C/EBPβ expression, whereas co-expression of constitute-active AMPK decreased C/EBPβ expression. Therefore, we assumed that modulation of C/EBPβ expression by AMPK regulates pancreatic β-cell mass. In vivo treatment with Vilda markedly ameliorated hyperglycaemia in TG mice and increased AMPK activity in pancreatic β cells. On the other hand, Met treatment in conjunction with Vilda did not considerably affect fed blood glucose levels. However, an oral glucose tolerance test revealed that treatment with Vilda and Met (Vilda, Met-TG) significantly improved glucose tolerance compared with only Vilda alone (Vilda-TG), with a further increase in AMPK activity. Immunostaining analyses of pancreatic islets showed that Vilda, Met-TG mice exhibited an increase in pancreatic β-cell mass compared with Vilda-TG mice; this result is consistent with the reduced C/EBPβ expression observed in this study.
Conclusion: Vilda might activate AMPK in pancreatic β-cells. Furthermore, treatment with Met in conjunction with Vilda led to a greater increase in AMPK activity compared with Vilda alone. As a result, C/EBPβ expression was suppressed to the proportion of AMPK activity. Therefore, these findings suggest that the modulation of C/EBPβ expression by AMPK could regulate pancreatic β-cell mass.

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The in-vitro effects of glucagon-like peptide-1 (GLP-1) and gastrin combination therapy on pancreatic beta cell proliferation and islet cell survival

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Background and aims: Combination therapy with GLP-1 and gastrin has been shown to control hyperglycaemia in diabetic mice. This improvement in glycemic control was shown to be accompanied by a significant increase in beta cell mass for combination treatment. Here we investigated the *in-vitro* effects of exendin-4 (Ex-4) + h[Leu15]gastrin17 (G-17) combination therapy in regulation of pancreatic beta cell proliferation and islets cell survival in isolated neonatal rat islets.
Materials and methods: Isolated neonatal rat islets from pancreases of 3-5 day-old Wistar rat pups were used in this study. The islets were incubated for 4 days (proliferation study) or for 2 days (apoptosis study) with either the combination of Ex-4 + G-17 (10 nM + 40 nM), or reference compounds Ex-4 (10 nM), or G-17 (40 nM). Human growth hormone (hGH, 20nM) was used as a positive control in the proliferation study. Proliferation was measured by 5-ethynyl-2'-deoxyuridine (EdU) incorporation in islets and calculated as the percentage of EdU^+ Pancreatic duodenal homeobox-1^+ (PDX1) cells of total $PDX1^+$cells. Apoptosis was induced with either cytokines (IL-1β, 150 pg/ml; IFN-γ, 100 pg/ml) or palmitate (1 mM) and measured by the presence of histone-associated DNA fragments in cell lysate (apoptosis) and supernatant (necrosis).
Results: As expected hGH significantly increased beta cell proliferation ($PDX1^+$) (4-fold increase, $^{***}p < 0.001$ vs. control islets). Neonatal rat islets cultured with Ex-4 + G-17 combination significantly increased the proliferation of beta cells (140 %, $^{***}p < 0.001$ vs. control islets). Treatment with G-17 or Ex-4 alone showed no significant increase in the proliferation of beta cells. The changes in the level of apoptosis can be seen in Figure 1. There was a 5-fold increase in the level of apoptosis in islets incubated with IL-1 β and IFN-γ. The effect of tested compounds on cytokine-induced apoptosis was not statistically significant; nonetheless there was a 33 % reduction in the level of apoptosis in islets cultured with Ex-4 + G-17 combination. Also, treatment with G-17 or Ex-4 alone decreased the level of cytokine-induced apoptosis by 14 % and 16 %, respectively. In response to palmitate, there was a 7-fold increase in the level of apoptosis in control islets. The combination treatment with Ex-4 + G-17 decreased palmitate-induced apoptosis by 47 %, as did treatments with Ex-4 (48 %) and G-17 (43 %) alone; however this reduction was not statistically significant.
Conclusion: In conclusion, the combination treatment with Ex-4 + G-17 significantly increased beta cell proliferation, and showed a trend toward reducing the levels of cytokine- and palmitate-induced apoptosis in neonatal rat islets, suggesting that beta cell regenerating and protective effects of GLP-1-gastrin dual agonism may provide an attractive option for the treatment and/or prevention of Type 2 diabetes.

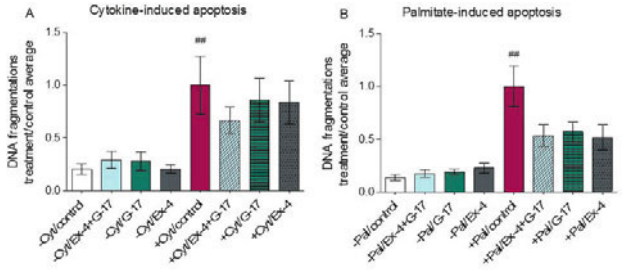

Figure 1. Effects of the combination treatment with Ex-4 and G-17 on pancreatic islets cell survival. A) Cytokine-induced apoptosis, B) Palmitate-induced apoptosis. All treatment conditions were normalized to the control condition in the presence of cytokines or palmitate. Data were analyzed using one-way ANOVA test followed by Dunnett's multiple comparison test, vs. +Cyt/control or +Pal/control; or Mann Whitney test ##p < 0.01 vs. -Cyt/control or -Pal/control. n=5-6. Data are mean ± SEM.

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Dopamine could be involved in the maintenance of rat pancreatic beta cells controlling insulin secretion and cellular proliferation

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Background and aims: Dopamine is a neurotransmitter that plays a critical role in neurological and psychiatric disorders and it is implicated in various physiological functions, including endocrinological modulation. In isolated islets, dopamine and its analogs have shown contradictory results on insulin release. These findings suggest that beta cells might be directly responsive to dopamine. Recent studies have shown the presence into beta cells of the enzymes responsible on synthesis or metabolization of dopamine. Thus it could be accepted that dopamine exerts an auto-paracrine regulation of insulin secretion from pancreatic beta cells. The aim of this study is to analyze whether dopamine is a regulator of proliferation of rat pancreatic beta cells in pancreatic isolated islets after glucose induced secretion of insulin.
Materials and methods: In order to analyze if dopamine inhibitory effect on secretion of insulin whether related to changes in the pancreatic beta cells populations an in vitro study on isolated pancreatic islets, obtained from adult and old rats, was carried out analyzing the effects of 10 µmol/l and 1 µmol/l dopamine from 1 to 12 hours. The secretion of insulin was determined by RIA, and the cellular proliferation of beta cells by double immunocytochemical labelling for PCNA (proliferating cell nuclear antigen) and insulin. Data were analyzed using the GraphPad Prism and ImageJ software.
Results: Without significant differences between both doses of dopamine assayed, dopamine inhibits significantly ($p<0.05$) the secretion of insulin from isolated islets as early as 1 hour after treatment. In adult and old rats, the percentage of insulin-positive cells in the islets decreases significantly ($p<0.05$) after 1 hour of treatment. These effects were sustained until 12 hours of treatment. However, the effects were significantly ($p<0.05$) more evident after treatment with 10 µmol/l dopamine than 1 µmol/l dopamine. The proliferation of insulin-positive cells in the islets decreased significantly ($p<0.01$) after treatment with dopamine. The higher effect was observed after 3 hours of treatments. After 6 and 12 hours of treatment with 1 µmol/l dopamine the inhibitory effects were significantly ($p<0.05$) lower that those observed after 3 hours. The inhibitory effects of 10 µmol/l dopamine were similar after 3 and 6 hours of treatment, so with this treatment the effect was more sustained in time than those observed after treatment with 1 µmol/l.
Conclusion: The results obtained in this study demonstrate that dopamine could modulate the proliferation of pancreatic beta cells and suggest that dopamine could be involved in the maintenance of pancreatic islets.
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Differentiation of human adult pancreatic duct cells into insulin-producing cells

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Background and aims: Adult human pancreatic duct cells have been reported to differentiate *in vitro* into insulin-producing cells. Glucagon-like peptide-1 (GLP-1) induces islet neogenesis in animal models, but little information is available on the effects of GLP-1 on human duct cells. The aim of this study was to investigate whether liraglutide, a human long-acting GLP-1 analog, could enhance the differentiation of human adult pancreatic duct cells into insulin-producing cells *in vitro*.
Materials and methods: Pancreas from 12 human cadaveric organ donors, 4 male, 56±3 y.o. (range: 33-79), BMI 27.8±1.62 (Kg/m^2), were processed as for islet isolation and the exocrine fraction was collected and dispersed into single cells. Duct cells were purified by magnetic cell sorting with CA19.9 antibody, and cultured in suspension for one month in differentiation serum-free medium with liraglutide (300nM) or without (control), and/or EGF (20ng/mL). Gene expression was determined by real time RT-PCR. Replication (BrdU) and protein expression were analysed by immunohistochemistry. Insulin and C-Peptide content and insulin secretion in response to glucose (20mM) were determined by ELISA.
Results: After 2-3 days in culture, the sorted cell population (82.7±1.01% duct cells, 0.40±0.11% beta cells) clustered into pancreatospheres (92.4±0.45% $PanCK^+$ cells; 0.19±0.07% $C\text{-}Peptide^+$ cells). Gene expression of *krt19* was reduced on days 3, 7 and 30 compared with post-sorting day ($P<0.05$). Duct cell replication was similar in liraglutide and control groups at all time points, and was similarly increased by EGF in both groups ($P<0.05$). After 30 days in culture, the expression of the islet hormone genes *ins* ($P<0.01$), *sst* ($P<0.001$), and *gcg* ($P<0.01$), and of *Pdx-1* ($P<0.01$) was increased compared with post-sorting day and culture day 3 in both groups. No significant differences were found between liraglutide and control treated groups. Insulin and C-Peptide content (corrected per DNA) was significantly increased on day 30 compared with post-sorting day ($P<0.05$), but remained very low compared with human islets (≈1%). It was similar in liraglutide and control groups. Insulin secretion did not increase in response to glucose stimulation.
Conclusion: The results support the hypothesis that adult human pancreatic duct cells can differentiate into insulin-producing cells. The GLP-1 analog liraglutide did not increase the efficiency of human adult duct cell differentiation into insulin-positive cells, and did not exert a mitogenic effect in human adult duct cells.
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DPP-4 (CD26) is expressed by ductal and islet cells in human adult pancreas

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Background and aims: Dipeptidyl peptidase-4 (DPP-4) or CD26 is a membrane glycoprotein and serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. DPP-4 plays a major role in glucose metabolism by degrading incretins such as GLP-1 that enhance insulin release and, at least in rodents, promotes growth of endocrine progenitor cells and insulin gene transcription. The class of oral anti-hypoglycemic drugs called DPP-4 inhibitors works by prolonging incretin action in vivo. DPP-4 is a marker of various cancers including pancreatic adenocarcinoma and it has been argued that its long-term inhibition may predispose to pancreatitis and pancreatic cancer. Expression of DDP-4 in situ has not been reported in the human adult pancreas. To identify the target cells of DDP-4 inhibition we measured its expression in organ donor pancreata.
Materials and methods: Pancreata were obtained, with informed consent of next-of-kin, from heart beating, brain dead organ donors. From each donor tissue a comparable region was cleaned of fat, embedded in OCT and snap-frozen. Cryosections from tissue of non-diabetic (n=15), type 2 diabetic (n=2) and type 1 diabetic (n=6) organ donors were stained with a DPP-4

monoclonal antibody (clone M-A261) using the APAAP technique. Beta cells were identified with the biotinylated anti-human proinsulin monoclonal antibody GS-9A8 and detected using horseradish peroxidase labelled streptavidin. Alpha cells were identified with the glucagon monoclonal antibody K79bB10. For co-localisation studies, sections were double stained for DPP-4 and proinsulin or glucagon and scanned using a Leica SP2 Confocal Microscope. Image analysis was performed with the ImageJ software.
Results: DPP-4 expression by both ductal and islet cells was detected in all specimens. 85% of islets (from 17 donors) contained cells expressing DPP-4, even in the absence of proinsulin expression. To identify DPP-4-positive cells, alpha and beta cells were visualised in the consecutive sections. DPP-4-positive cells had the same intra-islet location and morphology as glucagon-positive cells. In type 1 diabetic donors all remaining islet cells were DPP-4-positive. Co-localisation studies revealed overlapping expression of DPP-4 and glucagon.
Conclusion: Our findings show that ductal and alpha cells are potential direct target cells for DPP-4 inhibition. Expression of DPP-4 by alpha cells, which in humans are in proximity to beta cells, suggests a paracrine mechanism of incretin action on beta cells in human islets.
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Effects of pharmacological glucokinase activation on beta cell survival and function in rat islets cultured for one week in a low, intermediate or high glucose concentration
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Background and aims: The long-term effects of glucose on β cell survival and function in cultured rat islets are complex, a rise in glucose being beneficial between 5 and 10 mM and deleterious between 10 and 30 mM. The aim of this study was to evaluate the effects of moderate pharmacological glucokinase activation during prolonged culture at low, intermediate and high glucose concentrations on rat β cell gene expression, survival and function.
Materials and methods: Male Wistar rat pancreatic islets were cultured in RPMI medium containing 5g/L BSA and 5, 10 or 30 mM glucose (G5, G10, G30) with 3 μM of the glucokinase activator Ro 28-0450 (Ro) or vehicle alone (DMSO 1/1000). After culture, preproinsulin (*Ppi*), *Gadd153* and metallothionein 1a (*Mt1a*) to *Tbp* mRNA ratios were measured by real-time RT-PCR. Mitochondrial redox-sensitive GFP (mt-roGFP) oxidation was measured as a marker of oxidative stress. β cell apoptosis was measured by TUNEL assay on islet sections stained for insulin. The intracellular Ca^{2+} concentration (fura-PE3 microspectrofluorimetry) and the rate of insulin secretion (RIA) were measured in islets perifused with increasing glucose concentrations. Results are means±SE for at least 3 islet preparations. The significance of differences between groups was assessed by 2-way ANOVA and a post-test of Bonferroni.
Results: One week culture in G5, G10 and G30 affected rat β cell gene expression, survival and function as expected. While *Ppi/Tbp* mRNA ratio increased ~10-fold in G10 *vs*. G5 and only slightly decreased in G30 *vs.* G10, *Gadd153* and *Mt1a/Tbp* mRNA ratio decreased by 83% and 97% in G10 *vs.* G5 and were not different in G30 *vs.* G10. The proportion of TUNEL+ β cells after one week culture decreased from 12±1% in G5 to 3±1% in G10, but then increased to 6±1% in G30. Parallel changes in mt-roGFP oxidation were detected after 18h culture under the same conditions. In comparison with culture in G10 that optimally preserved glucose-induced Ca^{2+} rise and insulin secretion, culture in G5 lead to loss of glucose responsiveness. In contrast, culture in G30 increased the acute Ca^{2+} and secretion responses to 6 mM glucose while reducing those to 20 mM glucose. These functional alterations by culture in G5 and G30 were not corrected by addition of 3 μM Ro during the perifusion, despite the ability of the drug to acutely increase the glucose sensitivity of islets previously cultured in G10. In contrast, long term treatment with 3 μM Ro during culture in G5 fully mimicked the beneficial effect of culture in G10 on rat β cell insulin gene expression, survival and function, in parallel with a large reduction of mt-roGFP oxidation and of *Gadd153* and *Mt1a* mRNA levels. However, treatment with 3 μM Ro during culture in G10 mimicked the deleterious effect of culture in G30 on rat β cell survival and function, except for a lack of increase in mt-roGFP oxidation. No significant effect of Ro was observed on any parameter during long term culture in G30.
Conclusion: Pharmacological glucokinase activation during long term culture shifts to the left the glucose-response curve for changes in β cell gene expression, survival and function. Glucokinase activators may improve β cell survival under stress conditions leading a reduction of β cell glucose sensitivity, but they may also increase β cell glucotoxicity in normal β cells.
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Pancreatic stellate cells induce beta cell failure in vivo
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Background and aims: Beta cell failure plays a crucial role in T2DM, but the mechanisms have not been fully explained. Recently, several studies report that pancreatic stellate cells (PSCs) are observed in the islets of patients and animal models with T2DM. However, the exact effects remain unclear. In this study, we investigated the effects of PSCs on islets in vivo.
Materials and methods: PSCs were isolated from Wistar rats, and cell purity was assessed by morphology and cytofilament staining of vimentin, α-SMA and desmin. PSCs were transplanted into the pancreas of normal Wistar (untreated: WU, sham operation: WS and transplanted: WT) and diabetic GK rats (GU, GS, GT) by multi-point injection. 16W after transplantation, oral glucose tolerance test (OGTT) was performed and insulin and HbA1c were assayed. The area under the curve for glucose (AUC_g) and insulin (AUC_i) were calculated using a trapezoidal estimation from the values. The beta cell mass and islet fibrosis were showed by insulin immunofluorescent staining and Masson's trichrome staining respectively. Statistical significance was determined by one-way variance (ANOVA). $P<0.05$ was considered to be statistically significant.
Results: In the GT group, the peak blood glucose level was observed at 30 min, showing a statistical difference compared to the GU and GS group (GT: 30.06±4.52 vs. GU: 22.44±4.10 and GS: 23.20±1.92 mmol/l, P< 0.05). In addition, PSC transplantation significantly increased the AUC_g (GT: 2953.20±296.07 vs. GU: 2474.40±374.01 and GS: 2525.40±273.27 mmol/l·min, P<0.05), but decreased the AUC_i (GT: 0.551±0.156 vs. GU: 1.424±0.545 and GS: 1.283±0.279 mmol/l·min, P<0.05). HbA1c in the GT group was significant higher than the controls (GT: 9.80±1.37 vs. GU: 8.30±1.06 and GS: 8.34±1.00 %, P<0.05). The relative beta cell mass was the least in the GT group (GT: 0.085±0.032 vs. GU: 0.132±0.05 and GS: 0.129±0.05, P<0.05). And the islet fibrosis was aggrevated followed by transplantation (GT: 29.6±6.7% vs. GU: 22.4±7.6 and GS: 23.2±4.3, P<0.05). However, in our present study, PSCs transplantation did not influence the Wistar rats.
Conclusion: Our present study demonstrates that PSCs transplantation deteriorate the islet dysfunction, beta cell loss and islet fibrosis in GK rats, and the effects are environment dependent. Our data suggest PSCs may be the promoters, rather than initiators of beta cell failure in T2DM.
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Unfermented aqueous honeybush extract (*Cyclopia maculata*) attenuates STZ-induced beta cell cytotoxicity
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Background and aims: Streptozotocin (STZ), a glucosamine-nitrosourea, is commonly used to induce diabetes in rodents. The mechanism of STZ-induced β-cell cytotoxicity involves mitochondrial and cell dysfunction with increased oxidative stress (due to increased NO and ROS), ATP depletion, DNA alkylation and apoptosis. Several studies have demonstrated the ability of antioxidants [e.g. N-acetyl cysteine (NAC), and vitamins C and E] to preserve β-cell function. Plant-derived polyphenols have been described as possessing both anti-inflammatory and anti-oxidative properties and may protect against or ameliorate β-cell cytotoxicity. The aim of this study was to determine if an aqueous extract of unfermented honeybush (*Cyclopia maculata*) protects against STZ-induced β-cell cytotoxicity.
Materials and methods: Forty-eight adult, male Wistar rats were randomized into six groups, i.e. vehicle control, STZ control, metformin (125 mg/kg/d), NAC (125 mg/kg/d) and two extract-treated groups (30 and 300 mg/kg/d). The rats were pretreated with honeybush extract, metformin, NAC or vehicle

for 21 days. On day 16rats were injected with STZ (30 mg/kg i.p). An oral glucose tolerance test was performed prior to euthanisation on day 21. Blood was collected at termination and the following parameters assessed: plasma glucose, total serum triglycerides, serum insulin and plasma nitrite levels. To assess the *in vitro* effect of the extract on β-cell viability, ATP and MTT assays were performed on RIN-5F insulinoma cells exposed to 10 mM STZ.
Results: Treatment with both concentrations of honeybush extract reduced the OGTT glucose area under the curve compared to the STZ control (5264.38 ± 396.14 vs. 3114.13 ± 752.23, p<0.05; 2824.38 ± 536.00, p<0.01, respectively). The 300 mg/kg/day honeybush treated group showed a reduction in total triglycerides compared to the STZ control (1.48 mmol/l ± 0.48 vs. 0.40 mmol/l ± 0.04, p<0.05). Honeybush treated rats showed a reduction in the glucose:insulin ratio at the 300 mg/kg/day treated group (12.70 ± 2.34 vs. 2.89 ± 0.70, p<0.05) as well as plasma nitrite concentration at 30 and 300 mg/kg/day (0.563 ± 0.08 vs. 0.422 ± 0.01, p<0.05 and 0.421 ± 0.02, p<0.05, respectively) when compared to the STZ controls. *In vitro,* the honeybush extract improved the viability of RIN-5F insulinoma cells exposed to STZ, as measured using an MTT assay and by measuring cellular ATP (56.80% ± 1.49 vs. 91.03% ± 3.41, p<0.05; and 51.70% ± 2.32 vs. 112.84% ± 12.64, p<0.001, respectively).
Conclusion: Unfermented honeybush extract (*Cyclopia maculata*) demonstrated some protection against STZ-induced β-cell toxicity, hyperglycaemia and associated oxidative stress-induced nitrite production. This protective effect against STZ-induced β-cell cytotoxicity was verified *in vitro* using RIN-5F insulinoma cells.
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Effect of liraglutide on apoptosis and tissue factor activity on Rin-m5f, in response to microparticles: interest in islet transplantation
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Background and aims: IBMIR (Instant Blood Mediated Inflammatory Reaction) follows islet transplantation and is characterized by drastic cytokine secretion and the expression of tissue factor (TF) at islet vicinity. Microparticles (MPs) are plasma membrane fragments shed from stressed cells that act as cellular effectors. Recently, islet cytoprotection by incretinomimetics was proposed. We evaluate the protective effect of Liraglutide on β cell dysfunction mediated by MPs, using oxidative and inflammatory cell stress model.
Materials and methods: Rat β cells, Rin-m5f, were stimulated by 100µM H2O2 or 50U/ml IL-1β combined to 1000U/ml TNF-α. MPs generated by oxidative and cytokinic stress were isolated and applied to naive Rin-m5f for 24h. Effects of 1µM Liraglutide were assessed on insulin secretion, apoptosis by hypodiploïd DNA quantification and, MP release and TF activity by ELISA (n=9). MP cell membrane integration was probed by fluorescent dye.
Results: Direct protection by Liraglutide is revealed by a significant decrease in oxidative stress-induced apoptosis (10% vs. 18%) and by restored insulin secretion (oxidative stress: +55%, cytokinic stress: +25%). Indirect protection of β cell occurs through reduced MP shedding (oxidative: -25%, p=0,006; cytokinic -18%, p=0,01) and by counteracting the decrease in insulin secretion induced by MPs. By fluorescence probing, 50% of Rin-m5f integrate MPs and this integration is not modified by Liraglutide. Liraglutide reduces oxidative and cytokinic TF-induced activity with a significant decrease at Rin-m5f (oxidative: -18%; cytokinic: -17%) and MP surfaces (respectively: -31%; -15%).
Conclusion: In conclusion, Liraglutide shows a protective effect on β cell survival at different levels, on apoptosis, insulin secretion and TF activity. Furthermore, Liraglutide limits the deleterious message conveyed by MPs. Our data bring new hints on the mechanisms of cytoprotection by Liraglutide in islet transplantation and particularly during IBMIR.
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PS 031 ER stress and apoptosis

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Chaperones ameliorate hIAPP-induced ER stress in beta cells
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Background and aims: In type 2 diabetes, loss of beta cell function is thought to be due to several causes, one being the formation of toxic protein aggregates called islet amyloid, formed by accumulations of misfold human islet amyloid polypeptide (hIAPP). In many cases, misfolding is a natural consequence of protein biogenesis, but in T2D, it may contribute to β-cell dysfunction. In addition, accumulation of misfolding proteins perturbs the endoplasmic reticulum (ER) homeostasis activating the unfolded protein response (UPR). Molecular chaperones have been described to be important for regulation of ER signaling in response to ER stress, among others. The aim of the present work is to investigate the role of chaperones in ER stress response induced by hIAPP overexpression.
Materials and methods: Freshly isolated hIAPP transgenic mouse islets and rat pancreatic beta cell line INS1E stably expressing hIAPP (INS1E_hIAPP cells) were cultured with pharmacological (0.5µM thapsigargin) and non-pharmacological (25 mM glucose and 400µM palmitic acid) ER stress inducers in a time-dependent manner. To mimic the plausible protective role of endogenous chaperone BiP/GRP78 during ER stress, hIAPP transgenic mouse islets and INS1E_hIAPP cells were pre-treated with the chemical chaperone taurine-conjugated ursodeoxycholic acid (TUDCA) and phenylbutyric acid (PBA). Adenovirus mediated BiP/GRP78 overexpression was performed in isolated hIAPP transgenic mouse islets and INS1E_hIAPP cells. Gene expression and protein levels of ER stress markers (CHOP, ATF3 and spliced XBP1) and BiP/GRP78 were analyzed by real-time RT-PCR and Western Blot.
Results: Stressed beta cells showed a reduction in BiP/GRP78 protein levels when compared to control cells after exposure to 0.5µM thapsigargin or a combination of palmitic acid and high glucose. Interestingly, the expression of ER stress genes was higher in INS1E_hIAPP (CHOP 11.2-fold, ATF3 42.3-fold and spliced XBP1 8.3-fold) over untreated cells (p<0.001) compared to INS1E cells (CHOP 5.9-fold, ATF3 18.5-fold and spliced XBP1 4-fold) over untreated cells (p<0.001). The addition of 400µM palmitic acid to 25 mM glucose induced an increase of ATF3 and spliced XBP1 mRNA expression levels of 4.2-fold, and 3.5-fold, respectively compared to basal conditions. Overexpression of BiP/GRP78 in hIAPP-expressing beta cells, previously treated with thapsigargin or a combination of palmitic acid and high glucose, showed a reduction of ER stress genes such as spliced XBP1. In addition, treatment of stressed beta cells with chemical chaperones TUDCA or PBA showed a markedly decrease in protein levels of ATF3, spliced XBP1 and CHOP, and a significant reduction in ATF3 and spliced XBP1 genes (13%, p<0.05 and 18%, p<0.05 respectively) when compared to hIAPP-expressing controls treated with ER stress inducers.
Conclusion: Overexpression of hIAPP is sensed by ER and ultimately decreases chaperone BiP/GRP78 expression. In that way, overexpression of BiP/GRP78 or addition of chemical chaperones TUDCA and PBA ameliorates ER stress, suggesting that improving chaperone capacity can be important for the folding of hIAPP.
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The translocon, a functional reticular calcium leak channel in human islets: towards a new way of protecting pancreatic beta cells from lipotoxicity
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Background and aims: Accumulating evidence suggests that palmitate mediates beta cell dysfunction in vivo and in vitro by inducing endoplasmic reticulum (ER) stress and [Ca2+] perturbation. However, not many treatments are able to efficiently protect beta cells from lipotoxicity. [Ca2+]ER store de-

pletion by itself can induce both ER stress and apoptosis. A recent study on prostatic cancerous cells demonstrated that [Ca2+]ER depletion ocurred via a passive [Ca2+] leak channel, the translocon, an ER multiprotein complex involved in translation. We explored in this study the role of translocon in pancreatic beta cells. We further analysed the effects of its modulation on calcium homeostasis and insulin secretion after palmitate treatment.
Materials and methods: Using the FURA-2 AM probe, we measured on human islets and MIN6B1 murine beta cells, the resting [Ca2+] and [Ca2+]ER by addition of thapsigargin (1μM). Functional translocon was determined by [Ca2+]ER measurements after addition of anisomycin (200μM), a translocon inhibitor, and/or puromycin (200μM), a translocon activator. We further analysed the modulation of translocon on MIN6B1 treated by BSA or palmitate (200 mM) during 24h. Glucose stimulated insulin secretion (GSIS) was determined by ELISA, ER stress markers, Serca2 - a [Ca2+]ER pump- and SEC61a - a major translocon protein - by RT-qPCR and western-blot.
Results: Puromycin induced a significant [Ca2+]ER release (p=0.05 vs resting calcium), and anisomycin counteracted totally this effect in human islets (p<0.001) and MIN6B1 cells (p<0.001). Anisomycin reduced the total [Ca2+] ER release induced by thapsigargin in human islets (p<0.01) and MIN6B1 (p<0.01). These results demonstrated that the translocon is implicated in [Ca2+]ER leak in beta cells. Palmitate treatment induced in MIN6B1, mRNA overexpression of ER stress markers such as GRP78 (p<0.05 vs BSA), CHOP (p<0.001), and XBP-1S (p<0.05); puromycin and anisomycin were not able to prevent these effects. Palmitate increased protein expression of Grp78 (p<0.05 vs BSA), CHOP (p<0.05), pJNK (p<0.05), and decreased Serca2 protein levels (p<0.05). Moreover, we observed in presence of palmitate, higher mRNA and protein levels of SEC61a (p<0.05 vs BSA) proving that palmitate enhanced the translocon expression. Palmitate markedly reduced [Ca2+]ER (p<0,001 vs BSA) in MIN6B1 and addition of anisomycin totally prevented this effect (p=NS vs BSA). GSIS, not modified by addition of anisomycin and puromycin in basal conditions, was reduced after palmitate treatment (p<0.05) but was restored by anisomycin (p<0.05).
Conclusion: We demonstrate for the first time that the translocon is implicated in the calcium regulation in human and murine beta cells. We also point out that inhibition of the translocon is able to protect beta cells from lipotoxicity by restoring insulin secretion and calcium homeostasis, without significant decrease of ER stress. These results highlight the role of translocon in beta cell function and supported the hypothesis that inhibition of the translocon opens a new potential way of treatments in type 2 diabetes.
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Endoplasmic reticulum stress induces ATF4 and CHOP expression independently of PERK/eIF2alpha pathway in pancreatic beta cells
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Background and aims: Chronic endoplasmic reticulum (ER) stress causes an increase in the expression of the transcription factor C/EBP homologous protein (CHOP), which has been implicated in pancreatic β-cell dysfunction and death and hence in the development of type-2 diabetes mellitus (T2DM). It has been proposed that this increase in CHOP expression is mediated by activating transcription factor-4 (ATF4), whose expression is up-regulated by the PERK-dependent phosphorylation of eIF2α. The aim of this project was to re-examine the role of eIF2/PERK in ER stress induced ATF4 and CHOP expression in pancreatic β-cells.
Materials and methods: MIN6 cells and primary rat islets were treated with thapsigargin to induce ER stress. Changes in the expression of ATF4 and CHOP were determined using qRT-PCR, luciferase reporter assays and Western blotting. The role of individual UPR transducers and eIF2α phosphorylation in ER stress induced ATF4/CHOP expression were explored using pharmacological inhibitors, expressing dominate-negative (DN) mutants of PERK and IRE1, and expressing GADD34ΔN, which promotes the dephosphorylation of eIF2α. All results are representative of at least three independent experiments. Statistical analysis was carried out using 1 way ANOVA Bonferroni's Multiple Comparison Test.
Results: Chronic ER stress causes an increase in both ATF4 and CHOP mRNA and protein expression in clonal pancreatic β-cells. However, the inhibition of PERK activation or eIF2α phosphorylation had no significant effect on the expression of ATF4 and CHOP but chronic eIF2α de-phosphorylation led to a significant increase in β-cell death. On the other hand pharmacological inhibition of inositol requiring enzyme 1 (IRE1) or expression of dominant negative IRE1 led to a decrease ATF4/CHOP protein expression and a decrease in CHOP promoter activity.
Conclusion: It has been proposed that pharmacological inhibitors of PERK/eIF2α may have therapeutic potential in the treatment/prevention of T2DM. However, according to the results presented here we question the value to developing drugs which target PERK/eIF2α in the treatment of diabetes and are thus currently seeking alternative drug targets by determining how ER stress up-regulates CHOP and ATF4 expression via an IRE1-dependent pathway in pancreatic β-cells.

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HDAC3 inhibition rescues pancreatic beta cells from glucolipotoxicity and improves insulin secretion in obese diabetic rats
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Background and aims: Glucolipotoxicity activates oxidative-, endoplasmic reticulum- and inflammatory-stress pathways in pancreatic beta cells. Both inflammatory mediators (cytokines) and metabolites (acetyl CoA) affect gene expression by regulating epigenetic modifiers such as histone acetyl transferases (HATs) and deacetylases (HDACs). We have previously shown that inhibition of HDACs prevents cytokine-induced beta cell apoptosis. It is unknown if HDAC inhibition prevents beta cell glucolipotoxicity. Therefore we hypothesized that HDAC inhibition protects pancreatic beta cells against glucolipotoxicity in vitro and in vivo.
Materials and methods: Selective HDAC inhibitors were tested in INS-1E cells, primary rat and human islets and in Zucker diabetic fatty (ZDF) rats. High glucose (25mM) and palmitate (0.5mM) were used as in vitro glucolipotoxic condition.
Results: We screened 21 inhibitors with different selectivity against the classical HDACs 1-11 and novel HDAC3-selective small molecule inhibitors for their ability to protect beta cells against palmitate-induced apoptosis. Class I (HDAC1, -2, -3 and -8) and in particular HDAC3 selective inhibitors reduced palmitate-induced caspase-3 activity in INS-1E cells and glucolipotoxic apoptosis in INS-1E cells, rat and human islets. The protective effects of pharmacological HDAC3 inhibition were confirmed by siRNA-mediated HDAC3 knock-down in INS-1E cells. A novel HDAC3-selective small molecule inhibitor, BRD3308, also restored insulin-secretion in INS-1E cells exposed to glucolipotoxic conditions. Mechanistically, BRD3308 reduced JNK phosphorylation, CHOP transcription, reactive oxygen species (ROS) formation and caspase 9 activity, suggesting that HDAC3 mediates glucolipotoxic apoptosis via ER-stress-dependent activation of the intrinsic death pathway.
Conclusion: The HDAC3 inhibitor BRD3308 protects pancreatic beta cells against glucolipotoxicity in vitro and in vivo by interfering with ER stress-induced mitochondrial death pathways. These findings have considerable translational potential since orally active broad HDAC inhibitors are clinically well-tolerated and more specific HDAC inhibitors are being developed.
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Suppression of NO-induced beta cell death by (-)-DHMEQ via activation of Nrf2-ARE pathway and alteration of MAPK activity
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Background and aims: Pancreatic beta cells are considered to be degraded by inflammatory cytokines and mediators mainly secreted by activated macrophages in the onset of type-1 diabetes mellitus. These inflammatory reactions may be also important for type-2 diabetes mellitus. Among the inflammatory mediators excessive nitric oxide (NO) plays a pivotal role in the progression of beta cell apoptosis. Therefore, we have looked for the inhibi-

tors of NO-induced beta cell death using the beta cell like cell line, and also studied the mechanism of inhibition.
Materials and methods: We employed mouse insulinoma Min6 cells as a model of beta cells to screen low molecular weight compounds that inhibit NO-induced cell death. We prepared a luciferase reporter DNA for the evaluation of Nrf2 activity. Cell death was evaluated by Trypan blue dye exclusion and apoptosis by selective staining.
Results: We found that (-)-DHMEQ, an NF-kappa B inhibitor, rescued NO-induced apoptosis in Min6 cells. NO was shown to decrease the Akt activity and to increase the p38 activity. These changes were inhibited by (-)-DHMEQ. Moreover, (-)-DHMEQ activated Nrf2 and induced transcription of Nrf2-target genes following the increase of antioxidant response element (ARE) reporter activity. Similarly, tert-butyl hydroquinone (tBHQ), a known activator of Nrf2, inhibited NO-induced cell death activating Nrf2. RNAi-mediated knockdown of Nrf2 lowered the cytoprotective effect of (-)-DHMEQ against NO, suggesting that (-)-DHMEQ inhibited NO-induced cell death via Nrf2 activation. Bay11-7082, a known NF-kappa B inhibitor, also activated Nrf2. Furthermore, overexpression of Nrf2 rendered cells to be more resistant to NO, indicating that Nrf2 activation provides critical defense function against NO in Min6 cells.
Conclusion: (-)-DHMEQ inhibited NO-induced cell death in mouse beta cell-like Min6 cells. It may be a useful therapeutic agent for type-1 diabetes mellitus in the onset of disease protecting pancreatic beta cells from apoptosis.

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PKBalpha but not PKBbeta can protect human islets from IL-1beta-induced apoptosis

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Background and aims: Loss of PKBα or PKBβ in mice results in increased or decreased glucose tolerance, respectively, whereas loss of PKBγ has no effect. The regulation of β-cell mass is normal in these mouse models indicating that no single isoform is required *in vivo* in pancreatic islets. The aim of this study is to test how gain-of-function for PKB isoforms affects regulation of proliferation, growth, apoptosis, and insulin production in human islets.
Materials and methods: Human islets (three donors) were obtained from the Juvenile Diabetes Research Fund (JDRF) and the European Consortium for Islet Transplantation's (ECIT) "Islets for Research Distribution Program". Intact or dissociated islets were cultured on extracellular matrix (ECM)-coated dishes. Gain-of-function was induced by overexpressing PKBα or PKBβ (GFP as control) from adenoviral vectors. Proliferation and apoptosis were assessed by BrdU incorporation and TUNEL. Insulin secretion (GSIS) and insulin content were assessed with intact islets. Insulin was measured by ELISA. β-cell size was determined after dissociation of islets. At least 50 islets or 2000 single cells in triplicate were evaluated. Data were normalised to GFP control and presented as mean ± SE and were analysed by student's t test.
Results: In intact islets, only PKBα increased proliferation significantly (3.21 ± 0.49 fold). Both isoforms increased β-cell proliferation significantly after dissociation of islets (PKBα: 4.2 ± 0.13 fold; PKBβ: 3.58 ± 0.69 fold). Proliferation of non-β-cells was not significantly increased. β-cell size was significantly increased by PKBα (1.34 ± 0.07 fold) and PKBβ (1.38 ± 0.06 fold). In intact islets IL-1β increased apoptosis 2.36 ± 0.33 fold ($p<0.01$). PKBα prevented the IL-1β-induced increase in apoptosis ($p<0.05$), whereas PKBβ had no effect. After dissociation of islets IL-1β did not affect apoptosis (n=1), however, the overall rate of apoptosis was increased significantly over intact islets. PKBα decreased basal apoptosis of β-cells by 80% whereas PKBβ decreased it only 20%. After dissociation, only PKBα increased the fraction of insulin-positive cells by 6.34 ± 1.4%. PKBα and PKBβ increased insulin content significantly (1.54 ± 0.19 and 1.6 ± 0.13 fold), whereas GSIS remained unchanged.
Conclusion: Our findings indicate significant differences between intact and dissociated human islets, both with respect to the role of PKB isoforms and the potency of IL-1β to induce apoptosis, possibly due to cellular interactions in intact islets. Overall PKBα appears to be more potent than PKBβ in regulating human islet mass as it protects β-cells from apoptosis and increases the percentage of β-cells to non-β-cells.
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IL-17 increases the expression and release of pro-inflammatory chemokines by human pancreatic islets

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Background and aims: Type 1 diabetes (T1D) is an autoimmune disease characterized by selective pancreatic beta cell destruction in the course of islet inflammation (insulitis). Cytotoxic T-cells and macrophages are the most prevalent cells during insulitis. These cells contribute to beta cell destruction at least in part through the production of pro-inflammatory cytokines such as IL-1β, IFN-γ TNF-α. IL-17-secreting $CD4^+$ T-cells (Th17) are implicated in several autoimmune diseases, and we have recently shown activation of the IL-17 pathway in both circulating T-cells and pancreatic islets of T1D patients. IL-17 exacerbates IL-1β+IFN-γ and TNF-α+IFN-γ-induced apoptosis in human islets. We have now evaluated whether IL-17 also contributes to the local production of chemokines, thus contributing to the build up and aggravation of insulitis.
Materials and methods: Human islets (12 donors) were isolated in Pisa, Italy and sent to Brussels for the experiments described below. Islets from wt and STAT-$1^{-/-}$ mice were studied in parallel. Dispersed islets cells were left untreated or treated with IL-17 alone or together with IL-1β+IFN-γ or TNF-α+IFN-γ. After 48h of treatment supernatants were collected for measurement of chemokine secretion by ELISA, whereas cells were used to evaluate mRNA expression by qRT-PCR. For histological studies whole human islets were treated with the same cytokine combination and then paraffin-embedded. RNA interference was used to knockdown (KD) STAT-1 in human islets.
Results: IL-17 augmented IL-1β+IFN-γ and TNF-α+IFN-γ-induced CXCL1, CCL20 and IL-8 mRNA expression in human islets ($P<0.05$). ELISA confirmed that IL-17 exacerbated IL-1β+IFN-γ and TNF-α+IFN-γ-induced CXCL1, CXCL10 and IL-8 secretion ($P<0.05$), while CCL20 was increased by IL-17 when combined with IL-1β+IFN-γ and CCL2 when combined with TNF-α+IFN-γ. IHC indicated detectable islet protein expression of CXCL1 and CXCL10 after cytokine + IL-17 treatment. Double immunofluorescence confirmed that beta cells are at least in part responsible for chemokine production. KD of STAT-1 in human islets prevented IL-17 + cytokine-induced apoptosis and expression of some (e.g. CXCL9 and CXCL10) but not other chemokines. Similar observations were made in islets isolated from STAT-$1^{-/-}$ mice. These concordant observations suggest that STAT-1 and other mechanisms that remain to be discovered contribute to the pro-inflammatory effects of IL-17.
Conclusion: The present findings indicate that IL-17 exacerbates pro-inflammatory chemokine expression and secretion by human islets exposed to IL-1β+IFN-γ and TNF-α+IFN-γ. For some chemokines this is mediated via STAT-1 activation. These observations suggest that IL-17 contributes to the pathogenesis of type 1 diabetes by two mechanisms, namely increased local production of chemokines (potentially aggravating insulitis) and exacerbation of beta cell apoptosis.
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Identification of a JNK3 module required for adaptation of beta cells in response to obesogenic and gestational environment

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Background and aims: Activation of c-Jun amino terminal kinase (JNK) 1 and JNK2 is substantial for eliciting beta cells death in response to chronic exposure to diabetogenic stressors including pro-inflammatory cytokines and fatty acids. However, on the three JNKs present in beta cells, JNK3 is the most abundant and is rather required for protecting beta cells against pro-apop-

totic stimuli. The data suggests that the JNK signaling could also coordinate an anti-apoptotic signal under temporal and/or physiological circumstances. In this study we investigated the mechanism leading to JNK3 activation and the potential role of this signaling in beta cells under obese and pregnancy environment.
Material and methods: RNA and total protein were extracted from the insulin secreting INS-1E and MIN6 cells, isolated islets of pregnant rats at different stages, obese ob/ob mice and obese individuals with or without diabetes. Co-immunoprecipitation experiments were performed using MIN6 cells. The protein and mRNA contents were analyzed by Western blotting experiments and quantitative real-time PCR, respectively.
Results: We found that activation of JNK3 was orchestrated by the Dual Leucine bearing zipper Kinase (DLK) as the mitogen activated protein kinase kinase kinase within a module operated by the scaffold Islet brain 1 (IB1). DLK was only present in insulin-positive cells within islets and triggered PDX-1 activity. Suppression of DLK impaired JNK3 activation, leading to loss of PDX-1 activity, insulin expression and an increase in cytokines-induced apoptosis. Conversely, overexpression of DLK raised PDX-1 activity, resulting in an increase of expression and secretion of insulin, and protection of cells against cytokines-induced apoptosis. On the one hand, the DLK level was increased in isolated islets of pregnant rats, hyperinsulinemic obese mice and obese individuals without diabetes. The GLP-1 mimetic exendin 4 stimulated the expression of DLK and JNK3 activation, supporting a role of the signaling for adaptation of beta cells under obesity and pregnancy. On the other hand the expression of DLK was diminished in islets of obese with diabetes and in response to chronic incubation with palmitate or pro-inflammatory cytokines.
Conclusion: The DLK-JNK3 is required for beta cells tasks by controlling PDX-1 activity. This signaling could be a key for adaptation of islets triggered by GLP-1 under obesity and pregnancy. Dysfunction of this signaling could account for beta cells failure in diabetes.
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Effect of angiotensin-(1-7) on apoptosis of insulin-secreting cell line NIT-1 induced by high glucose
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Background and aims: The association of the renin-angiotensin system with diabetes has been demonstrated by studies. Angiotensin-(1-7) (Ang-(1-7)) an endogenous heptapeptide hormone formed by cleavage of angiotensinI and AngiotensinII (AngII), counter acts many actions of AngII. Recent findings suggested that Ang-(1-7) exerts positive physiological modulation of insulin actions. Earlier work from our team showed that Ang-(1-7) could reverse the inhibitory effect of AngIIon insulin-stimulated Akt-Ser phosphorylation in NIT-1 cells. The purpose of the present study was to examine the effects of Ang-(1-7) on the apoptosis of pancreatic β cell induced by high glucose.
Materials and methods: Mouse pancreatic β cell line NIT-1 was divided into five groups: control group, Ang-(1-7) (10^{-5}M) group, high glucose (HG, 35mM glucose) group, HG+Ang-(1-7) group and HG+Ang-(1-7) + A-779 (Mas receptor antagonist, 10^{-5}M) group. The cells apoptosis were evaluated by TUNEL and flow cytometry analysis (Annexin V/PI). The reactive oxygen species (ROS) was detected by DCFH-DA fluorescent staining assay. The malondialdehyde (MDA) was measured by TBA method. Western Blot was used for evaluating the expression of pro-apoptotic protein cleave caspase-3 and phosphorylation of JNK and p38MAPK. One way analysis of variance (ANOVA) and LSD test were used for data analysis.
Results: The results of TUNEL and flow cytometry analysis were showed as follows. Compared with control group, the percentage of apoptotic cells in HG group was significantly increased($p<0.05$), whereas there was no significant difference between Ang-(1-7) group and control group. The percentage of apoptotic cells in HG+Ang-(1-7) group was lower than that in HG group ($p<0.05$), but was higher than that in control group ($p<0.05$). The percentage of apoptotic cells in HG+Ang-(1-7) +A-779 group was significantly increased when compared with HG+Ang-(1-7) group ($p<0.05$), was not significantly different from that in HG group. Analogously, the expression of caspase-3 in HG group was significantly higher than that in control group ($p<0.05$). Whereas after co-incubated with Ang-(1-7) for 72 h, the expression of caspase-3 was significantly declined compared with that in high glucose treated cells ($p<0.05$). A-779 blocked the inhibitory effect of Ang-(1-7) on the expression of caspase-3($p<0.05$). Concentrations of ROS and MDA in HG group was higher than that in control group ($p<0.05$). Glucose-stimulated JNK and p38MAPK phosphorylation significantly increased in HG group compared to the control group($p<0.05$). After co-incubated with Ang-(1-7) for 72 h, both concentrations of ROS and MDA and phosphorylation of JNK and p38MAPK were significantly declined compared with those in high glucose treated cells HG group($p<0.05$). These inhibitory effect of Ang-(1-7) were reversed by the A-779 ($p<0.05$).
Conclusion: Our data indicated that Ang-(1-7) could attenuate the apoptosis of NIT-1 cells induced by high glucose. It also suggested that inhibitory effect of Ang-(1-7) on apoptosis mediated through Mas receptor,and probably through JNK and p38MAPK-mediated signaling pathways and blocking oxidative stress.
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Role of muscle PGC-1α in the effects of resveratrol and exercise training on high fat diet induced changes in pancreas in mice
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Background and aims: Obesity is associated with many adverse changes such as pancreatic dysfunction. It is suggested that both physical activity and resveratrol can prevent adverse metabolic effects of increased caloric intake, but the potential protective effects of physical activity and resveratrol on high-fat diet induced changes in pancreas have not been resolved. Furthermore, the molecular mechanisms underlying the beneficial effect of exercise training and resveratrol have not been fully elucidated, although muscle PGC-1α has been proposed to play a role. Therefore, the aim is to test the hypothesis that muscle peroxisome proliferator-activated receptor-Ɣ coactivator (PGC)-1α is required for mediating the beneficial effects of exercise and resveratrol on high fat diet (HFD) induced changes in pancreas in mice.
Materials and methods: Muscle-specific PGC-1α knockout (MKO) and littermate wildtype (WT) mice completed 16 weeks of intervention with either carbohydrate (chow), high-fat diet (HFD), HFD with resveratrol (RSV, 4g/kg), HFD+ exercise (EX) or HFD+RSV+EX. Glucose and insulin tolerance tests and magnetic resonance scans were performed at week 14-15. Mice were euthanized by cervical dislocation at week 16 and pancreases were removed.
Results: HFD worsened glucose tolerance (p≤0,05), but insulin tolerance was unaffected, which could indicate impaired glucose sensitivity of the pancreas. Neither RSV nor EX could prevent this change. The lack of muscle PGC-1α reduced glucose tolerance to the same level as for WT on HFD. HFD did not affect Cytochrome C (Cyt C) protein, but resulted in increased Cytochrome C Oxidase I (COXI) protein (p≤0,05) in pancreas. RSV enhanced this increase in COXI further (p≤0,05), but had no effect on Cyt C protein. EX alone and in combination with RSV had no effect. None of the interventions had an impact on PDH-E1α protein expression, while HFD increased PDK4 protein content (p≤0,05) in pancreas. HFD did not affect Bax protein, but increased Bcl2 protein (p≤0,05) in WT mice but not in PGC-1α MKO mice. EX in WT but not in PGC-1α MKO mice and RSV in both genotypes increased the Bcl2 protein (p≤0,05).
Conclusion: The main finding in the present study is that HFD induced impairment of glucose tolerance seemed to involve pancreatic changes opposite of expected this seemed to be associated with increased anti-appoptotic changes of pancreas, which may be due to compensatory regulation. RSV and EX training did not rescue HFD induced changes and muscle PGC-1α did not play a role.
Clinical Trial Registration Number: 2009 561 1689
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PS 032 Islet cell toxicity and survival

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c-Src regulates the phosphorylation status of STAT3 in pancreatic beta cells and modulates their response to interleukin-6

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Background and aims: Human type 2 diabetes is characterised by low grade systemic inflammation associated with elevated circulating levels of interleukin (IL)-6. Islet cells are responsive to IL-6 and this cytokine mediates its effects, in part, by promoting the phosphorylation of the transcription factor STAT3. Therefore, we have studied the regulation of STAT3 by IL-6 and, in particular, have considered the possibility that this may be influenced by the tyrosine kinase c-Src which is thought to modulate the activation of STAT3 in some cell types.
Materials and methods: INS-1E, HEK293T cells and isolated human islets were used. STAT3 phosphorylation was assessed in cell extracts by Western blotting with site-specific antisera. Transcriptional activity was measured using a STAT3 responsive dual-luciferase reporter construct. Cell viability was assessed by flow cytometry and nitrite levels were determined by Greiss assay.
Results: The IL-6 receptor comprises two subunits, IL-6Rα and GP130, and both were readily detected in INS-1E cells and human islets by RT-PCR. Exposure of INS-1E cells to IL-6 alone (20ng/ml) did not influence their viability but the cytokine enhanced the detrimental effects of a range of different cytotoxic stimuli, including IL-1β (IL-1β: 14.3 ± 0.2% dead cells; IL-1β + IL-6: 22.2 ± 0.7%, p<0.001), a pro-inflammatory cytokine mix (mix: 16.3 ± 0.3% dead cells; mix + IL-6: 22.7 ± 0.7%, p<0.001) and the saturated fatty acid, palmitate (palm: 37.4 ± 0.8% dead cells; palm + IL-6: 44.3 ± 0.3%, p<0.001). Nitrite production (an index of nitric oxide formation) was not elevated in cells treated with IL-6 alone, but the increase caused by IL-1β was potentiated in the presence of IL-6 (IL-1β alone: 17.5 ± 0.3 μM nitrite; IL-1β + IL-6: 21.8 ± 0.4 μM, p<0.001). These responses correlated with a rapid increase in the phosphorylation of STAT3 at Tyr705 upon addition of IL-6. The activity of a STAT3 responsive reporter was enhanced significantly in cells exposed to 20ng/ml IL-6 (by 59.9±24.6 fold above control; n=6; p<0.05). Phosphorylation of a second residue within STAT3, Ser727, was also detected under these conditions but this was seen in control cells and was only marginally influenced by IL-6. To study the possible role of the non-receptor tyrosine kinase, c-Src, cells were exposed to a selective inhibitor of the enzyme, Src-I (10μM). Upon exposure to IL-6, this agent reduced the phosphorylation of STAT3 at both Tyr705 and Ser727. Under these same conditions, STAT3 reporter activity was strongly inhibited (from 26.98±6.74 to 2.91±1.35; p<0.05). The inhibitor also markedly reduced STAT3 activity in control cells (to 0.18±0.04, relative to control; p<0.01) and this correlated with a selective attenuation of the phosphorylation of Ser727.
Conclusion: IL-6 augments the detrimental effects of various cytotoxic stimuli in pancreatic β-cells. This correlates with increased phosphorylation of STAT3 at Tyr705 and a rise in STAT3 transcriptional activity. STAT3 is also phosphorylated at a second site, Ser727, which is not enhanced upon exposure to IL-6. However, loss of the phosphate group from Ser727 occurred in cells exposed to a c-Src inhibitor, leading to attenuation of the response to IL-6. These data imply that the activity of c-Src may be required to facilitate the response of pancreatic beta cells to IL-6 and that inhibition of this enzyme attenuates the pro-inflammatory actions of IL-6.

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Long-term overexpression of c-Kit negatively impacts beta cell function and survival via capillary and inflammatory recruitment in the islet

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Background and aims: Diabetes progression is marked by a decline in pancreatic β-cell mass. We have shown that the receptor tyrosine kinase, c-Kit, plays an integral role in mediating beta cell mass, function and survival using c-KitWv/+ mutant and transgenic mice (c-KitβTg) overexpressing c-Kit specifically in beta cell. Our recent study demonstrated that c-KitβTg mice showed protective effect against short-term (4 weeks) high-fat diet (HFD) induced diabetes with significant activation of Phosphoinositide 3-kinase/Akt signaling pathway. However, this protective effect diminishes after a long-term HFD (20 weeks) and the mice developed progressive hyperglycaemia. This evidence suggests that overexpression of c-Kit in beta cells may affect its function and survival through a different regulatory mechanism under a long-term HFD. We propose that over-expression of c-Kit may promote HIF-1α and VEGF expression leading to capillary recruitment in the islets and an increase in inflammatory signals, which imposes a negative impact in beta cell function and survival.
Materials and methods: In vitro, insulinoma 832/13 (INS-1) cells were cultured with either stem cell factor (SCF) or c-Kit siRNA to examine the effect of SCF/c-Kit interaction in regulating HIF-1α and VEGF expression. In vivo, c-KitβTg and wild-type (WT) mice were fed with HFD for 20 weeks started at 6 weeks of age. Metabolic and morphological studies including islet microvasculature formation, islet inflammatory cytokines and beta cell function were characterized using qRT-PCR, western blot, ELISA and double immunofluorescence approaches.
Results: In vitro, SCF augmented VEGF production in INS-1 cell culture in a time-dependent manner. This up-regulation was significantly reduced after cells were transfected with c-Kit siRNA (P<0.05). A significant increase of HIF-1α (P<0.05) and VEGF (P<0.01) were detected in islets isolated from c-KitβTg mice, and decreased in c-Kitwv/+ islets, when compared to WT islets. Furthermore, isolated c-KitβTg islets cultured in 3D fibrin gel showed increased sprouting of endothelial-like cells and endothelial cell proliferation compared to WT islets. These results suggest that c-Kit upregulates expression of VEGF, which may lead to capillary recruitment. In vivo, c-KitβTg mice under long-term HFD showed a significant increase of body weight (P<0.05), elevated overnight fasting glucose level (P<0.05), together with glucose intolerance and reduced insulin secretion (P<0.05) as compared to WT mice. Significant increased beta cell mass was observed in the WT-HFD mice, but this HFD-induced compensation in c-KitβTg-HFD mice was diminished. In parallel, an increased islet capillary area and vascular dilation (P<0.05) in c-KitβTg-HFD mice was detected, which could result in the recruitment of macrophage and inflammatory cytokines in the islets.
Conclusion: The present study represents unique and integrated approaches to elucidate cellular mechanisms by which altered c-Kit expression in beta cell under the long-term HFD stress could negatively impact beta cell function and survival. This negative regulation is likely associated with VEGF-induced vascularization and subsequently leading to potent chemoattractant tracking for inflammatory process, which could induce the development and progress of type 2 diabetes.

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Fibrin modulates αvbeta3 integrin expression and FAK signalling to mediate islet cell differentiation, function, proliferation and survival

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Background and aims: Integrins are a class of cell adhesion molecules that integrate cells to the extracellular matrix (ECM). ECM-integrin interactions influence β-cell differentiation, function, proliferation and survival; yet, when ECM proteins are used in culture, progressive loss of islet function and structure occurs. Fibrin is a provisional ECM protein and contains Arg-Gly-Asp amino acid motifs that are bound by integrin receptors such as αvβ3. Fibrin has been used in multiple surgical specialties and supports human and rat adult islet function, but the molecular mechanisms by which this occurs is unknown. The present study examined the functional role of integrins and downstream signaling pathways during fibrin culture of human fetal islet progenitor cells and mature beta cells.
Materials and methods: Human fetal islet progenitor cells isolated from 2nd trimester of pregnancy and mature beta (INS-1) cells were cultured up to 4 weeks on tissue culture polystyrene (TCPS) and with fibrin in 2D and 3D. An anti-αvβ3 integrin blocking antibody was used to determine the necessity of integrins with respect to beta cell growth and intracellular signaling. Cultured cells were analyzed by scanning electron microscopy, quantitative RT-PCR, western blot, immunofluorescence and ELISA.
Results: Fibrin culture of human islet progenitors led to preserved expression of glucagon, insulin and PDX1 when compared to TCPS. Mature beta (INS-1) cells cultured with fibrin formed islet-like clusters and showed direct contacts with fibrin as determined by scanning electron microscopy. As well, fibrin improved basal insulin release (p<0.01), glucose-stimulated insulin secretion (p<0.05) and Pdx-1 expression (p<0.01) at 4 weeks of culture. Significant increases in integrin αvβ3 (p<0.01) and phosphorylated FAK, Akt and ERK1/2

protein levels (p<0.05-0.001) were observed in fibrin-cultured cells compared to controls. These changes were associated with significantly increased cyclin D1 expression levels and Ki67 labeling (p<0.01), as well as decreased cell apoptosis (p<0.05). Perturbation of αvβ3 integrin function affected beta cell spreading on fibrin gels and resulted in significantly decreased FAK phosphorylation and increased cleaved caspase-3 levels (p<0.05).
Conclusion: This study demonstrates that activation of FAK, Akt and ERK1/2 signaling pathways by fibrin-integrin interactions mediates differentiation, function, proliferation and survival of islet cells. Using fibrin during cell culture may assist in promoting long-term beta cell health, as well as supporting the differentiation of human fetal islet progenitor cells. In this manner, existing beta cell sources can generated and maintained for use in diabetic therapies.

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AMPK regulates ER morphology and function in stressed pancreatic beta cells via phosphorylation of DRP1

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Background and aims: Lipotoxicity designates the functional and physical demise of β-cells induced by metabolic stress in obesity and type 2 diabetes. Fatty-acid excess induces ER stress, accompanied by ER morphological changes. The energy sensor AMPK regulates metabolic stress; however its impact on ER morphology and function is unknown. We studied the regulation of ER morphology by fatty acids and AMPK in relation to β-cell function and survival.
Materials and methods: Mouse and human islets and INS-1E β-cells were treated with and without palmitate and/or the AMPK activators AICAR and metformin. AMPK was inhibited using a dominant-negative AMPK adenovirus. ER and mitochondrial morphology were analyzed by super-resolution confocal and electron microscopy. Islet proinsulin and insulin content and secretion were analyzed by ELISA and RIA, respectively. Apoptosis was monitored by Western blot for cleaved caspase 3 and by nucleosome ELISA assay.
Results: Palmitate induced marked ER expansion and mitochondrial fragmentation. We demonstrate that DRP1, a key regulator of mitochondrial fragmentation, is an ER resident and plays an important role in this process. Adenoviral vector expression of a dominant-negative DRP1 (Ad-K38A) in β-cells attenuated fatty acid-induced ER expansion and mitochondrial fragmentation. Stimulating AMPK inhibited DRP1 by phosphorylating aminoacid Ser637 and completely prevented the alterations in ER and mitochondrial morphology. Confocal microscope imaging and sub-cellular fractionation of palmitate-treated cells showed that DRP1 was localized in mitochondrial fission sites and in the ER. Further, palmitate-induced ER enlargement was associated with proinsulin retention in the ER, resulting in impaired proinsulin trafficking and processing. Stimulation of AMPK prevented these alterations and attenuated palmitate-induced apoptosis.
Conclusion: AMPK-DRP1 is a novel pathway regulating ER and mitochondrial morphology, thereby controlling the response of β-cells to metabolic stress. DRP1 may function as a node integrating signals from stress regulators, such as AMPK, to coordinate organelle shape and function.
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Pathways involved in the GLP-1-mediated protection against cytokine-induced beta cell dysfunction and death in human islets: a proteomic approach

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Background and aims: Type 1 diabetes (T1D) is characterized by cytokine-mediated beta cell destruction, resulting in life-long insulin dependency for the patient. The incretin hormone glucagon-like peptide-1 (GLP-1) has been shown to protect pancreatic beta cells against cytokine-induced dysfunction and destruction, thereby maintaining glucose homeostasis. Incretin-based compounds have emerged as a new class of agents for treatment of T2D while few reports have also described the effects of incretin-based agents in T1D. However, the mechanisms through which GLP-1 exerts its effects are complex and still poorly understood. The aim of this study was to analyze the protein expression profiles of human islets of Langerhans treated with cytokines in the presence or absence of GLP-1 in order to better understand the molecular pathways involved in GLP-1-mediated beta cell protection.
Materials and methods: Whole human islets of Langerhans were incubated for 72 h with IL1β (50U/ml) and IFNγ (1000U/ml) either alone, or in combination with GLP-1 (10nM). Beta cell apoptosis was determined by electron microscopy and beta cell dysfunction was evaluated by means of glucose-stimulated insulin secretion measurements. In parallel alterations in protein expression profiles were identified by 2-dimensional difference gel electrophoresis (2D-DIGE) combined with sophisticated protein interactome network analysis. Interesting results were confirmed by Western blotting.
Results: Cytokines induced an almost two-fold decrease in glucose-stimulated insulin secretion (n=3, $p < 0.05$) and increase in apoptosis from 1 ± 0.6 to 18 ± 3.5 % (n=3, $p < 0.05$), an effect which was almost completely restored by co-incubation with GLP-1. 2D-DIGE (n=4) and interactome network analysis revealed that GLP-1 alters in an integrated manner protein networks in cytokine-exposed islets. This showed that the cytoprotective effects of GLP-1 are mainly executed through counteracting the effects of cytokines on proteins form different functional classes such as restoration of actin cytoskeleton organization (e.g. ARP3 and CAPZB), normalization of chaperones levels (e.g. EIF3I), metabolic proteins (e.g. ALDH) and islet regenerating proteins (REG1A and REG1B).
Conclusion: GLP-1 alters in an integrated manner protein networks in cytokine-exposed human islets while protecting them against cytokine-mediated cell death and dysfunction. These data illustrate the beneficial effects of GLP-1 on human islets under immune attack and lead to a better understanding of the underlying mechanisms involved, a prerequisite for improving therapies for diabetic patients.
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Mechanisms underlying compensatory islet response to insulin resistance

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Background and aims: Compensatory islet response is a distinct feature of the pre-diabetic insulin resistant state in both rodents and humans. In this study, we aim to identify specific protein alterations in islet cell function, apoptosis and proliferation that characterize this adaptive response.
Material and methods: Islets from six-month-old male control, high-fat diet fed and obese Ob/Ob mice were analyzed by liquid chromatography coupled mass spectrometry (LC-MS). Accurate mass and time tag (AMT-Tag) and selected reaction monitoring (SRM) were applied to quantify and verify the proteomic response in islets.
Results: The LC-MS quantitative proteome profiling revealed ~1,700 islet proteins that were identified and quantified by at least two unique peptides with a false discovery rate <1%. Statistically significant alterations in protein abundances were observed for ~450 proteins between control and insulin resistant conditions. Our study reveals common exacerbated dysfunction in energy metabolism (e.g. ALDOA and PFKL), oxidative phosphorylation (e.g. ATP5I, COX5A and COX6A), hormone processing (e.g. PC1 and PC2) and secretory pathways (e.g. RAB7A and UCN3). Increased expression of actors involved in protein synthesis (such as EIF3 family members) and folding (e.g. ERP29 and ERP44) was observed and suggests endoplasmic reticulum stress response in insulin resistant islets. Compensatory cell survival and proliferation were revealed as the main mechanisms by which islet-cells compensate to increased metabolic needs during insulin resistance.
Conclusion: This study represents the first quantitative study of the islet proteome that is focused on the compensatory islet-cell growth in response to insulin-resistance without overt diabetes. The extensive dataset provides a resource of islet protein candidates to apprehend islet cell replenishment to increasing the number of beta cells in patients with diabetes.
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Reduced availability of glucose during culture attenuates hypoxia-induced reduction in cellular viability and function in pancreatic beta cells

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Background and aim: To improve the success of islet transplantation in type 1 diabetes patients one needs to minimize hypoxia-induced beta cell death. Here we used clonal beta cells and rat pancreatic islets to test by viability and functional parameters if decreasing cellular metabolism by in vitro exposure to a low glucose concentration could reduce the impact of experimental hypoxia and hence be a potentially beneficial part of a pre-transplantation protocol. Further, we tested for evidence of lingering metabolic adaption to hypoxia.
Materials and methods: INS-1-derived 832/13 cells were cultured in medium containing either 5.5 or 11 mM glucose for three days under normoxic conditions before exposure to hypoxia for 8 or 18 h. Control cells were grown in parallel at continuous normoxia. Subsequent re-oxygenation (overnight) was always carried out at 11 mM glucose. Rat pancreatic islets were subjected to similar conditions.
Results: Reduction of cell viability after 18 h of hypoxia followed by re-oxygenation was assessed by the MTT assay, by cell counting and by measurement of apoptosis/necrosis. Reduction was less pronounced following culture at 5.5 mM glucose: for MTT 46 ± 2.7 vs. 62 ± 2.9 %, $p < 0.001$ (n = 4), for cell counting 32.7 ± 6.4 vs. 47.6 ± 6.1 %, $p < 0.002$ (n = 5), for incremental apoptotic DNA 1.52 ± 0.14 vs. 2.42 ± 0.19, $p < 0.02$ (n = 8), and necrotic DNA 1.54 ± 0.27 vs. 2.31 ± 0.26, $p < 0.08$ (n = 8). A favorable effect of pre-culture at low glucose (on MTT) was confirmed in rat pancreatic islets. Previous hypoxia tended to increase basal insulin secretion regardless of glucose concentration during culture. Previous hypoxia decreased an index of stimulation (ratio of insulin released at 16.7 to secretion at 3.3mM glucose) for cells cultured at 11 mM glucose , but did not affect this index in cells cultured at 5.5 mM glucose. Basal oxygen consumption in cells cultured at 11 mM glucose was not affected by 8 h of previous hypoxia and subsequent re-oxygenation, whereas respiration in 5.5 mM glucose-cultured cells was modestly increased. Previous hypoxia reduced the ratio of oligomycin-inhibited/FCCP-induced oxygen uptake in cells pre-exposed to any of the two glucose concentrations during culture, implying induction of a lesser degree of mitochondrial uncoupling.
Conclusion: Our findings provide evidence that culture at low glucose lessens the impact of experimental hypoxia on cell viability and function. Further they suggest that cellular adaption takes place in response to hypoxia.
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Cytokines and histone deacetylase inhibition differentially regulate the expression of beta cell inflammasomes

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Background and aims: In type 1 and type 2 diabetes mellitus IL-1β is believed to contribute to beta cell failure and destruction. Histone deacetylase (HDAC) inhibitors protect against inflammatory beta cell demise. We recently demonstrated that HDACs regulate beta cell IL-1β production, but it is unknown which inflammasome is responsible for beta cell processing of proIL-1β. Here we wanted to examine the expression levels of the NALP1, NLRP3 and ASC inflammasome components, as well as expression levels of the purinergic p2x7 receptor that senses extracellular ATP as a danger-associated molecular pattern and mediates inflammasome activation via K+ efflux.
Materials and methods: INS-1 cells were preincubated with or without the HDAC inhibitor givinostat for 1h before exposure to 150 pg/mL IL-1β and 0.1 ng/mL IFNγ for 1, 3, 6, 12 or 24 h. Cells were lysed, RNA extracted using Nucleospin II, converted to cDNA using iScript, and mRNA levels were determined by qPCR using the 2ΔΔCt method.
Results: Here we show that cytokines and HDAC inhibition differentially regulated beta cell inflammasome expression. NLRP1 mRNA was induced by cytokines after 3 h ($p<0.001$), peaked at 6 h ($p<0.001$), and was down-regulated after 24 h ($p<0.01$), while NLRP3 and ASC expression was suppressed by cytokines after 6 and 12 h, respectively ($p<0.01$). HDAC inhibition down-regulated basal expressions of NLRP3 at all timepoints ($p<0.001$) and ASC at 12 and 24h ($p<0.001$), while cytokine induction of NLRP1 was attenuated by histone deacetylase inhibition at 3h ($p<0.001$) and 6h ($p<0.001$). P2x7 expression was downregulated after 1 h of HDAC inhibition ($p<0.01$) but was unaffected by cytokines.
Conclusion: We conclude that downregulation of beta cell p2x7 receptor and inflammasome expression may contribute to the protective effects of HDAC inhibition in inflammatory beta cell destruction, suggesting that HDAC inhibitors may be attractive novel antidiabetic drugs.
Supported by: EFSD/JDR/Novo Nordisk, NNF, UCPH

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NLRP3 inflammasome is expressed and regulated in human islets

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Background and aims: NRLP3 inflammasome is a protein complex playing an important role in innate immunity. This complex is activated in response to infection, inflammation and autoimmune processes and is involved in the maturation of IL1β by the cleavage of caspase-1. Blood level of IL1β was shown to increase in diabetic patients and NRLP3 inflammasome was suggested to mediate this effect. However, the real contribution of NRLP3 inflammasome in local islet production of IL1β has not been yet demonstrated. The aim of this study was to determine the expression and the regulation of the NRLP3 inflammasome in human islets.
Materials and methods: Human islets were enzymatically isolated from cadaveric donor pancreases. Isolated islets were stimulated or not with 1µg/ml LPS for 4h and successively with 5mM ATP for 30 min. This treatment was performed in the presence or absence of 200µM glyburide, an inflammasome inhibitor. Supernatants were collected and secreted IL1β was quantified by ELISA. The *NRLP3* and *IL1β* genes expressed in islets were studied by real time PCR and caspase-1 by western blot.
Results: *NRLP3* and *IL1β* were found to be expressed in untreated isolated human islets. In response to the LPS plus ATP treatment, *NRLP3* and *IL1β* increased 3.7±0.2 fold (n=4, p=0.02) and 45.5±9.8 fold (n=2), respectively. Glyburide prevented the augmentation of *NLRP3* (2.6±0.2 fold, p=0.03) and *IL1β* (23.4±2.9 fold, n=2). The LPS plus ATP treatment induced the cleavage of caspase-1, an essential enzyme for the maturation and secretion of IL1β. Human islets secreted 38.1±1.8 pg/ml IL1β (n=5, p=0.0097) in response to LPS plus ATP. Glyburide was shown to prevent the cleavage of caspase-1 and decrease IL1β secretion by 97.6% (0.92±0.28 pg/ml IL1β, n=5, p=0.0079).
Conclusion: These results show that the NRLP3 inflammasome is expressed and regulated in human islets in response to LPS plus ATP. The NRLP3 inflammasome could be a potential therapeutic target to prevent local IL1β production and the subsequent islet damages in both diabetes and islet transplantation.

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Human serum vs human serum albumin for human islet culture

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Background and aims: Human islets are usually incubated for 48 or 72 hours in medium supplemented with human serum albumin (HSA) before clinical transplantation. However, whole serum is preferred for standard tissue culture due to the presence of growth factors improving cell viability. There is conflicting evidence favouring both the use of HSA and of human serum (HS) as best strategy for culture of human islets. The aim of this study was to evaluate the effects of HS versus HSA supplementation of culture media on: a) in vitro beta cell viability and function; b) in vivo islet graft revascularization, beta cell death and metabolic outcome after experimental islet transplantation.
Material and methods: Human islets isolated from the pancreases of 15 cadaveric organ donors were cultured for 3 days in CMRL 1066 medium

supplemented with HSA (HSA group) or human AB serum (HS group) immediately after isolation. Beta cell apoptosis was quantified in islets double stained by TUNEL and insulin, and islet function as glucose-stimulated insulin secretion (GSIS) (basal: 2.8 mM; stimulated: 20 mM). In vivo viability and revascularization was determined in HS and HSA cultured islets transplanted in the anterior chamber of the eye of normoglycemic Balb/c mice (n= 14), using intravenous propidium iodide and rhodamine B isothiocyanate-dextran, respectively. Beta cell apoptosis was quantified in paraffin-embedded eyes double stained by TUNEL and insulin. Function of transplanted islets was determined as fed plasma glucose in streptozotocin (STZ)-diabetic nude mice transplanted with a 2000 IEQs of HS (n= 17) or HAS (n=16) cultured islets.
Results: After 3 days in culture, beta cell apoptosis was lower in HS group (2.45±0.59% vs 5.54±0.68%) (p= 0.008). Insulin content was similar in HS and HSA groups. GSIS was higher in HS group (37.3±13.5 ng/μg DNA vs 26.4±9.3 ng/μg DNA) (p= 0.027). The stimulation index was also higher in HS group (12.0±2.6 vs 5.26±0.50) (p= 0.026), and the ratio stimulated insulin secretion/insulin content tended to be higher in the HS group (4.82±1.59% vs 2.94±0.71%) (p=0.062). However, islets transplanted into the anterior chamber of the eye showed similar viability (intravenous propidium iodide: $5.67.10^{-5}$±$4.51.10^{-5}$ vs $4.00.10^{-5}$±$3.00.10^{-5}$ death cells/μm^2), and beta cell apoptosis (8/903 vs 0/528 apoptotic/total nuclei) (HS vs HSA group), 10-15 days after transplantation. Revascularization was observed in one graft (HS group) on day 10 after transplantation. Metabolic evolution was similar in STZ-diabetic nude mice transplanted with HSA and HS cultured human islets that showed comparable blood glucose levels and percentage of normoglycemic animals over time.
Conclusion: Human islets cultured in medium supplemented with HS showed higher in vitro islet viability and function, but this beneficial effect did not translate into an improved survival and metabolic evolution after transplantation in mice.
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PS 033 Experimental immunology

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Prevention of type 1 diabetes in NOD mice by targeting dipeptidyl peptidase IV is associated to modification of both central and peripheral T-cell subsets
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Background and aims: CD26 or dipeptidyl peptidase IV (DPPIV) is a multifunctional glycoprotein expressed ubiquitously, with T-lymphocyte costimulatory actions. We aimed to assess the effect of CD26 targeted treatment with a DPPIV inhibitor (MK0626) on the prevention of type 1 diabetes (T1D), the insulitis score and the percentage of T-lymphocyte subsets from both the thymus and the periphery in the *nonobese* diabetic mouse (NOD).
Materials and methods: 15 mice treated with MK0626 administered in the diet, started at 8 weeks of age, and 15 untreated (control group) were followed during 30 weeks. Insulitis score was determined by two blinded evaluators. Lymphocyte subsets were studied in another group of treated and control mice at 2, 4 and 6 weeks of treatment (n=6 at each time-point). Flow cytometry for characterization of lymphocyte subsets was performed using MoAbs (CD3-V450, CD4-APC-Cy7, CD8-V500,CD26-PerCP-Cy5, CD25-PE, FoxP3-FITC, CD44-FITC, CD45RB-PE, CD62L-PerCP-Cy5.5, CCR7-APC) and identified as: double positive (DP) ($CD4^+CD8^+$), double negative (DN) ($CD4^-/CD8^-$), single positive (SP) CD4 ($CD8^-CD4^+$), SP CD8 ($CD4^-/CD8^+$), naïve ($CD3^+CD4^+/CD8^+CD44^-CD62L^+CCR7^-$); central memory (CM)($CD3^+CD4^+$/ $CD8^+CD44^{high}CD62L^{+low}CCR7^+$); effector memory (EM) ($CD3^+CD4^+/CD8^+CD44^{high}CD62L^+CCR7^-$);Tregs($CD4^+CD25^+Foxp3^+$).
Results: MK0626 treatment decreased diabetes incidence by 45% (37.5% *vs* 68% controls, p=0.05). Insulitis score: At week 6, insulitis score significantly decreased in treated vs control mice (0.83±0.27 *vs* 1.52±0.26, p=0.02) with no changes at week 2 and 4. Lymphocyte subsets: at week 4 of treatment, treated mice showed in comparison with the control group: 1) Periphery: decreased percentage of CD4 and CD8 naïve T-cells (p<0.001, respectively), CD4 and CD8 CM T-cells (p<0.001, respectively), and increased percentage of CD4 and CD8 EM T-cells (p<0.001, respectively), 2) Thymocytes: decreased percentage of DN (p=0.002), SP CD4 (p=0.003) and SP CD8 (p=0.004) and increased percentage of DP (p=0.001) thymocytes. No differences were observed at week 2 nor at week 6 of treatment.
Conclusion: Treatment with the DPPIV inhibitor MK0626 in NOD mice decreases the incidence of T1D, reduces insulitis score and modifies the percentage of several T-lymphocyte subsets in the thymus and the periphery. The effect of this drug on both central and peripheral T-cell compartments may be involved in the development of immune tolerance, thereby preventing T1D in NOD mice.
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Changes in the circadian rhythmicity of energy metabolism and cerebral cFos expression at diabetes onset in NOD mice
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Background and aims: An endogenous rhythm generator located in the hypothalamic suprachiasmatic nucleus (SCN) controls the rhythmicity of metabolic and immunological processes. As the pathogenesis of type 1 diabetes is associated with dysregulated energy metabolism and immune reactivity it may be assumed that disturbances of circadian rhythms of these processes are involved in disease manifestation. We hypothesized that the onset of insulin-deficient diabetes in non-obese diabetic (NOD) mice coincides with alterations of the rhythmicity of metabolic processes and the circadian system.

Materials and methods: Female C57BL6, normoglycemic and newly diabetic (<3 d) NOD mice were kept at a 12-h light/12-h dark cycle. Respiratory quotients (RQ) were determined by indirect calorimetry. Locomotor activity was monitored by interruption of infrared beams ("counts"). Expression of the transcription factor cFos, reflecting neuronal activity, was analyzed in the SCN by immunohistochemistry.
Results: In light cycles all groups of mice show comparable spontaneous locomotor activity, whereas in dark cycles diabetic NOD mice have a lower locomotor activity (575 ± 84 counts) than C57BL6 (1164 ± 478, counts, $p<0.05$) or nondiabetic NOD mice (1169 ± 299 counts, $p<0.05$). Furthermore, diabetic NOD mice have a reduced RQ in light (0.82 ± 0.02) and dark cycles (0.84 ± 0.01) when compared to C57BL6 (light 0.88 ± 0.01, dark 0.94 ± 0.02, $p<0.001$) and nondiabetic NOD mice (light 0.88 ± 0.02, dark 0.95 ± 0.01, $p<0.001$). Cerebral cFos expression was about 40% higher in the SCN area of C57BL6 and nondiabetic NOD mice at ZT02 (2 h after lights-on) than at ZT14 (2 h after lights-off), but the total number of cFos positive cell nuclei was significantly decreased in the SCN of nondiabetic NOD mice at ZT02 (cFos positive cells in SCN: 87 ± 29) when compared to C57BL6 mice (cFos positive cells in the SCN: 196 ± 22). However, diabetic NOD mice showed no light/dark rhythmicity of cFos expression (cFos positive cells in SCN: ZT02: 73 ± 17; ZT14: 78 ± 16).
Conclusion: In conclusion, nondiabetic but diabetes prone NOD mice exhibit a reduced cerebral cFos expression when compared to C57BL6 mice without diabetes predisposition. In newly diabetic NOD mice this abnormality coexists with an altered energy metabolism pointing to a link between the SCN endogenous rhythm generator and the metabolic changes at or even before the onset of insulin-deficient diabetes.
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Differential requirement for endogenous IL-15 and IL-21 in activating diabetogenic CD8+ T cells in a virus-induced model of autoimmune type 1 diabetes
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Background and aims: We have shown that stimulation of potentially diabetogenic CD8+ T cells with the inflammatory cytokines IL-15 and IL-21 enables them to respond to weak TCR stimuli and induce autoimmune type 1 diabetes (T1D) in a transgenic mouse model. We have also shown that lack of IL-15 or IL-21 protects NOD mice expressing the highly pathogenic 8.3 transgenic TCR on CD8+ T cells from T1D. The aim of this study is to determine the role of endogenous IL-15 and IL-21 in activating potentially diabetogenic CD8+ T cells in a virus-induced T1D model.
Materials and methods: We introduced the null alleles of Il15 or IL21 into RIP-GP mice expressing the lymphocytic choriomeningitis virus (LCMV) glycoprotein in pancreatic beta cells under the rat insulin promoter (RIP). RIP-GP, Il15-/-.RIP-GP and IL21-/-.RIP-GP mice were infected with LCMV and evaluated for T1D development, insulitis and viral load. Lymphocytes from infected mice were characterized phenotypically and tested for the ability to transfer disease to Rag1-/-.RIP-GP mice.
Results: IL-15 deficiency hampered clearance of LCMV in RIP-GP mice, suggesting impaired immune response towards LCMV antigens. Accordingly, the proportion of CD8+ T cells with the CD44hiCD62Llo activated phenotype was significantly lower in Il15-/-.RIP-GP mice than in control mice. Despite this inability to mount protective immune response against LCMV, 50% of Il15-/-.RIP-GP mice developed T1D with marked destruction of pancreatic islets. On the other hand, Il21-/-.RIP-GP mice efficiently cleared LCMV and all mice developed T1D with similar kinetics as wildtype RIP-GP mice.
Conclusion: Our results support the notion that IL-15 is needed for efficient activation of diabetogenic CD8+ T cells. On the other hand, IL-21 is dispensable in LCMV-induced T1D in RIP-GP mice, although a recent study has reported requirement for IL-21 in LCMV-induced T1D in NOD mice expressing LCMV nucleoprotein (NP) in the islets. Our findings suggest that other genetic susceptibility factors may influence the pathogenic role of IL-21.
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Alterations of lipid homeostasis and insulin sensitivity in TLR4-deficient non-obese diabetic mice
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Background and aims: Studies in animal models demonstrate that the toll-like receptor 4 (TLR4) is involved in the maintenance of metabolic homeostasis and implicate a role of TLR4 in the development of autoimmunity. In non-obese diabetic (NOD) mice disturbed glucose and lipid homeostasis may promote the development of autoimmune-mediated diabetes. As glucose homeostasis depends on insulin availability and responsiveness we tested the effect of TLR4 expression on insulin release, insulin action and circulating fetuin A, a mediator of free fatty acid (FFA) binding to TLR4 in NOD mice.
Materials and methods: In female TLR4-expressing ($TLR4^{+/+}$) and TLR4-deficient ($TLR4^{-/-}$) prediabetic NOD mice (age 120 d) body weight and food intake were documented and body composition was analyzed by magnetic resonance imaging (MRI). Glucose-stimulated insulin release was determined in isolated pancreatic islets. Serum levels of insulin and of fetuin A were measured by ELISA. Fasted mice also underwent intraperitoneal insulin tolerance tests (ipITT) to assess insulin sensitivity.
Results: When compared to $TLR4^{+/+}$ NOD mice, $TLR4^{-/-}$ animals have a higher body weight ($TLR4^{+/+}$ 25.4 ± 1.3 g, $TLR4^{-/-}$ 28.1 ± 1.7 g, $p<0.01$), but comparable food uptake ($TLR4^{+/+}$ 0.15 ± 0.01, $TLR4^{-/-}$ 0.16 ± 0.01 g/g body weight per day). Furthermore, $TLR4^{-/-}$ animals have a greater total adipose tissue mass (13.0 ± 1.8 %) when compared to $TLR4^{+/+}$ NOD mice (10.9 ± 1.6 %, $p<0.05$) and increased free fatty acid levels ($TLR4^{+/+}$ 0.5 ± 0.1, $TLR4^{-/-}$ 0.7 ± 0.1 mmol/l, $p<0.01$) and triglyceride levels ($TLR4^{+/+}$ 66 ± 5, $TLR4^{-/-}$ 79 ± 9 mg/dl, $p<0.01$), but similar levels of fetuin A ($TLR4^{+/+}$ 110 ± 7, $TLR4^{-/-}$ 96 ± 12 µg/ml). Glucose-stimulated islets of $TLR4^{+/+}$ and $TLR4^{-/-}$ mice released comparable amounts of insulin (at 5.5 mM glucose: $TLR4^{+/+}$ 129 ± 9 pg/ml, $TLR4^{-/-}$ 105 ± 45 pg/ml; at 28 mM glucose: $TLR4^{+/+}$ 239 ± 16 pg/ml, $TLR4^{-/-}$ 314 ± 84 pg/ml). However, the response of blood glucose to insulin injection during ipITT was 56 % lower in $TLR4^{-/-}$ NOD mice than in $TLR4^{+/+}$ mice ($p<0.001$).
Conclusion: Our finding of disturbed glucose and lipid homeostasis in diabetes-prone TLR4-deficient NOD mice indicates that TLR4 deficiency may accelerate the progression of diabetes by impairing insulin sensitivity rather than by affecting the function of beta cells, the primary target of autoimmune reactivity.
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CD26/DPPIV inhibition alters the expression of immune related genes in the thymus of NOD mice
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Background and aims: It has been described that CD26/dipeptidyl peptidase IV (DPPIV) inhibition delays the onset of type 1 diabetes (T1D), and even, can reverse new-onset TID in *non obese* diabetic (NOD) mice. We recently observed that targeting CD26/DPPIV decreases the incidence of T1D and reduces insulitis in NOD mice, and alters T-lymphocyte subsets in the thymus and periphery, redirecting towards an immunoregulatory phenotype. In the current study, the effects of a DPPIV inhibitor (MK0626) in the transcriptome of the thymus were examined by microarray analysis.
Materials and methods: MK0626 treatment was administered in the diet to 8 wk old female NOD mice during 6 weeks. Microarray experiments were performed to identify differentially expressed genes after DPPIV inhibition in thymic glands of treated mice (n=5) compared to untreated group (n=5). Statistically significance for differential gene expression was considered for adjusted p- value ≤ 0.05 and $\log_2 FC > \pm 0.8$. Results were validated by RT-PCR.
Results: Transcriptome analysis of the thymus showed that 66 genes were significantly up-regulated in treated mice NOD when compared with controls. Immune response-related genes included those involved in 1) Innate immu-

nity *(Plunc, Ear1,Ear2, Ear11, RegIIIγ, Clec7a, Clec9a,Csf1r, Lbp*), 2) Immunoregulation (*Muc-1, Fcgr2b, Lilrb4*), 3) Chemotaxis (*Ccl11, Ccl6, Ccl3, Ccl9, Ccl27, Ccl21, C3ar1, Cxcl16*), 4) Antigen presentation and processing (*Lgmn)* and 5) Inflammation (*Scgb3a1,Sgb1a1, fstl1, Retnla*). Most of these genes are implicated in central tolerance mechanisms through several pathways: enhancing negative selection of autoreactive T cells, modification of thymic architecture, control of T cell activation and response, enhancement of Tregs expansion and function and, finally, stimulation of apoptotic cells clearance. Differential expression was confirmed in all selected genes by RT-PCR.
Conclusion: Treatment with the DPPIV inhibitor MK0626 in NOD mice alters immune response gene expression profile in the thymus, and potentially may enhance the efficiency of immunological central tolerance.
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Immunomodulatory effects of efferocytosis: a key role in the suppression of autoimmunity to beta cells
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Background and aims: Efferocytosis is a crucial process by which apoptotic cells are cleared by phagocytes, maintaining immune tolerance to self in the absence of inflammation. Type 1 diabetes, caused by the loss of tolerance to beta cells, can be prevented by the administration of tolerogenic dendritic cells (DCs) loaded with islet apoptotic cells in NOD mice. Our aim was to determine molecular mechanisms involved in tolerogenic features achieved by DCs after the uptake of apoptotic cells, thus explaining the prevention of autoimmunity.
Materials and methods: DCs from NOD mice, pulsed with apoptotic islet cells, were administered to pre-diabetic NOD mice and observed by in vivo imaging. The ability of these cells to induce autologous T cell proliferation and to suppress mature dendritic cell function was assessed, together with cytokine production. Microarray experiments were performed to identify transcriptome of DCs after efferocytosis. The role of Prostaglandin E2 -one of the differentially expressed genes- in tolerogenic functions of DCs was determined. For paired data, a non-parametric Wilcoxon test was performed. Otherwise, Mann Whitney test was used. A p-value < 0.05 was considered significant.
Results: In vivo imaging revealed the signal of tolerogenic DCs in the pancreatic lymph nodes three days after the injection. Efferocytosis causes significant molecular and functional changes in DCs: 1) Impaired ability to stimulate autologous T cell proliferation. 2) Cytokine profile typical for immature dendritic cells. 2) Suppressive ability of mature dendritic cell function. 3) Downregulation of antigen processing and presentation gene expression. 4) Increased Prostaglandin E2 production, responsible for immunosuppressive mechanisms.
Conclusion: The tolerogenic behaviour of DCs after efferocytosis points to a mechanism of silencing potential autoreactive T cells in the microenvironment of pancreatic islets through prostaglandin E2 production in the context of autoimmunity. Our results suggest that this is a viable strategy for a variety of autoimmune diseases.
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Correlation of islet infiltration to gene expression of blood mononuclear cells and pancreas draining lymph nodes in the LEW.1AR1-iddm rat model of autoimmune diabetes
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Background and aims: The LEW.1AR1-iddm rat is an animal model of spontaneous autoimmune diabetes. Islet infiltration occurs within a narrow time range resulting in progressive beta cell destruction and overt diabetes. It was the aim of this study to correlate haemograms and expression profiles of inflammatory genes in peripheral blood mononuclear cells (PBMC) to stages of islet infiltration and gene expression profiles in pancreas draining lymph nodes (PLN).
Materials and methods: Normoglycaemic LEW.1AR1-iddm and LEW.1AR1 control rats were killed at the age of 40, 45, 50, 55 and 60 days. Blood, pancreas, PLNs were asservated for histology and gene expression analysis. Differential haemograms were created from peripheral blood by the ADVIA 2120 system. Serial pancreatic sections were stained with Haematoxylin-Eosin (HE) to document the status of infiltration. RNA was isolated from PLNs and PBMCs. Gene expression was quantified for proinflammatory (TNFα, IFNγ, IL1β) and antiinflammatory cytokines (IL4, IL10), T-cell markers (CD25, CTLA4, NRP1), L-Selectin, TGFβ and FoxP3 by RT-PCR.
Results: The LEW.1AR1-iddm strain showed a 40 % lower lymphocyte content in peripheral blood than the diabetes-resistant LEW.1AR1 irrespective of the stages of islet infiltrations (day 40 - day 60). The early islet infiltration process in LEW.1AR1-iddm rats correlated with a significant higher level of monocytes and granulocytes (1.6-fold). At day 60 there was a significant increase of eosinophil granulocytes in rats with severe islet infiltration. The PBMC profile correlated with higher levels of proinflammatory cytokines TNFα, IFNγ, IL1β at the early stage of islet infiltration (day 40) indicating an activation of the innate immune system. At day 40 PLNs from rats with islet infiltration showed significantly higher levels of CD25 (1.9 fold) and L-Selectin (3.1 fold) but not of proinflammatory cytokines. With progression of islet infiltration (day 45) we observed an increase of IFNγ (1.9 fold) and TGFβ (1.3 fold) followed by an intermittent increase of the antiinflammatory cytokine IL4 (2.5 fold) at day 50. From day 55 there was a marked increase of IFNγ (3.1 fold), NRP1 (2.5 fold) and TGFβ (1.9 fold) in PLNs from infiltrated pancreas. At day 60 PLNs from infiltrated pancreata showed a significant increase of IFNγ (3.5 fold), TNFα and IL10 (1.8 fold) as well as the T cell markers CTLA4 (3.0 fold), CD25, FoxP3 and L-Selectin (1.6 fold).
Conclusion: Although the lymphocyte content is lower in diabetes prone LEW.1AR1-iddm rats this trait was not indicative for the process of islet infiltration. At the early stage of islet infiltration there is a clear activation of the innate immune system in PBMCs while PLNs showed no increase of proinflammatory cytokines and markers of T cell activation. With progression of islet infiltration there was a delayed increase of inflammatory markers in PLNs. Our data provide evidence that activation of the innate immune system is a hallmark of early islet infiltration either as trigger or bystander effect of local inflammation. Immune cell activation in PLNs during progression of beta cell destruction is not mirrored by corresponding activation in PBMCs. Only at late stages of islet infiltration there is a good correlation between immune cell activation markers in peripheral blood and PLN.
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In depth lipidomic characterisation of the early stages of the type 1 diabetes pathogenesis
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Background and aims: The early mechanisms regulating progression towards β cell failure in type 1 diabetes are poorly understood, but it is generally acknowledged that several factors such as genetic and environmental components are involved. The lipidomic phenotype is sensitive to minor variations in both, and may thus reflect changes that lead to the development of type 1 diabetes. We used a novel extraction technique and subsequent liquid chromatography electrospray ionisation tandem mass spectrometry to profile the lipididome in depth in a genetically modified murine model resembling the early stages of type 1 diabetes. We hypothesize that alterations in the lipidomic phenotypes characterize the early pathogenesis of T1D and by profiling of these provide new insight to the development of type 1 diabetes.
Materials and methods: For this study a transgenic non-diabetes prone C57BL/6 mouse expressing CD154 under the control of the rat insulin promoter (RIP) crossed into the immuno-deficient recombination-activating gene (RAG) knockout (-/-) C57BL/6 mice was used. These mice have reduced β-cell mass, but no lymphocytic infiltration is seen and full blown diabetes does not occur. We considered these mice a good model for addressing the lipidomic phenotype of the early stages of type 1 diabetes. We compared plasma and liver samples from age matched (12 weeks) groups of the RIP CD154 x RAG -/- with their background RAG -/- and C57BL/6 mice (n=10, n=11 and n=11, respectively). The lipidome of plasma and livers were assessed using a novel high through-put extracting technique and subsequent liquid chromatography electrospray ionisation tandem mass spectrometry based multiple reaction monitoring capable of detecting >400 different lipids.
Results: Comparison of the lipidome of the RIP CD154 x RAG -/- mouse to RAG -/- mice and C57BL/6 mice revealed alterations of >100 different lipids in plasma and livers, dominated by the lipid classes phosphocholine and

spingomyelin, with lower total levels seen in the RIP CD154 x RAG -/- mice p<0.05 (Bonferonni corrected). These two classes of lipids are considered donors of choline, where a lower level of this previously has been linked to development of type 1 diabetes. The group of plasmalogens that protects cells from oxidative damage was also significantly lower in the RIP CD154 x RAG -/- mice, p<0.05 (Bonferonni corrected).
Conclusion: These observations provide evidence that lipid disturbances precede the onset of type 1 diabetes, and could by closer examinations lead to earlier diagnosis of patients and identification of new pharmaceutical drug targets.
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Carbon monoxide-releasing molecule CORM-A1 ameliorates islet-directed autoimmunity in mice
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Background and aims: The inflammatory lesions within islets of those with type 1 diabetes (T1D) are characterized by a decrease (or absence) of insulin-producing β cells along with a pancreatic islet cell infiltrates composed of T lymphocytes, B lymphocytes, macrophages, and lesser numbers of other immune cells. Recent studies identified carbon monoxide (CO) as a potential therapeutic molecule due to its anti-inflammatory and anti-apoptotic abilities. CORM-A1 is a pharmacologically designed molecule that is capable of modulating physiological functions via liberating CO. However, its biological activity in T1D has not been examined so far. Therefore, in the present study we investigated possible therapeutic value of CORM-A1 in the model of diabetes induced in C57BL/6 mice by multiple low doses of streptozotocin.
Materials and methods: T1D was induced in C57BL/6 mice with multiple low doses of steptozotocin (MLDS, 5 x 40 mg/kg). CORM-A1 (2 and 4 mg/kg) was administered as prophylactic, or therapeutic treatment. The disease severity was evaluated by weekly measurement of blood glucose level and by histological analyses of the pancreas. To determine the effect of CORM-A1 within the local environment of the endocrine pancreas, infiltrated mononuclear cells were isolated from pancreata at day 8-10 of disease induction and phenotype of cell infiltrates was determined by flow cytometric analysis and real time PCR.
Results: Administration of CORM-A1 during diabetes induction, or even after the induction of the disease, improved clinical and histological signs of the disease. Flow cytometric analyses revealed reduction of percentage of CD4+, CD8+, B220+ (B cells) and pro-inflammatory M1 (F4/80+CD40+) macrophages while anti-inflammatory M2 (F4/80+CD206+) macrophage cell number was unchanged in pancreatic infiltrates of CORM-A1-treated mice compared to diabetic mice. Also, flow cytometric analyses showed reduced proportion of Th1 (CD4+IFN-γ+) infiltrated cells and increased proportion of Th2 (CD4+IL-4+) infiltrated cells in CORM-A1-treated mice compared to control MLDS-treated mice, as well as an expression of these cytokines per cell confirmed by mean fluorescence intensity. However, Th17 cells remained unchanged in both groups. In line with these results, mRNA expression of Th signature cytokines, IFN-γ and IL-4, was inversely regulated, resulting in lower IFN-γ/IL-4 ratio in CORM-A1-treated mice.
Conclusion: Our study suggests that CORM-A1 exerts its protective effect by preventing the infiltration of cells in the pancreas. Furthermore, CORM-A1 suppresses autoimmune response by shifting the balance towards protective Th2 cells. CORM-A1 may thus represent a novel treatment strategy that would operate through interfering with an islet-directed autoimmune response.
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Identification of a targetable pathway that controls plasmacytoid dendritic cells and the interferon response
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Background and aims: Plasmacytoid dendritic cells (pDCs) are crucial bridge between the innate and adaptive immune system. pDCs constitutively express TLR7 and TLR9, the toll like receptors that recognize ssRNA and unmethylated DNA, and in response to pathogen associated nucleic acids, pDCs release copious amounts of type I interferon (IFN), which initiates an inflammatory response that recruits and facilitates T cell and B cell immunity. pDCs play a well defined role in the development of autoimmune diseases, such as lupus erythematosus, and recently pDCs were shown to be crucial in the early induction of type 1 diabetes (T1D). Owing to their role T1D pathogenesis, there is a major medical need for strategies that can modulate pDCs and the interferon response.
Materials and methods: Here we set out to delineate the molecular pathways that control pDC's response to nucleic acids and their production of IFN, and to identify genes within these pathways that can be targeted to subdue pDCs during the early stages of T1D development.
Results: We have discovered that the microRNA miR-126 is highly and specifically expressed in mouse and human pDCs, but not other immune cells. Phenotypic analysis revealed that in the absence of miR-126, pDCs undergo early apoptosis and have a diminished capacity to become activated and produce IFN in response to ssRNA and CpG DNA, and miR-126-/- mice injected with TLR7 or TLR9 agonists have a major impairment in their IFN response. Unexpectedly, we found that vascular endothelial growth factor receptor 2 (VEGFR2) is downregulated on pDCs in the absence of miR-126. VEGFR2 is the main receptor for the angiogenic factor VEGF and has, until now, considered to be expressed only in endothelial cells. However, we found that VEGFR2 is highly expressed on mouse and human pDCs, but not other hematopoietic cells, and that knockout of VEGFR2 specifically in DCs impairs their ability to produce IFN similar to the loss of miR-126. While the VEGF pathway has long been known to be key in angiogenesis, these studies uncover a previously unappreciated role for the miR-126/VEGFR2 axis in the innate response to nucleic acids through multiscale control of pDC development and function.
Conclusion: These results link the VEGFR2/miR-126 axis to control of the innate immune response, and, since anti-VEGF and anti-VEGFR2 drugs are already in the clinic, our findings also provide a potential therapeutic means of modulating the response. Elevated VEGF levels and IFN have been associated with early myeloid infiltration in islets during the stages of pre-diabetes. We are now investigating if anti-VEGF drugs can decrease pDC infiltration and IFN production both in NOD mice and *db/db* mice to control T1D and T2D development.
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Insulin resistance, beta cell function and the effect of non-HLA genetic variants in Finnish DIPP study children with HLA-conferred risk for type 1 diabetes

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Background and aims: Like in type 2 diabetes (T2D), insulin resistance (IR) has been hypothesised to play a role in the pathogenesis of type 1 diabetes (T1D). The association of different beta cell-specific autoantibodies with various HLA haplotypes and non-HLA polymorphisms is characteristic for the immune mechanisms leading to T1D. The aims of this study were to characterize in a longitudinal manner: 1. Whether markers of insulin resistance show association with diabetes-associated autoantibodies; 2. How different genetic variants known to be associated with insulin resistance and T2D or T1D affect insulin secretory capacity and development of insulin resistance in children at increased genetic risk for T1D; 3. How these factors are related to emergence of various autoantibody patterns.
Materials and methods: Type 1 Diabetes Prediction and Prevention Project in Finland (DIPP) is a population-based follow-up study of genetically susceptible children to T1D. To date, a total of 1101 intravenous glucose tolerance tests (IVGTTs) have been performed to 492 children in the Turku DIPP center. 92 of these children have been diagnosed with T1D. Insulin resistance values were measured using HOMA-IR (homeostasis model assessment) and beta cell function was evaluated by first phase insulin response (FPIR). As part of regular follow-up visits, diabetes-associated autoantibodies were analysed. Autoantibody measurements were carried out using techniques standardized according to DASP recommendations. Selected non-HLA genetic variants (n=23) were analysed in a subcohort of study children. Statistical analyses were performed using RM ANOVA for log-transformed values. The models included the child's progression to diabetes, autoantibody level, genetic variants and their interaction. In case of positive interaction the progression groups were analysed separately.
Results: The relationship between IAA and fasting insulin and HOMA-IR levels differed between progressors and non-progressors (p=0.002 for fasting insulin and p=0.003 for HOMA-IR).Progressors' fasting insulin had a strong positive correlation with IAA (β(se)=0.11(0.032), p<0.001). This association of fasting insulin and IAA can also be seen in HOMA-IR (β(se)=0.12(0.036); p=0.002). The non-progressors showed no correlation between IAA levels and fasting glucose or HOMA-IR. Other antibodies (IA2, GAD) showed no association with fasting insulin. Fasting glucose did not associate with any of the antibodies. Analyses of genetic variants are ongoing and results will be reported in the meeting.
Conclusion: Our preliminary findings imply that there is a relationship between insulin resistance and serum IAA levels in children who later progress to T1D.
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Better glycaemic control but similar diabetes associated complications in LADA compared to type 2 diabetes in the DPV cohort in Germany and Austria

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Background and aims: We characterized German/ Austrian LADA (latent autoimmune diabetes of adult) in comparison to type 1 (T1D) and type 2 diabetes (T2D) for anthropometry, HbA1c, associated complications, insulin therapy.
Materials and methods: Data were analyzed from anonymized longitudinal electronic records of 190,703 people with diabetes manifestation > age of 35 yrs from the German/Austrian multicenter Diab Pat Verlaufsdokumentation (DPV) database. Patients were categorized "LADA" if islet antibodies were present and without insulin therapy for at least 6 months after diabetes onset. T2D patients were islet antibody negative, while T1D patients were islet antibodies positive and received insulin at diabetes onset. Anthropometric data, diabetes duration, diabetes associated complications and insulin dose per kg body weight were compared for the most recent year of follow-up. Analyses were adjusted for age, sex, BMI and diabetes duration.
Results: LADA patients (n=238) were significantly older (median age 59.05yrs) than T1D (n= 1128, age 53.65 yrs) and slightly younger than T2D (n=2744, age 60.75yrs) (p<0.000001). Diabetes duration in LADA (6.6 yrs) was longer compared to T1D (2.80yrs) and similar to T2D (6.50 yrs) (p<0.000001), BMI of LADA (29.38 kg/m2) was higher than in T1D (25.48 kg/m2) and similar to T2D (30.10kg/m2) (p<0.000001). Upon adjustment for age, sex, BMI and diabetes duration, HbA1c was significantly lower in LADA (8.0%) compared to T1D (8.4%) and also to T2D (8.2%) (p<0.05). 72% of LADA and 63% of T2D were treated with insulin. Insulin dosage did not differ between LADA (0.61U/kg body weight), T1D (0.62) and T2D (0.66) (p>0.05). Severe hypoglycaemia was more frequent in T1D (0.38 events/ patient-year) versus LADA (0.12; p< 0.0001) or T2D(0.038; p < 0.0001). Prevalence of hypertension was similar in LADA (73 %) and T2D (73%), significantly more frequent compared to T1D (57%, (p<0.0001). Dyslipidemia occurred more often in T2D (78%) or LADA (78%) compared to T1D (64%, p<0.0001). Microalbuminuria was less frequent in T1D (26%) compared to LADA (37%) and T2D (37%, p<0.003)) , similar to macroalbuminuria (T1D: 1.6%, T2D: 3.9%, LADA 4.5% (p<0.02/p<0.001). Non-proliferative (NPR) and proliferative retinopathy (PR) was diagnosed with comparable frequencies in T1D (NPR 15%; PR 3.9%) , LADA (15% /2.3%) and T2D (15%/4.3%).
Conclusion: LADA patients showed better glycemic control with more severe hypoglycemic events compared to patients with T2D. LADA and T2D did not differ for hypertension, dyslipidemia, albuminuria and retinopathy. Type 1 diabetes patients showed HbA1c similar to T2D and were diagnosed less frequently with hypertension, dyslipidemia or albuminuria, but similar rates of retinopathy.
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Incidence of autoimmune antibodies in diabetes type 1 patients with long diabetes duration: the JEVIN-Trial

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Background and aims: The JEVIN-Trial started as a cross-sectional survey in 1989/90 on the quality of diabetes care of insulin treated patients with diabetes (DM) type 1 and 2 (n=190; age 16-60y), registered in Jena. Since then, the population with DM1 was followed up every 5 years until 2009/10. The aim of the present investigation was to detect the incidence of autoimmune antibodies like insulin auto-antibodies (IAA), thyreoperoxidase (TPO)

antibodies, glutamate decarboxylase (GADII) auto-antibodies and parietal cell antibodies (PCA) in patients with DM1 and long diabetes duration. We detected the prevalence of clinical autoimmunethyreoiditis and vitamin B12 deficiency.
Materials and methods: 131 patients with DM1 were analyzed in 1989/90. Of the living population in 2009/10 (n=104) 83 (79,8%) patients were identified and 76 (92%) patients were screened regarding the IAA; pathologically with a titre of >0,4U/ml, TPO antibodies with >60U/ml, GADII antibodies with >0.9U/ml and positive PCA antibodies with >160. Mean age of the population was 58.1y; women 34%, diabetes duration 35,1y (20-68), BMI 26,56kg/m2, blood pressure 144/83mmHg, A1c 6.9% (DCCT adj.).
Results: Of all patients 71% (n=54) had at least one increased antibody titre. Patients with increased antibodies had significant shorter DM-duration (33y vs 42y;p=0.03) and higher A1c (7% vs 6.5%;p=0.015). Most patients had pathologically elevated IAA with 52.6% (n=40), mean titre 9.9U/ml ±10.51 (min: 0.6; max: 36.5U/ml). These patients were by trend younger (56y vs 61y;p=0.052) and had by trend shorter DM-duration (33y vs 38y;p=0.087). GAD II antibodies were elevated in 30.3% (n=23), mean titre 33.2U/ml ±51.78 (min: 1.0; max: 166.2U/ml). These patients had significant shorter DM-duration (30y vs 38y;p=0.001) and by trend higher A1c (7.2% vs 6.7%;p=0.07). Elevated TPO antibodies were detected in 24% (n=18), mean titre 1306.7 U/ml ± 1999.22 (min: 65; max: 8283.4U/ml) and 7 patients had clinical autoimmunethyreoiditis. No difference between patients with pathologically and patients with normal TPO antibody titre were found regarding age, DM duration, BMI, blood pressure and A1c. PCA antibodies were present in 5.3% (n=4) with a mean of 880.0 ±1120.0 (min: 320.0; max: 2560.0). These patients had by trend a higher A1c as patients without increased PCA-antibodies (7.1% vs 6.4%:p=0.062). No one had clinical vitamin B12 deficiency.
Conclusion: About two third of patients with long term diabetes type 1 had at least one positive autoantibody. Only 1/3 of all patients had still pathologically increased GADII antibodies. Of patients with elevated TPO antibodies, less of half had clinically autoimmunethyreoiditis.

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Subgroup analysis of DIA-AID 1, an immune intervention phase 3 study in newly diagnosed type 1 diabetes patients
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Background and aims: DIA-AID 1, a phase 3 clinical trial in newly diagnosed type 1 diabetes (T1D) adult patients demonstrated preservation of endogenous insulin secretion and clinical benefits after 2 years of treatment with DiaPep277®. Analysis of the modified intent-to-treat (mITT) population data revealed that DiaPep277® significantly preserved C-peptide levels compared to placebo (treatment effect of 23.4%, p=0.037). This preservation was supported by clinical outcomes such as a significantly higher percentage of patients who maintained HbA1c levels ≤ 7% at study end. In addition, a significantly higher proportion of treated patients were in partial remission at study end. DiaPep277® treated patients experienced fewer hypoglycemic events with a significant decrease in the rate of hypoglycaemia. The aim of this analysis was to define the profile of patients who better responded to treatment.
Materials and methods: We conducted analyses of 10 subgroups looking for a treatment effect higher than that observed in the entire mITT population. These included baseline insulin dose, fasting C-peptide, HbA1c, age, gender, BMI and body weight, seasonality, HLA genotype and Northern European phenotype.
Results: The baseline characteristics that mostly impacted the treatment effect of DiaPep277® were the insulin dose, age, Northern European phenotype and HLA genotype. In 164 patients who were randomized with baseline insulin ≤ 0.35 IU/kg/day, the treatment effect was 34.5% (p=0.009) compared to 7.6% in patients randomized with baseline insulin > 0.35 IU/kg/day (144 patients). Among 152 patients older than 27 years, the treatment effect was 44.4% (p=0.009). In 99 patients from Northern European countries with high prevalence of T1D, the treatment effect was 34% (p=0.046). In 142 patients with moderate and high risk HLA genotype, the treatment effect was 35.4% (p=0.017). There were too few patients to establish an effect in the low risk HLA genotype group. Analysis of subgroups according to their baseline fasting C-peptide or their baseline HbA1c, gender, BMI, body weight and seasonality did not show a difference in treatment effect from the entire mITT population.
Conclusion: Adult patients requiring lower insulin doses at diagnosis could potentially benefit the most from DiaPep277®. The geographical and HLA analyses indicate that patients with a higher genetic predisposition to T1D responded well to treatment.
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Long term safety and efficacy of DiaPep277® - initial results of an extension study to DIA-AID 1
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Background and aims: DIA-AID 1, a phase 3 clinical trial in newly diagnosed type 1 diabetes (T1D) adult patients demonstrated preservation of endogenous insulin secretion and clinical benefits after 2 years of treatment with DiaPep277®. An open-label, active-treatment extension study to DIA-AID 1, to evaluate long-term safety and efficacy of DiaPep277® is ongoing in 3 medical centers in Israel.
Materials and methods: Patients eligible for the extension study were those who completed the DIA-AID 1 study and had stimulated C-peptide levels ≥0.2 nmol/l regardless of their original allocation, DiaPep277® or Placebo. Patients are being treated with 1 mg of the drug administered for 2 years at 3 month intervals in addition to insulin. Forty-three patients were enrolled in the extension study, of which 28 were originally treated with DiaPep277® (DiaPep277® - DiaPep277® treated) and 15 with Placebo (Placebo -DiaPep277® treated).
Results: Preliminary results from 32 patients (17 DiaPep277® - DiaPep277® treated and 15 Placebo - DiaPep277® treated) who completed the 2 year extension study indicate that there are no safety concerns. Out of 81 adverse events reported by 31 subjects, only two were related to the study drug, both of which were resolved. Only one severe adverse event was reported which was not considered related to the study medication. The most common non-related adverse events were infections and infestations (35/81, 43%) followed by gastrointestinal disorders (9/81, 11%). A higher proportion of patients who were treated for 4 years with DiaPep277® maintained fasting C-peptide ≥ 0.2 nmol/l at the end of the 2-year extension study, 42% compared to only 17% of the Placebo -DiaPep277® treated patients. Similarly, a higher proportion of patients who were treated for 4 years with DiaPep277® maintained target HbA1c ≤ 7% at the end of the extension study, 59% vs 27% of the Placebo - DiaPep277® treated patients.
Conclusion: These results suggest that 4 years treatment with DiaPep277® is safe and well tolerated. The extended treatment maintains patients in better glycemic control and preserves beta cell function. The full results of this study will be available by the end of 2013.
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Serum vitamin D may modulate fetuin-A, cytokines and insulin resistance across the spectrum of glycaemia
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Background and aims: Vitamin-D is believed to modulate glucose homeostasis and cytokines. Fetuin-A (FetA) is believed to modulate insulin resistance (IR) and inflammation, by being the principle ligand for free fatty acid induced activation of toll like receptor-4 and NF-κB. TNFα, IL1β and IL6 are the principle cytokines implicated in IR. IL1ra is a predominantly anti-inflammatory cytokine. This study aimed to evaluate the relation between vitamin-D, FetA, cytokines and IR in normal individuals (NI), individuals with prediabetes (IPD) and newly diagnosed treatment naïve type-2 diabetes (T2DM).

Material and methods: 30 NI (Group-I), 57 IPD (Group-II) and 37 T2DM (Group-III) who had persistent normoglycaemia, IFG and/or IGT and blood glucose in diabetes range respectively, over 2 OGTTs over a week, without any co-morbidities underwent estimation of insulin, 25-hydroxyvitamin-D (25OHD) (CLIA, Architect, USA), IL6, TNFα, IL1ra, IL-1β and FetA (ELISA, Ray Biotech, USA).
Results: 75% and 41.1% individuals (M:F=57:67) had 25OHD ≤30 and ≤20ng/ml respectively. Mean age of NI, IPD and T2DM was 39.7±7.7, 42.4±8.8, 42.2±6.7 years respectively. Significantly increased FetA (489.2±103.7 vs 443.8±116.6ng/ml; P=0.02), hsCRP (3.2±1.9 vs. 2.3±1.8mg/L; P=0.005), IL1β (7.9±7.7 vs. 6.1±2.9pg/ml; P=0.03), fasting glucose (112.4±22.4 vs. 105.5±22.8mg/dl; P=0.04) and IR (HOMA2IR; 1.5±0.99 vs. 1.1±0.8; P=0.01) was observed in individuals with metabolic syndrome (MetS; IDF criteria; n=46) compared to those without (n=78). In NI, HOMA2-IR had positive correlation with BMI (r=0.57; P=0.02), IL1β (r=0.52; P=0.02), hsCRP (r=0.48; P=0.06) and FetA (r=0.49; P=0.04). Serum 25OHD had inverse correlation with IL6 (r=-0.47; P=0.006), IL1β (r=-0.56; P=0.01), hsCRP (r=-0.46; P=0.01) and positive with IL1ra (r=0.42; P=0.02). BMI positively correlated with IL1ra (r=0.45; P=0.01). Among IPD, HOMA2-IR had positive correlation with BMI (r=0.39; P<0.001), hsCRP (r=0.23; P=0.06) and TNFα (r=0.23; P=0.03). Serum 25OHD had inverse correlation with HOMA2-IR (r=-0.31; P=0.001), TNFα (r=-0.28; P=0.003), IL6 (r=-0.26; P=0.006), IL1β (r=-0.27; P=0.02), FetA (r=-0.31; P=0.01) and positive correlation with IL1ra (r=0.33; P=0.006). IL1ra was negatively correlated with TNFα (r=-0.3; P=0.01) and IL6 (r=-0.28; P=0.03). In T2DM, 25OHD had positive correlation with IL1ra (r=0.36; P=0.02) and negative with FetA (r=-0.35;P=0.03).
Conclusion: The inverse correlation of 25OHD with inflammatory cytokines along with positive correlation with IL1ra supports anti-inflammatory role of vitamin-D. It may be hypothesized that vitamin-D may exert its beneficial effects on IR through decreased inflammation and modulation of FetA, as evidenced by significant inverse correlation of 25OHD with FetA in IPD and T2DM.

Comparison of clinical, anthropometric, glycemic, insulin resistance, inflammatory cytokines, fetuin

Parameter	Group-I (Normoglycaemia) (n=30)	Group-II (Prediabetes) (n=57)	Group-III (Diabetes) (n=37)	P-value
BMI (kg/m2)	24.69±2.79	26.4±4.92	26.21±4.97	0.18
HbA1c (%)	4.95±0.68	6.14±0.59	7.65±1.34	<0.001
HOMA2-IR	0.77±0.35	1.38±0.91	1.33±0.91	0.001
HOMA2-beta	97.1±37.08	83.44±38.41	51.67±33.27	<0.001
TNF-a (pg/ml)	23.87±22.5	55.68±33.7	64.97±20.51	<0.001
IL-6 (pg/ml)	3.46±0.58	6.74±0.36	6.91±0.15	<0.001
hs-CRP (mg/L)	1.36±0.75	2.96±2.05	3.6±1.66	<0.001
IL-1β(pg/ml)	3.10±0.99	7.33±6.55	8.84±2.46	<0.001
IL-1ra (ng/ml)	0.25±0.05	0.25±0.13	0.21±0.05	0.001
FetA (μg/ml)	418.49±81.19	460.15±135.04	506.75±70.28	0.003
25OHD (ng/ml)	21.46±8.05	23.28±12.08	23.31±8.76	0.55

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25-hydroxy vitamin D and thyroid autoimmunity in patients with type 2 diabetes mellitus and controls

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Background and aims: Although the association between type 1 Diabetes Mellitus and thyroid autoimmunity (TAI) is established, relevant evidence is inconclusive in the setting of type 2 Diabetes Mellitus (T2DM). Several lines of evidence suggest the role of vitamin D in the regulation of the immune system. Thus, we aimed to explore the putative association between T2DM and TAI focusing on the role of 25-hydroxy Vitamin D [25(OH)D].
Materials and methods: Subjects with an established diagnosis of T2DM were consecutively recruited from the outpatient diabetes clinic of a tertiary reference hospital during 2012. Community-dwelling individuals from the same region were also recruited as controls. Medical history were retrieved and blood samples were drawn for the measurement of 25(OH)D levels and titers of autoantibodies against thyroid peroxidase (TPOab) and thyroglobulin (TGab). A subject was designated as TAI positive if either TPOab or TGab was above 100 U/mL. To explore the potential association between 25(OH)D and thyroid autoimmunity while controlling for potential confounders - namely age, gender, body mass index (BMI), and presence of T2DM, multivariate logistic regression analyses were undertaken. Standardized values (z-scores) of the natural logarithms for all continuous variables were used. All analyses were undertaken within Stata 10.0.
Results: A total of 498 participants (264 patients with T2DM and 234 healthy controls) constituted the study population. Patients with T2DM were younger (mean age: 67.6 versus 72.2, Mann-Whitney $p < 0.001$), had significantly lower 25(OH)D levels (Mann-Whitney $p < 0.001$) and higher anti-TPO titers (Mann-Whitney p: 0.005) compared to controls. TAI positivity was detected in 18.8% patients with T2DM and 14.3% controls. Multivariable logistic regression analyses adjusting for age, gender, body mass index and presence of T2DM suggested that 25(OH)D levels were significantly associated with the presence of thyroid autoimmunity [Odds ratio = 1.49, 95% Confidence Interval (CI) = 1.06 - 2.1, $p = 0.023$]. Presence of T2DM and female gender were also significant predictors.
Conclusion: In an unselected general elderly population, 25(OH)D levels are independently associated with the presence of thyroid autoimmunity. Each unit (expressed in standard deviations, corresponding to approximately 12 ng/mL) increase in 25(OH)D levels is associated with a 50% increase (in odds) for thyroid autoimmunity.

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Expression of circulating CD4+CD5+FOXP3+ regulatory T cells in obese patients

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Background and aims: $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) are natural T cells, which play an important role in immunopathogenesis and maintenance of immunologic balance. Recently, a few studies found $CD4^+CD25^+Foxp3^+$Treg and it's highly expressed molecule cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) were associated with insulin resistance. We carried this study to further investigate the expression of circulating $CD4^+CD25^+Foxp3^+$ Treg in obese patients and its association with obesity and insulin resistance.
Materials and methods: 20 obese patients without diabetes and 20 obese patients with newly diagnosed diabetes whose body mass index (BMI) were higher than or equal to 28kg/m^2 were enrolled. 20 non-obese healthy subjects were also enrolled in control group. The frequencies of circulating $CD4^+CD25^+Foxp3^+$Treg and the expression levels of CTLA-4 on these cells were analyzed by flow cytometry. The relationship between $CD4^+CD25^+Foxp3^+$Treg and insulin resistance were analyzed by correlation analysis and stepwise multiple regression. Insulin resistance was measured by HOMA-IR.
Results: $CD4^+CD25^+Foxp3^+$Treg in obese patients without/with diabetes were(3.86±0.27)% and(3.76±0.25)% respectively, which were both significantly lower than that in healthy control group(6.36±0.21)% (P<0.05). No statistic difference was found between obese diabetic patients and obese non-diabetic patients. The expression levels of CTLA-4 in $CD4^+CD25^+Foxp3^+$ regulatory T cells in obese patients without/with diabetes were (77.27±2.81)% and (60.32±2.48)% respectively, which were all significantly lower than that in controls(80.09±1.69)% (P<0.05). And obese patients with diabetes was lower than that in non diabetes (P<0.05). In addition, BMI, WC and HOMA-IR were all significantly affecting expression numbers of $CD4^+CD25^+Foxp3^+$Treg (Beta= -0.322, -0.344, -0.331, F = 78.519, P<0.001).
Conclusion: $CD4^+CD25^+Foxp3^+$Treg and expression of CTLA-4 were significantly decreased in obese patients, especially in obese diabetic patients. It may play a role in obesity and insulin resistance.

PS 035 Islet transplantation

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Acute hypoxia impacts similarly on encapsulated and non-encapsulated human pancreatic islets

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Background and aims: Human islet transplantation as a treatment for type 1 diabetes is hampered by the need of life-long immunosuppression to avoid islet graft rejection. Microencapsulation could provide immunoprotection to transplanted islets but also represents an extra barrier between islets and the blood oxygen supply. This could possibly increase the risk of islet damage caused by the hypoxic environment that encounter the islet graft during the early post-transplantation period. Here we compared the impact of hypoxia on microencapsulated vs. non-encapsulated islets to hypoxia.
Materials and methods: Human islets (University of Illinois, Chicago), alginate microencapsulated or non-encapsulated, were exposed to experimental hypoxia (0.1-0.3 % O_2) for 8 h followed by 14-18 h of re-oxygenation. Islet viability was then tested by the MTT assay and by O_2 consumption measurements (O-2k, Oroboros), and islet function was assessed by insulin secretion.
Results: Hypoxia reduced viability as measured by MTT by 33.8 ± 3.5 % in encapsulated islets and by 42.9 ± 5.2 % in non-encapsulated islets ($p < 0.2$ for difference, n = 12). Basal oxygen consumption (at 5.5 mM glucose) was reduced by 22.0 ± 6.1 % in encapsulated islets and by 24.8 ± 5.7 % in non-encapsulated islets ($p < 0.8$ for difference, n = 5-6). FCCP-induced oxygen uptake (reflecting total oxidative capacity) was for encapsulated islets 20.4 ± 4.4 pmol/s/mill cells and for non-encapsulated islets 15.0 ± 3.7 pmol/s/mill cells ($p < 0.4$ for difference, n = 5-6). Previous hypoxia did not affect these values. The stimulation index, i.e. the ratio of stimulated (by 16.7 mM glucose) over basal secretion was decreased less by hypoxia in encapsulated islets (by 29.0 ± 9.7 % vs. 66.0 ± 7.5 % in non-encapsulated islets, $p < 0.02$, n = 8).
Conclusion: Microencapsulation of human islets does not increase susceptibility to negative effects by acute hypoxia. This is a positive finding in relation to potential use of encapsulation for islet transplantation.
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Improved siRNA delivery to pancreatic islets using R3V6 peptide carrier

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Background and aims: Transplantation of isolated pancreatic islets is a promising approach to the treatment of type 1 diabetes mellitus. To enhance survival rate of the transplanted islets, target gene silencing and cytoprotective drug treatment of islet cells have been attempted. However, the efficient delivery of siRNA and hydrophobic drugs into intact islets is a bottleneck. R3V6 peptides, composed of 3 arginines and 6 valines, form self-assembled micelles with hydrophobic cores in an aqueous solution, and have potential as an efficient carrier of siRNA and hydrophobic drug combination delivery. The aim of this study is to improve transplanted islet cell viability through iNOS gene silencing and beta-estradiol (E2) delivery using novel R3V6 peptide carrier.
Materials and methods: Isolated mouse islets were transfected with siRNA-5'FITC using E2-loaded R3V6 carrier or lipofectamine 2000, and transfection efficiency and cytotoxicity were analyzed by confocal microscope and flow cytometry. After transfection of mouse islets with E2-loaded R3V6 peptides carrying siRNA for iNOS (R3V6-E2-iNOS siRNA), iNOS gene expression was assessed by real time RT-PCR. The effect of gene silencing was assessed with inflammatory cytokine-induced cell death (AV-PI flowcytometry) and marginal mass islet transplantation into renal subcapsular space of diabetic syngeneic mice.
Results: The E2-loaded R3V6 carrier delivered siRNA-5'FITC to mouse islets more efficiently than lipofectamine 2000 without cytotoxicity. Transfection of mouse islets with R3V6-E2-iNOS siRNA silenced iNOS gene expression by >60% and significantly reduced apoptotic cell death from cytokine-induced damage *in vitro*. Six weeks after TPL of 160 IEQs, diabetes cure rate with islets transfected R3V6-E2-iNOS siRNA was significantly higher than that with mock-transfected islets (77% *vs*. 36%).
Conclusion: This study suggests that the R3V6 peptides delivered siRNA for iNOS and beta-estradiol with high efficiency into isolated mouse islets leading to islet cell protection from cytokine-induced damage *in vitro* and improved diabetes cure rates after transplantation. Therefore, from a therapeutic viewpoint, target gene silencing of pancreatic islets combined with hydrophobic cytoprotective drug delivery using R3V6 peptide carrier may be a promising method of safe and effective *ex vivo* gene therapy in advance of islet transplantation.
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Zinc oxide nanoparticles for the protection of human beta cells

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Background and aims: Beta cell inflammation may contribute to the onset and progression of diabetes as well as to graft failure after islet transplantation. Whereas several approaches have been proposed to tackle this problem, here we describe the use of anti-inflammatory zinc oxide nanoparticles for human beta cell protection in-vitro.
Materials and methods: INS-1E beta cells, human pancreatic islets [isolated from 5 non-diabetic cadaveric organ donors (age: 68 ± 11 years, BMI: 24.1 ± 2.7 kg/m2)], and peripheral blood mononuclear cells (PBMCs, prepared from buffy coats of healthy blood donors) were used. Dose (up to 50 μg/ml zinc oxide) and time (up to 7 days) response curves were perfomed and cells were studied in terms of survival, morphology, ultrastructure, insulin secretion and cytokine release.
Results: Light and electron microscopy demonstrated well maintained INS-1E and human beta cells up to 7 days of incubation with zinc oxide, with no evidence of changes in survival and preserved beta cell ultrastructure; electron microscopy also showed the presence of zinc oxide nanoparticles within the cytoplasm. Glucose-stimulated insulin secretion, expressed as stimulation index, was 3.5±2.4 in control human islets and 3.2±2.7 in zinc oxide treated samples. Incubation with zinc oxide dose dependently decreased the viability of PBMCs, with significant reduction (from 808±38 to 416±132 pg/ml; $p<0.01$) of MCP-1 release.
Conclusion: These results show that zinc oxide nanoparticles could represent a novel tool to influence inflammation phenomena in beta cell microenvironment, potentially useful in experimental and preclinical islet cell studies.
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Role of oxidative stress in the pro-angiogenic effect of liraglutide on rat pancreatic islets

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Background and aims: The formation of microvascularization by capillary sprouting at site of pancreatic islet implantation is crucial for survival of the graft. Vascular Endothelial Growth Factor (VEGF), a major angiogenic factor, may be a key protein in modulating the angiogenesis of islets after transplantation. Increased of VEGF by Deferoxamine *via* HIF-1α pathway has been shown to be beneficial for islet survival. Previous *in vitro* study realized in the laboratory showed that liraglutide stimulated VEGF secretion significantly in islets and improved their function. Thus, this angiogenic property could explain the benefic effect of liraglutide on the graft. The aim of this work was to determine mechanisms which could explain the pro-angiogenic effect of liraglutide on Rat islets model focusing on hypoxia and oxidative stress pathways.
Materials and methods: Rat islets were incubated in presence of 10 μmol/L of liraglutide during 0, 12, 24 and 48h. HIF-1α translocation and mTOR ex-

pression were assessed using western blotting. mTOR activation was evaluated with the ratio between the expression of phosphorylated mTOR and mTOR. Total antioxidant capacity of liraglutide was performed by a trolox equivalent antioxidant capacity method (ABTS) test from islets supernatant. Finally, the level of intracellular reactive oxygen species (ROS) was measured by immunofluorescence using dihydroethidium (DHE) on frozen sections of islets.
Results: 10 µmol/L of liraglutide did not induce a stimulation of nuclear HIF-1α expression in comparison with control with respectively 0.285±0.049 and 0.322±0.048 of protein/β-actin after 24h of culture (n=4). However, mTOR activation was significantly increased after 24h of treatment with liraglutide (0.962±0.233 vs 0.423±0.037, n=6; p<0.05). Concerning oxidative stress, the level of intracellular ROS was transiently and significantly enhanced in the presence of liraglutide after 12h of culture. Finally, these results were correlated with the ABTS test with a significant increase of the total antioxidant capacity at 12h with liraglutide (control: 4.624±0.11, n=4; lira glutide: 5.537±0.27, n=6; p<0.05).
Conclusion: The mechanism involved in the pro-angiogenic effect of liraglutide seems to be related to oxidative stress inducing overexpression of VEGF and the increase of mTOR activation. Finally, this study showed that it is possible to improve islets vascularization and graft survival using oxidative stress. In that purpose, strategies need to be developed to control oxidative stress.

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Islet survival and function following intramuscular vs intraportal autotransplantation in the minipig

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Background and aims: Islet transplantation is an efficient therapy of diabetes mellitus. The classic intraportal way may not be optimal due to an instant blood-mediated inflammatory response (IBMIR), and low revascularization of islets. Therefore, intramuscular islet transplantation (IMIT) offers an attractive alternative, based on its simplicity, easier access for graft imaging and cell explantation. The aim of our work was to compare survival and function of intramuscular and intraportal autotransplanted islets in the minipig.
Materials and methods: Using the intramuscular injection technique in the minipig (n=30), we demonstrated, by histological evidence, the rapid revascularization of islets autotransplanted into the gracilius muscle. Islet survival assessment was performed using immunohistochemistry staining for insulin and glucagon up to a period of 6 months. Furthermore, we showed the crucial role of minimizing technical trauma to the myofibers and limiting exocrine contamination.
Results: Graft function was confirmed by documenting the acute insulin response (AIR) to intravenous glucose in 5/11 totally pancreatectomized animals. Graft function after IMIT remained however significantly lower than the function measured in 12/18 minipigs who received a similar islet volume in the liver. The mean AIR observed after IMIT was significantly inferior to the level observed after intraportal islet transplantation (2.8 +/- 1.7 mU/L vs 6.2 +/- 1.3 mU/L, p=0.02). As expected, AIR was lowered in both groups when compared to the levels observed in healthy controls (35.9 +/- 9.1 mUI/L, p<0.01 vs intramuscular and intraportal islet recipients).
Conclusion: Collectively, these results suggest, in a clinically relevant preclinical model, that IMIT can become a promising alternative to intraportal infusion.
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Coronary artery disease in diabetic patients long-term after simultaneous pancreas and kidney transplantation compared with kidney transplantation alone

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Background and aims: Improved long-term glycaemic control protects against development of cardiovascular disease and death in type 1 diabetic patients. We aimed to determine whether long-term normoglycaemia (>8 years), as achieved by successful pancreas and kidney transplantation, would delay development of coronary artery disease (CAD) as compared with successful kidney transplantation alone (KTA).
Materials and methods: Twenty-eight type 1 diabetic patients who had received simultaneous pancreas and kidney (SPK) grafts were compared with eighteen single live donor kidney (LDK) recipients. Unlike KTA recipients from deceased donors, recipients from live donors are younger and more comparable in comorbidity with SPK recipients. At follow-up 8-12 years later, the patients underwent coronary angiography (SPK; *n*=23, LDK; *n*=18) and computed tomography (CT) examination (SPK; *n*=25, LDK; *n*=14) to obtain coronary artery calcium scoring (CACS). The Mann-Whitney *U* (Wilcoxon) and χ^2 tests were used to compare continuous and categorical variables, respectively. Binary logistic regression was used to correlate CACS with coronary artery stenosis (>50% diameter stenosis).
Results: Age (mean±SD) at transplantation was 41.3±6.7 and 40.5±10.0 years in the SPK and LDK groups, respectively (*p*=0.59). Duration of diabetes at engraftment was 29.1±7.2 and 28.1±10.0 years for the SPK and LDK recipients, respectively (*p*=0.26). Follow-up after transplantation was 12.1±4.3 years in the SPK group and 10.6±2.5 years in the LDK group (*p*=0.39), and HbA1c was 5.7±0.5% vs 8.5±1.8%, respectively (*p*<0.001*)*. At follow-up there was no difference in coronary artery lumen narrowing between the groups. Twenty-two out of twenty-three (96%) SPK recipients and sixteen out of eighteen (89%) LDK recipients had coronary irregularities (*p*=0.57). Among those, fifteen (65%) SPK and eight (44%) LDK recipients (*p*=0.18) had significant coronary artery stenosis (>50% diameter stenosis). Agatston score (mean±SD) **o**f coronary arteries with CT scan was 2335±2133 and 1701±1657 for the SPK and LDK groups, respectively (*p*=0.33). There was no significant correlation (OR 1.000, CI 1.000, 1.001; *p*=0.054) between high CACS and coronary lumen narrowing (>50% diameter stenosis).
Conclusion: The appearance of CAD as judged by coronary angiography as well as CACS was similar in SPK recipients compared with LDK recipients in spite of more than 8 years with normoglycaemia in the SPK group. This pilot study may suggest that hyperglycaemia is not the main reason for progression of stable CAD in diabetic patients.

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Long-term effects of pancreas transplantation in type 1 diabetes

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Background and aims: Long-term efficacy and safety results of pancreas transplantation (PTx) in the treatment of type 1 diabetic (T1D) patients are matter of debate. We report the outcomes of a single centre whole PTx programme from April 1996 to March 2013.
Materials and methods: Data were analyzed retrospectively taking into account the following milestones: 1996, first simultaneous cadaveric pancreas-kidney transplantation (SPKTx); 2000, first pancreas transplantation alone (PTA); 2001, first simultaneous cadaveric pancreas-living kidney transplantation (SPLKTx); 2002, first pancreas after kidney transplantation (PAK); 2010, first laparoscopic, robotic-assisted PTx.
Results: Overall, 348 PTx were performed in 331 patients (mean age 39.5 yrs; range 22-60) according to established recommendations; of them, 198 were SPKTx, 28 SPLKTx, 91 PTA and 31 PAK. In the group of 226 combined transplantations, 78 (34.5%) were performed on T1D uremic patients preemptively. PTx were systemic-bladder drained in 39 cases, systemic-enteric drained in 139 cases and portal-enteric drained in 170 cases. Immunosuppressive regimen was quadruple for each procedure: induction (ATG=130; Basiliximab=218) + calcineurin inhibitor (CSA=66; TAC=282) + antimetabolite (AZA=2; MMF=346) + low dose steroids. Relaparotomy rate was 14.1% (49/348). Overall, 1, 3, 5 and 10 yr patient actuarial survival rate was 96, 95, 94 and 92%. The respective insulin-independence rate was 88, 85, 83 and 81% in SPKTx and 87, 79, 76 and 75% in PTA. At the same times, grafted kidney survival rate was 92, 90, 88 and 85%. Twelve pancreases were lost due to vascular thrombosis, and the remaining for acute or chronic rejection. Successful PTA was also associated with improved blood pressure and lipid parameter values, and better echocardiographic measures. Seven SPKTx and 2 PTA recipients developed tumors during the follow-up.
Conclusion: PTx, including PTA, associates with favourable long-term clinical outcomes in T1D patients.

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Alpha-1 antitrypsin is associated with long term graft function markers after clinical islet transplantation

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Background and aims: Gluco and lipotoxicity induce oxidative stress and up-regulate inflammatory cytokines, leading to neutophil activation and ß cell damage. Oxidative stress results in disruption of the balance between 1) pro-oxidant (malondialdehyde (MDA) issued from free fatty acid (FFA) peroxidation) and antioxidant (superoxide dismutase (SOD)) on the one hand, 2) neutrophil activation producing proteases, and protease inhibitors such as alpha-1-anti-trypsine (A1AT) and anti-elastase activity (PAE), on the other-hand. Beneficial effects of A1T1 are suggested in islet transplantation (PNAS 2012). Nevertheless, no assessment has been performed in clinical islet transplantation. This work describes the evolution of oxidative markers after islet transplantation.
Materials and methods: SOD, MDA, FFA, total antioxidant status (TAS)), A1AT, PAE, tacrolimus and sirolimus blood levels, metabolic parameters, kidney markers, and ß score, an index of graft function ranging from 0 (no function) to 8 (excellent function) were measured before and yearly over a 10-year period in 31 islet transplanted patients either alone (ITA: n=18) or after kidney (IAK: n=13).
Results: A1AT and PAE significantly differed according to the level of ßscore (groups 0, 1-2, 3-4, 5-6, 7-8) (p< 0.0001), at the difference with other oxidative markers. A1AT and PAE differed between group 0 and all other ßscore groups including 1-2 (p<0.01). A1AT and PAE, strongly correlated (r=0.936, p<0.0001), and both correlated with graft duration (only A1AT given: r=0.325, p=0.001), BMI (r=-0.207, p<0.04), fasting blood glucose (FBG) (r=-0.188, p=0.0074), C-peptide (r=0.176, p= 0.011), and sirolimus (r= 0.280, p<0.0001), but not tacrolimus. From the 6 oxidative markers, only TAS differed between ITA and IAK groups (p=0.003), the 2 groups also differing by creatinine, MDRD and microalbuminuria levels (p<0.0001).
Conclusion: Neutrophil activation markers (A1AT, PAE) are the only oxidative markers consistently influenced by graft function: a higher level of protease inhibitors is associated with lower FBG, higher C-peptide and ßscore, but also with sirolimus, suggesting a specific role of mTOR inhibitors on oxidative stress. These results confirm the association between high level of A1AT and successful graft function in clinical islet transplantation. Nevertheless, it does not foresee the efficiency of exogenous A1AT administration after islet transplantation.
Clinical Trial Registration Number: NCT00446264; NCT01123187
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Improvement of sensory nerve conduction correlates with CGMS mean glucose and variability reduction five years after islet transplantation for type 1 diabetes

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Background and aims: Long-term benefit-risk ratio of islet transplantation remains unclear.
Materials and methods: This study describes the evolution of peripheral and autonomic neuropathy 5 years after islet transplantation with the Edmonton protocol in type 1 diabetic patients. Twenty-one consecutive patients (13 islet-alone, 8 islet-after-kidney), transplanted for at least 5 years, underwent biological evaluation, continuous blood pressure and glucose monitoring (CGM), lower-limb electrophysiological and cardiovascular autonomic testing (R-R variation with paced breathing, Valsalva ratio, postural heart rate and blood pressure changes) before transplantation and yearly during 5 years.
Results: Ten/21 patients were insulin-independent 5 years post-transplantation with a median (IQR) A1c at 6.0 (5.8-6.7) vs. 7.8 (6.9-8.3)% in the insulin-requiring group p<0.001). Three lost their islet graft, but were analyzed in intention-to-treat. The medians of sensory action potential (p<0.05) and both sensory and motor nerve conduction velocities (p<0.01) improved between 0 and 5 years. All 4 parameters significantly correlated negatively with CGM mean glucose and all except sensory nerve conduction velocity negatively with triglycerides (p≤0.01). Sensory conduction velocity correlated negatively with glucose variability (SD) on CGM (p<0.01). Tacrolimus levels negatively correlated with motor conduction parameters (p≤0.02). All 4 parameters correlated positively with ß score or post-prandial C-peptide level (p<0.05). Cardiovascular reflex testing did not change over the 5-year follow-up.
Conclusion: Islet-alone or after-kidney transplantation improved significantly sensory nerve conduction parameters. As previously demonstrated, mean glucose was the main factor influencing this improvement.
Clinical Trial Registration Number: NCT00446264/01123187

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Reduction of fat mass and leptin with the mTOR inhibitor, sirolimus, in humans: From transplantation to lipodystrophies?

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Background and aims: Sirolimus inhibits adipocyte differentiation. This study compares weight and fat markers in two groups of patients treated or not with sirolimus, before and after transplantation.
Materials and methods: 19 islet-alone transplanted patients treated with sirolimus and 7 islet-alone or liver-transplanted patients NOT treated with sirolimus were compared 1 year after transplantation in terms of weight, fat mass (equation of Body Fat and percentage of fat mass by DEXA), and metabolic parameters. 14/19 islet-alone transplanted patients treated with sirolimus were reassessed 5 years post-transplantation.
Results: Before transplantation, the metabolic and weight/fat parameters were similar in the 2 groups except for C-peptide. One year post-transplantation, Body Fat and leptin reduction was more important in the sirolimus than in the NON-sirolimus group (p<0.05). Leptin levels were lower in the sirolimus group (p=0.01). Compared to pre-transplant values, one year post-transplantation, weight, fat mass and metabolic parameters did not change in the NON-sirolimus group while the sirolimus group showed a significant reduction in weight (p<0.001), BMI (p<0.001), Body Fat (p<0.001), percentage of body (p<0.05) and truncal (p<0.05) fat mass, HbA_{1c} levels (p<0.001) and ßscore (p<0.01). Compared to pre-transplant values, the sirolimus group showed a significant reduction of leptin level one (p=0.004) and five years (p=0.01) post-transplantation, as well as a persistent reduction of HbA_{1c} (before: 8.4 (1.5); 1-year: 5.8 (1.7) ; 5-years 6.7 (1.7)% and ß score (before: 0.0; 1-year: 7.0 (3.5) ; 5-years 4.0 (4.0)) (p< 0.05). Sirolimus correlated with leptin (r=-0.22, p=0.018) and body fat mass (r=-0.18, p=0.03).
Conclusion: These results suggest that sirolimus modulates the amount and/or the quality of adipose tissue and innate immunity, opening new perspectives both in the choice of immuno-suppressant, and the treatment of nucleopathies, especially lipodystrophies.
Clinical Trial Registration Number: NCT14144660ID

PS 036 Insulin is not the only islet player

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Specific measurement of the proglucagon-derived peptide glicentin in human samples

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Background and aims: Preproglucagon is processed into glucagon like protein-1, glucagon like protein-2, oxyntomodulin and glicentin in endocrine L-cells in the intestine. The biological actions of glicentin are similar to those of glucagon, including stimulation of insulin secretion, inhibition of gastric acid secretion and regulation of gut motility. Until today it has been difficult to measure plasma concentrations of glicentin alone because of its shared sequence with glucagon and oxyntomodulin. In vitro studies have suggested that glicentin may be cleaved by peptidase DPP-4. Our aim was to develop a specific ELISA for measurement of glicentin in human samples. Different methods of sample preservation were also investigated.
Materials and methods: Monoclonal antibodies against human glicentin were generated in mice. A solid phase two-site enzyme immunoassay was developed, using a C-terminal anti-glicentin clone for capture and a peroxidase-conjugated N-terminal clone for detection. Calibrators were prepared by synthetic glicentin. Specificity was evaluated against oxyntomodulin and glucagon. Selectivity and linearity was evaluated by recovery upon dilution and addition. Glicentin preservation in 4 different sample types was examined: 1 = serum, 2 = EDTA, 3 = EDTA + aprotinin, 4 = EDTA + protease + esterase + DPP-4 inhibitor.
Results: There was no detectable cross-reactivity to oxyntomodulin or glucagon at concentrations up to 3000 pM of each tested peptide. The recovery upon dilution was 88 - 132% (mean 107%). The recovery upon addition was 83 - 100% (mean 90%). The glicentin concentration in 20 apparently healthy donors was in the range 8.8 pM - 75 pM. Stability studies indicates that EDTA + protease + esterase + DPP-4 inhibitor is the best method of sample preservation ($p < 0.05$, sample type 4 vs. samples types 1, 2 and 3, ANOVA).
Conclusion: ELISA can be used as a method for specific determination of glicentin in human samples. Sample preservation must to be considered when collecting blood for glicentin determination. Specific measurement of glicentin is needed to further understand the physiological function of this proglucagon-derived peptide.
Supported by: Mercodia AB

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A novel glucagon receptor antagonist improves hyperglycaemia in both hyperinsulinaemic and relatively insulin-deficient db/db mice

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Background and aims: In patients with type 2 diabetes, an imbalance of insulin/glucagon levels leads to hyperglycemia *via* the excessive production of glucose in the liver. Many agents for type 2 diabetes improve insulin secretion or insulin sensitivity; i.e., those are insulin dependent agents. A Diabetes Outcome Progression Trial (ADOPT) revealed that the effect of treatment with the insulin-dependent agent sulfonylurea or thiazolidinedione was attenuated with a reduction of pancreatic beta cell function in some years. Several clinical trials revealed that glucagon receptor (GCGR) antagonists lowered blood glucose levels in diabetic patients. However, it remains unclear whether GCGR antagonists improve hyperglycemia in diabetes with advanced reduction of beta cell function. The glucose-lowering effects by the suppression of glucagon action were insulin-independent; therefore, GCGR antagonists would be effective for various diabetic patients, including those with advanced reduction of beta cell function. To confirm this hypothesis, we examined the difference in the anti-hyperglycemic effects of GCGR-A, our novel GCGR antagonist, between hyperinsulinemic and relatively insulin-deficient db/db mice.
Materials and methods: GCGR-A was synthesized at our laboratories. For the GCGR binding assay or cyclic AMP assay, we used 293 cells overexpressing the human or mouse glucagon receptor. To examine glucose-lowering effects, GCGR-A was orally administered as a single dose to ob/ob mice and orally administered to young (6 weeks) or old (12 weeks) db/db mice once a day for 2 weeks.
Results: GCGR-A inhibited glucagon binding to human GCGR with a Ki value of 170.2 nmol/l (95% CI, 100.9-286.9 nmol/l), and inhibited glucagon-induced cyclic AMP production with an IC_{50} value of 82.5 nmol/l (59.4-114.6) for human GCGR and 15.7 nmol/l (5.3-46.5) for mouse. Single treatment with GCGR-A lowered plasma glucose levels ($AUC_{0\text{-}6\ h}$: vehicle, 2476.5 ± 228.6; 0.1 mg/kg, 2089.8 ± 118.6, NS; 0.3 mg/kg, 1705.8 ± 135.6, $p < 0.01$; 1 mg/kg, 1740.3 ± 139.9 mg·h/dl, $p < 0.01$) without a change in plasma insulin levels in ob/ob mice. In the next study, we examined the antihyperglycemic effect of GCGR-A using hyperinsulinemic young db/db mice and relatively insulin-deficient old db/db mice (plasma insulin levels in the fed state were 15.4 ± 1.4 and 4.3 ± 0.3 ng/ml, respectively). Chronic treatment with 1 mg/kg GCGR-A improved hyperglycemia not only in young db/db mice but also in old db/db mice ($AUC_{0\text{-}8\ h}$: young, 6007.6 ± 372.9 *vs* 6962.0 ± 182.6 (vehicle), $p < 0.05$; old, 6280.0 ± 215.1 *vs* 7246.2 ± 100.3 mg·h/dl, $p < 0.01$; mean difference *vs* vehicle: young, 954.4 ± 372.9; old, 966.2 ± 215.1 mg·h/dl). On the other hand, the chronic effect of 10 mg/kg pioglitazone in old db/db mice was attenuated compared with that in young db/db mice (young, 5294.5 ± 319.0 *vs* 6962.0 ± 182.6, $p < 0.01$; old, 6759.9 ± 167.7 *vs* 7246.2 ± 100.3 mg·h/dl, $p < 0.05$; mean difference *vs* vehicle: young, 1667 ± 319.0; old, 486.3 ± 167.7 mg·h/dl). These results show that GCGR-A is effective for hyperglycemia independent of beta cell function.
Conclusion: GCGR-A is a novel oral GCGR antagonist and will be beneficial in various diabetic patients, including those with advanced beta cell dysfunction.

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A euglycaemic clamp pilot study assessing the effects of the glucagon receptor antagonist LY2409021 on 24-hour insulin requirement in patients with type 1 diabetes mellitus

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Background and aims: Recent research has shown that blocking glucagon action prevents lethal metabolic effects seen in mice with type 1 diabetes mellitus (T1DM). LY2409021 (LY), a potent, selective antagonist of the human glucagon receptor, is being investigated as a treatment for type 2 diabetes mellitus. To test its effects in T1DM, we assessed whether single oral doses of LY could result in clinically meaningful reductions of the 24-hour insulin requirement in patients with T1DM.
Materials and methods: Twenty T1DM patients had euglycemia (5.6 mmol/L) maintained overnight by a glucose-controlled insulin infusion system (Biostator) after their usual insulin regimens were discontinued. On Day 1, euglycemia was maintained using regimens of variable intravenous insulin and standardized meals. These regimens were readministered on Day 2, but patients, randomized 1:2:2, also received a placebo or a 100-mg or 300-mg single dose of LY before breakfast.
Results: Patients had a mean age of 43.0 years (SD, 10.3 years), a mean diabetes duration of 19.0 years (SD, 13.8 years) and a mean baseline HbA1c of 7.6% (SD, 0.7%). The placebo-corrected 24-hour insulin dose needed to maintain euglycemia was reduced by a mean of 17.0% (95% confidence interval [CI], -33.7% to -0.4%; *P*=.046) and a mean of 19.6% (95% CI, -35.0% to -4.3%; *P*=.019) in 100-mg and 300-mg dose groups, respectively. Group mean glucose values were well matched and maintained near euglycemia throughout the clamp procedure. Although LY led to an expected 2- to 3-fold dose-dependent increase in plasma glucagon levels, no significant changes from placebo values were observed for other pharmacodynamic parameters, including levels of glucagon-like peptide-1 (total and active), C-peptide, lipids, and β-hydroxybutyrate. No clinically significant differences between LY and placebo groups were observed in hypoglycemia or adverse event frequency during and after the clamp procedure.
Conclusion: Results of this pilot study suggest that glucagon antagonism can reduce insulin requirements in T1DM.
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Mice deficient in glucagon gene exhibit impaired glucose tolerance and the resistance to obesity on high-fat diet feeding

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Background and aims: Glucagon is produced in pancreatic α-cells from the precursor proglucagon that is encoded by glucagon gene (*Gcg*), and plays critical roles in glucose homeostasis. Blockage of glucagon receptor signaling has been shown to improve glucose metabolism in mice fed high fat diet (HFD). In addition, glucagon receptor knockout mice were resistant to diet-induced obesity. *Gcg*-deficient mice that are homozygous for a *Gcg*-GFP knock-in allele ($Gcg^{gfp/gfp}$) cannot produce proglucagon-derived peptides (PGDPs) such as glucagon, glucagon-like peptide-1 (GLP-1) and GLP-2. We have recently reported that $Gcg^{gfp/gfp}$ mice displayed improved glucose tolerance and enhanced insulin secretion, and that ectopic GIP expression in β-cells maintains insulin secretion. Here we studied glucose and energy metabolism in $Gcg^{gfp/gfp}$ mice with HFD-induced obesity.
Material and methods: $Gcg^{gfp/gfp}$ and $Gcg^{gfp/+}$male mice were fed either a normal chow diet (NCD) or an HFD for 15-18 weeks. We investigated glucose tolerance, insulin secretion, morphometrical analysis of pancreatic islets, energy balance and gene expression.
Results: Fasting glucose levels were increased in both $Gcg^{gfp/gfp}$ and $Gcg^{gfp/+}$ mice fed HFD compared to those fed NCD. Intraperitoneal glucose tolerance test (IPGTT) showed that glucose tolerance in HFD-fed mice were impaired compared to NCD-fed mice regardless of the genotype, however, glucose level 120 min after glucose loading was significantly lower in $Gcg^{gfp/gfp}$ mice fed HFD than $Gcg^{gfp/+}$mice fed HFD. Increase in ß-cell mass was observed in $Gcg^{gfp/+}$mice fed HFD, but not in $Gcg^{gfp/gfp}$ mice fed HFD ($Gcg^{gfp/+}$, 1.59 ± 0.35; $Gcg^{gfp/gfp}$, 0.68 ± 0.11 %, p < 0.05). These results indicate that mice deficient in glucagon do develop diet-induced diabetes in the combined deficiency in other PGDPs. HFD feeding increased body weights in both $Gcg^{gfp/gfp}$ and $Gcg^{gfp/+}$ mice during the experimental period. The ratio of weight gain was obviously lower in $Gcg^{gfp/gfp}$ mice than $Gcg^{gfp/+}$mice ($Gcg^{gfp/+}$, 46.9 %; $Gcg^{gfp/gfp}$, 21.3 %). Oxygen consumption was enhanced in $Gcg^{gfp/gfp}$ mice fed HFD compared to $Gcg^{gfp/+}$mice fed HFD in light phase. HFD feeding significantly increased uncoupling protein 1 (UCP1) mRNA expression in brown adipose tissue of $Gcg^{gfp/gfp}$ mice. These findings suggest that PGDPs play some roles in regulation of brown adipose tissue function through regulating the expression of UCP1.
Conclusions: Considering that GLP-1 levels were elevated in animal models depleted in glucagon receptor signaling, our results suggest that deficiency in GLP-1, but not glucagon contributes to glucose intolerance in $Gcg^{gfp/gfp}$ mice fed HFD, and that deficiency in glucagon signaling enhances oxygen consumption in HFD-fed mice, resulting in resistance to diet-induced obesity.

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Secretion and effects of GLP-1 on pancreatic alpha cell

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Background and aims: An intra-islet incretin system has been recently suggested most likely through modulation of the expression and activity of proconvertase 2 and 1/3 (PC1/3, PC2) in pancreatic alpha-cell accounting for local release of GLP-1. Little is known, however, whether this alpha-cell activity can be affected by the typical metabolic alteration occurring in type 2 diabetes.
Materials and methods: AlphaTC1/6 (TC1/6) cells from a mice pancreatic cell line were incubated in the presence of different glucose (G) concentration (5.5, 11.1, 16.7 and 22.2 mM) from 0 to 120 h and expression of PC1/3, PC2, and GLP-1R mRNA determined by RT-PCR. GLP-1-receptor protein (GLP1R) was determined by immunofluorescence using specific antibodies, and GLP-1 secretion measured by ELISA. Effect of GLP-1 on alpha cell viability was determined by MTT assay following incubation in the presence of glucose or fatty acid (FA, 2:1 palmitate:oleate, 10^{-5}, $0.5x10^{-4}$, 10^{-4}, $0.5x10^{-3}$ M) with or without 10^{-9} M exendin 4 (Ex-4), a GLP-1 analog resistant to DPP-IV degradation.
Results: Upon 16.7 mM G incubation, PC2 expression increased 3.2±0.6 and 3.2±0.9 times after 16 and 24 hr, respectively, returning to basal values after 48hr; PC1/3 expression also increased at 16hr with no decrease with prolongation of incubation. The 16hr increment in PC1/3 expression was accompanied by increased GLP-1 secretion into the medium(313±67% of basal, $p<0.05$) progressively returning to basal values over the ensuing 5 day incubation. After 16hr incubation at 16.7 mM G, GLP-1R mRNA expression increased 19.6±3.4 folds. GLP-1R expression was also documented by immunofuorescence after 24hr incubation at 16.7 mM G. Ex-4 increased viability in cells incubated for 5days at 11.1 to 16.7 mM G but not at lower G (11.1 mM=168±8%, +Ex-4=187±10% of basal, $p<0.05$; 16.7 mM=203±9%, +Ex-4=236±14% $p<0.02$). No effect was apparent for shorter incubations (24 or 48hr). Cell viability decreased after 4h FA incubation at all concentration tested, which was partially prevented with 2hr pretreatment with Ex-4 (10^{-5}M FA=99±4% *vs* Ex-4=125±8% of basal, $p<0.01$; $0.5x10^{-4}$M=89±3% *vs* +Ex-4=110±9% of basal, $p<0.03$, 10^{-4}M= 72±2% *vs* +Ex-4=85±5% of basal, $p<0.04$).
Conclusion: These data suggest that under hyperglycemic conditions, alpha cells can increase expression PC1/3 and activate GLP-1 secretion, which may contribute protecting both alpha and beta-cells from glucose and partially from lipotoxicity.

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Exploring the Somatostatin axis in primary murine mixed islet cultures

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Background and aims: Somatostatin (Sst) is a hormone implicated in paracrine suppression of a number of endocrine cells, including alpha- and beta-cells, secreting insulin and glucagon respectively. In the endocrine pancreas, Sst is secreted from delta-cells, which are not readily distinguishable from other islet cells on morphological grounds. We therefore made a new transgenic mouse model expressing Cre-recombinase under the control of the somatostatin promoter. In this model live Sst-expressing cells can be identified when crossed with fluorescent Cre-reporter strains. As Sst release from pancreatic delta cells is stimulated by increased extracellular glucose, we sought to characterize the mechanisms underlying glucose-stimulated Sst release and also investigated a potential role of GLP-1 in modulating delta cell excitation.
Materials and methods: Trangenic mice carrying a ~200kbp construct derived from a bacterial artificial chromosome in which the Sst-coding region has been replaced by iCre-sequence were crossed with Rosa26-reporter mice, either expressing a red fluorescent protein (tdRFP) or a genetically encoded calcium indicator, GCaMP3, after Cre-mediated recombination. Fluorescently tagged delta cells were purified by fluorescence-activated cell sorting for gene expression analysis. Mixed primary cultures established from pancreatic islets were used to monitor real-time responses in identified delta cells during perfusion with different stimuli on a microscope stage, measuring NAD(P)H auto-, Fura-2 or GCaMP3 fluorescence.
Results: Immunohistochemistry carried out on the tdRFP-crossed mice verified specific expression of the reporter in Sst-positive cells. Gene expression analysis revealed that pancreatic delta cells express the canonical beta-cell glucose-sensing components-glucokinase, Kir6.2, and SUR1-as well as the GLP-1 receptor. Monitoring Ca^{2+} in cultures derived from GCaMP3-reporter mice after loading with Fura2-AM verified similar responsiveness of both reporters in delta cells with 1.14-, 1.20- and 1.27 fold increases compared to baseline Ca^{2+} at 1 mM glucose when glucose was raised to 3, 5 and 10 mM respectively (n>7, p<0.05). Addition of the glucokinase activator GKA50 (3 μM) to 1 mM glucose elicited a 1.62-fold increase in Ca^{2+} (n= 4, p<0.01), which was significantly greater than the effect of raising the glucose concentration to 10 mM (p<0.05). GKA50 also increased the metabolic rate as a 1.16-fold increase in NAD(P)H autofluorescence was observed relative to the baseline at 1 mM glucose (n= 7, p<0.001). Addition of 100 nM GLP-1 increased metabolism and intracellular Ca^{2+}, but the 1.03-fold (n= 5, p<0.01) increase in NAD(P)H and 1.26-fold (n= 3, p<0.05) increase in Ca^{2+}-sensor fluorescence were more modest in comparison to the effects of pharmacological glucokinase activation.
Conclusion: We have established a transgenic mouse model enabling live-cell imaging from pancreatic delta cells. Delta cells respond to changes in ambient glucose using similar molecular machinery to pancreatic beta cells. Metabolic flux seems to be strongly controlled by glucokinase activity and as delta cells express the GLP-1 receptor and exhibit increased metabolism in response to this peptide it seems likely that GLP-1 modulates Sst secretion at least in part through activation of glucokinase in delta cells. As most pancreatic alpha cells

do not express the GLP-1 receptor, this might be an important component of the glucagonostatic effect of GLP-1.
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GPR120 is expressed preferentially in the delta cells of murine islets of Langerhans and regulates glucose-induced somatostatin secretion
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Background and aims: The expression and functional roles of the lipid-responsive G-protein coupled receptor, GPR120, in the endocrine pancreas remain controversial. Some studies have concluded that GPR120 is not expressed in cells of the islets of Langerhans while others imply that it is present in both human and rodent islets. To clarify this situation, we have employed a novel GPR120 knock-out/β-galactosidase knock-in mouse to establish the localisation of this receptor within islets and to determine its functional role.
Materials and methods: A global GPR120 knock-out (KO)/β-galactosidase (β-gal) knock-in (KI) mouse was generated by replacement of the sequence encoding exon 1 of the GPR120 gene with the lacZ gene. This placed β-gal under the control of the GPR120 promoter, thereby allowing detection of β-galactosidase as a surrogate for GPR120. β-gal expression was then studied in pancreas sections from wild type (WT) and GPR120 KO animals either by reporter gene assays or by direct immunofluorescence with anti-β-gal antibodies. Islet endocrine cells were identified with relevant antibodies. Somatostatin (SSN) secretion was studied using islets isolated from WT and GPR120 KO animals treated with three selective, synthetic, GPR120 agonists (designated as compounds A, B or C).
Results: LacZ reporter assays revealed the expression of β-gal (and, by inference, GPR120) in a population of cells distributed peripherally in the islets of GPR120 KO/β-gal KI mice. Staining was absent from WT islets. Dual immunofluorescence staining revealed that β-gal co-localised predominantly with SSN, whereas it was not detected in insulin-positive cells. Treatment of WT islets with each of 3 selective GPR120 agonists resulted in marked inhibition of glucose (16.6mM) -induced SSN secretion (16.6mM glucose alone: 10.4 ± 1.1 pmol/islet/h; 16.6mM glucose plus either compound A : 5.7 ± 0.6 pmol/islet/h; compound B: 4.5± 0.7 pmol/islet/h; compound C: 5.0 ± 0.7 pmol/islet/h; $p<0.01$ in each case) while the agonists had no effect on glucose-induced insulin secretion. None of the GPR120 agonists altered basal SSN secretion (at 3.3mM glucose). The effects of compounds A, B or C on SSN release were similar to those observed with the cholinergic agonist, carbachol; a known inhibitor of glucose-induced SSN secretion. The inhibitory effects of the GPR120 agonists on SSN secretion were lost in islets from GPR120 KO mice. Pretreatment of islets from WT mice with pertussis toxin (Ptx) antagonised the inhibitory effects of compound A on SSN secretion at 16.6mM glucose (control islets: 16.6mM glucose alone: 13.5 ± 1.1 pmol/islet/h; 16.6mM glucose plus compound A: 6.3± 0.5 pmol/islet/h; Ptx-treated islets: 16.6mM glucose alone: 11.3 ± 1.9 pmol/islet/h; 16.6mM glucose plus compound A, 9.0 ± 0.8 pmol/islet/h; $p<0.05$) suggesting that the receptor may mediate its effects via Gαi.
Conclusion: We conclude that GPR120 is expressed predominantly in the delta-cells of mouse islets and that activation of this receptor leads to inhibition of glucose-induced SSN secretion. This response is mediated, at least in part, by coupling of GPR120 to the G-protein, Gαi.
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Human bone marrow- and pancreatic islet- derived mesenchymal stem cells: effects of hyperglycaemia and gastrointestinal peptides
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Background and aims: In the context of diabetes research, mesenchymal stem cells (MSCs) have been shown to endowed with several possibilities to be exploited. MSCs can generate insulin-producing cells, counteract autoimmunity, enhance islet engraftment, improve metabolic control in animal models for type 2 diabetes and treat chronic diabetic complications. Further, MSC derived from pancreatic islets show different characteristics compared to bone marrow derived counterparts, possibly reflecting spontaneous differentiation and commitment. Glucose toxicity is not solely restricted to beta cells, but affects also survival, proliferation of other cells, such as endothelial cells, contributing to beta cell function impairment and loss. In the present study we aimed to evaluate the effects of high glucose conditions on human MSCs derived from different sources, bone marrow and pancreatic islets. Further, in the light of studies indicating a cytoprotective role for ghrelin gene-derived peptides and obestatin on beta and islet endothelial cells, we evaluated whether these peptides may exert their effects also on MSCs in high glucose conditions.
Materials and methods: Human MSCs were obtained from bone marrow (BM-MSCs) and from pancreatic islets (HI-MSCs), characterized and cultured in normal glucose or in 14 mmol/L and 28 mmol/L glucose concentrations for 3 and 7 days. Gene expression of the ghrelin receptors, GHS-R1a and GPR-39, was assessed by RT-PCR. Cell Proliferation and survival were analysed by BrdU (Roche) and MTT assays, respectively. Apoptosis (assessed as Annexin V expression), cell cycle and cell viability were also evaluated using Muse™ Cell analyzer by flowcytometry. High glucose cultures were also treated with ghrelin and obestatin (10 nM).
Results: The RT-PCR analysis showed that both BM-MSCs and HI-MSCs expressed GHS-R1a and GPR-39 receptors. Under high glucose conditions, from 3 to 7 days, proliferation of BM-MSCs and HI-MSCs progressively decreased (respectively of 35 and 44%) compared to normal glucose culture. Early and late apoptosis, assessed as Annexin-V expression, increased in high glucose conditions in both cell lines (in BM-MSCs from 20% to 24%, and in HI-MSCs from 19% to 32%). Preliminary treatment with ghrelin and obestatin in high glucose, increased proliferation and survival in both cell lines, having obestatin the greatest protective effects (increasing proliferation of approximately 42% comparing to high glucose conditions).
Conclusion: These results suggest that HI-MSCs are more affected by high glucose conditions, compared to their bone marrow-derived counterparts, thus affecting potential reparative effects within pancreatic islets. Moreover, the ghrelin gene-derived peptides and obestatin appear to exert protective effects on both cell lines, rendering these peptides an appealing therapeutic tools to regulate islet fate *in vivo*.

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Intravenous injection of valsartan augmented pancreatic blood flow without affecting in vivo insulin secretion in male GK rats
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Background and aims: Results of NAVIGATOR (The Nateglinide and Valsartan in Impaired Glucose Tolerance) demonstrated that treatment with Valsartan for 5 years reduced the incidence of type 2 diabetes Mellitus (T2DM) by 14% in subjects with impaired glucose tolerance (IGT), but the underlying mechanisms remain elusive. Studies with captopril and irbesartan confirmed that blockade of RAS system regulated islet microcirculation and improved islet β cells function in both normoglycaemic and hyperglycaemic rodents models. Since islet microcirculation is important to preserve islet βcells function, and in vivo insulin secretion can be rapidly tuned by changes in islet microcirculation,it is conceivable that the prevention of newly onset diabetes in IGT patients by valsartan might be related to its regulation of islet microcirculation.In this setting, we chose male diabetic GK rats with elevated islet blood flow (IBF) as subjects and treated them with intravenous injection of large dose of Valsartan,and measured pancreatic blood flow (PBF) and IBF,and in vivo insulin secretion.
Materials and methods: Adult male GK rats were randomly divided into two groups. Either Valsartan dissolved in 1ml sodium bicarbonate (3mg•kg-1) or vehicle alone was injected intravenously. A non-radioactive microsphere technique was adopted to measure the regional tissue blood flow. Blood glucose concentrations were measured with test reagent strips, serum insulin concentrations were measured by ELISA.In intravenous glucose tolerance test, 1ml 30% D-glucose solution was injected 10 minutes after the i.v. injection of valsartan(3mg•kg-1). Blood samples were drawn immediately before and 2, 4, 6, 8 and 10 min after glucose administration. Data was analysed by SPSS16.0,and values were given as means±SEM.A difference was defined as significant when $P< 0.05$.
Results: 1.Intravenous injection of valsartan significantly reduced mean arterial blood pressure of male GK rats($P<0.05$).2.Pancreatic blood flow (PBF) was markedly increased by valsartan ($P<0.05$,Fig.1),while neither islet blood

flow (IBF) nor fraction of IBF out of PBF (fIBF) was affected by valsartan treatment. Kidney blood flow was markedly enhanced by intravenous injection of valsartan(P< 0.05).3.Acute treatment with valsartan changed neither GK rats' blood glucose concentration nor insulin secretion.4.Intravenous injection of valsartan did not change blood glucose concentration in intravenous glucose tolerance tests in GK rats.

Conclusion: In diabetic rats with lower blood pressure, both PBF and KBF was significantly enhanced, while elevated IBF was not further increased, indicating that regulation of islet microcirculation involved in the protection of islet β cell and might contribute to its prevention of diabetes in IGT patients.

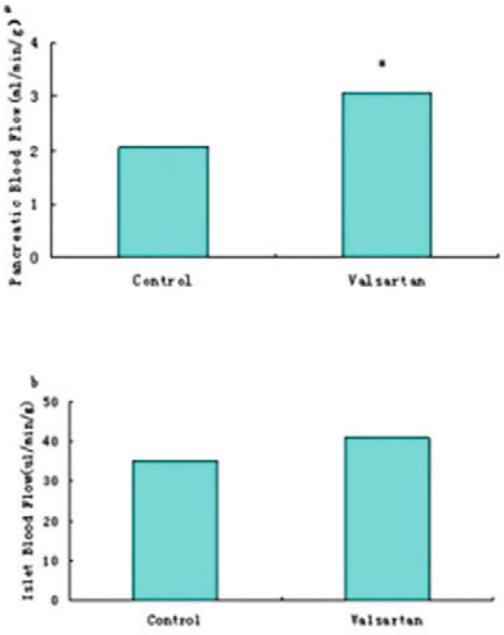

Fig. 1 Pancreatic blood flow was increased by valsartan. *P<0.05 vs control

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Human islet amyloid polypeptide improves hepatic insulin signalling

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Background and aims: Islet amyloid polypeptide (IAPP) is a pancreatic β cells-derived hormone, which is co-secreted with insulin. IAPP plays a role in glycemic regulation by slowing gastric emptying, lowering body weight, and thereby prevents post-prandial spikes in blood glucose levels. IAPP analog has been used clinically for glycemic control. However, the mechanism underlying the beneficial effects of IAPP is not fully understood. As liver plays a critical role in glucose homeostasis, we investigated whether hepatic insulin sensitivity was regulated by IAPP.

Materials and methods: We used heterozygous IAPP transgenic mice with elevated serum IAPP concentration by 2~3 folds. Huh 7 hepatoma cells were cultured in DMEM with 10% FBS and starved in serum-free medium for 16hours before IAPP treatment.

Results: Compared with wild-type (WT) mice, body weight was decreased by 6% in IAPP transgenic mice. Insulin tolerance test revealed that glycemic regulation in IAPP transgenic mice were more sensitive to insulin. IAPP transgenic mice had higher hepatic glycogen content as shown by PAS staining. Hepatic phosphorylation of AKT and GSK3β in IAPP transgenic mice were much stronger than that in WT mice in response to insulin stimulation through portal vein injection. Moreover, we found that both full length and cleaved active sterol regulatory element-binding protein-1 (SREBP-1) levels were down-regulated in IAPP transgenic mice, while with SREBP-1 changes, insulin receptor substrate-2 (IRS-2) levels were increased. *In vitro* experiments in Huh 7 hepatoma cells also demonstrated that IAPP enhanced insulin-stimulated AKT phosphorylation in a dose-dependent manner. Consistently with *in vivo* experiments, similar changes of SREBP-1 and IRS-2 were observed with IAPP treatment.

Conclusion: Our results demonstrated that IAPP improved hepatic insulin signaling both *in vivo* and *in vitro*. SREBP-1 and IRS-2 signal cascade may be involved in the regulation of IAPP on insulin sensitivity. These results provided novel evidences for clinical usage of IAPP analog for the treatment of diabetes and related hepatic disorder.

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PS 037 Clinical hypoglycaemia

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Nocturnal hypoglycaemia: effect of insulin analogues compared to human insulin in type 1 diabetic patients prone to severe hypoglycaemia

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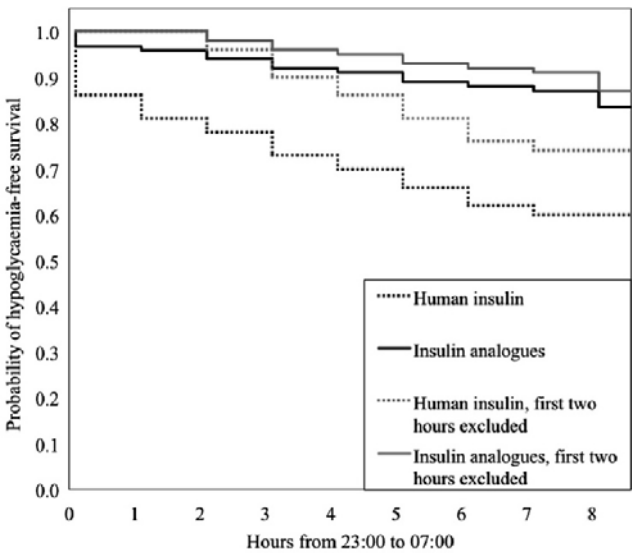
Background and aims: Nocturnal hypoglycaemia is a critical limitation of insulin therapy in type 1 diabetes. Insulin analogues reduce the risk of nocturnal hypoglycaemia in subjects at low risk of severe hypoglycaemia. We tested whether treatment based on insulin analogues compared to human insulin reduces occurrence of documented nocturnal hypoglycaemic episodes in patients with recurrent severe hypoglycaemia (two or more events during the last year).
Materials and methods: 72 participants (46 males, age 54±12 years (mean±SD), HbA1c 8.1±1.1%, duration of diabetes 30±14 years) in a two-year randomised, open-labelled, crossover, multicentre trial of basal-bolus therapy with insulin detemir / insulin aspart or human NPH insulin / human regular insulin stayed overnight at our Diabetes Center for two nights during each treatment arm. Venous blood was drawn hourly from 23:00-07:00 for later plasma glucose measurements. Primary endpoint was occurrence of hypoglycaemia ≤ 3.9 mmol/l (ADA threshold).
Results: Valid data were obtained in 217 nights. One night was excluded due to lack of measurements at bedtime yielding 100 nights in the human insulin arm and 116 in the analogue arm. Kaplan-Meier analysis showed a nocturnal "hypoglycaemia-free survival" of 73%. A higher incidence of nocturnal hypoglycaemia was present in the human insulin arm (40 nights (40%)) than in the insulin analogue arm (19 nights (16%)) (p<0.001) (figure). Cox regression revealed a hazard ratio (HR) of 0.41 (95% CI 0.2-0.6; p<0.001). A possible "carry-over effect" of dinner-time human short-acting insulin was tested by excluding hypoglycaemic events at bedtime (23:00) and midnight (00:00). After exclusion of these events "hypoglycaemia-free survival" was 82%. The incidence rates of hypoglycaemia were still lower in the analogue arm (14 nights (13%)) than in the human insulin arm (21 nights (26%)) (p=0.015) (figure). The number needed to treat with insulin analogues to avoid one episode of nocturnal hypoglycaemia is one patient in approximately 5 nights. No nocturnal peak occurrence of hypoglycaemia was observed in any treatment arm.
Conclusion: Treatment with long-acting insulin analogues significantly reduces the occurrence of nocturnal hypoglycaemia in type 1 diabetic patients prone to severe hypoglycaemia. Dinner-time short-acting human insulin involves a notable "carry-over effect" on the incidence of early nocturnal hypoglycaemia.



Clinical Trial Registration Number: NCT00346996

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Effect of insulin analogues on frequency of mild hypoglycaemia in patients with type 1 diabetes and recurrent severe hypoglycaemia: the prospective, controlled HypoAna trial

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Background and aims: Hypoglycaemia is a constant threat for people with type 1 diabetes. Episodes of mild hypoglycaemia (MH) are not always benign because they may impair physiological and behavioural defences against subsequent hypoglycaemia. The HypoAna Trial tested whether insulin analogues in comparison with human insulin are advantageous in reducing the incidence of hypoglycaemia in patients at high risk of severe hypoglycaemia. Here we report the results on MH.
Materials and methods: The study was an investigator-initiated two year, prospective, randomized, open-labelled, blinded endpoint (PROBE) multicenter trial including 159 patients with type I diabetes and two or more episodes of severe hypoglycaemia in the preceding year as the major inclusion criteria. Patients were randomized to treatment with basal-bolus therapy based on analogues (aspart/detemir) or human insulin (regular/NPH) in a balanced cross-over design. Patients were instructed to measure 7-point blood glucose profiles during daytime twice a week and to measure nocturnal blood glucose at 03 a.m. once every month. Episodes of documented MH defined by a measured plasma glucose concentration ≤ 3,9 mmol/L +/- typical symptoms were recorded and compared between the two treatments. Occurrence of MH was evaluated in the last 9 months of each treatment arm.
Results: A total number of 9360 episodes of MH were recorded; 52% in the human insulin arm and 48% in the insulin analogue arm, corresponding to 1,12 and 1,05 episodes per patient-week, respectively (mean). 16% of MH was nocturnal (23-07). The per protocol analysis demonstrated a 6% rate reduction (95% CI: 2-9%; p=0.0077) in the total number of episodes of MH in patients being treated with insulin analogues compared to human insulin. The reduction in MH was solely due to reduction in the rate of nocturnal MH (38%; 95% CI: 31-44%; p=0.0001), whereas daytime occurrence of MH was similar in the two treatment arms (p=0.32). The intention-to-treat analysis demonstrated similar results. The results were obtained during maintenance of baseline glycaemic control (HbA1c: 8.0±1.0% (64±11 mmol/mol) (mean±SD)) throughout both treatment arms.
Conclusion: Insulin aspart/detemir significantly reduces the rate of nocturnal MH in patients with type 1 diabetes and recurrent severe hypoglycaemia compared to regular/NPH insulin. The reduction appears mainly to be due to use of the long-acting insulin analogue.
Clinical Trial Registration Number: NCT00346996
Supported by: Novo Nordisk

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Patients with type 1 diabetes treated with insulin pumps do not experience a reduced risk of severe hypoglycaemia in a real life setting

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Background and aims: The main target in diabetes treatment is adequate lowering of HbA1c to avoid late complications. However, people with type 1 diabetes on intensive insulin treatment are at daily risk of severe hypoglycaemia (SH). SH is feared by patients and relatives and associated with reduced

quality of life. Our aim was to evaluate, in a real life setting, the impact of insulin pump therapy (CSII) on the frequency of SH compared with conventional insulin treatment (CIT).
Materials and methods: A questionnaire on hypoglycaemia and related subjects posted from six Danish diabetes clinics to 6112 unselected adult patients with type 1 diabetes was filled in by 3861 patients (63 %). In a subset of 1728 patients (45%) supplementary clinical and laboratory data was available. Parametric and non-parametric testing was used as appropriate. For multivariate analysis a log-linear negative binomial model was applied with adjustment for age, awareness status, duration of diabetes and sex, and in the subpopulation also for HbA1c and plasma creatinine.
Results: 3813 patients with type 1 diabetes (CSII vs. CIT: 211 vs. 3602 patients (83% on ≥4 injections/day), men 37% vs. 54%, age 45±14 vs. 48±15 years (mean±SD), duration of diabetes 25±13 vs. 23±14 years, awareness status (aware/impaired/unaware) 38/47/15 vs. 46/42/12 %) were eligible. The number of episodes of SH was 1.3±4.3 (range: 0-40) and 1.2±5.0 (range: 0-112) per patient-year in the CSII and CIT groups, respectively (p=0.8). The adjusted multiple regression analysis confirmed that no difference in the frequency of SH existed between the groups while occurrence of SH increased with age (p=0.01) and decreasing hypoglycaemia awareness (p<0.001). In the subpopulation (CSII vs. CIT: 112 vs. 1616 patients (82% on ≥4 injections/day), men 43 vs. 53%, age 48±14 vs.51 ±15 years, duration of diabetes 27±12 vs.26±14 years, awareness status (aware/impaired/unaware) 38/46/16 vs. 44/42/14 %, HbA1c 7.6±0.9 vs. 8.0±1.0 % (p<0.001), p-creatinine 71±16 vs. 80±48 µmol/l (p=0.031)), the frequency of SH was 1.2±4.1 (range: 0-30) and 1.2±5.0 (range: 0-112) episodes per patient-year (p=0.2). This finding was confirmed in the adjusted regression analysis where occurrence of SH was associated with increasing plasma creatinine (p=0.01) and decreasing hypoglycaemia awareness (p<0.001).
Conclusion: In this large questionnaire study occurrence of SH the last year, in a real life setting, was similar in patients treated with CSII and with other, primarily basal-bolus, insulin regimens. Confounding by indication may be involved. However, indications for starting CSII and whether CSII was started during the last year or earlier are not known. To further elucidate these aspects supplementary clinical and laboratory data on a larger proportion of the CSII patients are needed.
Supported by: Novo Nordisk

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Relationship between glycaemic control and hypoglycaemia using insulin glargine versus premixed insulin in type 2 diabetes: a subanalysis of GALAPAGOS

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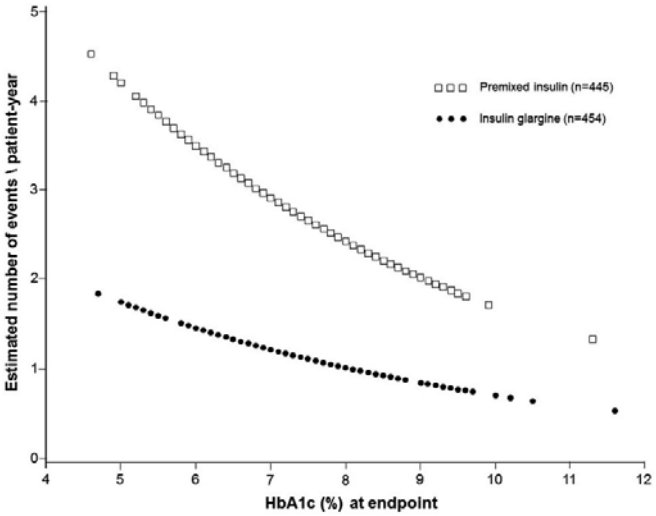
Background and aims: The GALAPAGOS study investigated the efficacy and safety of insulin glargine (GLA) QD ± glulisine (GLU) QD at the main meal compared to premixed insulin (PRE QD or BID) in insulin-naïve type 2 diabetes (T2DM) patients uncontrolled on OADs. A similar percentage of patients in the overall GLA and PRE groups reached the primary endpoint (HbA1c <7% and no symptomatic confirmed hypoglycaemia [PG ≤3.1 mmol/L] at 24 weeks), failing to demonstrate superiority, but demonstrating non-inferiority of GLA-based treatment. Since hypoglycaemia is an important safety factor and may also impact patient adherence to therapy, we performed a *post-hoc* analysis of the relationship between glycaemic control and symptomatic confirmed hypoglycaemia [PG ≤3.1 mmol/L] in the 2 groups.
Materials and methods: GALAPAGOS was a 24-week, open-label, randomised, multinational, superiority trial. Patients were initially randomised to GLA QD or PRE (QD or BID) while continuing OADs (metformin ± insulin secretagogue [IS]). A second PRE injection could be added any time in PRE QD patients; one injection of GLU at main meal could be added in GLA QD patients with HbA1c ≥7% and FPG <7 mmol/L from week 12 onwards. IS was stopped with the addition of a second injection. Insulin titration targeted FPG ≤5.6 mmol/L. The relationship between HbA1c at study end and event rate of hypoglycaemia throughout the study was analysed using negative binomial regression.
Results: There were a total of 256 documented confirmed overall symptomatic hypoglycaemic events [PG ≤3.1 mmol/L] with GLA and 623 events with PRE, and an incidence rate of 22% for GLA and 35% for PRE (p<0.001). The estimated event rate (episodes/patient-yr) of documented confirmed overall hypoglycaemia [PG ≤3.1 mmol/L] was 1.2 for GLA and 2.9 for PRE; rate ratio was 0.41 [95% CI: 0.30, 0.56, p<0.001] and NNT=8 to have one less patient to experience a hypoglycaemia within 6 months with GLA rather than PRE therapy. When the estimated number of events/patient-year was plotted against HbA1c at study end, there was a greater risk of overall hypoglycaemia with PRE vs. GLA treatment at all HbA1c levels (Figure). Similar results were obtained for nocturnal hypoglycaemia [PG ≤3.1 mmol/L] with 79 confirmed events with GLA and 221 events with PRE; incidence rates were 7% and 19%, respectively. Estimated rates were 0.4 and 1.0 episodes/patient-year, respectively; rate ratio: 0.35 [0.22, 0.56; p<0.001] and NNT=9.
Conclusion: In this analysis, the use of insulin glargine or premixed insulin produced a similar HbA1c lowering, but the use of insulin glargine was associated with a lower rate of confirmed symptomatic hypoglycaemia at all levels of HbA1c.



Clinical Trial Registration Number: 01121835
Supported by: Sanofi

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The effect of vildagliptin relative to sulphonylureas in Muslim patients with type 2 diabetes fasting during Ramadan: the VIRTUE study

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Background and aims: The VIRTUE study was a prospective, observational study that assessed the risk of hypoglycemic events (HE) in patients with type 2 diabetes mellitus (T2DM) who fast during Ramadan.
Materials and methods: The study enrolled Muslim patients with T2DM in two cohorts: vildagliptin (n=684) or sulphonylurea (SU; n=631), both given either as monotherapy or dual therapy with metformin. Patients were recruited from 10 countries in the Middle East and Asia. Mean HbA1c was 7.39% and mean BMI was 28.99kg/m^2. The primary endpoint was the proportion of patients with ≥1 HE during Ramadan. Secondary endpoints included change in HbA1c, change in body weight, treatment adherence and overall safety.
Results: Significantly (p<0.001) fewer patients experienced 1 or more HE with vildagliptin (n=36/669, 5.4%) compared with SU (n=123/621, 19.8%). No patients reported a grade 2 HE (requiring third-party assistance) with vildagliptin compared with four patients receiving SUs (p=0.053). The mean change in HbA1c, pre- to post-Ramadan, was -0.24% in the vildagliptin group compared with +0.02% in the SU group (-0.26% between treatment difference; p<0.001). Greater body weight reductions were observed with vildagliptin compared with SU (-0.76 vs. -0.13 kg; -0.63kg between treatment difference; p<0.001). Treatment adherence was high with a similar number of missed doses between cohorts. A higher proportion of SU-treated patients

experienced adverse events compared with vildagliptin (22.8% vs. 10.2%), with the difference driven by hypoglycemia, which was reported as the most common adverse event.
Conclusion: Vildagliptin therapy was associated with significantly fewer patients experiencing hypoglycemia compared with SU therapy in this large cohort of fasting patients with T2DM. This outcome is particularly meaningful when viewed in the context of good glycemic and weight control accompanied by favorable tolerability observed in vildagliptin-treated patients who fasted in this study.
Supported by: Novartis Pharma AG

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Hypoglycaemia is associated with retinopathy in French type 2 diabetic patients at inclusion in the Gerodiab cohort
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Background and aims: The observational Gerodiab cohort study aimed to describe the mortality and morbidity and associated factors in 987 French type 2 diabetic patients aged 70 years and over, during a five-year period. This study looked for factors associated at baseline with the frequency of hypoglycemia during the previous 6 months.
Materials and methods: Hypoglycemia was assessed on clinical events and self-monitoring. Data (mean±SD) were analyzed using t-test and chi² test; multivariate analyses used the logistic model.
Results: Patients with hypoglycemia (33.6%) had a longer duration of diabetes (21±11 vs.16±10 yr; P<0.001) but a similar age (77±5 vs. 77±5 yr; NS). They had a lower BMI (29±5 vs. 30±5 kg/m²; P<0.05), DBP (73±11 vs. 75±10 mmHg; P<0.05), LDL-cholesterol (2.3±0.8 vs. 2.6±1.0 mmol/L; P<0.001), MDRD (65±22 vs. 69±23 ml/min; P<0.01) and a slightly but not significantly lower HbA1c (58±11 vs. 60±15 mmol/mol [7.5±1.0 vs. 7.6±1.4%]; NS). They had a higher frequency of retinopathy (36 vs. 21%; P<0.001), nephropathy (54 vs. 44%; P<0.05) and peripheral neuropathy (36 vs 24%; P<0.01), whilst the frequency of strokes, heart failure and cerebral involvement was similar in both groups. Scores of geriatric scales (Mini-Mental State Examination, Mini-Nutritional Assessment, Instrumental Activity of Daily Living and Activity of Daily Life) were similar in both groups with the exception of the mini-Geriatric Depression Scale score which was more frequently impaired (42 vs. 31%; P<0.01). Insulin was the only treatment associated with increased frequency of hypoglycemia (75 vs. 48%; P<0.001). Using stepwise logistic regression, hypoglycemia was associated with retinopathy (P<0.01), LDL-cholesterol and the mini-GDS score (P<0.05), successively (concordance 44%; P<0.001).
Conclusion: These results underline the association between hypoglycemia and retinopathy in elderly type 2 diabetic patients and support the importance of individualizing goals in order to prevent hypoglycemic events.
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Glucose variability and hypoglycaemic excursions in elderly type 2 diabetic patients treated with insulin
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Background and aims: Continuous glucose monitoring (CGM) systems provide new opportunities for estimation of glucose variability (GV) and detection of hypoglycemia in diabetic patients. It is widely discussed that increased GV and unrecognized hypoglycemia may be associated with cardiovascular complications, particularly in elderly patients. The aim of this study was to estimate determinants of CGM-defined GV and hypoglycemic episodes in elderly insulin-treated type 2 diabetic patients.
Materials and methods: 76 patients ≥65 years of age were observed. Medical history included diabetic retinopathy (87%), peripheral artery disease (83%), coronary artery disease (61%), heart failure (55%), obesity (45%) and chronic kidney disease (CKD, 46%). Use of basal insulin (47%), premixed insulin (16%) or basal-bolus insulin regimen (37%) was followed by metformin (58%), glimepiride (18%) and DPP-IV inhibitors (18%). 3-days CGM was performed in all patients. During CGM, 3 fasting and 3 2-h postprandial finger-prick glucose values were obtained daily with glucometer. Data are presented as median, percentile 25-75.
Results: GV (SD of mean glucose during monitoring) was similar in patients on basal, premixed insulin and basal-bolus regimen (2.3, 1.8-2.8; 2.3, 2.0-2.8; 2.3, 1.7-3.3 mmol/l respectively; Kruskal-Wallis ANOVA: p=0.76) and did not depend from used oral antihyperglycemic agents. GV correlated significantly with mean monitored glucose (r=0.45, p=0.00005), but not with HbA1c (r=0.22, p=0.06). No associations were found between GV and diabetes complications. Hypoglycemic excursions (<3.3 mmol/l) were detected in 43 (57%) patients by CGM system and in 10 (13%) patients by glucometer (p=0.0001). Most of hypoglycemic episodes detected during CGM (72%) were not recognized by patients. Subjects with hypoglycemia as compared to the other patients demonstrated higher GV (2.7, 2.1-3.1 vs 1.9, 1.5-2.2 mmol/l, p=0.0001) and a tendency to lower mean interstitial glucose (7.0, 6.4-8.3 vs 7.9, 6.8-8.5 mmol/l, p=0.06). Hypoglycemia duration correlated positively with GV (r=0.43, p=0.0001). No significant differences were found between patients with and without hypoglycemia in HbA1c (7.7, 6.9-8.2 vs 7.5, 6.6-8.9% respectively, p=0.94) and daily insulin dose (0.52, 0.38-0.71 vs 0.49, 0.28-0.63 IU/kg, p=0.35). We also failed to find any differences between these groups in age, BMI, diabetes duration, treatment modality, prevalence of heart failure and CKD. The duration of insulin therapy tended to be longer in patients with hypoglycemic episodes (p=0.05). In discriminant analysis mean monitored glucose and GV, but not clinical parameters, were predictors for hypoglycemia with a classification accuracy of 81%.
Conclusion: The results indicate that asymptomatic hypoglycemia is a common complication in elderly type 2 diabetic patients treated with insulin. Traditional risk factors are weak predictors for hypoglycemia in this patient cohort. Glucose variability, but not HbA1c, is associated with incidence and duration of hypoglycemic episodes in elderly insulin-treated patients.

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Hypoglycaemia among 3048 insulin-treated patients in real life: frequency and predictive factors: results from the prospective DIALOG study
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Background and aims: Few data are available on hypoglycaemia rates in real life. The observational, prospective, multicentre, French DIALOG study examined the frequency of hypoglycaemia in patients with type 1 (T1DM) or type 2 (T2DM) diabetes, insulin-treated for >1 year.
Materials and methods: Endocrinologists and general practitioners consecutively enrolled patients and gave them a prospective questionnaire for self-recording hypoglycaemia over 1 month (using the ADA hypoglycaemia definition). The primary endpoint was frequency of total confirmed hypoglycaemia. Predictive factors (PF) were analysed by a multivariate regression model in 1304 T1DM and 1631 T2DM patients.
Results: Characteristics for the 3048 patients with T1DM / T2DM were: age: 49.7±15.9 / 66.8±10.6 years, BMI: 25.4±4.3 / 31.0±5.9 kg/m², HbA_{1c}: 7.8±1.2 / 7.8±1.1%, duration of diabetes: 20.9±13.0 / 17.7±9.3 years. Hypoglycaemia rates are shown in the table. Main PF in T1DM were previous history of hypoglycaemia (odds ratio [OR] 8.16), >2 daily insulin injections (OR 2.75), BMI <30 kg/m² (OR 2.14), insulin therapy duration 10 years (OR 2.00) and age >65 years (OR 1.86). In T2DM, main PF were previous history of hypoglycaemia (OR 3.52), insulin therapy duration >10 years (OR 1.72), >2 daily insulin injections (OR 2.69), use of insulin secretagogues (OR 1.54) and absence of coronary artery disease (OR 1.45).
Conclusion: DIALOG is one of the largest prospective studies of hypoglycaemia in real life which demonstrates a high frequency of confirmed hypoglycaemia in both T1DM and insulin-treated T2DM. Previous history of hypoglycaemia appears to be the main predictive factor for hypoglycaemia.

	Type 1 (N=1317)		Type 2 (N=1731)	
	Frequency (% of patients)	Events/ patient/ month	**Frequency (% of patients)**	Events/ patient/ month
All hypoglycaemia	85.3	6.3	43.6	1.6
Severe hypoglycaemia (requiring assistance of another person)	13.4	0.2	6.4	0.1
Confirmed (< 3.9 mmol/L) non severe hypoglycaemia	84.4	6.1	41.7	1.5
Confirmed (< 3.9 mmol/L) asymptomatic hypoglycaemia	27.9	0.9	7.7	0.2
Diurnal hypoglycaemia	82.7	5.2	40.7	2.6
Nocturnal (0.0-0.6h) hypoglycaemia	40.2	0.7	11.0	0.2

Clinical Trial Registration Number: NCT01628341
Supported by: Novo Nordisk

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Global prevalence of hypoglycaemia: association to treatment factors, self-management education and quality-of-life measurements

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Background and aims: Hypoglycemia is limiting patients achieving optimal glycemic control. The 2nd Diabetes Attitudes, Wishes and Needs (DAWN2) allows a global analysis of the incidence of severe hypoglycemia (SH) and its psychosocial correlates, and the role of diabetes education in the prevention of hypoglycemia.
Materials and methods: In each of the 17 countries across 4 continents, a sample of 500 adults, stratified by diabetes type and treatment, completed a questionnaire asking about the number of SH (defined as requiring assistance of a 3rd party for recovery) over the past 12 months, and about diabetes education received. The 5-Item Problem Areas in Diabetes (PAID-5) and the WHO-5 well-being index were completed. The impact of diabetes education on change in relative risk of SH was analyzed by logistic regression models.
Results: Of the 1368 adults with types 1 (T1DM) and 7228 type 2 diabetes (T2DM), 33% self-reported ≥1 severe SH during the past 12 months. The overall incidence was 1.64 episodes per year (epy). Persons with T1DM were more affected by SH than those with T2DM (54.0% vs 28.9%), and had a higher incidence of events/yr (2.86 vs 1.4). More insulin users than non-insulin users reported SH (42.3% vs 25.0%). Highest SH rates were reported in Turkey (3.71 epy) and lowest in Japan (0.31 epy). Compared to those without SH, individuals with SH reported more diabetes-related distress (PAID-5 score +9.2 and +13.2, respectively; p<0.0001) and lower well-being (WHO-5 score -4.3 and -6.8, respectively; all p<0.0001). When comparing countries, for every 5% more participants having received diabetes education, the risk of SH was reduced by 12% in T1DM (OR 0.88; 95% CI 0.79-0.97) and by 13% in T2DM (OR 0.87; 95% CI 0.77-1.0).
Conclusion: This global study shows a high incidence of hypoglycemia in persons with both T1DM and T2DM. There are expectable differences in the incidence of hypoglycemia between T1DM and T2DM and in T2DM between insulin users and non-users. But since only subsets of PWD report hypoglycemia problems, the existence of risk groups for SH is likely. However, the fact that these incidence data rely solely on self-report must be acknowledged. Interestingly, hypoglycemia is associated with elevated diabetes-related distress and poorer well-being. Large differences in incidence of SH between countries were found. Countries with more participants in diabetes education had a lower incidence of SH. Thus, participating in diabetes education seems to be important for individuals with either T1DM or T2DM to prevent or ameliorate hypoglycemia problems.

PS 038 Hypoglycaemia mechanism and prevention

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Hypoglycaemia promotes thrombosis and inflammation for at least one week in patients with type 2 diabetes

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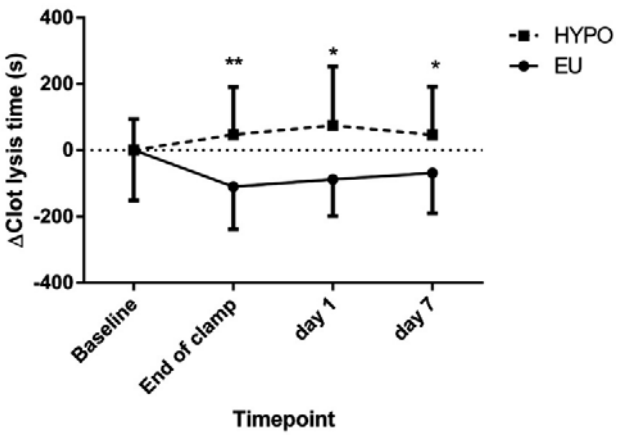
Background and aims: Increased cardiovascular (CV) mortality has been reported in trials of intensive glycaemic control in individuals with type 2 diabetes (T2DM). There is strong evidence that hypoglycaemia confers excess mortality in the weeks subsequent to the event. Altered fibrin structure characteristics and subclinical inflammation have been implicated in the predisposition to these CV events. We hypothesise that low glucose creates a thrombotic/inflammatory environment, contributing to increased mortality following hypoglycaemia. Our aim was to examine the effect of hypoglycaemia on fibrin network structure/fibrinolysis as well as plasma C-reactive protein (CRP) and fibrinogen following an experimental hypoglycaemic episode.
Materials and methods: Ten patients with T2DM with mean (±SD) age of 50±8 years, duration of diabetes 9.5±4.6 years and HbA1c 8.7±2.1% were recruited. All completed paired hyperinsulinaemic clamp studies separated by at least 4 weeks. Glucose was maintained at hypoglycaemia (2.5mmol/L) or euglycaemia (6mmol/L) for two 60 minute periods. Fibrin network properties and fibrinolysis of plasma samples were investigated using a dynamic turbidimetric assay. Clot maximum absorbance (an indicator of fibrin network density) and time from full clot formation to 50% lysis (an indicator of fibrinolysis potential) were analysed. Plasma fibrinogen levels were measured using the Clauss assay, and high sensitivity CRP (hs CRP) was determined via an immunoturbidemetric assay. Four time points were analysed: baseline, end of clamp, day 1 and day 7 post clamp in both arms.
Results: Clot maximum absorbance increased after hypoglycaemic clamps reaching a peak at day 7, whereas a decrease was detected in euglycaemic clamps (Δ 0.040±0.093 and Δ-0.035±0.080 AU, respectively compared with baseline; p = 0.01). Clot lysis time increased after hypoglycaemic clamps, a change that persisted at day 7, in contrast to euglycaemic clamps that were associated with reduced lysis times (Δ64±119 vs Δ-51± 64 s; p = 0.02, see Fig). CRP levels increased significantly comparing baseline with day 7 following hypoglycaemia, whereas a reduction was noted after euglycaemic clamps (Δ 0.80±0.98 vs Δ-0.89±0.80 mg/L, p<0.0001). Fibrinogen levels were higher day 7 after hypoglycaemia compared with euglycaemic control (Δ1.09±2.58 vs Δ 0.17 ±1.25 mg/ml, p = 0.05).
Conclusion: Hypoglycaemia induces prothrombotic changes in the fibrin network and aggravates subclinical inflammation, effects that are sustained for at least 1 week. By contrast, hyperinsulinaemic euglycaemia was associated with anti-thrombotic and anti-inflammatory changes, by unclear mechanisms, that persisted after the intervention. These data provide a possible explanation for the observed increase in CV mortality weeks after a hypoglycaemic event. Moreover, hypoglycaemia may override the benefits of intensive glucose control by creating a thrombotic and inflammatory milieu.

Fig.



Supported by: NIHR Biomedical Research Fellowship

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Experimental hypoglycaemia decreases cardiac vagal function for between 7 to 30 days in patients with type 2 diabetes

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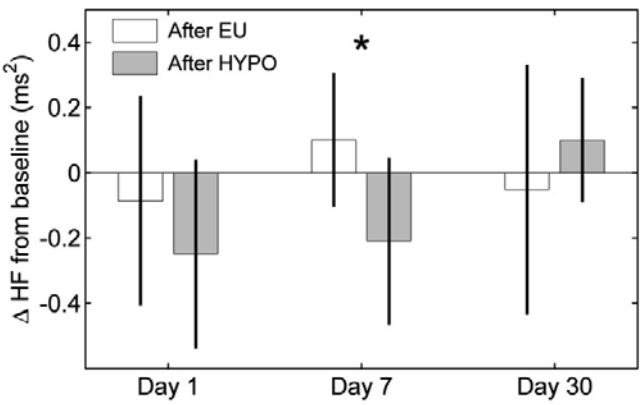
Background and aims: Intensive glycemic therapy is associated with excess cardiovascular (CV) death in type 2 diabetes patients at high CV risk and severe hypoglycaemia predicts increased cardiovascular mortality in the weeks following the event. Since decreased cardiac vagal function predisposes to arrhythmias and sudden cardiac death, we explored the effect of hypoglycaemia on autonomic balance for up to 4 weeks following an experimental hypoglycaemic episode.
Materials and methods: 11 adults with type 2 diabetes and no CVD (mean age 50±8 years, 8 male, duration of diabetes 9.5±4.6 years) underwent paired hyperinsulinaemic clamps at least one month apart. Glucose was maintained at euglycemia (6mmol/l) and hypoglycaemia (2.5mmol/l) for two 60 minute periods in each arm. Heart rate variability (HRV) measures in time and frequency domains were obtained one, seven and 30 days after clamps from 5-minute ECG recordings. Spectral analysis of heart rate variability was performed based on Task Force guidelines. Low frequency (LF: 0.04-0.15Hz) and high frequency (HF: 0.15-0.4Hz) powers were calculated. A value of $p<0.05$ was considered statistically significant.
Results: Mean heart rate at day 7 was significantly higher after hypoglycaemia (HYPO), mean difference 5.4 (95% CI 0.2 to 10.6) bpm, compared with no change after euglycaemia (EU) 0.0 (95% CI -3.9 to 3.9) bpm, $p=0.04$. Mean HF power increased at day 7 after EU clamp 0.10 (95% CI -0.10 to 0.30) ms^2 but decreased after HYPO clamp -0.21 (95% CI -0.46 to 0.05) ms^2, $p=0.02$, indicating reduced vagal tone (see figure). In contrast, the LF/HF ratio at day 7 decreased after EU -2.14 (95% CI -0.49 to -2.79) and increased after HYPO 0.35 (95% CI -1.59 to 2.3), $p=0.02$, suggesting diminished and increased sympathetic contribution respectively. All HRV measures recovered to baseline at day 30 and there were no differences between the two groups.
Conclusion: Hypoglycaemia impaired cardiac vagal function, an effect that persisted beyond the episode for at least 1 week. There were moderate improvements in vagal function following hyperinsulinaemic euglycaemia that may be protective. Our data demonstrate that in type 2 diabetes patients a single moderate hypoglycaemic episode can perturb autonomic function beyond the acute episode for between 7 and 30 days. This work identifies a mechanism which could contribute to increased CV risk following hypoglycaemia.



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Insulin prevents counter-regulatory glucagon secretion by stimulation of somatostatin release

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Background and aims: Hypoglycaemia is a major complication of insulin therapy in diabetic patients. This results from impaired counter-regulation

of glucagon secretion. The underlying mechanism is not known but it has been proposed that insulin directly inhibits glucagon secretion. Here we have tested this hypothesis by measurements of insulin-induced effects on pancreatic islet hormone secretion.
Materials and methods: Islet hormone release was measured in static incubations of groups of 10-20 size matched human or mouse pancreatic islets. Effects of insulin on the cytosolic calcium concentration ([Ca2+]i) was measured by confocal microscopy. Insulin receptor distribution was documented by immunocytochemistry.
Results: Insulin (100 nM) inhibited glucagon secretion as strongly as an increase in glucose from 1 to 6 mM. The inhibitory effect of insulin was seen only at 1 mM glucose and at 6 mM glucose, insulin actually stimulated glucagon secretion. Insulin had a weak stimulatory effect on C-peptide release but strongly (>3-fold) stimulated somatostatin release in islets exposed to 1 mM glucose. In mouse islets, insulin also potentiated somatostatin secretion. The inhibitory effect of insulin on glucagon secretion was antagonized by the insulin receptor S961. Interestingly, the SSTR2 antagonist CYN154806 prevented the suppressor effect of insulin on glucagon secretion in mouse islets (in which insulin likewise inhibits glucagon secretion and stimulates somatostatin secretion). The presence of insulin receptors in somatostatin-releasing delta cells was verified by immunocytochemistry. Measurements of [Ca2+]i in human islets revealed that insulin evoked [Ca2+]i oscillations at 1 mM glucose in a small subset of cells. A similar stimulation by insulin was seen in mouse delta cells. Insulin tolerance tests revealed improved insulin tolerance in mice in which insulin was co-administered with CYN154806.
Conclusion: Our data suggest that the suppressor effect of insulin on glucagon secretion, rather than being mediated by a direct effect on the alpha cells, results from stimulation of somatostatin secretion from the delta cells which then inhibits glucagon secretion by a paracrine effect. These findings highlight the significance of paracrine signalling in (human) pancreatic islets and suggest that insulin-induced hypoglycemia in diabetic patients may be possible to control with somatostatin receptor antagonists.
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Pharmacokinetics and pharmacodynamics of a chemically stable micro-dosed glucagon in a diabetic swine model of type 1 diabetes
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Background and aims: In order to create a bihormonal bionic endocrine pancreas that provides both insulin and glucagon replacement therapy for type I diabetics, a pumpable glucagon formulation is needed that is bioactive on the liver and chemically stable for up to three days at near body temperatures.
Materials and methods: We analyzed the pharmacokinetics (PK) and pharmacodynamics (PD) of a stable, non-aqueous, concentrated (5 mg/ml), pumpable, liquid glucagon formulation in an animal model of type 1 diabetes. Both fresh and aged (~7-8 days stored in a pig's backpack) doses of the glucagon were administered subcutaneously in separate experiments using an insulin pump connected to an infusion set inserted in Yorkshire swine after streptozoticin (STZ) treatment. Experiments began ~5 hours postprandially and after blood glucose had been regulated to between 60 and 140 mg/dl. Two consecutive glucagon doses (separated by ~60-120 minutes) were administered from the same pump reservoir for each experiment. Venous blood glucose concentration was measured in real time every 10 minutes (GlucoScout, International Biomedical) in order to assess glucagon PD. To assess glucagon PK, plasma samples were drawn every 10 minutes for plasma glucagon concentration, measured by immunoassay (Millipore). Identical experiments were conducted with freshly reconstituted commercially available glucagon formulation (Eli Lilly), which is chemically stable in solution for only a few hours.
Results: The first dose showed less of a PD response due to the higher plasma insulin levels that persisted from the insulin therapy in the pre-experimental period. Consequently, only the second dose response for each experiment (n = 3 experiments) was considered when calculating the increase in blood glucose that accompanied each glucagon dose. On the other hand, every dose response (2 doses per experiment, n = 3 experiments) was considered in the calculation of glucagon Tmax. The average Tmax for fresh and aged non-aqueous glucagon was 9 and 12 minutes, respectively (n = 6 experiments for both) and 10 minutes for freshly reconstituted Lilly glucagon (n = 4 experiments). The average increase in blood glucose during the second dose response for fresh and aged non-aqueous glucagon was 13.7 and 17.6 mg/dL/10g, respectively (n = 3 experiments), compared with 18.5 mg/dl/10g for freshly reconstituted Lilly glucagon (n = 2 experiments).
Conclusion: These preliminary results suggest that non-aqueous glucagon shows comparable efficacy, both aged and fresh, to freshly reconstituted Lilly glucagon in STZ-treated diabetic pigs. This swine model translates well to humans and paves the way for an upcoming first-in-human study using a stable pumpable glucagon in the bihormonal closed-loop artificial pancreas.

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Preclinical pharmacology study of stabilised liquid glucagon
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Background and aims: Severe hypoglycemia remains a significant unmet medical need. Currently approved glucagon rescue products (Lilly, Glucagon™ for Injection; Novo Glucagen®) are based on lyophilized formulations which require reconstitution, complicating ease of administration in emergency situations. A simple, ready-to-use, soluble glucagon formulation has been developed based on biocompatible, non-aqueous solvents that effectively suppress the fibrillation of glucagon typically observed in aqueous solutions. Upon characterization with both RP-HPLC and bioassay, this non-aqueous formulation has demonstrated superior chemical stability after six months of storage at room temperature and accelerated conditions. With a formulation exceeding CMC criteria required for the target product profile, *in vivo* pharmacology of the drug product was established in a rodent model.
Materials and methods: A comparative pharmacology study was comprised of 4 treatment groups of SD male (n=10) and female (n=10) rats. Rats were injected subcutaneously (SC) with 2.5 μg, 5 μg, or 50 μg non-aqueous glucagon, or with 5 μg Lilly Glucagon™ reference drug. Blood was collected from each animal pre-dosing (t=0 min), and 2.5., 5, 10, 15, 30, 45 and 60 min post-dose for determination of blood glucose and plasma glucagon concentrations.
Results: The study demonstrated that at *half the dose*, SC injected non-aqueous glucagon resulted in similar ($p > 0.05$) pharmacokinetic endpoints (AUC, C_{max}, $T_{max,\ and}$ $T_{1/2}$) to 5 μg SC doses of the Lilly Glucagon™ reference drug. Injection of 2.5 μg non-aqueous glucagon showed rapid absorption (T_{max} ~ 5 min), and marked elevation of blood glucose levels in male rats (TBG_{max} ~ 11.5 min). BG_{max} was normally distributed (p=0.2007) between groups. There was a proportional relationship between the non-aqueous glucagon injection dose (2.5 μg, 5 μg, or 50 μg) and the measured plasma glucagon concentrations.
Conclusion: The statistical analyses the study reported herein showed the non-aqueous glucagon formulation for SC injection to have a favorable non-clinical pharmacokinetic and pharmacodynamic profile when compared with the non-clinical *in vivo* behavior of the Lilly Glucagon™ reference drug. In a concurrent 14-day GLP safety/toxicology study, there were no statistically significant histopathological findings indicative of toxicity of the non-aqueous glucagon, again as compared to Lilly Glucagon™. Overall these data support development of the non-aqueous glucagon as a room-temperature stable, ready-to-use rescue drug for severe hypoglycemia, as well as a glucagon formulation suitable for a bi-hormonal (insulin-glucagon) infusion pump.
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Effects of RAS blockade on ECG and EEG during hypoglycaemia in patients with type 1 diabetes
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Background and aims: Increased heart rate and QT prolongation are associated with hypoglycaemia in type 1 diabetes, potentially leading to sudden death from ventricular tachyarrhythmia. High renin-angiotensin system (RAS) activity increases the risk of severe hypoglycaemia in type 1 diabetes, and RAS is implicated in ventricular tachyarrhythmia. Moreover, high spon-

taneous RAS activity may result in lower cerebral activity at the EEG and a more pronounced cognitive impairment during mild hypoglycaemia in type 1 diabetes, thus conferring an increased risk of severe hypoglycaemia. We therefore studied the effect of RAS blockade on ECG and EEG during hypoglycaemia in patients with type 1 diabetes.
Material and methods: Nine patients with type 1 diabetes and high RAS activity were included in a double-blind, randomised, balanced, cross-over study of the effect of 32 mg candesartan or placebo for one week on ECG (QT interval, heart rate) and EEG during two hyperinsulinaemic hypoglycaemic clamps separated by at least 4 weeks. Heart rate and QT intervals were calculated from five 12-lead ECG recordings of 10 sec duration obtained at the beginning and end of the baseline and hypoglycaemic periods. ECG analysis was done semi-automatically and QT analysis was performed by a trained cardiophysiologist. The QT interval was corrected for differences in heart rate (HR) according to Bazett's (QTcb) and Fridericia's (QTcf) formulas. A 20-lead EEG was obtained during a 5-min resting period at baseline and hypoglycaemia. The mean centroid frequency (5-11 Hz) was used to evaluate the effect of hypoglycaemia on the five brain areas: frontal cortex, cortex of the central sulcus, temporal cortex, parietal cortex, and occipital cortex.
Results: Heart rate increased from baseline to hypoglycaemia on the placebo day (p=0.01) but remained unchanged on the candesartan day (p=0.65). Heart rate on candesartan was 3.3 (0.8-5.8) (mean (95%CI)) beats per min lower than on placebo (p=0.02). QTcb and QTcf were increased by approximately 2.5% during hypoglycaemia (p<0.008) with similar effect during candesartan and placebo treatment. EEG was not affected by candesartan in any of the different brain areas (p=0.2-0.4).
Conclusion: Candesartan possesses a stabilising effect on heart rate during hypoglycaemia but has no effect on length of the QT interval. No effect on centroid frequency was observed in any brain area at candesartan treatment compared to placebo.
Clinical Trial Registration Number: NCT01116180
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Effects of 6 weeks treatment with sitagliptin on counterregulatory and incretin hormones during acute hypoglycaemia in patients with type 1 diabetes

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Background and aims: Within a few years of onset of type 1 diabetes the glucagon response to hypoglycaemia is severely diminished. Inhibitors of the enzyme dipeptidyl peptidase 4 (DPP-4), which under normal circumstances inactivates the incretin hormones, enhance glucagon secretion during hypoglycaemia in patients with type 2 diabetes. The aim of this study was to assess whether the DPP-4 inhibitor sitagliptin enhances the glucagon response to hypoglycaemia in patients with type 1 diabetes.
Materials and methods: We conducted a single-centre, randomised, double-blind, placebo-controlled, three-period cross-over study. We studied 16 male patients with type 1 diabetes and intact hypoglycaemia awareness (median [interquartile range] age 32 [27-40] years, diabetes duration 11 [6-15] years and HbA1c 66 (61-71) mmol/mol (8.1 [7.7-8.6] %)). Participants received sitagliptin (100 mg/day) or placebo as outpatients for 6 weeks and attended the hospital for three acute hyperinsulinaemic hypoglycaemia studies (at baseline, after sitagliptin treatment and after placebo). During the experiments arterialized blood glucose was stabilized for 30 minutes at 5.0 mmol/l (initialisation phase). Thereafter glucose infusion was discontinued until the patient developed objective evidence of an autonomic reaction (defined by an increment of 20% or more in heart rate and/or systolic blood pressure) or until blood glucose fell below 2.0 mmol/l. Following autonomic reaction the insulin infusion was terminated and an infusion of 20% glucose was commenced at a rate of 50 ml×h-1. We measured plasma concentrations of glucagon and other hormones during the hypoglycaemia studies at the middle of the initialisation phase, during autonomic reaction, and 10, 20 and 40 minutes after the onset of autonomic reaction.
Results: No significant differences were observed for the glucagon or adrenergic counterregulatory response during the three hypoglycaemia studies, but absolute growth hormone concentration at 40 minutes after occurrence of autonomic reaction was significantly lower after sitagliptin treatment (median [interquartile range] 23.0 [0.2-211.0] mEq/l) when compared to placebo (90.0 [8.8-180] mEq/l) (p=0.008), but not significantly lower when compared to baseline (64.0 [4.0-205.0] mEq/l) (p=0.16). The change in growth hormone concentration from the initialisation phase to 40 minutes after onset of autonomic reaction was also significantly lower after sitagliptin treatment 23.0 [1.5-73.4 mEq/l] than after placebo (82.6 [35.3-137.5] mEq/l) (p=0.001), but not when compared to baseline (37.1 [7.5-95.7 mEq/l]. No differences in growth hormone responses were observed between the three hypoglycaemia studies at other time points.
Conclusion: Sitagliptin does not enhance the glucagon counter-regulatory response in patients with type 1 diabetes.
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Risk factor assessment for irreversible brain damage in patients with drug-induced severe hypoglycaemia

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Background and aims: Type 2 diabetes patients who are treated with insulin or sulfonylurea drugs (SU) commonly experience severe hypoglycemia (SH) as a common adverse effect. Recently, many studies have shown a relationship between SH and cardiovascular events, but few have examined the risk factors for irreversible brain damage (IBD) due to SH.
Materials and methods: We retrospectively analyzed the risk factors for IBD in patients with drug-induced SH. We investigated 189 type 2 diabetes patients (age 73.8 ± 10.9 years) who visited our hospital's emergency department because of drug-induced SH between November 2008 and October 2012. The Glasgow Coma Scale (GCS) was used to determine the level of consciousness. IBD was defined as a GCS below 14 at day 14. We examined the following risk factors: age, gender, diabetic medication, plasma glucose level, HbA1c level, and GCS on arrival at the hospital and estimated maximum duration of coma (MAXC duration) before treatment. MAXC duration was defined as the interval between the time the patient last confirmed clear sensorium and the first care given in the hospital; duration of protracted hypoglycemia was defined as the interval between the time care was first given and the first stable capillary glucose level of >100 mg/dL.
Results: The patients' plasma glucose levels (34.4 ± 11.2 mg/dL) correlated with GCS on arrival at the hospital ($p < 0.001$). The mean HbA1c level was 6.70 ± 1.13%. One hundred and fourteen patients had been treated with SU; 50 with insulin; 13 with both SU and Dipeptidyl peptidase 4 (DPP4) inhibitors; 11 with both SU and insulin; and 1 with both insulin and DPP-4 inhibitors. Ten patients had IBD. Logistic analysis revealed that the risk factors for IBD were low plasma glucose levels on arrival (odds ratio [OR]: 0.81; 95% confidence interval [CI]: 0.62-0.94, $p < 0.01$), low GCS score on arrival (OR: 0.58; 95% CI: 0.25-0.95, $p < 0.05$), and MAXC duration (OR: 1.28; 95% CI: 1.13-1.65, $p < 0.001$). MAXC duration of less than 8 hours was likely to be associated with recovery of consciousness, but when the duration was longer than 12 hours, recovery was unlikely. Hypoglycemia due to SU use was associated with a longer duration of protracted hypoglycemia compared with the duration associated with hypoglycemia due to insulin use (7.89 ± 5.31 hours vs. 2.70 ± 3.02 hours, $p < 0.001$).
Conclusion: Our data suggest that MAXC lasting longer than 12 hours before treatment is the most reliable predictor for the development of IBD. Long-acting insulin and SU are likely to be the causes of IBD.

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Hypoglycaemia related events due to falls in elderly diabetics (H.E.A.L.E.D) study in US veterans

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Background and aims: Fall-related events in the elderly are associated with increased morbidity and mortality. This retrospective cohort study aimed to examine the association between hypoglycemia and fall-related events among elderly veterans with type 2 diabetes mellitus (T2DM).
Materials and methods: Electronic medical and pharmacy records were obtained for patients (N=149,053) with at least 2 records of T2DM diagnosis (ICD-9-CM codes: 250.x0 and 250.x2) from the Veterans Integrated Service Network (VISN) 16 data warehouse from 01/01/2004 to 06/30/2010. The VISN 16 covers Arkansas, Louisiana, Mississippi, Oklahoma, and parts of Alabama, Florida, Missouri, and Texas. Patients with type 1 diabetes were excluded (ICD-9-CM codes: 250.x1 and 250.x3). Subjects were required to be ≥65 years at cohort entry (01/01/2004) and have continuous enrolment and pharmacy benefits throughout the study period. The date of the first recorded hypoglycemia (ICD-9-CM codes: 250.8, 251.0, 251.1 and 251.2) was defined as the index date for a case. Cases with the index date from 01/01/2005 to 07/01/2009 were included to ensure one-year baseline and one-year follow-up. Controls (no hypoglycemia recorded) were randomly matched with cases by age (+/-5 years), gender, race, and location of the VA medical center. Fall-related events (i.e., fractures and head injuries) were defined as ICD-9-CM codes between 800.x - 995.x, with a fall being the external cause (ICD-9-CM E-codes between E880 - E888), which were recorded within 2 days before and after the fall event. Chi-square tests were used to compare the post-index fall-related events within 30 days, 90 days, 180 days and 365 days between the two cohorts. Generalized estimating equation (GEE) models were fit to compare fall-related events over 365 days in terms of the point estimate of the adjusted odds ratio (aOR) and the 95% confidence interval (95% CI), controlling for baseline characteristics and comorbidities. Two subgroup analyses (ages of <75 years and >=75 years) were also conducted.
Results: A total of 4,274 cases were matched with 4,274 controls. Over one year after the index hypoglycemia, patients with hypoglycemia consistently had higher fall-related events: 30 events (0.70%) among the cases and 2 events (0.05%) among the controls within 30 days; 53 events (1.24%) and 6 events (0.14%) within 90 days; 70 events (1.64%) and 15 events (0.35%) within 180 days; and 98 events (2.29%) and 31 events (0.73%) within 1 year. All cross-cohort differences were statistically significant in elderly veterans and by age group (all p-values <0.05). Correspondingly, the GEE models confirmed the elevated risk of fall-related events over 365 days (aOR=1.93, 95% CI=1.25-2.97) for the elderly veterans, and for the >=75 years group (aOR=1.95, 95% CI=1.15-3.34) and <75 years group (aOR=1.87, 95% CI=0.92-3.83; p=0.085).
Conclusion: Hypoglycemia in elderly diabetic veterans was associated with an almost 2 fold increase in the risk of major fall related events. Physicians treating elderly diabetics need to provide patient education and choose medications that minimize the risk of hypoglycemia and the ensuing complications of falls.
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PS 039 Insulin resistance improvement

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Cocoa flavonoids attenuate high glucose-induced insulin signalling blockade and enhance glucose uptake and production in human HepG2 hepatic cells

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Background and aims: Insulin resistance is the primary characteristic of type 2 diabetes, which results in insulin signalling defects. Flavonoids have been found to possess beneficial effects on health and have drawn attention because of their safety. Cocoa and its main flavanol, (-)-epicatechin (EC), display some antidiabetic effects, but the mechanisms for their preventive activities related to glucose metabolism and insulin signalling in the liver remain largely unknown. In the present work, the effect of EC and a cocoa polyphenolic extract (CPE) on insulin signalling is studied in insulin-responsive human HepG2 cells treated with high glucose.
Material and methods: HepG2 cells treated for 24 h with physiological concentrations of EC (10 μM) and CPE (1 μg/mL) were further exposed to 30 mM glucose for 24 h. Tyrosine phosphorylation of IR and its substrates (IRS-1 and -2) were analysed by Western blot in cellular immunoprecipitates. Total levels of IR, IRS-1 and -2 and key proteins of insulin signalling such as AKT, GSK3, GS, AMPK and PEPCK were assayed by Western blot. Glycogen content and glucose uptake were evaluated by using a commercial fluorometric kit and 2-((7-nitro-2,1,3-benzoxadiazol-4-yl)amino-2-deoxy-D-glucose (2-NBDG) assay, respectively. Glucose production was also determined by evaluating glucose levels after incubating the cells in glucose production buffer.
Results: Pre-treatment of cells with EC or CPE reverted the decreased tyrosine-phosphorylated and total levels of IR, IRS-1 and -2 induced by glucose (30 mM). EC and CPE pre-treatment also prevented the inactivation of the PI3K/AKT pathway induced by glucose, showing a glycogen content similar to those of control cells. Furthermore, glucose induced a diminution of p-AMPK levels, which was avoided by EC and CPE. Pre-treatment of cells with EC and CPE also prevented the increased PEPCK levels and glucose uptake provoked by 30 mM glucose and returned to control values the enhanced levels of glucose production. GLUT-2 expression levels were unaffected by any treatment.
Conclusion: EC and CPE at concentrations that are not toxic to hepatic cells and are reachable through the diet, possess an insulin-like activity. EC and CPE prevented the decrease of the tyrosine phosphorylated and total IR, IRS-1 and IRS-2 levels, as well as the inhibition of PI3K/AKT and AMPK pathways induced by high glucose. Further efforts are needed to define the precise role of EC and cocoa in the regulation of the insulin pathways in liver cells, but it could be suggested that a diet rich in EC and/or cocoa may be a potential chemopreventive tool useful for the management of diabetes.
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Can carotid sinus nerve resection be a therapeutic approach for the treatment of insulin resistance?

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Background and aims: The carotid bodies (CBs) are peripheral chemoreceptors which respond to its classical stimulus, hypoxia, increasing the action potential frequency in their sensorial nerve, the carotid sinus nerve (CSN), leading to an increase in minute ventilation and sympathetic outflow. Recently, our lab demonstrated that CB is involved in aetiology of insulin resistance (IR) and that CSN resection prevents the development of IR in rats fed with hypercaloric diets through sympathoadrenal overactivation. Herein, we tested if blockade of CB activity through CSN resection reverses IR induce by high sucrose (Hsu) diet.
Materials and methods: Four groups of Wistar rats, age 9-12 weeks were used. The control group was fed a sham diet ((7.4% fat+75% carbohydrate (4% sugar) + 17% protein) and the HSu group was fed 35% sucrose in drinking water. CSN bilateral resection in HSu rats was performed after 28 days

of hypercaloric diet under ketamine (30mg/kg)/xylazine (4mg/kg) anaesthesia and brupenorphine (10g/kg) analgesia. These animals were maintained under the hypercaloric diet after CSN denervation. Rats submitted to CSN bilateral resection were compared with animals submitted to the same surgical procedure but in which CSN was left intact (sham). Fasting glycemia and insulin sensitivity were evaluated in conscious rats prior to CSN denervation and once a week after CSN resection, through an insulin tolerance test (ITT). After 3 weeks, rats were anaesthetized with pentobarbital and blood pressure, body weight, visceral and total fat were determined. Also, blood was collected by heart puncture to quantify insulinemia, free fatty acids and triglycerides.
Results: Sham procedure did not modify any of the parameters evaluated. Insulin sensitivity diminished in HSu rats as the constant of the insulin tolerance test (KITT) decreased significantly to 2.46±0, 30 from a control value of 4, 39± 0,29. Basal glycemia was significantly increased in HSu rats (control = 83.33±1.81 mg/dL; HSu = 114.6±12.6 mg/dL). One week after CSN resection, insulin sensitivity increased in HSu rats and hyperglycemia was completely reversed to control values. Two weeks after CSN resection normoglycemia was maintained and insulin sensitivity was completely restored to control values. From the 2nd to 3rd week normoglycemia and insulin sensitivity were maintained, though the animals continued to be submitted to the hypercaloric diet. Weight, total and visceral fat were not modified by CSN resection. HSu diet significantly increased blood pressure and CSN resection reversed this increase.
Conclusion: Our results demonstrate that CSN resection reverses diet-induced HT and IR and may represent a potential therapeutic approach for the treatment of the metabolic syndrome.
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GRK2 is a novel modulator of insulin cardioprotective pathway during ageing and diet-induced insulin resistance
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Background and aims: The heart is a constitutive energy-demanding organ. Although mitochondrial lipid oxidation is the principal energy source, the maintenance of glucose utilization is necessary for normal cardiac function and alterations in cardiac energy metabolism downstream neurohormonal stimulation play a crucial role in the pathogenesis of heart failure. G protein-coupled receptors (GPCRs) are essential regulators of cardiovascular physiopathology that are under the control of G protein-coupled receptor kinases (GRKs). In particular, the GRK2 isoform is a key regulator of cardiac physiology and increased levels of cardiac GRK2 correlate with cardiac dysfunction and heart failure in humans. Previous works have described the potential role of GRK2 as a modulator of insulin signaling in several tissues, including the heart of young animals. In this work we explore the effect of lowering GRK2 levels in adult mice and under insulin resistance-promoting conditions in order to mimic two different situations in which humans start to develop heart complications.
Materials and methods: We have used GRK2 hemizygous mice ($GRK2^{+/-}$) as a model to assess the effects of a sustained systemic inhibition of GRK2 in heart tissue. Using microarray RNA expression techniques we have compared the transcriptional profile of the cardiac tissue of 9 months old wild-type and $GRK2^{+/-}$ C57BL6 mice, and also characterized the hearts of these animals by echocardiographic and morphometric analysis. We also studied cardiac insulin sensitivity with age and under high fat diet (HFD) by Western Blot.
Results: In 9 month-old $GRK2^{+/-}$ mice we detected changes in the gene expression profile of genes reported to be modulated by insulin or beta-agonists as well as in genes regulated by exercise and physiological heart hypertrophy that were in line with data obtained from the morphometrical studies. We also find that in $GRK2^{+/-}$ mice insulin sensitivity was preserved with age and after a high fat diet feeding. Consequently, we have found that GRK2 levels are increased in the heart of C57BL6 mice fed with HFD as well as in obese (ob/ob) mice.
Conclusion: These results demonstrate the importance of maintaining GRK2 levels under a certain physiological level to preserve insulin sensitivity in cardiac tissue. These data help explain the cardioprotective effects described for the inhibition of this kinase.
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Therapeutic properties of the vanadium compound, $VO(dmpp)_2$, by ex vivo and in vivo studies in diabetic GK rats
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Background and aims: Intensive research has been carried out to find compounds to substitute insulin in treatment of diabetes. The bis(1,2-dimethyl-3-hydroxy-4-pyridinonato)oxovanadium (IV), $VO(dmpp)_2$, has shown anti-diabetic effects by *ex vivo* study in Wistar (W) rats and *in vivo* study in obese Zucker rats. We aimed to confirm the therapeutic properties of $VO(dmpp)_2$ in non-obese diabetic Goto-Kakizaki (GK) rats.
Materials and methods: The effects of $VO(dmpp)_2$ on glucose uptake were assessed in W and GK rat adipocytes using [3-^{3}H]-glucose radioactive assay and compared to the effects of insulin and bis(maltolato)oxovanadium (IV), BMOV. W and GK rats were treated daily, during 21 days, with $VO(dmpp)_2$ (44 μg/kg) to show its effects on glycemia, OGTT and insulin signalling pathway using SDS-PAGE.
Results: In adipocytes from both W and GK rats the increase of glucose uptake, relative to basal value, achieved by 100 μmol/l $VO(dmpp)_2$ (193 ± 20% and 254 ± 21%, respectively) and by 500 μmol/l BMOV (152 ± 23% and 219 ± 37%, respectively) were similar to an insulin concentration of 10 ng/ml (176 ± 14% and 201 ± 29%, respectively) considered as the normal blood insulin concentration after a meal. Non-toxic concentrations of 100 and 500 μmol/l of $VO(dmpp)_2$ promoted, respectively, glucose uptake enhancement of 193 ± 20% and 322 ± 16% (1.9 and 3.2 times higher, respectively, $p<0.01$ and $p<0.001$, relative to basal value) in W adipocytes and 254 ± 21% and 424 ± 37% (2.5 and 4.2 times higher, respectively, $p<0.001$ and $p<0.001$, relative to basal value) in GK adipocytes. The same concentrations of BMOV produced a lower glucose uptake effect in both types of adipocytes (100 μmol/l: 111 ± 20%, not significant; 500 μmol/l: 153 ± 23%, $p<0.05$, relative to basal value for W adipocytes, vs 100 μmol/l: 145 ± 26%, $p<0.05$ and 500 μmol/l: 219 ± 37%, $p<0.01$, relative to basal value for GK adipocytes). Thus, $VO(dmpp)_2$ shows in W and GK rat adipocytes a better efficiency on glucose uptake compared to BMOV ($p<0.01$ or less), which is similar or even higher than that of insulin. *In vivo* study shows that after 8 days of treatment, $VO(dmpp)_2$ improved glycemia in GK rats compared to GK rats treated with placebo (8.4 ± 0.3 vs 10.1 ± 0.2 mmol/l, $p<0.001$). After 21 days of treatment, the body weights of W and GK rats were not changed by $VO(dmpp)_2$ but this compound improved glucose tolerance profile in GK rats compared to GK rats treated with placebo (13.1 ± 0.5 vs 20.6 ± 0.7 mmol/l/min, $p<0.001$), despite no increase in plasma insulin levels before and during OGTT. In W rats, OGTT was not changed by $VO(dmpp)_2$ treatment, however, plasma insulin levels were significantly lower in animals treated with this compound when compared to placebo group (0 min: 7.1 ± 1.8 vs 20.6 ± 3.1, $p<0.01$; 30 min: 29.8 ± 3.7 vs 50.9 ± 7.0, $p<0.05$; and 120 min: 7.6 ± 1.0 vs 21.2 ± 5.5 μU/ml, $p<0.05$). In W and GK rats $VO(dmpp)_2$ significantly promoted IRS2 expression ($p<0.05$) and phosphorylated AKT ($p<0.001$ and $p<0.05$, respectively, relative to respective controls) and in GK rats reduced the increase of PTP1B expression ($p<0.001$, relative to GK treated with placebo) which indicates that these proteins are targets of $VO(dmpp)_2$ action.
Conclusion: $VO(dmpp)_2$ shows anti-diabetic properties by improvement on glucose uptake, glycemia and OGTT by interaction with the insulin signalling pathway. These therapeutics properties show that $VO(dmpp)_2$ is a promising molecule for novel therapy of type 2 diabetes.

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Insulin receptor-sensitising polyaminosterols as a novel therapeutic approach for the treatment of type 2 diabetes

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Background and aims: Insulin governs glucose homeostasis by regulating glucose synthesis by the liver and the uptake of glucose by muscle and fat. A disturbed regulation can lead to type 2 diabetes (T2D), which is characterised by insulin-resistance in muscle, fat, and liver and the production of an amount of insulin that is insufficient to compensate for this resistance. In the present study, we aim to assess the potential therapeutic value of a novel class of polyaminosterols for the treatment of T2D.
Materials and methods: Various polyaminosterols were analyzed for their effect on GLUT4 translocation, glucose uptake, and insulin signalling in 3T3-L1 adipocytes and/or L6 myoblasts. One of the compounds that displayed the highest insulin-sensitizing effect was tested in lean and DIO mice (IP administration) to evaluate its action on glucose tolerance and insulinemia.
Results: In 3T3-L1 adipocytes, certain novel polyaminosterols enhanced insulin action on GLUT4 translocation, glucose uptake, and lipolysis. Moreover, these sterols reduced the half-maximal dose (ED50) of insulin regarding GLUT4 translocation (from 1.6 to 0.3 nM; P<0.0001), while their insulin-enhancing effect was maintained in insulin-resistant adipocytes. In 3T3-L1 adipocytes as well as in L6 myoblasts, these molecules induced an increase in the phosphorylation and activation of the insulin receptor (IR). This sensitization appeared to be a direct action of the sterols on the IR and to be distinct from the mode of action of the well-known aminosterol trodusquemine (MSI-1436). Trodusquemine, described to increase IR activation through inhibition of phosphatase PTP1B, did not sensitize the IR in our cells. Treatment of lean mice as well as insulin-resistant DIO mice with one of our polyaminosterols ameliorated glucose tolerance for all doses tested, without causing hypoglycemia. This amelioration was not accompanied by increased insulin levels suggesting that the sterol acted directly on the insulin-sensitive tissues.
Conclusion: We have identified novel polyaminosterols that increase insulin action in vitro and in vivo, under normal as well as under insulin-resistant conditions. Given the essential role of insulin-resistance in T2D, we speculate that these polyaminosterols could have potential for the development of a novel therapeutic avenue for the treatment of T2D.
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Dapagliflozin improves muscle glucose uptake and increases hepatic glucose production in type 2 diabetes mellitus individuals

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Background and aims: Chronic elevation in plasma glucose concentration increases the risk of microvascular complications and aggravates the core defects of T2DM, i.e. insulin resistance and beta cell dysfunction. Studies in experimental animals have demonstrated that lowering the plasma glucose concentration with phlorizin improves insulin sensitivity in diabetic animals. The aim of the present study was to examine whether lowering the plasma glucose concentration by inhibiting SGLT2 with dapagliflozin improves insulin sensitivity in T2DM individuals.
Materials and methods: 11 males with T2DM (age= 51±3; BMI= 31.1±1.6) received: (i) 75-gram OGTT; and (ii) euglycemic hyperinsulinemic clamp. After completing the baselines studies, subjects were admitted for 3 days to the CRC for the measurement of hepatic glucose production (HGP) rate and starting the dapagliflozin treatment. On day 1, HGP was measured after an overnight fast with ^{3}H-glucose infusion. On day 2, the ^{3}H-glucose infusion was started after an overnight fast and after 3-hour equilibration period, subjects ingested 10 mg dapagliflozin and the ^{3}H-glucose infusion was continued for additional 4 hours. Subjects received dapagliflozin (10 mg/day) for additional 6 days, and at day 3 the HGP was repeated and on days 6 and 7 the OGTT and the euglycemic clamp were repeated.
Results: Dapagliflozin increased urinary glucose excretion by 69±7 gram/24 hours and this glucosuria was accompanied by a 28±6 mg/dl and 72±11 mg/dl decrease in the FPG and 2-hour plasma glucose concentrations, respectively, (p<.001 for both). The decrease in plasma glucose concentration was accompanied by 28% increase (from 4.6±0.6 to 5.9±0.5 mg/kg.min, p<0.001) in the whole body glucose disposal (TGD) rate, and the increase in TGD (19%, p<0.01) remained significant after correcting for urinary glucose loss during the insulin clamp. Surprisingly, the decrease in the plasma glucose concentration was accompanied by 16% (p<0.05) increase in the rate of HGP and 23% increase in plasma glucagon concentration. Dapagliflozin also improved beta cell function. Following dapagliflozin treatment, there was ~2-fold increase in the insulin resistance/insulin sensitivity (disposition) index.
Conclusion: Lowering the plasma glucose concentration with SGLT2 inhibitor, dapagliflozin, improves both core defects of T2DM, insulin resistance and beta cell dysfunction.
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ISIS PTP-1B$_{RX}$, a novel protein tyrosine phosphatase antisense inhibitor, improves both insulin and leptin action and increases HMW adiponectin levels in obese subjects

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Background and aims: PTP-1B is a negative regulator of both insulin and leptin action. Since reduction of PTP-1B activity enhances insulin and leptin sensitivity in preclinical models, PTP-1B inhibitors could be promising therapeutics for T2DM and obesity. However, very few studies have examined the role of PTP-1B in human insulin and leptin resistance due to a lack of specific PTP-1B inhibitors. We developed a specific PTP-1B antisense inhibitor (ISIS PTP-1B$_{Rx}$) and investigated the safety, tolerability and PD of ISIS PTP-1B$_{Rx}$ administered subcutaneously in obese subjects.
Materials and methods: In the present randomized (3:1) double-blind, placebo-controlled Phase 1 study, single or multiple dose levels of a specific PTP-1B antisense inhibitor, ISIS-PTP-1B$_{Rx}$, (50, 100, 200 and 400 mg) were administered subcutaneously to 48 healthy subjects (age=49±11yrs, BMI=30±2.6) over a 4 week treatment period. Primary pharmacodynamic (PD) parameters were analyzed on Day 29 and Day 36.
Results: Treatment with ISIS-PTP-1B$_{Rx}$ for 4 weeks resulted in a dose-dependent increase in HMW adiponectin, with > 4-fold increase in HMW adiponectin in the 400 mg group. As compared to placebo, statistically significant reduction of leptin levels was also observed in ISIS PTP-1B$_{Rx}$ cohorts, with a maximal reduction from baseline of 14% (p=0.0295 vs. placebo). Furthermore, placebo-corrected plasma insulin levels as well as HOMA-IR were significantly reduced with no changes in FPG. ISIS PTP-1B$_{Rx}$ was well tolerated and no SAEs were observed. No clinically significant effects on vital signs, ECG, hepatic or renal function and no hypoglycemia were observed.
Conclusion: These data suggest that PTP-1B may be a novel therapeutic target for T2DM and obese patients and support further development of ISIS-PTP-1B$_{Rx}$ in these populations.

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The novel myokine YKL-40 is increased by exercise and inhibits TNFalpha-induced inflammation and insulin resistance in human skeletal muscle cells

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Background and aims: The glycoprotein YKL-40 is a member of the 18 glycosyl hydrolase family, but has no enzymatic activity. It is known to play a role in tissue remodeling and inflammatory processes and elevated serum levels of YKL-40 are found in patients with e.g. cardiovascular disease, insulin resistance and type 2 diabetes. Based on proteomic profiling of secreted proteins from primary human skeletal muscle cells (hSkMC) we identified YKL-40 as a novel myokine. However, the physiological role of YKL-40 remains largely unknown. The aim of this study was to investigate the effect of muscle

contraction on YKL-40 expression and release *in vitro* and *in vivo*. Furthermore, we investigated potential autocrine effects of YKL-40 in hSkMC.
Materials and methods: HSkMC were *in vitro* differentiated and electrical pulse stimulation (EPS) (1 Hz, 2 ms, 11.5 V) was applied for 24h. RNA, protein and supernatants were obtained, and YKL-40 expression and release was analyzed by quantitative RT-PCR, ELISA and Western Blot. In a human study, blood samples were obtained before and after 60 min of cycling at 70% VO_2max and analysed by ELISA. Furthermore, hSkMC were treated with TNFα in combination with YKL-40, and RNA and supernatants were collected. Effects on IL-6, IL-8 and MCP1 expression and secretion were analyzed by RT-PCR and ELISA. In addition, insulin signaling after acute TNFα treatment +/- YKL-40 was assessed at the level of Akt and GSK3 phosphorylation using Western Blot analysis.
Results: *In vitro* contraction of hSkMC induced by EPS significantly increased YKL-40 mRNA expression (~2fold) as well as secretion (~1.25fold). In addition, we observed a significant increase of YKL-40 serum concentrations after 60 min cycling. The concentrations increased from 15.2±0.8 ng/ml before exercise to 20.1±0.9 ng/ml immediately after exercise. In the post exercise period YKL-40 serum concentrations were significantly reduced after 120 min and normalized close to the basal level at 15.8±0.7 ng/ml. Stimulation of hSkMC with TNFα increased MCP1, IL-6 and IL-8 mRNA expression and secretion. These TNFα-induced effects were prevented by chronic treatment with YKL-40. Moreover, YKL-40 treatment inhibited TNFα-induced insulin resistance at the level of Akt and GSK3 phosphorylation while YKL-40 itself did not impact on Akt or GSK3 activation.
Conclusion: YKL-40 is upregulated by muscle contraction and able to prevent TNFα-induced inflammatory processes by affecting MCP1, IL-8 and IL-6 mRNA expression and secretion. In addition, YKL-40 treatment blocks TNFα-induced insulin resistance. In conclusion, the novel myokine YKL-40 inhibits harmful effects of TNFα and may hence represent an autoprotective factor of skeletal muscle tissue that is upregulated by exercise.
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The effects of long-term valsartan treatment on skeletal muscle fatty acid handling in humans with impaired glucose metabolism
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Background and aims: Blockade of the renin-angiotensin system reduces the incidence of type 2 diabetes in humans with impaired glucose metabolism (IGM). Nevertheless, underlying mechanisms remain to be established. The objective of this study was to investigate the effects of the angiotensin II type 1-receptor blocker valsartan (VAL) on skeletal muscle FA handling in subjects with IGM.
Materials and methods: In this randomized, double-blind placebo-controlled trial, fasting and postprandial skeletal muscle FA handling were assessed at baseline and after 26 weeks of treatment with VAL or placebo in 26 subjects with IGM. Fasting and postprandial skeletal muscle FA handling were determined by combining the forearm balance technique with stable isotopes of palmitate. [$^2H_2$]-palmitate was infused intravenously to label endogenous triacylglycerol (TAG) and NEFA in the circulation, and [U-^{13}C]-palmitate was incorporated in a high-fat mixed meal (2.6 MJ, 61% energy from fat) to label chylomicron-TAG. Muscle biopsy samples were taken to determine intramuscular TAG, diacylglycerol (DAG), FFA and phospholipid content, their fractional synthetic rates and degree of saturation, and mRNA expression of oxidative genes.
Results: VAL decreased saturation of intramuscular TAG and DAG fractions but did not affect net muscle uptake of [$^2H_2$]-palmitate, very low-density lipoprotein ([$^2H_2$])-TAG and chylomicron ([U-^{13}C])-TAG, and muscle mRNA expression. VAL decreased FA spillover, as estimated by circulating [U-^{13}C]-palmitate, and FFA rate of appearance, and tended to decrease chylomicron-TAG concentrations.
Conclusion: VAL treatment for 26 weeks decreased saturation of skeletal muscle TAG and DAG stores, suggesting altered intramuscular lipid partitioning of FA. The VAL-induced reduction in postprandial FA spillover, endogenous lipolysis, and chylomicron-TAG concentrations indicate improved adipose tissue lipid buffering capacity.
Clinical Trial Registration Number: NTR721; ISRCTN42786336
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Low dose pioglitazone-induced reduction in skeletal muscle TACE activity and TNFalpha is associated with an improvement of glycaemic control in type 2 diabetic patients
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Background and aims: Pioglitazone (PIO) is a PPARγ-agonist employed in the treatment of patients with type 2 diabetes mellitus (T2DM) at dosage ranging from 15 to 45 mg/day. PIO treatment has been shown to improve insulin sensitivity, glycemic control and inflammatory state. TNFα is an inflammatory cytokine whose plasma levels are elevated in insulin resistance related disorders. TNFα Converting Enzyme (TACE) is the enzyme producing TNFα from its precursor, and it is physiologically inhibited by Tissue Inhibitor of Metalloproteases 3 (TIMP-3). T2DM is associated with higher TACE activity and lower TIMP-3 levels in skeletal muscle (SKLM). In this study we examined the effect of low-dose PIO (15 mg/day) on TIMP-3/TACE dyad in T2DM.
Materials and methods: Twenty type 2 diabetic patients treated with metformin and/or sulfonylurea were randomized to receive PIO 15 mg/day or placebo (PLC) for 6 months. At baseline and after 6 months of treatment the subjects underwent to: (I) OGTT, (II) euglycemic hyperinsulinemic clamp, (III) SKLM biopsy to evaluate expression levels of insulin signaling cascade proteins, TNFα and TIMP-3/TACE by Western Blot, (IV) TACE activity evaluation on SKLM extracts by an *in vitro* fluorometric assay.
Results: Fasting and 2-hours post-load plasma glucose and HbA1c levels decreased by 10 to 15% ($p<0.05$) after PIO and did not change significantly after PLC. M/I value and insulin secretion/insulin resistance (disposition) index were significantly increased by 25% in PIO ($p<0.05$) but not in PLC group. SKLM TNFα protein levels decreased by 30% (0.26±0.05 to 0.18±0.05 A.U; $p=0.02$) in PIO but not in PLC treated patients. At baseline, TACE and TIMP-3 expression was similar between PLC and PIO group, and did not change in either group after 6 months of treatment. At the end of the study, TACE activity was significantly decreased by 80% as compared to baseline (0.29±0.07 to 0.05±0.01 A.U, $p=0.005$) in PIO whereas in PLC group TACE activity was reduced by ~ 36% in comparison to baseline (0.22±0.1 to 0.14±0.07 A.U, $p=0.07$). In addition, in PIO group the decrease in TACE activity correlated with the changes of HbA1c ($r=0.87$, $p=0.01$) and fasting plasma glucose ($r=0.88$, $p=0.01$). Interestingly, at the end of the study Insulin Receptor β (IRβ) expression was significantly increased by 22% in comparison to baseline in PIO group ($p=0.028$) but not in PLC group. The delta change of IRβ levels was significantly higher in PIO than PLC group (+22% vs. -9%, $p=0.008$ respectively). IRS1, AKT, p-AKT Ser^{473}, Jun N-terminal kinase (JNK), p-JNK Thr^{183}/Tyr^{185}, Extracellular signal-regulated kinase (ERK) and p-ERK Thr^{202}/Tyr^{204} levels were not different at baseline in either group and no changes were observed after 6 months.
Conclusion: The improvements in insulin sensitivity and glycemic control observed in type 2 diabetic patients treated with low-dose PIO (15 mg/day) were paralleled by increased SKLM IRβ expression, reduced TACE enzymatic activity and lower TNF-α protein levels, suggesting an important role of sub-inflammation in regulating insulin sensitivity in humans.
Clinical Trial Registration Number: NCT01223196
Supported by: Takeda

PS 040 Muscle metabolism

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Effect of exercise on fatty acid and glucose metabolism in cultured human myotubes

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Background and aims: Physical activity has an important place in prevention and treatment of type 2 diabetes. In this study we examined fuel handling in skeletal muscle cells (myotubes) isolated from subjects with normal and abnormal glucose metabolism before and after 12 weeks of extensive endurance and strength training.
Material and methods: Skeletal muscle cells were isolated from m. vastus lateralis of male volunteers before and after an intervention period, including two endurance and two strength training sessions of 6Yuan0 min. per week. Subjects with normal as well as abnormal glucose metabolism were included. The muscle cells were cultured and differentiated into multinucleated myotubes. Lipid and glucose metabolism were studied using radiolabeled 1-[14C] oleic acid and D-[14C(U)]glucose, respectively. In the preintervention experiments the cells were incubated with the PPARδ agonist GW501516 for the last 4 days of the differentiation period. Gene expression was studied using qPCR.
Results: Both groups of subjects showed an increased strength capacity (measured by three different strength tests), increased endurance capacity (measured by VO2 max) and increased insulin sensitivity (measured by clamp GIR). The group with abnormal glucose metabolism had the largest increases, except for in two of the strength tests. Myotubes isolated after a training period of 12 weeks showed an increased oxidation of oleic acid as well as glucose compared to myotubes isolated before the 12 weeks training period. The total uptake of oleic acid in myotubes was also increased after the exercise intervention period. Preliminary data indicated that the expression of some genes important for lipid metabolism and mitochondrial function/biogenesis, such as CD36, PGC1α, Cyc1 and PPARδ, were increased in myotubes after the exercise intervention period.
Conclusion: Exercise have a positive effect on glucose oxidation and fatty acid uptake and oxidation that appears to be conserved in human myotubes.
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DNA demethylation enhances myoblasts hypertrophy during the late phase of myogenesis activating the IGF-1 pathway

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Background and aims: Skeletal muscle damage is linked with metabolic syndrome. Cachexia is the dramatic weight loss and muscle atrophy seen in patients with chronic illness including diabetes mellitus. Cachexia causes dramatic loss of skeletal muscle and eventually leads to immobility. During muscle differentiation, myoblasts withdraw from the cell cycle and form myotubes. This process is principally governed by muscle regulatory factors (MRFs) and muscle specific protein such as Myosin Heavy Chain (MyHC). Instead, the end of myogenesis is lead by Myostatin (Mnst). Muscle hypertrophy is an increment of existing muscle fibers size, associated with an enhanced protein accumulation. Insulin Growth Factor 1 (IGF-1) plays an important role in hypertrophy induced by Growth Hormone (GH) treatment or exercise. Also the epigenetic mechanisms of DNA methylation are crucial in transcriptional control of genes involved in myogenesis. However, the specific impact of DNA hypomethylation on late phase of differentiation and on muscle mass regulation is not complete understood. To clarify DNA methylation role in muscle differentiation, we studied the effects of the most common DNA-demethylating agent 5-azacytidine (AZA) on the late phase of muscle differentiation and early stage of hypertrophy.
Materials and methods: The epigenetic process involved in myogenesis was studied with the DNA-demethylating agent 5-azacytidine (AZA). We induced muscle differentiation in C2C12 mouse myoblasts in presence of 5 μM AZA and growth (GM) or differentiation (DM) medium for 48, 72 and 96 hours. To study a potential AZA hypertrophic effect, we stimulated 72h differentiated myotubes with AZA for 24h. Unstimulated cells were used as control. By Western blot and Immunofluorescence analysis, we examined AZA action on myogenic regulatory factors expression, hypertrophic signaling pathway and myotubes morphological features.
Results: During differentiation, protein levels of myogenic markers, Myf6 and Myosin Heavy Chain (MyHC), were higher in AZA stimulated cells compared to control. Myostatin and p21 analysis revealed morphological changes which reflect a tendency to hypertrophy in myotubes. In AZA stimulated neo-formed myotubes, we observed that IGF-1 pathway, kinases p70 S6 and 4E-BP1 were activated. Furthermore, AZA treatment increased MyHC protein content in stimulated neo myotubes. Statistical evaluation was performed using a *t*-test. Data are presented as means ± SD. Results were considered statistically significant if $p \leq 0.05$.
Conclusion: Our work demonstrates that DNA demethylation could play an important role in promoting the late phase of myogenesis, activating endocellular pathways involved in protein increment and stimulating the hypertrophic process. These results constitute a proof of principle of an hypothetical clinical use of this agent in conditions of muscle mass impairment/hypotrophy in chronic disease (diabetes mellitus, metabolic syndrome). Furthermore, the demonstration that AZA-induced DNA demethylation accelerates skeletal muscle differentiation and hypertrophy might constitute a new model to study the regeneration process against atrophy damage in diabetic muscle cachexia.

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Knockout of STAT3 in skeletal muscle does not enhance insulin action

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Background and aims: Activation of signal transducer and activator of transcription 3 (STAT3) is increased in skeletal muscle from insulin resistant patients. Accordingly, STAT3 activation has been implicated in the development of skeletal muscle insulin resistance, though its precise role, in vivo, remains to be elucidated. Thus, the purpose of this study was to determine whether knockout of STAT3 in skeletal muscle enhances insulin-stimulated glucose uptake, and also, whether it would prevent insulin resistance in a high-fat (HF) diet-induced obesity mouse model.
Materials and methods: Mice with skeletal muscle-specific knockout of STAT3 were generated by crossing mice containing loxP sites flanking exon 22 of STAT3 (a region required for phosphorylation and activation) with mice harboring Cre recombinase under the control of the muscle creatine kinase promoter. Beginning at 10 weeks of age, male mice with muscle-specific knockout of STAT3 (mKO) and their floxed/wildtype (WT) littermates were fed a low-fat (LF; 10% of calories from fat) or HF (60% of calories from fat) diet for 20 days. Body composition and energy expenditure were measured using magnetic resonance imaging and indirect calorimetry, respectively. Basal and insulin-stimulated (0.36nmol/L [60μU/mL]) glucose uptake were measured using a 2-deoxyglucose uptake (2DOGU) assay in isolated soleus and extensor digitorum longus (EDL) muscles.
Results: STAT3 protein abundance was reduced by ~50% in soleus and EDL muscles of mKO vs. WT mice. Body weight and body fat percentage did not differ in WT vs. mKO mice on LF diet, though these parameters were significantly increased by HF diet. Energy expenditure did not differ between WT and mKO mice on either a LF or HF diet. Interestingly, insulin-stimulated 2DOGU (insulin 2DOGU minus basal 2DOGU) in LF-fed mice did not differ between genotypes in soleus (WT = 0.37±0.05; mKO = 0.32±0.05 μmol/20 min/g muscle) or EDL (WT = 0.29±0.02; mKO = 0.30±0.05 μmol/20 min/g muscle). In addition, HF diet significantly decreased insulin-stimulated 2DOGU to the same extent in mKO and WT soleus and EDL muscles (WT = 0.21±0.04; mKO = 0.20±0.04 and WT = 0.16±0.03; mKO = 0.15±0.02 μmol/20 min/g muscle, respectively).
Conclusion: Although activation of STAT3 is increased in skeletal muscle from insulin resistant patients, knockout of STAT3 does not enhance skeletal muscle insulin action. Furthermore, STAT3 knockout does not protect against HF diet-induced insulin resistance in skeletal muscle.
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Determination of glucose metabolic fluxes in live myoblasts by microfluidic nanosensing and data analysis

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Background and aims: Glucose is the main energy source for cells in an organism and its blood concentration is tightly regulated in healthy individuals. However impaired glycemia control has been found in metabolic syndrome and diabetes. Dissecting the dynamics of the different phenomena involved in glucose handling is relevant to identify which mechanisms are disrupted under disease conditions. In this work we performed an integrated quantitative study of glucose processing in living myoblast cultures.
Materials and methods: Murine myoblasts were cultured in a perfused microfluidic chip that allowed dynamic detection with minimal culture perturbation. Medium was continuously collected at the outlet of the culture chamber and its glucose concentration measured via enzymatic assay. Glucose uptake was then determined from the difference of concentration between inlet and outlet. Myoblasts were also transfected with the FRET nanosensor FLIPglu-600μΔ13V for determining cytosolic glucose concentration through live cell imaging. Experiments were carried out at multiple flow rates and inlet glucose concentrations. Mathematical modeling assisted experimental data analysis for the determination of the kinetic constants.
Results: We found a linear relationship between glucose concentration at the inlet (3-13 mM) and at the outlet, where it was reduced due to glucose consumption by the cell population. These measurements were used to derive myoblast glucose uptake rate, which was calculated equal to 34 ± 9.7 fmol/min/cell when the inlet glucose concentration was 5.6 ± 0.3 mM. We estimated that the actual glucose concentration in the immediate vicinity of the cell membrane was about 84% of the inlet one in our experimental conditions. Using an intra-cellular glucose FRET-based nanosensor, we obtained the intracellular glucose concentration. We found that the cytosolic glucose concentration was a linear function of the extracellular one, in the range 0.5-5 mM. In particular, it was approximately 12% of the extracellular concentration in the immediate vicinity of cell membrane. By integrating the results above with a simple mathematical model, we calculated the characteristic times of glucose transport across the membrane, equal to 3.7 s, and of phosphorylation, 0.5 s. From these data we derived the respective kinetic constants of the two processes: 0.27 s^{-1} and 2.01 s^{-1} in living cells. The model was then used to calculate the time a cell needed to reach a new steady state after a change in extracellular glucose conditions, which was about 2 s.
Conclusion: We show a methodology for quantitatively dissecting the contribution of glucose diffusion from the bulk of medium to the cell surface, of single-cell glucose uptake and of glucose phosphorylation kinetics in living cells. Thanks to the minimal perturbation of culture conditions during the measurements and to the precisely controlled microfluidic microenvironment, this system can be suitable to study dynamic alteration in glucose handling also under pathological conditions, i.e. hyperglycemia, hyperinsulinemia or rapid pulses of them.

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High fat diet induces selective alterations in microRNA expression profile in rat skeletal muscle

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Background and aims: Skeletal muscle is the major tissue responsible for insulin mediated glucose disposal, and is a principal site for the development of lipid-induced insulin resistance. MicroRNAs (miRNAs) are small non-coding RNAs that bind to the 3'-UTR of specific target mRNAs, resulting in reduced RNA stability or inhibition of translation. MiRNAs have widespread effects to regulate cellular processes and altered expression of specific miRNAs including miR-29, miR-107, miR-143 and miR-126, have been linked to obesity, insulin resistance (IR) and type 2 diabetes (T2D). However, few systematic assessments of miRNA expression in diet-induced IR in muscle have been performed. Here we aimed to identify miRNAs that were dysregulated in muscle from high fat diet (HFD)-fed rats and to predict the effects using bioinformatic tools.
Materials and methods: Six week old male Wistar rats were fed ad lib with HFD or chow for 5 or 20 weeks and following euthanasia, tibialis cranialis (TC) muscles were snap-frozen and total RNA was extracted (n=9-10 per group). Candidate miRNAs for expression screening were identified on the basis of evidence of association with IR, insulin signalling, metabolism or T2D in the literature. Relative quantitation of candidates (20) was performed using a probe based Real-time PCR with normalisation to small nucleolar RNA expression using proprietary kits. Two-way ANOVA was used to test for differences in expression levels between the groups, with factors of diet and study duration. We used TargetScan to predict the effects of the differentially expressed miRNAs on relevant target protein expression.
Results: Expression of miR-126*, miR-22 and miR-187 were all reduced by HFD-feeding (p=0.046, p=0.0011 and p=0.0020 respectively), with the magnitude of each effect tending to be bigger after 20 weeks of diet. There was also a similar trend for miR26a expression (p=0.086). Interestingly, miRs-26a, 185, 21, 29c, 22, 29a, 143 and 451 all showed increases in expression as a result of study duration, implying that relatively small differences in age might have widespread effects on miRNA expression programmes (rats were 15 weeks older in the 20 week-fed than the 5 week-fed groups). In contrast, miR-10b expression was reduced in the older rats. In the case of miR-29a, an age-related up-regulation in expression was reduced by HFD-feeding (interaction p=0.0108). Other miRNAs were not detectably regulated by either factor. TargetScan analysis identified a number of relevant miR-22 predicted targets, including Sirtuin-1 (an energy sensor and a regulator of PPAR-γ and PGC-1 in mitochondria), phosphatase and tensin homologue (PTEN; an AKT phosphatase) and SMAD4 (a key component of the TGF-β signalling pathway). In addition, miR-29a predicted protein targets include insulin receptor substrate-1, PTEN, and PIK3R1 (the p85 regulatory subunit of PI3-kinase).
Conclusion: Our analysis has demonstrated that HFD-induced IR is associated with altered expression of several miRNAs and that these candidates possess putative protein targets with major roles in insulin signalling and metabolism. Using interventional studies, we will now seek to establish whether these miRNAs can determine insulin sensitivity in skeletal muscle through regulation of these or other protein targets.
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Debio 0930, a novel direct AMPK activator, improves glycaemic control and lipid profile in metabolic disease models

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Background and aims: AMP-activated protein kinase (AMPK), a key cellular energy sensor, is a promising target for the treatment of metabolic disorders. This study describes the in vivo metabolic effects of a novel direct AMPK activator, Debio 0930, which is under preclinical development for type 2 diabetes.
Materials and methods: Debio 0930, a small molecule, activated at least two recombinant human AMPK heterotrimers containing the ß1 subunit in a submicromolar rang (5-12 fold stimulation). In human HepG2 hepatocytes, Debio 0930 promoted AMPK activation without any changes in AMP/ATP ratio, supporting a direct mechanism of action. Debio 0930 was also found to have attractive DMPK properties with a favorable in vitro safety profile.
Results: In vivo efficacy of the compound was examined in diet-induced obese (DIO) mice and dyslipidemic hamsters. Following 4-week oral repeat dosing in the DIO mice, Debio 0930 at 60 mg/kg BID reduced fasting plasma glucose and hepatic glucose production, and ameliorated insulin resistance (HOMA-IR). In addition, the treatment demonstrated marked improvement in liver lipid content (TG, Chol, FFA). In dyslipidemic hamsters, oral administration of Debio 0930 at 60 mg/kg for two weeks lowered fasting blood glucose and enhanced the HDL/LDL ratio. Plasma lipoprotein analysis demonstrated that Debio 0930 caused a significant reduction in VLDL and LDL and a substantial rise in HDL compared to vehicle treated animals. Food intake was not affected by Debio 0930 in either study.
Conclusion: In conclusion, Debio 0930 is a novel direct AMPK activator that improves both glycemic control and lipid profile, and potentially could be a new oral agent for the treatment of type 2 diabetes and dyslipidemia.

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Effects of chronic variable stress on insulin sensitivity, inflammation and plasma hormone levels

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Background and aims: Stress is a state of threat to homeostasis by intrinsic or extrinsic forces. Studies show that chronic stress and posttraumatic stress disorder contribute to metabolic diseases such as visceral obesity, metabolic syndrome and type 2 diabetes. However, the mechanisms linking stress to metabolic impairment are still unclear. Our aim was to characterize how chronic variable stress (CVS) affects insulin sensitivity in mice. Furthermore, cytokines and hormones, potentially regulating stress-induced changes in insulin sensitivity, were analyzed.
Material and methods: Age- and body weight (BW)-matched male C57BL/6 mice (n=9-11) were exposed to a random series of stressors for 15d (CVS), the unstressed controls (Ctrl) were housed separately. Body composition was analyzed with NMR. After CVS, mice were fasted for 4h and blood was collected (09:00-10:30am) for corticosterone measurements by RIA. Subsequently, insulin sensitivity was analyzed with hyperinsulinemic-euglycemic clamps. At the end of clamps, mice were euthanized and blood and tissues were collected for cytokine (n=8-10) and hormone (n=9-10) analysis by multiplex immunoassay and gene expression (n=7-10) with RT-PCR. Values were analyzed with two-tailed unpaired t-tests, $p<0.05$ was considered significant.
Results: Compared to Ctrl, CVS decreased BW (24.7±0.5, Ctrl: 26.6±0.6 g; $p<0.05$) and lean mass (21.5±0.4, Ctrl: 23.6±0.5 g; $p<0.01$) and increased fat mass (2.6±0.1, 1.9±0.1g; $p<0.0001$). Accordingly, plasma levels of corticosterone were higher in the CVS group (186.2±47.5, Ctrl: 36.6±8.3 ng/ml; $p<0.01$). Basal endogenous glucose production (EGP) was decreased after CVS (19.2±0.9, Ctrl: 23±1 mg/kg/min; $p<0.05$). Whole-body insulin sensitivity was not affected by CVS (glucose infusion rate: 65.4±2.3, Ctrl: 64.3±3.7 mg/kg/min). However, suppression of EGP by insulin was impaired (92±6, Ctrl: 117±7%; $p<0.05$), while insulin stimulation of glucose turnover in peripheral tissues was increased as indicated by the glucose rate of disappearance (Rd) (356±20, Ctrl: 276±9%; $p<0.05$) after CVS. CVS increased IL-10 (300±38, Ctrl: 212±19 pg/ml; $p<0.05$) and decreased RANTES (143±40, Ctrl: 281±32 pg/ml; $p<0.05$) in plasma. Ghrelin (9.85±0.62, Ctrl: 14.13±1.90 ng/ml; $p<0.05$) and glucagon (0.24±0.01, Ctrl: 0.31±0.02 ng/ml; $p<0.05$) plasma levels were decreased after CVS, while leptin was increased (1.72 ±0.22, Ctrl: 1.21±0.10 ng/ml; $p<0.05$). CVS decreased the expression of hepatic *Gck* ($p<0.05$) and *Fasn* ($p<0.05$). In muscle, CVS increased the expression of *Ucp3* ($p<0.05$).
Conclusion: CVS decreases hepatic glucose production and impairs hepatic insulin sensitivity, while glucose turnover in peripheral tissues is improved. As a result of these two opposite effects whole-body insulin sensitivity is not changed by CVS. In addition, CVS decreases in the liver and increases in muscle the expression of glucose and lipid metabolism genes. Furthermore, the changes in IL-10 could also play a role in the regulation of insulin sensitivity by stress.
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Glucose as a significant predictor of irisin levels in children

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Background and aims: Irisin is a recently identified novel myokine which increases energy expenditure and glucose tolerance and is thus associated with improvements in glucose homeostasis in humans. Coupled with this is that secreted irisin induces white adipocytes into 'beige' or 'brite' adipocytes. Taken together irisin has potential therapeutic applications for weight loss to improve glucose metabolism, insulin resistance in subjects with obesity and type 2 diabetes Mellitus (T2DM). Additionally as a metabolic biomarker studies note, in adults, irisin positively correlates with hyperglycemia, BMI and ghrelin in humans, whilst negatively correlated with insulin, cholesterol as well as adiponectin, with the suggestion of a compensatory role of irisin in metabolic regulation. Although how early irisin in human life irisin may impact as a metabolic biomarker is unknown. Therefore the aim of this study was to establish whether there were (1) significant relationships between serum irisin and metabolic profiles in children as noted in adults; (2) gender differences in association of irisin and metabolic profiles.
Materials and methods: In this cross-sectional study in a cohort of 154 Saudi children (80 Boys age: 12.4(mean±SD) 3.2yr; BMI: 19.5±5.9 and 74 girls: (age: 12.9 (mean±SD) 3.2yr; BMI: 20.6±5.2) fasted bloods, anthropometric and biochemical data was collected. Irisin was assessed by an enzyme-linked immunosorbent assay (ELISA: intra-assay % CV<10% and inter-assay variability <15%) with in house validation and correlated with biochemical and anthropometric data.
Results: There was a significant negative correlation between irisin levels and fasting blood glucose (FBG, r= -0.35, $p<0.001$), sagital abdominal diameter (SAD) (r=0.34, $p<0.001$) and HDL (r=0.17, p=0.04) across the entire cohort studied (n=154). Notably, in girls homeostasis model assessment-estimated insulin resistance (HOMA-IR) was found to be negatively correlated with irisin levels (r=-0.32, p=0.02) as noted in adults. Whilst after a stepwise linear regression analysis it was observed that FBG was the sole predictor of serum irisin levels (R2 =0.16) followed by SAD. Multivariate linear regression analysis after controlling for potential confounders such as age, BMI and gender identified that irisin levels was independently associated with FBG (β=-0.34, p=0.01) particularly in girls.
Conclusion: In the present study, serum irisin level correlated negatively with HOMA-IR, whilst also being independently associated with blood glucose levels, in girls, suggesting that irisin may play a crucial role in glucose intolerance and metabolism, as early as age 12, in females. Irisin levels are raised by modest increases in exercise and, as obesity are increasingly more prevalent in females, improving glucose homeostasis and energy expenditure via increased exercised induced irisin levels, at an early age, may delay onset of obesity and T2DM.

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A single mechanism underlies glucose kinetics in euglycaemic and hyperglycaemic clamps and oral glucose tolerance tests

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Background and aims: It is known that for a given insulin level glucose clearance (GCL) depends on glucose concentration. This makes GCL assessed in heterogeneous tests such as euglycemic and hyperglycemic clamps and oral glucose tolerance tests (OGTT) not comparable. Currently, no unifying quantitative representation is available to describe these heterogeneous tests. This study aims at modeling glucose kinetics (GK) in all these conditions according to a unique mechanism.
Materials and methods: Data from three studies in nondiabetic subjects were used in the analysis: a three-level hyperglycemic clamp (N=8), a two-level euglycemic hyperinsulinemic clamp (N=8), and paired OGTTs and euglycemic hyperinsulinemic clamps in the same subjects (N=8). In all tests, a glucose tracer was used. Literature data (whole body and forearm) from clamps at various glucose and insulin levels were also included in the analysis. Glucose and insulin ranges in the tests were wide (5-24 mM and 20-10000 pM, respectively). A model was developed based on a circulatory model of GK and basic notions of glucose transport. Glucose utilization was modeled as a Michaelis-Menten function of glucose concentration with constant K_m and insulin-controlled V_{max}. V_{max} was expressed as a Hill function of insulin at the site of action, related to plasma insulin by a first-order delay model. Model parameters were estimated using a population approach, assuming log-normal parameter distributions.
Results: The model was able to fit the tracer data well in all tests. The median K_m of the glucose model was 22.6 mM; the insulin K_m was 630 pM; the insulin delay half-time was 48 min. In a representative subject, GCL at an insulin concentration of 600 pM was reduced from 592 to 458 ml $min^{-1}m^{-2}$ when glucose was raised from 5 to 10 mM. The model reproduced a characteristic feature of GCL calculated with standard tracer methods, i.e., the lack of increase when hyperinsulinemia was accompanied by hyperglycemia, observed both in the hyperglycemic clamp and the OGTT. Insulin sensitivity, defined as GCL at 5 mM glucose and 600 pM insulin, was fully consistent when calculated from the OGTT and the euglycemic clamp (r=0.89, $p<0.002$). In all tests, insulin sensitivity was inversely correlated with BMI, as expected (r=-0.62, $p<0.001$).
Conclusion: We have shown that GK in euglycemic and hyperglycemic clamps and OGTTs can be described with a unifying mechanism, consistent

with the notions of glucose transport. In contrast to classical models ignoring the effects of glucose on GCL, our model reproduces specific features observed with concomitant hyperinsulinemia and hyperglycemia. The model could allow better understanding of glucose metabolism and improved glucose homeostasis simulators, which are relevant e.g. for drug development.
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Insulin-mediated suppression of lipolysis in adipose tissue and skeletal muscle of obese type 2 diabetic and normal glucose tolerant men

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Background and aims: Impaired regulation of lipolysis and accumulation of lipid intermediates in non-adipose tissues may contribute to obesity-related insulin resistance and type 2 diabetes mellitus (T2D). The aim of the present study was to compare insulin-mediated suppression of lipolysis in abdominal subcutaneous adipose tissue (AT) and skeletal muscle (SM) of obese normal glucose tolerant (NGT) and obese T2D men.
Materials and methods: 11 NGT and 9 long-term diagnosed T2D men (7±1y), matched for age (58±2 vs. 62±2 y), BMI (31.4±0.6 vs. 30.5±0.6 kg/m^2) and $V0_2$ max (28.9±1.5 vs. 29.5±2.4 mL/kg.min) participated in this study. Interstitial glycerol concentrations in AT and SM were assessed using microdialysis during a 1-h basal period and a 6-h step-wise hyperinsulinemic euglycemic clamp (8, 20 and 40 mU.m^{-2}.min^{-1}). AT and SM biopsies were collected to investigate underlying mechanisms.
Results: Hyperinsulinemia suppressed interstitial SM glycerol concentrations by 27±9 and 53±8% in T2D compared with NGT men ($p<0.01$). This was accompanied by increased circulating fatty acid and glycerol concentrations, a lower glucose infusion rate (21.8±3.1 vs. 30.5±2.0 μmol.kg body weight^{-1}.min^{-1}, $p<0.05$), a higher hormone-sensitive lipase (HSL) serine 660 phosphorylation, increased saturated diacylglycerol (DAG) lipid species in the muscle membrane and increased PKC activation in T2D versus NGT men. No significant differences in the insulin-mediated reduction in AT interstitial glycerol were observed between groups.
Conclusion: Our results suggest that a blunted insulin-mediated suppression of SM lipolysis might promote membrane saturated DAG accumulation, which may worsen insulin resistance, at least in part mediated by PKC. This might represent an important mechanism involved in the progression of insulin resistance towards T2D.
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Characterisation of PIMT KO mice: a model of deficient protein repair

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Background and aims: Post-translational protein modifications may create new antigenic epitopes and elicit autoimmunity. The enzyme PIMT (Protein-L-isoaspartate (D-aspartate) O-methyltransferase), encoded by PCMT1, repairs isomerised Asn and Asp residues and previous data suggest that it could be involved in the pathogenesis of type 1 diabetes (T1D). We have reported that PIMT KO mice show similar glucose responses to an oral glucose tolerance test (OGTT) to their wildtype (Wt) littermates, but a lower nadir and slower recovery from hypoglycaemia during an insulin tolerance test (ITT). Our aim was to assess glucose-stimulated insulinemia and counter-regulatory hormone response to hypoglycaemia, as well as to further characterize PIMT KO mice, with special focus on pancreatic islets and beta-cell mass.
Materials and methods: A total of 12 mice (50% KO/Wt; 50% males) were studied. Relevant clinical features were recorded. At a mean age of 60 days, insulin was measured during an OGTT and glucagon and corticosterone, during an ITT at 0, 15, 30, 60 and 90 min, using commercial ELISA kits. Total and relative organ weights were registered at necropsy. Tissues were collected and frozen/kept in tissue embedding compound. Beta cell mass was estimated assessing islet and beta cell area comparisons, and PIMT expression was evaluated on Wt pancreas by immunohistochemistry (IHC) (antibodies: goat anti-insulin, Santa Cruz; rabbit anti-PIMT, ProteinTech). To assess the effect of age-dependent insulin resistance, OGTT, ITT (120 and 360 days) and HbA1c (120 days) were performed in heterozygous (Het) and Wt mice. KO mice were not assessed at that age, due to short survival. Groups were compared using Mann-Whitney's U or Student's t test ($p<0.05$).
Results: PIMT KO mice showed growth-delay, kyphosis, macrocephaly, cachexia, and abnormal behavioural patterns such as anhedonia, although some heterogeneity was observed. No significant differences were found for corticosterone secretion [AUC Ko vs Wt = 205.85 (±84.57) vs 147.35 (±47.27); $p=0.27$] during the ITT. Insulin and glucagon concentrations fell below the standard curve. Thus, new analyses are ongoing. No differences were found in total body weight [KO vs WT: 19.92 g (±4.17) vs 21.33 (±5.72); $p=0.59$], or relative liver [5.15% (±0.72) vs 4.68 (±0.59); $p=0.46$], pancreas [1.06% (±0.17) vs 1.05 (±0.19)], $p=0.77$] and paragenital fat [1.20% (±0.46) vs 1.08 (±0.19); $p=0.23$)] weights. No significant differences were found at 120 or 360 days between Het and Wt in the OGTT, ITT or HbA1c. IHC revealed colocalisation of PIMT and insulin in the Wt mice.
Conclusion: PIMT KO mice show growth delay and anhedonia, compared to their Wt littermates. Corticosterone response does not explain their slower recovery from hypoglycaemia. Heterozygotes did not show differences from Wt in glucose metabolism or insulin sensitivity. Ongoing analysis may shed additional light on the potential role of PIMT in T1D.
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PS 041 Insulin resistance biomarkers

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Search for biomarkers that predict the development of type 2 diabetes

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Background and aims: Metabolomics is a powerful tool to investigate physiological status, diagnose diseases, and identify potential biomarkers. Several metabolites, such as branched chain amino acids (valine, leucine, and isoleucine) and aromatic amino acids (tyrosine and phenylalanine), have been reported to be potential predictors of the development of type 2 diabetes (T2D) in Caucasians. However, the targets of intervention and the mechanisms involved remain to be elucidated. In this study, we performed metabolome analysis of longitudinal plasma changes in an animal model of non-obese T2D, the spontaneously diabetic Torii (SDT) rat.
Materials and methods: The SDT (n=11) and control Sprague-Dawley (SD) (n=11) rats were phenotyped for body weight and blood glucose level from 6 to 25 weeks of age. Fasting plasma samples were collected at 6, 8, 12, 16, 20, and 24 weeks of age. Non-targeted metabolome analysis was performed on the plasma samples using gas chromatography-mass spectrometry (GC-MS). Principal component analysis (PCA) was used as a multivariate analysis method, and fold change analysis and t-test were used as univariate analysis methods.
Results: The SDT rats developed T2D as early as 14 weeks of age, reaching 100% incidence by 20 weeks of age. At 12 weeks of age, before the onset of T2D, the SDT rats exhibited glucose intolerance with impaired insulin secretion upon intravenous glucose tolerance test. On the GC-MS-based metabolomics platform, we obtained about 150 hydrophilic metabolites in plasma samples, including amino acids, carbohydrates, sugars and organic acids. At 12 weeks of age, eight metabolites (adenylosuccinic acid, galacturonic acid, glycine, kynurenine, N-alpha-acetyllysine, palmitoleic acid, taurine, and tryptophan) showed significant differences between SDT and SD rats (nominal p-value at t-test <0.05; fold change <0.67 or 1.5<). In particular, several metabolites in the tryptophan metabolism pathway (e.g., tryptophan and kynurenine) were decreased in SDT rats at 12 weeks of age and later, suggesting that the tryptophan metabolism pathway is involved in the development of T2D in the SDT rat. The lower tryptophan and kynurenine levels were confirmed by an additional longitudinal plasma metabolome analysis on an animal model of obese T2D, the Otsuka Long-Evans Tokushima Fatty (OLETF) rat.
Conclusion: Our data suggest that the longitudinal metabolome analysis of plasma samples of animal models is useful for investigation of the pathophysiology of T2D and for identification of biomarkers of the disease and that the tryptophan metabolism pathway might be a potential target of intervention in T2D.
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Elevated ferritin in skeletal muscle and insulin resistance in patients with type 2 diabetes mellitus

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Background and aims: Insulin resistance (IR) is a major characteristic of type 2 diabetes mellitus (T2DM). Despite numerous studies, the pathogenesis of IR has not been well established. Ferritin is known to be associated with IR, but little is known about the possible sites (e.g., muscle, liver, adipose tissue) where ferritin may induce IR. It has also been suggested ferritin may be an underlying factor in the development of oxidative stress in skeletal muscle. Thus we hypothesized that ferritin in human skeletal muscle may play a significant role in insulin resistance.
Materials and methods: We recruited 16 T2DM subjects and 12 subjects with normal glucose tolerance (NGT) for this study. Whole-body insulin-mediated glucose uptake was determined using a euglycemic hyperinsulinemic clamp test. We performed biopsies on the vastus lateralis muscle and used immunoblotting to determine levels of 3-nitrotyrosine, ferritin heavy chain, ferritin light chain,transferrin receptor-1(TfR-1), divalent metal transporter-1(DMT-1), and ironregulatory protein-1(IRP-1) in skeletal muscles from both T2DM subjects and NGT subjects.
Results: Tests on T2DM subjects resulted in a higher protein oxidative damage as assessed by detecting 3-nitrotyrosine, when compared with muscles from the NGT group (p-value <0.05). An elevated expression of ferritin, (heavy chain, p-value <0.01; light chain, p-value < 0.001), an important iron storage protein, was associated with T2DM. The iron transport protein, TfR-1, demonstrated a tendency to increase (P =0.07) and the level of DMT-1 protein expression was significantly increased in muscle from the T2DM group (P =0.01). Protein levels of IRP-1 were significantly decreased (P =0.01) in the T2DM group.
Conclusion: In conclusion, altered iron metabolism and iron accumulation in skeletal muscle may be associated with insulin resistance in T2DM patients.
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Uric acid is a biomarker of beta cell function in patients with newly diagnosed type 2 diabetes

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Background and aims: The relationship between uric acid and insulin resistance has been widely studied, whereas little is known about a potential interaction between uric acid and beta-cell function. Goal of this study was to investigate the relationship, if any, between uricaemia and beta-cell function in patients with newly diagnosed type 2 diabetes.
Materials and methods: In 569 GAD negative, drug naive patients (median [interquartile range]: age 60 [52-66] years, BMI 29.3 [26.6-32.9] kg/m^2, HbA1c 6.6 [6.1-7.4]%, uric acid 0.32 [0.27-0.37] mmol/L) with newly diagnosed type 2 diabetes we assessed insulin sensitivity by the euglycemic insulin clamp (M clamp: 605 [381-845] μmol·min^{-1}·m^{-2} BSA) and beta-cell function by state-of-art modelling of glucose/C-peptide curves during an oral glucose tolerance test (OGTT). There are two main outputs of the model: derivative control (DC: amount of insulin secreted in response to the rate of plasma glucose increase; median [interquartile range]: 444 [66-929] [pmol·m^{-2} BSA]/[mM·min^{-1}]) and proportional control of beta-cell function (PC: stimulus-response curve linking glucose concentration to insulin secretion rate; mean ± SD at the preselected glucose concentrations of 5.5, 8.0, 11.0, 15.0 and 20.0 mM: 158±67, 228±124, 376±229, 602±397, 889±623 pmol·min^{-1}·m^{-2} BSA).
Results: In univariate analysis, serum uric acid concentration was positively related to both DC (p<0.01) and PC (p<0.01) of beta-cell function. In multiple regression analysis, after adjusting for age, gender, BMI, insulin sensitivity and glomerular filtration rate, this positive relationship stayed statistically significant (p<0.01 e p<0.01 for DC and PC respectively). Consistently with this result, uricaemia was inversely correlated to HbA1c (p<0.01), fasting glucose (p<0.01), 1-hour and 2-hour OGTT glucose (p<0.01 and p<0.01 respectively). Patients in the 3rd tertile of uric acid had a 37% increase in DC (p<0.01) and a 21-30% increase in PC (p<0.01) of beta-cell function, when compared to those in the 1st tertile.
Conclusions: In patients with newly diagnosed type 2 diabetes there exists a strong positive correlation between serum uric acid concentration and beta-cell function. This finding might reflect antioxidant activity of uric acid. However, to determine whether uric acid improves beta-cell function per se or through other factors, mechanistic studies will be required.
Clinical Trial Registration Number: NCT01526720

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Plasma soluble leptin receptor levels are associated with beta cell function in patients with type 2 diabetes

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Background and aims: A soluble form of the leptin receptor (sOb-R) is the main binding protein for leptin in circulation and modulates its bioactivity. Plasma sOb-R levels are positively correlated with leptin sensitivity to body weight and energy expenditure, and inversely with the risk of developing type 2 diabetes (T2D). Hyperinsulinemia observed in a mouse model lacking leptin signaling in β-cells (Pancreas-Ob-R knockout) indicates that leptin acts on β-cell to inhibit insulin secretion. In this study, we investigated the clinical association of plasma sOb-R levels with β-cell function in T2D patients.
Materials and methods: Two hundred and eighty nine T2D patients without renal dysfunction or insulin therapy were included in this study (mean age, 61 years; duration of diabetes, 7.8 years; BMI, 25.4 kg/m^2). Fasting plasma leptin and sOb-R concentrations were measured by ELISA.
Results: The median (interquartile range) of plasma leptin or sOb-R level was 3.5 (1.8 - 6.6) or 23.7 (19.7 - 28.5) ng/ml. Plasma sOb-R was negatively correlated to HOMA-R ($r = -0.232$, $p < 0.0001$), HOMA-β ($r = -0.507$, $p < 0.0001$), and C-peptide index (CPI) ($r = -0.514$, $p < 0.0001$), whereas plasma leptin was positively to each index ($r = 0.615$, $p < 0.0001$, $r = 0.556$, $p < 0.0001$, and $r = 0.425$, $p < 0.0001$, respectively) in simple regression analysis. Multiple regression analyses including age, sex, duration of diabetes, BMI, blood pressure, serum creatinine, glycated hemoglobin, lipid profile, leptin, and sOb-R as independent variables revealed that sOb-R independently and negatively contributed to HOMA-β ($\beta = -0.206$, $p < 0.001$) and CPI ($\beta = -0.341$, $p < 0.0001$), but not to HOMA-R ($\beta = 0.004$, $p = 0.940$), whereas leptin did positively to HOMA-R ($\beta = 0.667$, $p < 0.0001$), HOMA-β ($\beta = 0.531$, $p < 0.0001$), and CPI ($\beta = 0.489$, $p < 0.0001$). These data indicate that plasma sOb-R is associated with decreased β-cell function, whereas plasma leptin is associated with insulin resistance and hyperinsulinemia, independently of obesity and other metabolic parameters, in T2D patients.
Conclusion: Plasma sOb-R levels are associated with β-cell function in T2D.

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Plasma concentrations of the methylglyoxal metabolite D-lactate are independently associated with insulin resistance: the CODAM study

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Background and aims: Methylglyoxal (MGO) is a highly reactive dicarbonyl compound generated by the spontaneous degradation of glycolytic intermediates during glycolysis. MGO is a major precursor for advanced glycation endproducts (AGEs) and can potentially disrupt cellular functions. MGO can be detoxified by the glyoxalase system into D-lactate. Experimental studies have shown that increased levels of MGO are associated with insulin resistance, but this has not yet been confirmed in human studies. Because MGO is difficult to measure, we investigated if plasma D-lactate levels, as a marker of MGO metabolism, are increased in individuals with impaired glucose metabolism (IGM) and type 2 diabetes mellitus (T2DM), and if they are associated with markers of insulin resistance in a large cohort study.
Materials and methods: We investigated 513 of the 574 participants in the baseline assessments of the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM1) study, who were not on insulin treatment and who had complete data on the (co)variables of interest. The study sample consisted of 278 individuals with normal glucose metabolism (NGM, 61% men, 58.6±7.3 years), 118 with IGM (61% men, 59.8±6.5 years) and 117 with T2DM (69% men, 60.7±6.2 years). Plasma D-lactate was measured with ultra-performance liquid chromatography-tandem mass spectrometry and insulin resistance was determined with the Homeostasis Model of Assessment (HOMA2-IR) calculator. Both these variables were $\log_e$ transformed prior to analyses to meet analytical assumptions. We used ANCOVA to first compare the levels of plasma D-lactate (adjusted for age and sex) across categories of glucose metabolism (NGM as referent). With linear regression we analysed the cross-sectional association of plasma D-lactate with HOMA2-IR in the whole study sample. This analysis was first adjusted for age and sex, and further for plasma L-lactate levels, HbA_{1C}, glucose metabolism status, smoking status, prior cardiovascular disease, use of medication (glucose-, lipid-, and blood-pressure-lowering), estimated glomerular filtration rate and waist circumference.
Results: Plasma D-lactate levels (geometric means in μmol/L) were significantly higher in individuals with IGM [8.93 (95%CI: 8.22-9.69)] and T2DM [10.97 (95%CI: 10.09-11.92)] than in individuals with NGM [8.01 (95%CI: 7.60-8.46)], age and sex adjusted: $p=0.03$ and $p<0.001$, respectively. In addition, plasma D-lactate concentrations were positively associated with HOMA2-IR, in both the model adjusted for age and sex [standardized regression coefficient $\beta=0.43$ (95%CI: 0.35-0.51)], as well as in the fully adjusted model [$\beta=0.15$ (95% CI: 0.05-0.24)], $p<0.01$ in both models.
Conclusion: Plasma D-lactate levels, as a marker of MGO metabolism, are increased in patients with IGM and T2DM, and are associated with HOMA2-IR, independently of putative confounders. These results suggest that higher levels of MGO may play a role in the aetiology of insulin resistance. Direct measurement of MGO in cohort studies is necessary to confirm the findings from our current study.
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The association between serum prothymosin-α concentrations and insulin resistance in subjects with or without diabetes mellitus

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Background and aims: Prothymosin-α is a ubiquitous acidic small protein participating in a variety of physiological process including cell proliferation, chromatin remodeling, transcriptional activation, as well as cytokine-like function. One recent study showed that Prothymosin-α exerted immunomodulation actions through binding to Toll-like receptor 4 (TLR-4) to stimulate the production of interferons in cells. In addition, the activation of TLR-4 could impair insulin sensitivity, and disrupt the homeostasis of blood glucose. However, the effects of Prothymosin-α on glucose homeostasis and insulin sensitivity are still unknown.
Materials and methods: A total of 93 age- and sex-matched subjects with normal glucose tolerance (NGT, n=46) and newly diagnosed diabetes (NDD, n=47) were recruited. The anthropometric parameters (body mass index and waist circumference) were recorded. Fasting plasma glucose, serum lipid profiles, biochemistry, insulin, and adiponectin levels were measured. Insulin resistance was estimated by homeostasis model assessment (HOMA-IR). Serum Prothymosin-α concentrations were determined by enzyme-linked immunosorbent assay (ELISA). All subjects received a standard 75-g oral glucose tolerance test and diabetes was diagnosed according to recommendations of American Diabetes Association.
Results: NDD group had higher serum Prothymosin-α concentrations (525 ± 116 vs. 241 ± 46 mg/mL, $p=0.027$) and HOMA-IR (0.90 ± 0.12 vs. 0.53 ± 0.08, $p=0.015$) than subjects with NGT. In addition, subjects with NDD had higher fasting and 2-h postload plasma glucose, A1C, C-reactive protein (CRP), triglyceride, but lower HDL cholesterol than NGT ones. However, there were no significant differences in body mass index, waist circumference, blood pressures, creatinine, total cholesterol, and adiponectin. To explore the effect of Prothymosin-α on insulin resistance, multiple linear regression analysis using HOMA-IR as the dependent variable were performed. Age ($p<0.05$), fasting plasma glucose ($p<0.05$), body mass index ($p<0.05$), and Prothymosin-α ($p<0.05$) were the positively associated factors of HOMA-IR, after adjusting for age, anthropometric indices, lipid profile, systolic blood pressure, creatinine, CRP, and adiponectin.
Conclusion: Prothymosin-α was associated with increased risk of insulin resistance after adjusting for cardiometabolic risk factors. Elevated Prothymosin-α levels might have a clinical implication in the pathophysiology of diabetes and its complications.

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ZEBD3 3 is associated with obesity and insulin resistance in humans

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Background and aims: GWAS have shown that Zbed3 is associated with T2DM. To date, no report has demonstrated the relationship of Zbed3 with insulin resistance and T2DM in humans. To gain insight into the physiological role of circulating Zbed3 in humans, we report here that Zbed3 is a secreted factor detectable in human serum and evaluate whether Zbed3 correlates with obesity and insulin resistance by cross-sectional and interventional studies on anthropometric, metabolic, and hormonal predictors of circulating Zbed3 in humans.
Materials and methods: To determine whether the Zbed3 is a secreted protein in the vitro, we tagged the Flag peptide sequence to the coding region of Zbed3 (Zbed3-F) at the carboxy terminus. 113 healthy subjects, 102 impaired glucose tolerance (IGT) and 109 newly diagnosed T2DM (nT2DM) were studied. We also investigated the Zbed3 mRNA and protein expressions in muscle and adipose tissues in 15 healthy and 15 T2DM subjects. Finally, we examined whether circulating Zbed3 levels were affected by fasting (a low-insulin and low-glucose state) and oral glucose tolerance test (OGTT, a high-glucose state), or euglycemic- hyperinsulinemic clamp (EHC, euglycemic-hyperinsulinemic state).
Results: In vitro, Zbed3 is detected in both conditioned medium and lysates of HEK- 293T cells transfected with overexpressed vector. In the clinical study, there were significantly higher circulating Zbed3 levels in IGT and nT2DM relative to NGT. Zbed3 correlated positively with BMI, WHR, FAT%, blood pressure, FBG, TG, HbA1c, FIns and HOMA-IR, and inversely with HDL-C. Increasing levels of Zbed3 showed a significant linear trend and were independently associated with IGT and T2DM. Zbed3 mRNA and protein in muscle and fat were significantly increased in T2DM patients. Moreover, there was a concentration-dependent effect of glucose on Zbed3 release, whereas insulin exhibited an inhibitory effect on Zbed3 levels.
Conclusion: We identified, for the first time, Zbed3 as a novel secreted protein. More important, we present novel data of increased circulating Zbed3 levels in both IGT and T2DM subjects, increased expressions of Zbed3 mRNA and protein levels in muscle and adipose tissues of T2DM patients, and in vivo regulation of Zbed3 by glucose and insulin. However, the physiologic and pathologic significance of our findings remain to be elucidated.
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TIMP3 regulates metabolism acting on different pathways: studies with a metabolomics approach

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Background and aims: Timp3 down-regulation is associated to insulin resistance, glucose intolerance and atherosclerosis in human subjects and experimental models via up-regulation of inflammatory pathways in WAT, liver and vasculature. We used metabolomics to identify pathways associated to metabolic inflammation in the TIMP3KO mice.
Materials and methods: TIMP3KO and WT mice were subjected to diet induced obesity for 16 weeks. IPGTT, IPITT, LC-MS/GC-MS metabolomics, olecular biology and immuno-fluorescence techniques were used to phenotype mice.
Results: TIMP3KO mice after 16 weeks of HFD were glucose intolerant and insulin resistant compared to WT ($P<0.01$, n=16 per group). TIMP3 deletion exerted negative defects compared to WT mice independently from body weight since both 40 g were comparable in terms of glucose intolerance. We performed metablomics studies to gain insight the effect of TIMP3. Random Forest classification of metabolomics results in wild-type and TIMP3KO serum from animals either on a chow diet or on a high fat diet gave a predictive accuracy of 100 %. TIMP3 had an effect on glycolysis intermediates and Pentose Phosphate Pathway with increased Ribulose and Ribulose-5P and intermediates of the tricarboxylic acid cycle such as alpha-ketoglutarate and malate ($P<0.05$ for all, n=6 per group) independent of the diet. Upon a HFD TIMP3KO exhibited increased alpha-hydroxyisovalerate, alpha-hydroxyisocaproate and 2-hydroxy-3-methyòvalerate ($p<0.05$, n=6 per group), generally associated with a deficiency in branched-chain keto-acid dehydrogenase (BCKDH). On the other hand, levels of isovalerylglycine and isovalerylcarnitine ($P<0.05$ for all, n=6 per group), which are produced distal to BCKDH, were decreased in TIMP3KO serum. Together, this pattern of changes strongly suggests that loss of Timp3 function leads to changed BCKDH activity, expression, or regulation. A number of metabolites derived from the aromatic amino acids tryptophan and tyrosine appeared in the RF Importance Plot as factors separating HFD-fed WT and T3-KO; in fact TIMP3KO exhibited significant increased amounts of indolelactate, 3-hydroxysulfate, p-cresol-sulfate, phenolsulphate and 3-(4-hydroxyphenyl)lactate. Since these compounds are of gut microbiome metabolic origin or contribution we analyzed intestinal inflammation which revealed a significant increased expression of the inflammatory cytokine such as Interleukin 1 beta (IL-1β), Tumor Necrosis Factor alpha (TNFα), CD11b and CD11c ($p\leq 0.05$) and a trend for increased Interleukin 6 (IL-6), F4/80, MHC and CD3.
Conclusion: Metabolomics studies in TIMP3 KO mouse revealed alterations in multiple pathways in association with insulin resistance and low-grade inflammation and particularly related to restricted branched-chain keto-acid dehydrogenase activity suggested by changes in branched-chain AA intermediates and to compounds with gut microbiome metabolic origin or contribution in association to increased intestinal inflammation.
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Improvement of metabolite risk profile for type 2 diabetes and cardiovascular disease by weight loss and weight maintenance

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Background and aims: Branched-chain amino acids (BCAA) and aromatic amino acids are associated with obesity, type 2 diabetes (T2D), and cardiovascular disease (CVD). Recently, we showed that a signature composed of three of these amino acids, the DM-AA score, strongly predicted future T2D and CVD. Here, we investigated if this risk-score could be modified in the long-term by weight loss induced by a low-calorie diet (LCD) followed by a six month weight maintenance period in human adult obese subjects.
Materials and methods: The DM-AA score is composed of the sum of the blood plasma concentrations of isoleucine, tyrosine and phenylalanine and is based on results from a subset with matched controls from the Malmö diet and cancer (MDC) cohort (n=805). Metabolite profiling data from the weight loss program (n=12; 59 metabolites) and additional measurements of HDL, LDL, cholesterol, glucose, triacylglycerols, insulin and adiponectin were obtained at three sampling occasions: Before (BMI 43±1.7kg/m^2) and after (BMI 36±2.0kg/m^2) weight loss (101±26days) as well as after a six month (167±37days) period with maintained weight (BMI 36±1.9 kg/m^2). The profile of each metabolite during the course of the weight loss program was calculated using orthogonal projections to latent structures discriminant analysis (OPLS-DA).
Results: The DM-AA score showed a significant association to both waist circumference and BMI even when adjusting for sex, age, hypertensive treatment, systolic blood pressure, homeostasis model of assessment of insulin resistance, smoking status and diabetes status. During the weight loss study, 85% of the metabolite levels were altered at some point during the program. Moreover, 58% of the metabolites were altered in the long-term, i.e. after weight maintenance. Many of the metabolites followed the profiles of clinical markers. The amino acids in the DM-AA score decreased during the weight loss program but the levels of the BCAA and aromatic amino acids were differentially regulated during the weight loss program: The levels of isoleucine and leucine decreased during weight loss and retained their levels during weight maintenance, while valine returned to baseline level during weight maintenance. Phenylalanine, tryptophan and tyrosine decreased during weight loss and increased slightly during weight maintenance, but remained below baseline concentrations.
Conclusion: Our results illustrate that the obesity-associated risk for future T2D and/or CVD indicated by the DM-AA score is modifiable by weight loss

and remains decreased after weight maintenance. Meanwhile, the effect of weight loss and weight maintenance on metabolites seems to be differential as illustrated by the return to baseline of the third BCAA valine. This information can likely be used for evaluation of the potential benefit of weight loss programs.
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Gene-metabolite networks reveal the regulation of clock genes by insulin
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Background and aims: Recently genetic studies suggested that the circadian system is tightly coupled with mechanisms controlling metabolism. This regulation consists of complexly integrated regulatory loops, as circadian regulators of metabolic pathways are themselves regulated by different nutritional and metabolic signals. Secretion of insulin and insulin sensitivity are on circadian control. Here we tested hypothesis, that insulin directly or via metabolic-induced changes may regulate circadian clocks.
Materials and methods: In the randomized single-blind cross-over study on male moderate overweight subjects (n=14) effect of insulin on clock genes were tested in two conditions: in the saline infusion (NaCl-INF) and in the euglycemic-hyperinsulinemic clamp (EC). Adipose tissue biopsies and plasma samples were taken before and after start of saline/insulin-infusions (t = -40, 240 min). Subcutaneous adipose tissue biopsies were analysed by microarray (Agilent 60-mer Whole Human Genome (4x44K) single-color DNA microarrays) and genes of interest were additionally studied by quantitative RT-PCR. To address the great complexity of hypothesized regulatory loops, we used results from gene expression analyse and metabolic profiling (GC-TOF/MS technique) for the reconstruction of integrated gene-metabolites networks of both experiments. For the human adipocyte culture, mesenchymal stem cells were differentiated in vitro into adipocytes and treated with 100nM insulin for 4 hours. The real-time bioluminescence detection was used for monitoring rhythms of clock gene expression in cell/tissue cultures received from PER2::LUC knockin mice.
Results: Five of eighteen core clock genes were significantly changed in experiments. Circadian genes were closely and direct connected to insulin in the NaCL-INF network and to insulin and glycerol in the EC network. Period circadian protein homolog 2 (PER2) was included in the NaCL-INF network but it was disintegrated from the molecular information exchange in the EC-network. In addition, the PER2 gene expression was significantly changed in the subcutaneous adipose tissue samples of NaCL-INF but not in the EC experiments, suggesting that insulin regulates its expression. In the next step, we observed the upregulation of PER2 gene expression by insulin in the human adipocytes in vitro. Using bioluminescence assays of cell/tissue cultures received from PER2::LUC knockin mice we confirmed insulin effects on clock gene expression in adipose tissue and hepatocytes but not in macrophages and lungs.
Conclusion: Our results demonstrate that insulin directly regulates circadian clock machinery in the insulin-dependent tissues in vivo and in vitro. This data contribute to the further understanding the complexity of the interplay between circadian clock and metabolism.
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PS 042 Weight loss and caloric modulation

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Effect of an isocaloric reduced protein intake on glucose metabolism and fatty liver in ovariectomised rat
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Background and aims: Moderate protein undernutrition (MPU) is frequently observed in postmenopausal women. Moreover, estrogen withdrawal favours fatty liver that is strongly linked with insulin resistance. Therefore to explore the impact of MPU on hepatic and glucose metabolism in a stage of estrogen deficiency, we fed a moderate decreased protein intake to ovariectomized rats.
Materials and methods: Seven-month-old female rats were ovariectomized (OVX) and fed an isocaloric moderate 5% casein diet (MCD; N=10) or an adequate 14% casein diet (ACD; N=10) for 13 weeks. At the end of the study, fasting blood glucose, triglycerides (TG), insulin, IGF-1 and glucagon were measured by enzymatic assays, ELISA or RIA. Hepatic mRNA gene expressions of IGF-1, Peroxisome Proliferator-Activated Receptor alpha (PPARα), PPARγ coactivator 1-alpha (PGC1α) and myostatin receptor Activin receptor type-2B (Acvr2b) were assessed by real-time RT-PCR. Hepatic steatosis was observed by Oil Red O staining of cryostat sections. Just before sacrifice, body composition was evaluated by EchoMRI. Soleus and gastrocnemius muscles as well as tibia were collected.
Results: While MCD didn't induce any changes in body weight and composition, it induced an increase in fasting glycemia (+53% vs. OVX-ACD $p<0.001$) and triglyceridemia (+23% vs. OVX-ACD $p=0.04$) which was associated with hepatic steatosis confirmed by macrovesicular Oil Red O staining. These results were accompanied by reduced hepatic gene expression of PPARα (-40% vs. OVX-ACD $p=0.04$) and PGC1α (-43% vs. OVX-ACD $p=0.007$), two key transcription factors involved in lipid oxidation and mitochondrial biogenesis. While fasting insulinemia didn't change, fasting glucagonemia was markedly increased in the OVX-MCD group (+78% vs. OVX-ACD $p=0.0002$). MCD also decreased hepatic IGF-1 gene expression (-20% vs. OVX-ACD $p=0.004$) while circulating IGF-1 was not changed. Interestingly, a strong correlation ($r=0.74$ $p=0.0002$) was observed between the hepatic gene expression of IGF-1 and that of the hepatic myostatin receptor Acvr2b.
Conclusion: Collectively, these results suggest that MPU impairs glucose and TG metabolism in the first instance by increasing the fasting glucagon, a stimulator of the hepatic glucose production, and secondly by exacerbating the hepatic steatosis in association with a decreased gene expression of PPARα and PGC1α. Moreover, the correlation between IGF-1 and myostatin receptor gene expressions suggests that MPU may interfere with the muscle-liver axis. This tissue interrelationship is under investigation. Finally this study is the first to point out the deleterious impact of MPU on glucose and hepatic metabolisms in an animal model mimicking the postmenopausal phenotype.
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Middle-aged overweight male south Asians exhibit a different metabolic adaptation to short-term caloric restriction compared to Caucasians
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Background and aims: South Asians (SA) develop type 2 diabetes at a younger age and lower BMI than Caucasians (C). The underlying cause is still poorly understood but might be related to differences in energy/nutrient-sensing signaling pathways leading to insulin resistance (IR). The present study aimed to compare the metabolic adaptation to short-term caloric restriction (CR) in SA and C.
Materials and methods: 12 middle-aged overweight male SA and 12 matched C (age 44.6±0.8 years, BMI 28.3±0.3 kg/m^2) were studied before and after an 8-day very-low-calorie-diet (VLCD, ~450 kcal/day). Endogenous glucose

production (EGP), glucose disposal rate (R_d), substrate oxidation (ventilated hood) and non-oxidative glucose disposal (NOGD) were determined basal and during a 2-step hyperinsulinemic euglycemic clamp (insulin infusion rates 10 and 40 mU/m²/min). In addition, skeletal muscle biopsies were obtained basal and after 30 min of clamp step 2.
Results: Basal and clamp EGP rates were comparable between groups before the VLCD, despite higher insulin levels in SA, indicating greater hepatic IR. In both ethnicities, hepatic insulin sensitivity (IS) significantly improved after the VLCD. Insulin-stimulated R_d and NOGD (step 2) were lower in SA compared to C before the VLCD (p=0.001) in spite of higher insulin levels in SA, indicating lower peripheral IS. After the diet, R_d improved in SA (p=0.039) despite lowered insulin levels, whereas R_d was not affected in C (p=0.198; between groups p=0.019). This beneficial effect in SA was entirely due to enhanced NOGD (p<0.001). Furthermore, SA mostly relied on glucose oxidation whereas C switched to lipid oxidation after the diet. At the molecular level, no obvious differences were observed in insulin signaling between groups. However, phosphorylation of AMPK and of key proteins involved in the mTOR-pathway was significantly increased after the VLCD only in SA. Furthermore, while protein expression of mitochondrial respiratory-chain complex 2 was not affected by the VLCD in SA, it significantly increased in C.
Conclusion: Middle-aged overweight male SA had lower hepatic and peripheral IS before CR than matched C. After an 8-day VLCD, R_d improved in SA, but not in C. However, SA relied on glucose oxidation, whereas C switched to lipid oxidation. This was reflected in a differential diet effect on the phosphorylation state of AMPK and the mTOR-pathway and on protein expression of respiratory-chain complex 2. We conclude that SA exhibit a different metabolic adaptation to short-term CR.
Clinical Trial Registration Number: NTR 2473
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Obesogenic effect of hypercaloric diet on early undernourished rats: changes in central and peripheral insulin sensitivity
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Background and aims: Nutritional imbalances during growth stages have an impact on immature tissues and lead to increased risk of several diseases later in life. A correlation between low birth weight and higher risk of obesity has been documented by several studies. However, there are divergences in the published results probably due to disparities in the definition of: onset, severity and duration of the food restriction. Many people all over the world were poorly nourished from immaturity and have experienced a shift toward Western eating habits in adult life. Animal models are helpful to understand this open enquiry replete with important social and health implications. We used a model of early undernutrition followed by nutritional rehabilitation with "cafeteria" diet to study some of those harmful effects.
Materials and methods: Wistar rats were fed *ad libitum* with a commercial chow (3% lipid/w; 2.9 Kcal/g) and a model of severe undernutrition was developed from intrauterine period until 70 days of life. From this adult stage, we started the rehabilitation during 200 days with a "cafeteria" diet (13% lipid/w; 4.6 Kcal/g) prepared by adding complementary ingredients to chow and supplied *ad libitum*. Four animal groups were established: control rats continuing with the normal chow (C), controls given a "cafeteria" diet (CC), undernourished rats kept under restriction (U) and undernourished transferred to "cafeteria"(UC). We measured weekly intakes and food efficiency. Visceral (VAT) and subcutaneous (SAT) adipose tissue were assessed by RMI. Plasma levels of insulin, leptin, triglycerides and cholesterol were determined by RIA, ELISA and enzymatic reaction, respectively. Triglycerides and cholesterol contents were also analyzed in liver and muscle. Hyperinsulinemic-euglycemic clamp was applied to evaluate whole insulin sensitivity. After direct icv injections of insulin and leptin, we analyzed both signalling pathways.
Results: Transfer to "cafeteria" formula elicited 17% and 160% increases of VAT in CC and UC rats, respectively, although the final proportions of this type of fat were similar in both groups. Liver triglycerides were: 12.0 and 19.0 μg/ g protein in CC and UC rats, respectively (p<0.01); as regards skeletal muscle, triglyceride contents were also lower in CC (5.3 μg/g protein) than in UC (20.0 μg/g protein). Insulin hypersensitivity associated with undernutrition was abolished after moving to "cafeteria" diet. Plasma leptin reached a peak at 10 days in control rats, not found in the age-matched undernourished animals. At 70 days, undernourished rats showed hypothalamic resistance to both insulin and leptin.
Conclusion: Our results show that feeding on a "cafeteria" diet leads to a large increase in the whole fat mass, irrespective of the previous nutritional condition. However, the increase in visceral fat is proportionally higher in the UC group. Some deleterious consequences of "cafeteria" diet, such as hyperlipemia and ectopic lipid accumulation, are also more markedly pronounced in UC than in CC. This situation is linked to hyperphagia associated, in turn, with hypothalamic resistance to anorexigenic factors (e.g. insulin and leptin).
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Effects of a hypercaloric snacking diet on liver fat and insulin sensitivity in lean men
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Background and aims: Hepatic steatosis predisposes to fatty liver disease and is associated with visceral obesity. Also, fat accumulation in the liver reduces hepatic insulin sensitivity which increases plasma glucose concentrations. In addition to ectopic fat accumulation, excessive intake of dietary sugars and fat has been suggested to stimulate triglyceride storage in the liver. In the present study we evaluated the effect of increasing meal size (S) or meal frequency (F) while on a hypercaloric high sugar (HS) or hypercaloric high fat high sugar (HFHS) diet on liver fat, hepatic- and peripheral insulin sensitivity in lean men.
Materials and methods: We included 36 lean healthy men (age: 22.1±2.5yrs; BMI: 22.3± 1.4 kg/m2) and randomised them into 1 of the 4 hypercaloric diet interventions groups or a control group (N=5). Subjects followed the diet for 6 weeks. The caloric intake was 40% above a weight maintaining diet based on energy expenditure assessed with indirect calorimetry. Before and after the diet intervention, we performed a two-step hyperinsulinemic euglycemic clamp with a stable glucose isotope tracer to assess both hepatic- and peripheral insulin sensitivity. We performed MRSpectroscopy (1H-MRS) to measure liver fat.
Results: Weight was significantly increased in all 4 diet groups with no difference between the four diet groups, yet almost all subjects remained within the normal BMI range (20-25 kg/m2). Liver lipid volume was significantly increased in the HFHS- and the HS group randomised to an increase in meal frequency, but not in the groups randomised to increased meal size. A trend towards a decrease in hepatic insulin sensitivity was observed in the HFHS group with increased meal frequency (p=0.08), but not in the HS group. Peripheral insulin sensitivity was unchanged in all groups. None of the measurements were changed in the control group.
Conclusion: Thus, 6 weeks of hypercaloric snacking results in an increase in liver fat and high-fat high-sugar snacking affects hepatic insulin sensitivity. This suggests that a constant nutrient flow to the liver perturbs hepatic glucose metabolism independent of an increase in body weight.
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Forced catch-up growth after early food-restriction alters the entero-insular axis programme leading to glucose intolerance in adult female rats
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Background and aims: Obesity and related diseases have become a major health issue in modern society and the trend is mirrored in developing nations that are transitioning to first world economies and lifestyles. The recent rate at which these diseases have increased suggests that environmental and behavioural influences, rather than genetic causes, are triggering the present epidemic. A relationship has been established between adverse fetal and early-infant phases of life and the subsequent development of adult obesity and type 2 diabetes. Under this scenario, we have performed a study focused on

the relevance of the diet on early stages of life, on the development of obesity and related metabolic disorders in adulthood.
Materials and methods: The experiments were carried out in female Wistar rats aged of six months, belonging to four diet groups: control (C), fed *ad libitum* with a standard chow diet, undernourished (U), fed 35% of the daily control food intake, control high-fat (CHF) and undernourished high-fat (UHF), both fed *ad libitum* with a high-fat diet (HFD) from weaning onwards. To evaluate the impact of the diet, body weight and food intake were assessed weekly, pancreatic and gut histology were studied and visceral fat pad was analysed. Oral glucose tolerance tests (OGTT) were performed to evaluate insulin, GLP-1 and GIP secretion and HOMA-IR values were calculated. Functionality of pancreatic islets was evaluated *in vitro* and gut incretin content was determined.
Results: After weaning, all rats gained weight when fed the HFD, being UHF rats significantly more hyperphagic than CHF animals. Adiposity measures showed that HFD increased visceral fat accumulation and adipocyte size, which were more marked in the UHF population. Although both populations under HFD developed glucose intolerance, UHF rats displayed increased GIP and GLP-1 secretion during OGTT as compared to CHF, showing enhanced glucose-stimulated insulin secretion *in vivo*. Nevertheless, glucose intolerance was more severe and not compensated in UHF rats, suggesting insulin resistance, as shown by HOMA-IR values. Surprisingly, following HFD, the *in vitro* response of beta cells was impaired not only in C rats but also in the U group. Moreover, although islet size and beta-cell volume increased in both HFD-fed rats, UHF rats showed fewer islets compared with their C counterparts. When addressing the effects of HFD on the enteroendocrine K and L cells, we observed a greater impact in U rats. Thus, whereas UHF rats showed a significant hyperplasia in both cell types as compared with U rats, gut histology did not revealed significant differences between C and CHF rats. This result was corroborated by measuring gut GIP and GLP-1 content, which were significantly increased in the UHF population.
Conclusion: The results of this study stress the importance of the nutrition during early life and its relevance in the development of long-term metabolic disorders. Thus, the greatest effects occur in offspring of food-restricted dams submitted to long-term HF feeding, which developed a metabolic syndrome-like phenotype, characterised by increased adiposity, glucose intolerance and an insulin resistance state, highlighting the adverse consequences associated with catch-up growth.
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Effects of phentermine and topiramate extended-release (PHEN/TPM ER) on weight loss (WL) and glycaemic parameters by gender, race, and baseline body mass index (BMI)
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Background and aims: In obese individuals, gender, race, and baseline BMI can affect WL and the risk of developing type 2 diabetes mellitus (T2DM). PHEN/TPM ER has previously demonstrated significant WL in the EQUIP study of obese subjects and in the CONQUER study of obese/overweight subjects with ≥2 weight-related comorbidities. The aim of this post-hoc analysis was to evaluate changes in percent WL, fasting glucose (FG), and annualized incidence of T2DM in subjects based on gender, race, and baseline BMI.
Materials and methods: Data from 2 double-blind, placebo-controlled Phase 3 studies (EQUIP: BMI ≥35 kg/m^2; CONQUER: BMI ≥27 to ≤45 kg/m^2 with ≥2 weight-related comorbidities, including T2DM) were pooled and subjects were stratified by gender (male vs female), race (white vs nonwhite), and baseline BMI (<35 vs ≥35 kg/m^2). Subjects in the intent-to-treat population were randomized to placebo (n=1477), PHEN 3.75 mg/TPM ER 23 mg (3.75/23; n=234), PHEN 7.5 mg/TPM ER 46 mg (7.5/46; n=488), or PHEN 15 mg/TPM ER 92 mg (15/92; n=1479). All subjects received lifestyle modification counseling based on the LEARN program.
Results: In the overall population at baseline, 74.3% (n=2731) of subjects were female, 84.3% (n=3099) were white, mean weight was 107.4±20.1 kg, BMI was 38.4±5.8 kg/m^2, and FG was 5.6±1.1 mmol/L; these values were comparable among subjects across race categories. Mean weight was slightly higher in male subjects than in female subjects. At week 56, subjects receiving PHEN/TPM ER experienced significantly greater least-squares (LS) mean percent WL, regardless of gender, race, or baseline BMI (*P*<.0001 vs placebo for 15/92, all comparisons; Table). FG was decreased in PHEN/TPM ER-treated subjects versus those receiving placebo across race, gender, and BMI categories (Table). In the CONQUER study, the annualized incidence rate of T2DM decreased in female subjects (34% to 64% reduction compared with placebo), in male subjects (4% to 51% reduction compared with placebo), in both race categories (white: 37% to 52%; nonwhite: 42% to 52% reduction compared with placebo), and in both BMI categories (BMI <35: 26% to 70%; BMI ≥35: 39% to 45% reduction compared with placebo). Common adverse events were constipation, dry mouth, and paraesthesia.
Conclusion: PHEN/TPM ER produced significant dose-related WL vs placebo regardless of gender, race, or baseline BMI ≥35. This WL was accompanied by improvements in FG and a lower annualized incidence rate of T2DM, regardless of race and baseline BMI. Whereas glycemic benefits were seen in both genders, this was only significant in female subjects vs placebo. These findings suggest that PHEN/TPM ER, as an adjunct to lifestyle modifications, may be an effective WL treatment for obese and overweight patients, regardless of gender, race, or baseline BMI.

Table. Changes in Percent Weight Loss and Fasting Glucose by Gender, Race, and BMI (ITT-LOCF).

		LS Mean Change in Weight, % (SE)				LS Mean Change in FG, mmol/L (SE)			
		Placebo	3.75/23	7.5/46	15/92	Placebo	3.75/23	7.5/46	15/92
Gender	Male	-2.1 (0.4)	-5.0 (1.1)*	-7.6 (0.6)†	-9.3 (0.4)†	-0.12 (0.04)	-0.25 (0.13)	-0.14 (0.07)	-0.19 (0.04)
	Female	-1.6 (0.2)	-5.0 (0.5)†	-8.8 (0.4)†	-11.1 (0.2)†	0.01 (0.02)	-0.17 (0.05)*	-0.11 (0.04)*	-0.20 (0.02)†
Race	White	-1.9 (0.2)	-5.4 (0.5)†	-8.6 (0.4)†	-10.7 (0.2)†	-0.03 (0.02)	-0.21 (0.06)*	-0.12 (0.04)	-0.19 (0.02)†
	Nonwhite	-1.0 (0.4)	-3.7 (1.0)*	-7.5 (0.8)†	-10.2 (0.4)†	0.03 (0.04)	-0.13 (0.10)	-0.12 (0.08)	-0.22 (0.04)†
BMI, kg/m^2	<35	-1.7 (0.4)	-10.2 (5.0)	-8.1 (0.5)†	-10.2 (0.4)†	-0.16 (0.04)	-0.19 (0.54)	-0.30 (0.05)*	-0.24 (0.04)
	≥35	-1.8 (0.2)	-5.1 (0.5)†	-8.6 (0.4)†	-10.7 (0.2)†	0.02 (0.02)	-0.16 (0.05)*	-0.02 (0.05)	-0.18 (0.02)†

*P<.05 vs placebo; †P≤.0001 vs placebo
ITT=intent to treat; LOCF=last observation carried forward; LS=least-squares; SE=standard error

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Lorcaserin HCl phase 3 trials in obese and overweight patients: week 52 outcomes for those achieving at least 5% weight loss at week 12: a per prescribing information analysis
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Background and aims: Lorcaserin, a selective 5-HT2C agonist, was recently approved by the US FDA for chronic weight management in conjunction with lifestyle modification in obese patients (BMI ≥30), and overweight patients (BMI ≥27) with at least one co-morbidity.
Materials and methods: Three randomized, double-blind, placebo-controlled trials of lorcaserin HCl as an adjunct to lifestyle modification were conducted in patients with diabetes (BLOOM-DM) and without diabetes (BLOOM and BLOSSOM). BLOOM (N=3182) and BLOSSOM (N=4008) enrolled non-diabetic patients, ages18-65 years with BMI 30-45 kg/m^2, or 27 -29.9 kg/m^2 with at least one obesity-related co-morbidity. BLOOM-DM (n=604) enrolled patients without type 2 diabetes (T2DM) with inadequate glycemic control (HbA1c 7-10%) and BMI 27 - 45 kg/m^2.
Results: In those without diabetes, 47% of patients randomized to lorcaserin HCl lost ≥5% of initial body weight at Week 52, compared to 23% of patients randomized to placebo; the values for absolute weight loss were 5.8 kg and 2.5 kg, respectively (MITT-LOCF). In those with type 2 diabetes mellitus (T2DM), results were 38 vs.16% and 4.7 vs. 1.6 kg, respectively. Using AUC for ROC analysis, at least 5% weight loss by Week 12 was identified as the optimal early criterion for predicting at least 5% weight loss at Week 52; patients meeting this criterion at Week 12 were called Responders. In those without diabetes, 49.3% of patients randomized to lorcaserin HCl and 22.6% of those randomized to placebo were Responders. At Week 52, lorcaserin patients without diabetes who were Responders lost on average 10.6 kg, with 85.5% achieving at least 5% weight loss and 49.8% achieving at least 10% weight loss. In those with diabetes, 35.9% of patients randomized to lorcaserin HCl and 11.5% of those randomized to placebo were Responders. At Week 52, lorcaserin patients with diabetes who were Responders lost on average 9.3 kg, with 70.5% achieving at least 5% weight loss and 35.9% achieving at least 10% weight loss. At Week 52, lorcaserin Responders with diabetes showed reduc-

tions in fasting plasma glucose (29.3 mg/dL) and HbA1c (1.2%). At Week 52, lorcaserin Responders without diabetes showed reductions in systolic and diastolic BP and heart rate of 3.4 mmHg, 2.5 mmHg, and 2.5 bpm, respectively and lorcaserin Responders with diabetes had reductions of 2.6 mmHg, 1.9 mmHg, and 3.2 bpm, respectively.
Conclusion: In patients with and without diabetes, Week 12 weight loss was highly predictive of a robust response to lorcaserin in weight loss and improvements in cardiovascular and glycemic parameters at Week 52. At least 5% weight loss by Week 12 provides an early criterion for identifying those who will respond very favorably to lorcaserin.
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Beneficial oxytocin effects in adipose tissue of obese/diabetic leptin-deficient mice

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Background and aims: Oxytocin has been demonstrated to be effective in the treatment of obesity, glucose intolerance and insulin resistance present in diet-induced obese rats and mice. The aim of this study was to examine its effectiveness in a more marked model of obesity and diabetes (leptin-deficient *ob/ob* mice).
Materials and methods: *ob/ob* (O) and lean (L) control mice were treated with oxytocin (T) or vehicle (Unt) subcutaneously for 2 weeks. Pair-fed animal groups were included when the treatment decreased food intake (pair-fed animals received the same daily amount of food than the oxytocin-treated mice). Food, water, body weight and glycemia were measured daily; body composition was determined by magnetic resonance imaging before and after the treatment, and glucose tolerance was assessed at the end of the treatment. Gene, protein and enzyme activities were measured in the adipose tissue and the hypothalamus. At last, adipose tissue inflammation was studied through the quantification of macrophage infiltration by immunofluorescence and gene expression measurements.
Results: In lean mice, the treatment (LT) decreased body weight in comparison with the untreated group (LUnt) only during the first day, the difference being maintained thereafter (overall delta body weight gain: LUnt=3.1g, LT=2.3g, $p<0.05$). In the obese group, the treatment prevented the animals from gaining weight (overall delta body weight gain: OUnt=5.6g, OT=1.4g, $p<0.05$) and fat mass (delta fat mass: OUnt=3.9g, OT=1g, $p<0.0001$) throughout the treatment. The effects in obese mice were partially due to a decrease in food intake (cumulative food intake: OUnt=83.2g, OT=68.5g, $p<0.0001$) and also related to a decreased expression of key genes involved in lipid uptake (*Lpl*) and lipogenesis (*Fas*) and to an increase in lipolysis (*Hsl*). The enzyme activity of Fas was also found to be decreased. Adipose tissue inflammation was reduced (decrease in Mac-2 positive macrophage population OUnt=21.9%, OT=13.6% Mac2+/total cells, $p<0.05$) through a decrease in the M1 pro-inflammatory macrophage population (reduction in *Emr1a*, *Itgam* and *Cd11c* gene expression), without any modification of the M2 anti-inflammatory population (no change in *Mgl1* and *Cd206* gene expression). All these changes were "obesity-dependent", but "food intake-independent", as they were neither present in lean-treated mice, nor in the obese pair-fed group. Although oxytocin treatment decreased food intake in obese mice, no change was observed in the gene expression level of the hypothalamic neuropeptides synthetized within the first-order neurons (*Pomc*/*Cart* and *Npy*/*Agrp*). Surprisingly, although obese-treated mice were leaner, with less fat mass and lower adipose tissue inflammation than untreated mice, their glucose tolerance failed to be improved by the treatment.
Conclusion: Oxytocin effects in lean mice are limited to the first day of the treatment, whereas in leptin-deficient mice, they include prevention of body weight and fat mass gain throughout the treatment. Such oxytocin effects in obese mice are leptin-independent and they are partially due to a decrease in food intake and partially to direct effects on adipose tissue.
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A long-acting oxyntomodulin derivative exerts superior body weight lowering to GLP1R agonism in monkeys

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Background and aims: Analogs of the incretin hormone glucagon-like peptide 1 (GLP-1) stimulate insulin secretion and suppress glucagon levels. Such therapy improves the management of T2D and is often accompanied by modest weight loss. This has prompted the search for alternative approaches to enhance such efficacy. Oxyntomodulin (OXM) is a peptide hormone secreted by the gut following nutrient ingestion that is a dual agonist at the GLP1R and glucagon receptor (GCGR) *in vivo*. OXM causes weight loss in obese man and improves glucose metabolism in T2D patients. We have previously reported that simultaneous activation of the GLP-1 receptor and GCGR can improve glucose metabolism and exhibit superior weight loss when compared to GLP1R agonism in obese rodents. We now report the translation of these observations to spontaneously obese rhesus monkeys, which were treated with a protease-resistant long-acting OXM derivative.
Materials and methods: Body weight and food intake were monitored in twenty-four male and female obese rhesus monkeys randomized for body weight, age and sex and treated daily for 21 days with a long-acting OXM derivative (3 μg/kg s.c., n=8), liraglutide (20 μg/kg s.c., n=8) or placebo (n=8). A meal tolerance test (MTT) was performed in a sequential study in six diabetic rhesus monkeys treated with placebo, liraglutide (10 μg/kg s.c.), placebo and the long-acting OXM derivative (1 μg/kg s.c.).
Results: Daily administration of OXM at a nearly sevenfold lower dose and exposures to the GLP1R agonist liraglutide resulted in superior weight loss (-8% vs -2.5% placebo-corrected). The impact on glycemic control and the potential hyperglycemic risk of GCGR activation was assessed in diabetic rhesus monkeys. Long-acting OXM improved fasting plasma glucose (152 ± 5 vs 122 ± 3 mg/dL, OXM vs. placebo, $P < 0.05$) and glucose tolerance during the MTT in the absence of changes in body weight and food intake.
Conclusion: These results are the first report of the superior pharmacology of a long acting OXM derivative (GLP1R/GCGR dual agonist) in a translational model, deepening our belief that this approach may offer a promising new mechanism in the treatment of T2DM.

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Differences in the metabolic response to an oral glucose tolerance test between adult lean and obese individuals

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Background and aims: Obese individuals often display impaired glucose tolerance during an oral glucose tolerance test (OGTT). Consequently obesity is one of the strongest risk factors for developing type 2 diabetes (T2D). Both obesity and diabetes are conditions associated with a malfunctioning metabolism and it may be of importance to gain a better understanding of the dynamic changes occurring in the metabolome during an OGTT. Currently little is known about difference in metabolic response to an OGTT between obese and healthy lean people. By using a metabolomics approach, we aimed to detect such potential differences to distinguish between metabolites with a disturbed profile that could be used either as markers for future risk or benefit of weight loss to prevent future T2D.
Materials and methods: Serum samples were collected from 14 non-diabetic obese volunteers (body mass index [BMI] 44±1.5 kg/m^2, mean±SEM) at time-points 0, 30, 120 min during a 75 g OGTT. Targeted metabolite profiling was performed by gas chromatography connected to a mass spectrometry (GC/MS). The dataset was compared with data previously obtained from lean volunteers (BMI 22.4±2.4 kg/m^2, mean±SEM) by principal component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) to find metabolite patterns discriminating between the OGTT time-points.
Results: As expected, the obese participants displayed impaired glucose tolerance due to lower insulin secretion and thereby unable to suppress blood glucose levels at 120 min during the OGTT compared to lean participants. More than 50 metabolites were identified in the obese and lean group respec-

tively, 35 of these metabolites were found in both groups and could therefore be compared. There was a distinct and significant separation between the patterns of metabolite concentrations from the different OGTT time-points between obese and lean participants. Several of the amino acids that were identified (asparagine, glutamate, taurine, tyrosine and isoleucine/leucine) showed an increased level in the obese compared to the lean during the first 30 min. At 120 min, these amino acids levels were decreased compared to baseline in the blood in both obese and lean individuals. The normal rapid insulin-mediated fatty acid suppression seen in the lean people was partially defect in the obese: The fatty acids identified (palmitic acid, lauric acid, oleic acid, pentadecanoic acid and stearic acid) showed a delayed response in obese individuals and suppressed only after 120 min.
Conclusion: In addition to the expected differences in glucose and insulin levels between lean and obese participants the OGTT is also characterized by other marked differences in the metabolic profiles. The altered OGTT-induced response for amino acids may be an example of an early pre-diabetic change in obese individuals. In addition, the delayed fatty acid response in the obese might serve as a helpful tool to identify the degree of a failing insulin sensitivity response. By finding metabolites with a differential response during an OGTT, it may be possible to distinguish obese people at risk for developing T2D and/or evaluate the effect of preventive measures such as weight loss and thereby become useful in clinical practice.
Supported by: VR

PS 043 Beta cell function in man

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In patients with newly-diagnosed type 2 diabetes beta cell function is an independent predictor of glucose control evolution over 18 months
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Background and aims: We asked the question whether the metabolic phenotype at baseline and/or a number of type 2 diabetes mellitus (T2DM) risk genes may predict the evolution of glucose control (GC) within the first 18 months after diagnosis of the disease.
Materials and methods: 593 GAD-antibodies negative patients with newly diagnosed T2DM (age: 59±0.4 yrs; BMI: 30.0±0.2 kg/m^2) were studied with: 1. Prolonged (5-hours) frequently sampled OGTT to assess beta cell function (BCF) by state of art mathematical modelling of glucose and C-peptide; 2. Standard euglycemic insulin clamp to assess insulin sensitivity (SI); 3. Genotyping the common T2DM risk variants of the following genes: ADAMTS9, CDKAL1, FTO, G6PC2, GCK, GCKR, GNPDA2, HHEX, HNF1B, IGF2BP2, IRS1, JAZF1, KCNJ11, MTNR1B, NOTCH2, PPARG, SCL30A8, TCFL2, THADA, TMEM18, TSPAN and WFS1. GC evolution was defined as the difference between HbA_{1c} at diagnosis (7.0±0.1%) and HbA1c at 18 months (6.5±0.1%).
Results: 141 patients were lost to follow-up, thereby leaving 452 patients for evaluation. In all multivariate regression models, basal HbA1c (standardized beta [stBETA]: 0.92, p<0.0001) was the strongest positive predictor of favourable GC evolution (i.e. the higher HbA_{1c} at diagnosis, the greater its fall within 18 months). No role for T2DM risk gene variants, either as a single SNP or as a genetic score derived from all SNPs, could be detected. BCF (stBETA: 0.26) and eGFR (stBETA: 0.08), but not age nor BMI nor SI nor pharmacological therapy, were positive independent predictors of favourable GC evolution (p<0.001 e p<0.01, respectively).
Conclusion: Thus, better BCF at diagnosis, but not SI nor the T2DM genotype assessed in this study, is an independent predictor and a putative determinant of more desirable short-term (18 months) GC evolution.
Clinical Trial Registration Number: NCT01526720

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Cross-sectional and longitudinal analyses of factors contributing to the progressive loss of beta cell function in type 2 diabetes
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Background and aims: Type 2 diabetes is a progressive disease characterized by insulin resistance and impaired insulin secretion. A gradual decline in insulin secretory capacity is a major obstacle to prevent the development of chronic vascular complications. In this study we assessed factors contributing to the insulin secretory defect in patients with type 2 diabetes by cross-sectional and longitudinal analyses.
Materials and methods: Subjects of this study were 327 inpatients with type 2 diabetes, consisted of 173 males and 154 females, aged 58 ± 13 years, with BMI of 24.3 ± 5.2. The duration of type 2 diabetes was 10.2 ± 8.5 years. The diagnosis of type 2 diabetes was established based on the EASD and ADA criteria for diabetes and the absence of pancreatic autoimmune markers including GAD antibodies and IA-2 antibodies. We excluded patients with renal failure, severe liver disease, or chronic pancreatitis. The lucagon stimulation test (GST) was performed to assess pancreatic β-cell function. None of the subjects were administered with incretin-related drugs. Among the subjects, 22 male and 28 female patients, aged 55.8 ± 9.7 years with BMI of 24.2 ± 4.5, underwent GST again after 4-9 years.
Results: A simple liner regression analysis of the cross-sectional data showed that ΔCPR (6-min postglucagon increment in CPR) decreases at the rate of 0.019 nmol/L/year. In a multiple regression model with gender, age, the duration of diabetes, BMI, the family history of diabetes, habitual alcohol intake, the presence of retinopathy, serum creatinine, basal plasma glucose, HbA1c, total cholesterol, HDL cholesterol, and log transformed triglyceride levels as independent variables, BMI and log(TG) were positively, and the duration of diabetes, the family history of diabetes, and the presence of retinopathy were inversely correlated with ΔCPR. In the 50 patients who underwent GST

twice, ΔCPR decreased from 0.755 ± 0.490 nmol/L to 0.573 ± 0.361 nmol/L over a period of 6.5 ± 0.9 years (p<0.0001). Thus ΔCPR declined at the ratio of 0.028 ± 0.045 nmol/L/year. Baseline BMI was positively associated with the declining rate of ΔCPR during the period between two GSTs. However, in a multiple regression analysis including baseline ΔCPR as an independent variable, baseline ΔCPR and the average of monthly HbA1c levels during the period were positively, and BMI was inversely associated with the declining rate of ΔCPR. Administration of sulfonylurea, biganide, alpha-GI, or insulin was not significantly correlated with the declining rate.
Conclusion: The progressive loss of insulin secretory capacity was demonstrated by both cross-sectional and sequential analyses of patients with type 2 diabetes. Thus the β-cell failure may be attributable to long-term metabolic disturbances. Furthermore, the presence of retinopathy was an independent predictor of insulin secretory defect, suggesting that intra-islet hypoperfusion could be involved in the β-cell dysfunction in patients with advanced microangiopathy. The longitudinal analysis showed that adequate glycemic control is a critical factor in preserving residual insulin secretory capacity in type 2 diabetes.

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Predictors of beta cell dysfunction in type 2 diabetes: the Beta Decline study

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Background and aims: Beta-cell dysfunction is an early step in the natural history of type 2 diabetes. However, its progression rate is variable and potentially influenced by several factors, including hypoglycaemic drugs. *BetaDecline* is an Italian multi-center prospective study, which has the aim to evaluate the association of beta-cell dysfunction with several clinical variables in a large cohort of type 2 diabetic subjects on stable treatment with oral hypoglycaemic drugs (OHAs) or diet only for more than one year.
Materials and methods: Clinical and lifestyle data were collected in all participants**.** Beta-cell dysfunction has been evaluated by the proinsulin/insulin ratio (P/I) and HOMA-B index; the degree of insulin resistance by $HOMA_{IR}$ model; common metabolic parameters, as well as fasting serum levels of NEFA, IL-6 and hsCRP were also measured.
Results: Overall, type 2 diabetic subjects participating to our study (n=508, 58.7% men and 41.3% women; mean age 63 years) were overweight (mean BMI 29.2 kg/m^2, mean waist circumference 102 cm) with an acceptable metabolic control (mean HbA_{1c} 7.2 %), and a mean diabetes duration of 8.8 years. Of these, 84% was currently treated with metformin, alone (39%) or in combination with other OHAs (45%); 35% was on sulphonylureas (SU), 10% on glytazones, 16% on glinides, 2% on acarbose and 5,5% of them was on diet only. At baseline, the PI/I ratio showed an inverse linear association with male gender, BMI, total cholesterol (T-C), LDL-C, HDL-C and hsCRP serum levels, and a positive linear association with HbA_{1c}, FBG and triglycerides levels (P trend<0.05 for all comparisons); whereas no significant differences were noted in IL-6 and NEFA concentrations, as well as in systolic and diastolic blood pressure values across quartiles of P/I ratio. Both HOMA-B and $HOMA_{IR}$ values progressively decreased across the increasing P/I ratio quartiles (P<0.0001, both). Secretagogues (SU e glinides) use was significantly greater in the highest P/I ratio quartile (P<0.0001), whereas no differences in the distribution of other hypoglycaemic, ipolipidemic and/or anti-hypertensive drugs according to the degree of beta-cell dysfunction were noted. At multivariate analysis, the risk for having a P/I ratio in the top quartile was four- times higher in diabetic subjects on secretagogues drugs (OR=4.2; IC95% 2.6-6.9), and greater in men (OR=1.8; IC95% 1.1-2.9).
Conclusion: In conclusion, in this large cohort of type 2 diabetic outpatients, secretagogues use is independently associated with a decrease of beta-cell secretion, which is also more frequent in diabetic men.
Supported by: MSD Italy

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CSII therapy should be stopped for accurate evaluation of beta cell function in type 2 diabetic patients

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Background and aims: It is well accepted that insulin secretagogues should be stopped before evaluation of β-cell function, because their stimulation of insulin secretion may lead to inaccurate assessment. Unlike insulin secretagogues, insulin therapy, especially continuous subcutaneous insulin injection (CSII),has been proved to have effect on "β-cell rest", which means endogenous insulin secretion may decrease during insulin therapy. No study investigates whether insulin should be stopped for evaluation of β-cell function in type 2 diabetic patients during CSII treatment. This study was designed to evaluate the impact of CSII on β-cell function assessment.
Materials and methods: Patients with type 2 diabetes (T2DM) admitted to our University Hospital treated with CSII were enrolled between September 2012 and April 2013. When patients achieved the target of fasting capillary glucose lower than 7.0mmol/L,serum samples were obtained 0min,30mins and 120mins after a fixed meal to measure C-peptide level. Then CSII were stopped at 10pm on the same day. The same tests were repeated on the next day.
Results: 49 patients with mean age of 52.7±12.4years,BMI 25.0±4.0Kg/m^2,HbA1c 10.8±2.8%,achieved the glycaemic target in 8.7±3.1days on 0.67±0.25 insulin(U/d/kg).C-peptide levels measured before stopping CSII were 0.21±0.10 nmol/L at 0min,0.33±0.18 nmol/L at 30mins, and 0.64±0.49 nmol/L at 120mins after the fixed meal. After CSII was stopped, C-peptide levels significantly increased (0min:0.38±0.11nmol/L;30mins:0.67±0.25nmol/L;120mins:1.36±0.65)(p<0.05). The percentages of C-peptide improvement [(C-peptide after CSII stopping - C-peptide before CSII stopping)/C-peptide before CSII stopping] were 133%, 147% and 208% at 0min,30mins and 120mins,respectively.The AUC of C-peptide increased from 103.66±61.33 noml/L×min before CSII stopping to 214.65±82.09noml/L×min after CSII stopping (p<0.05).
Conclusion: Effect of "β-cell rest" induced by intensive insulin therapy may bias β-cell function assessment in type 2 diabetic patients. Therefore, CSII therapy should be stopped for accurate evaluation of β-cell function in type 2 diabetic patients.

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Liver transplantation improves both beta cell function and insulin sensitivity and may induce reversal of type 2 diabetes mellitus in cirrhotic patients

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Background and aims: Diabetes mellitus develops in a variable proportion of patients undergoing orthotopic liver transplantation (OLT). The roles played by insulin secretion and action in determining changes in glucose tolerance after OLT are imperfectly known. Aim of this study was to monitor glucose tolerance, beta cell function and insulin sensitivity in a cohort of patients undergoing OLT in an effort to unveil the determinant(s) of changes in glucose homeostasis.
Materials and methods: 48 patients patients with liver cirrhosis, 33M/15F, age 52 ± 16 years, were studied before and 3, 6, 12 months after OLT with a frequently sampled oral glucose tolerance test (fsOGTT). Indication to, and treatments before and after OLT were as per ongoing protocols in our Institution, and are strictly adherent to international guidelines. Patients were planned to be classified as NonConverters (NC), i.e. patients who would maintain either nondiabetic or diabetic glucose regulation, or Converters to diabetes mellitus (C), or Regressors to nondiabetic glucose regulation (R). Beta cell function was assessed by state-of-art modelling of glucose/C-peptide curves during the fsOGTT. There are two key outputs of the model: derivative control (DC: amount of insulin secreted in response to the rate of plasma glucose increase; units: [pmol·m^{-2} BSA]/[mM·min^{-1}]) and proportional control of beta-cell function (PC). response to glucose concentration per se, presented as the stimulus-response curve linking glucose to insulin secretion rate at the

preselected glucose concentrations of 5.5, 8.0, 11.0, 15.0 and 20.0 mM; units: pmol·min^{-1}·m^{-2} BSA). The OGIS index, a robust estimate of insulin sensitivity, was computed with the glucose/insulin data of the fsOGTT.
Results: 45% of patients were found to be diabetic before OLT. After transplantation, glucose tolerance improved progressively (p<0.0001), and diabetes prevalence decreased to 40.3%, 30.2% and 24.3% at 3, 6, 12 months, respectively. No C were found. In multivariate analysis, shorter duration of liver disease and higher HDL-cholesterol levels at baseline were significant predictors (p<0.05 for both) of being R. R (p<0.02), but neither NC group (p=0.29-086), showed a significant improvement in the DC of beta cell function. Both in nondiabetic NC and in R, but not in diabetic NC, there were progressive improvements in the DC of beta cell function (p=0.02-0.03, p=0.001-0.02, and p=0.46-0.97 in nondiabetic NC, R and diabetic NC, respectively) and in the OGIS index (p=0.02, p=0.0001 and p=0.74 in nondiabetic NC, R and diabetic NC, respectively). Influences of pre- and post-OLT treatments were found to be minor.
Conclusion: In cirrhotic patients, OLT most often is associated with progressive, remarkable improvements in both beta cell function and insulin sensitivity, which in a number of diabetic patients result into reversal of diabetes. Diabetic patients with a long story of liver disease and low HDL-cholesterol levels are resistant to the beneficial effects of OLT on glucose metabolism.

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Modelled response to a meal is useful method to characterise beta cell function (BCF) across the glucose tolerance (GT) spectrum: correlation with FSIGT
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Background and aims: It is important to easily measure BCF and IS in longitudinal clinical trials and show similar responses with methodology like the FSIGT. Mixed meal tolerance tests (MMTT) are appealing due to simplicity and relevance to enteric physiology, yet complex due to variable absorption. The goal of this study is to measure BCF and IS to a standard, commercially available 450 kCal meal across the spectrum from normal GT (NGT) to prediabetes (PDM) to type 2 DM (T2DM) and to compare the BCF and IS responses to those from the FSIGT.
Materials and methods: All subjects were tested after an overnight fast on 3 separate days. Each subject had 2 MMTTs and 1 FSIGT. Parameters for BCF were estimated using published methods. The table summarizes sample size, BMI, and respective MMTT/FSIGT measures for: IS (Si); insulin release (Φtot and AIRg); and disposition index (DItot and DI) by group. MMTT parameters and FSIGT Si summarized as geometric means (95% CI); all others as arithmetic means (95% CI).
Results: The table below summarizes the results of the study. For MMTT and FSIGT, Si, Φtot and AIRg, and DI, values decline from NGT to T2DM (all P<0.001). Correlation analysis for each MTT/FSIGT parameter pair ACROSS ALL 3 GROUPS: Si (r=0.69); Φtot/AIRg (r=0.73); and DItot and DI (r=0.74), suggesting that values from the tests tracked similarly across GT states.
Conclusion: Modeled results from the MMTT correspond to FSIGT-derived parameters across range of responses of GT spectrum.

			Insulin Sensitivity		Insulin Secretion		Disposition Index	
	N	BMI (kg/m2)	MMTT Si (10^{-4}/((μU/ml)(min))	FSIGT Si (10^{-4}/((μU/ml)(min))	MMTT Φtot (10^{-9}/min)	FSIGT AIRg (μU min/ml)	MMTT DItot (10^{-13}/((μU/ml)(min^2))	FSIGT DI
NGT	23 12M/11W)	31.5 (30.3-32.7)	4.5[a] (3.83-5.33)	1.6[a] (1.23-2.08)	102.6[a] (89.0-118.4)	914[a] (594-1235)	464[a] (375-573)	1339[a] (970-1709)
PDM	8 (2M/6W)	33.0 (31.0-35.0)	2.3[b] (1.75-3.04)	1.2[a] (0.9-1.59)	108.4[a] (93.8-125.3)	412[b] (91-733)	250[a] (190-329)	520[b] (124-915)
T2DM	22 (11M/11W)	32.8 (31.1-34.5)	1[c] (0.74-1.35)	0.49[b] (0.29-0.8)	15.4[b] (12.8-18.6)	10.4[b] (-8.7-29.6)	16.8[b] (11.3-24.9)	9.3[b] (-13.8-32.5)
	ANOVA across populations (P) Superscripts that differ from one another are statistically separate for that parameter, $P<0.05$.		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Clinical Trial Registration Number: NCT01663220
Supported by: Funders of the FNIH Biomarkers Consortium Beta Cell Project Team

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Pramlintide improves insulin action and worsens beta cell responsivity in healthy adults: a minimal model study
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Background and aims: Use of Pramlintide to treat type 1 diabetes (T1DM) within closed loop artificial pancreas systems is currently being explored. However, its effects on postprandial insulin sensitivity and glucose kinetics are relatively unknown.
Materials and methods: We studied healthy individuals (n=7; 2 males; age 36±4 years; BMI 24±1 kg/m^2; %body fat 29±4%; HbA1c 5±0.2%) with scintigraphically measured normal gastric emptying on two occasions in random order with or without 30mcg Pramlintide administered at start of a mixed meal. The oral glucose and C-peptide minimal models were used to measure insulin action and secretion, respectively.
Results: Although peak plasma glucose concentration was delayed with pramlintide (40.7±2.8 vs. 100.7±5.4 min; p<0.05) postprandial glucose excursions over six hours did not differ. In contrast, postprandial insulin excursion was lower (4904.3±464.6 vs. 3603.2±322.0 μU/ml/6 h; p<0.05) with pramlintide. Insulin sensitivity (S_I) was higher (16.9±3.0 vs. 24.9±2.5 10^{-4} dL/kg/min per μU/mL; p<0.05), β-cell responsivity lower (16.1±1.3 vs. 13.4±0.8 10^{-9} min^{-1}; p<0.05) and disposition index higher (428.0±42.4 vs. 541.9±35.4 10^{-14} dL/kg/min^2 per pmol/L; p<0.05) with pramlintide.
Conclusion: Pramlintide improves insulin sensitivity in healthy adults. In contrast, beta cell responsivity was reduced with disposition index higher with pramlintide. Triple tracer studies were also done in the same subjects and will be reported later. Similar studies are necessary in T1DM before application of pramlintide into future informed closed loop control systems.
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Adaptive insulin response to increasing caloric load in healthy subjects
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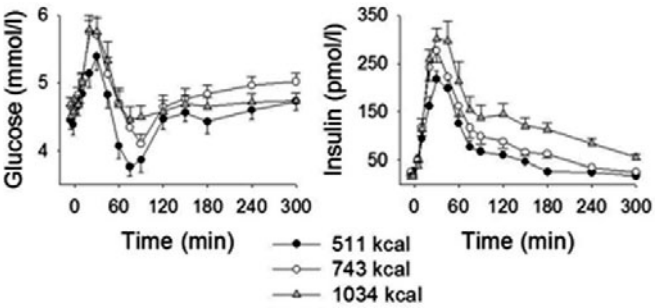
Background and aims: The dynamic interaction between insulin demand and insulin levels is a key factor for glycemic control and of importance for the fine-tuned insulin response to ingestion of meals. However, little is known

on the insulin adaptation to different caloric loads in meals of more regular daily life size and at other times of the day than in the morning after an overnight fast. We therefore examined the insulin adaptation to demands created by ingestion of increasing caloric loads in identical meals at lunch time after a standard breakfast in healthy subjects.
Materials and methods: Twenty-four healthy lean subjects (12 M, 12F, mean age 25 yrs, BMI 22.2 kg/m2) ingested a standard 500 kcal meal as breakfast after an overnight fast followed by a standardized lunch exactly four hours later. The caloric content of the lunch was 511, 743 or 1034 kcal and had identical nutrient composition (protein 18E%, fat 32E% and carbohydrate E50%). Blood samples for analysis of glucose, insulin and C-peptide were taken. Insulin secretion was evaluated by an insulinogenic index, calculated as the 120 min area under the curve (AUC) for C-peptide divided by 120 min AUCglucose (IGIC-peptide, pmolC-peptide/mmolglucose), insulin sensitivity with the composite insulin sensitivity index (ISIcomp) and insulin clearance by hepatic insulin extraction (HE) from insulin and C-peptide data.
Results: Plasma glucose rose rapidly after all three meals to a peak at 30 min. The small meal had a post-peak reduction below baseline to a nadir of 3.8±0.1mmol/l after 75min ($p<0.001$). For the two larger meals, glucose levels slowly declined after the peaks to reach baseline after 90 min. Plasma insulin concentrations increased after all three meals with 30 min peak levels being significantly higher with 743 and 1034 kcal meals than with 511kcal meal (both $P<0.02$) but with no significant difference between the two larger meals. IGIC-peptide increased by increasing caloric load and was 0.17±0.01, 0.19±0.01 and 0.21±0.01 after the meals, respectively ($P<0.001$). HE was reduced by increasing caloric load (62±2% with 511 kcal, 58±3% with 743 kcal and 50±3% with 1034 kcal $p\leq0.032$ between meals). There was no significant difference in ISIcomp between the three meals (58±7, 58±8 and 58±7, respectively).
Conclusion: Lunch meals elicit a caloric dependent adaptation in beta cell secretion and reduction in insulin clearance, whereas dynamic insulin sensitivity does not depend on caloric intake. The increased insulinemia at the two larger meals perfectly adapts to the caloric demand, resulting in identical glucose excursions, whereas after a lower caloric lunch (511 kcal) the insulin response is inappropriately high resulting in post peak suppression of glucose below baseline. We suggest that the insulin adaptation to caloric demands after lunch meals in healthy humans involves both beta cell secretion and insulin clearance and is perfect after meals of medium or large size, whereas lower caloric intake results in an overshoot of insulin, which may elicit post-lunch hypoglycemia.



Clinical Trial Registration Number: NCT01366781
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PS 044 Incretins: secretions and action

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CART is expressed in L-cells and K-cells in humans, regulates GIP and GLP-1 expression in vitro, and potentiates glucose stimulated GIP secretion in vivo in mice
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Background and aims: Cocaine- and amphetamine-regulated transcript (CART) is a regulatory peptide expressed in the nervous system, adrenal medulla, pancreatic islets, adipose tissue, and neuroendocrine tumors. CART stimulates insulin secretion and inhibits glucagon secretion and CART-/- mice have blunted insulin secretion in vitro and in vivo. We have recently shown that CART is expressed in human enteroendocrine cells, including L- and K-cells in the human duodenum and jejunum. Furthermore, we have shown that CART plasma levels increase after a meal in humans. The main objective of the present study was to assess the relationship between CART and the incretins glucagon like peptide 1 (GLP-1) and glucose dependent insulinotropic peptide (GIP) using in vitro and in vivo model systems.
Materials and methods: Human specimens of duodenum and jejunum (n=10) were double-stained for CART and GIP or GLP-1 to quantify the co-expression of CART with incretin hormones. GLUTag and STC-1 enteroendocrine cell lines were used as L- and K-cell models respectively to study regulation of CART gene expression in vitro. Adenoviral overexpression of human CART in GLUTag and STC-1 cells was used in order to study the effect of CART on incretin gene expression. To test the effect of CART on GIP secretion in vivo in mice CART was administered i.v. during an oral glucose-tolerance test (OGTT).
Results: The majority of CART expressing endocrine cells in human duodenum and jejunum were identical to GIP-producing K-cells (75±8 % and 54±17% respectively). CART was also expressed in minor proportions of GLP-1-producing L-cells in both duodenum and jejunum. Expression of CART was also evident in GLUTag and STC-1 cell lines; therefore these cell lines were used to study CART gene regulation. In both STC-1 and GLUTag cells CART mRNA levels were positively regulated by GIP ($P<0.05$), but not by GLP-1. However, adenoviral overexpression of CART increased GLP-1 and GIP mRNA levels ($P<0.05$) in STC-1, but not in GLUTag cells. In addition, intravenous administration of CART 54-102 during an OGTT provoked elevated GIP secretion at 10 and 20 min ($P<0.01$ and $P<0.001$) and AUC for GIP at 0-30 min ($P<0.01$).
Conclusion: CART is co-expressed with GIP and GLP-1 in the human duodenum and jejunum. CART mRNA is regulated by GIP in enteroendocrine cell lines. CART regulates transcription of GLP-1 and GIP in STC-1 cells. CART potentiates glucose-stimulated GIP secretion in vivo in mice. Thus, our data suggest that CART is a regulator of incretin synthesis and secretion. Whether CART is co-secreted with the incretins needs further investigation.
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Identification of novel GPR39 agonists that stimulate insulin and GLP-1 secretion in vitro using StaR® technology
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Background and aims: Activation of GPR39, a GPCR expressed in the gastrointestinal tract, adipose tissue, liver and pancreas, by zinc couples to Gαs and Gαq to increase intracellular cAMP and(or) Ca^{2+} levels. Genetic studies have implicated a key role for GPR39 in metabolic regulation and pancreatic islet function. Using proprietary stabilised receptor (StaR®) technology and a homology modelling based approach, agonists for GPR39 have been identified and used to examine the role of GPR39 in insulin and incretin secretion *in vitro*.
Materials and methods: cAMP (Gαs) and IP_1 (Gαq) accumulation in response to agonist stimulation was assayed in HEK293 cells transiently expressing hGPR39. The secretion of insulin or GLP-1 in response to GPR39 agonism was evaluated using the pancreatic β-cell line, NIT-1, or primary

mouse intestinal epithelial cells (mIECs). Endpoints were assayed using HTRF cAMP, IP_1, insulin or active GLP-1 detection kits (Cisbio).
Results: Zn^{2+} and compounds (G#01, G#07, G#50 and G#89) stimulated Gαs and Gαq activity in HEK293 cells expressing hGPR39 (table 1). Mock transfected HEK293 cells were unresponsive to Zn^{2+} or compound. G#01, G#50 and G#89 acted as positive allosteric modulators of Zn^{2+}, significantly improving the $EC_{50(cAMP)}$ of Zn^{2+} from 245.3 ± 13.4 nM to 59.6 ± 6.1, 56.1 ± 5.2 and 37.9 ± 3.7 nM, respectively (all $P < 0.001$). Compounds stimulated GLP-1 secretion from mIECs and glucose-dependently stimulated insulin secretion from NIT-1 cells above the E_{max} for Zn^{2+} (table 1; $P < 0.001$). siRNA knockdown of GPR39 inhibited the insulin secretory activity of Zn^{2+} and all compounds, and inhibited basal insulin release from NIT-1 cells by 34 ± 8.7 % ($P < 0.001$). The insulinotropic activity of G#01, G#07, G#50 and G#89 (100 μM) was driven by a combination of Gαs and Gαq since it was partially inhibited by 5 μM H-89 (a PKA inhibitor) and also partially inhibited by both 5 μM nitrendipine (a voltage-gated Ca^{2+} channel inhibitor) and 50 nM LY333531 (a PKC inhibitor). GLP-1 secretion mediated by these compounds (100 μM) was inhibited by both 5 μM nitrendipine and 50 nM LY333531 indicating a Gαq-mediated component, and secretion by G#01 and G#89 was also inhibited by 5 μM H-89, showing that Gαs contributes towards the GLP-1 response raised by these compounds.
Conclusion: In summary, GPR39 agonists identified using StaR® technology can stimulate insulin and GLP-1 secretion from native/primary cell systems *in vitro*. GPR39 agonists which can stimulate Gαs and Gαq signalling pathways to elicit gut hormone responses and increase glucose-dependent insulin secretion positions GPR39 agonists as potential efficacious agents for metabolic disease and may have therapeutic benefits.

Table 1. In vitro properties of GPR39 agonists Zn2+, G#01, G#07, G#50 and G#89

agonist	HEK293 cells transiently expressing hGPR39				mIECs		NIT-1 cells	
	cAMP accumulation (1)		IP1 accumulation (2)		GLP-1 secretion (3)		Insulin secretion (4)	
	EC_{50} (nM)	E_{max} (% Zn^{2+})	EC_{50} (nM)	E_{max} (% Zn^{2+})	EC_{50} (nM)	E_{max} (% Zn^{2+})	EC_{50} (nM)	E_{max} (% Zn^{2+})
zinc	844.0	100.0	598.1	100.0	1588.0	100.0	256.5	100.0
G#89	2389.0	101.5	32600.0	43.4*	3431.4	161.9*	449.9	151.3*
G#50	1292.0	58.0*	25860.0	97.2	116.2	147.8*	387.1	141.8*
G#07	2170.0	63.9*	22410.0	31.9*	172.8	170.8*	368.1	145.6*
G#01	4380.0	157.7*	14710.0	96.7	1183.7	270.8*	938.7	262.9*

Stimulation of cAMP (1) or IP_1 (2) accumulation in HEK293 cells transiently expressing hGPR39 or stimulation of active GLP-1 secretion (3) from mouse IECs or insulin secretion (4) from NIT-1 cells by GPR39 agonists, Zn^{2+} or compound G#01, G#07, G#50 or G#89. Statistical significance was determined using Students *t* test vs. Zn^{2+} E_{max} where * $P < 0.001$

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Peptide sensing by glucagon-like peptide-1 secreting L cells
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Background and aims: L-cells are enteroendocrine cells scattered along the length of the intestinal tract. They secrete several metabolically active peptides, most notably glucagon-like peptide-1 (GLP-1) which enhances postprandial insulin secretion, reduces appetite and promotes β-cell survival and proliferation. GLP-1 is secreted physiologically in response to ingestion of a variety of macronutrients, including carbohydrates, protein and lipids. It remains unclear, however, how protein digestion products trigger GLP-1 secretion, and the aim of this project was to investigate the mechanisms underlying L-cell detection of small peptides.
Materials and methods: A transgenic mouse model in which GLP-1 producing cells were labelled with the yellow fluorescent protein, Venus, was utilised to identify and purify L-cells. Primary murine colonic cultures were stimulated with di/tripeptides and protein hydrolysate, and GLP-1 secretion was measured. Ratiometric [Ca2+]i imaging experiments were carried out on identified L-cells in mixed epithelial cultures loaded with Fura-2AM.
Results: The di- and tripeptides, glycine-phenylalanine (GF), leucine-glycine-glycine (LGG) and the non-hydrolysable dipeptide glycine-sarcosine (GSar) stimulated GLP-1 secretion from primary intestinal cultures (1.5±0.4, 1.5±0.3, 1.5±0.1 fold stimulation respectively, compared to baseline, $p<0.05$) accompanied by reversible rises in L-cell Ca^{2+} levels (measured as 1.3±0.1 fold for GLL and 1.5±0.1 fold for GSar increases of the 340/380 nm fluorescence ratio compared to baseline, $p<0.01$). GSar-triggered GLP-1 secretion was abolished by the Ca^{2+} channel blocker nifedipine. Consistent with the involvement of voltage gated Ca^{2+} channels, GSar did not elicit a Ca^{2+} response in Ca^{2+} free buffer. Dipeptides, including GSar, are established substrates for the proton-coupled transporter PepT1. To investigate the potential involvement of PepT1 in GLP-1 secretion, experiments were performed at pH 6.5, 7.4 or 8. GSar-triggered GLP-1 release was pH dependent, with highest responses evident at pH 6.5 (1.9±0.1 fold above baseline, $p<0.001$) and lowest responses at pH 8 (1.2±0.1 fold above baseline, $p<0.05$). At pH 8, the Ca^{2+} response to GSar was also impaired. The non-translocated PepT1 inhibitor 4-Aminomethylbenzoic acid (AMBA) significantly reduced GSar stimulated GLP-1 release ($p<0.05$ vs GSar alone). Consistent with a role for PepT1 in protein sensing, GSar triggered GLP-1 release was impaired in colonic cultures from PepT1-/- mice ($p<0.01$ vs secretion from $PepT1^{+/+}$ mice). Protein hydrolysate (1, 5 or 50mg/ml), dose dependently enhanced GLP-1 secretion (1.6±0.1, 3.6±0.3, 10.3±0.4 fold vs baseline respectively, $p<0.001$) and these responses were unaffected by AMBA. A selective Ca^{2+}-sensing receptor antagonist (NPS2143) significantly attenuated protein-hydrolysate triggered GLP-1 secretion ($p<0.01$).
Conclusion: Our findings suggest that the stimulation of GLP-1 secretion from primary murine cultures by protein digestion products involves mechanisms employing PeptT1 and the Ca^{2+}-sensing receptor. Although GLP-1-mimetics and DPP4 inhibitors are widely used in the treatment of type 2 diabetes, stimulating pathways involved in endogenous GLP-1 release could offer new therapeutic strategies for diabetes and obesity.
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Distribution of fatty acid binding receptors in GIP-secreting K cells in the small intestine
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Background and aims: Gastric inhibitory polypeptide (GIP) is an incretin secreted from enteroendocrine K-cells after nutrient uptake and has a role in compensatory insulin secretion in obese state and fat storage in adipocytes. GIP secretion is increased under high-fat diet (HFD) feeding. We reported that long-term high-fat diet (HFD) feeding increases GIP mRNA and content in K-cells of GIP-GFP knock-in (GIP-GFP) mice which enable us to visualize K-cells by EGFP (Suzuki K, et al. J Biol Chem 2013). On the other hand, it is unknown how single-dose HFD-administration increases GIP secretion. Several fatty acid binding receptors are reported to be involved in GLP-1 secretion, however their expressions in K-cells remains unclear. In this study, we evaluated expressions of these receptors in K-cells.
Materials and methods: Tissue distribution of K-cells and K-cell number in gastrointestinal (GI) tract were evaluated by flow cytometry analysis and immunohistochemistry using GIP-GFP heterozygous mice. After collecting GFP-positive cells and GFP-negative cells as K-cells and non-K-cells, respectively, from GI tract by flow cytometer, RT-PCR was performed to evaluate expressions of GIP and fatty acid binding receptors (GPR40, GPR41, GPR43, GPR119, and GPR120).
Results: Immunohistochemical and flow cytometry analysis showed that GFP-positive cells were expressed in upper (US) and lower (LS) small intestine, but not in stomach and colon. The number of GFP-positive cells was significantly higher in US than those in LS (0.052% vs. 0.028% of epithelial cells; $P<0.05$). GIP mRNA expression and GIP content was 4-hold and 2-hold higher in K-cells of US than that in LS, respectively. GPR120 mRNA expression was exclusively high in K-cells of US, while GPR40 and GPR43 mRNA expressions were exclusively high in K-cells of LS. GPR41 and GPR119 mRNA expressions tended to be increased in K-cells of LS, but there was no significantly difference between K-cells and non-K-cells. Because GPR40, GPR43, and GPR120 belong to Gq-protein-coupled receptor, we examined inositol trisphosphate receptor (IP3R) expression and found that IP3R isoform 3 was mainly expressed in K-cells of US and LS.

Conclusion: There is a difference in not only K-cell number but also GIP expression in K-cells between upper and lower small intestine of GIP-GFP mice. Furthermore, fatty acid binding Gq-protein-coupled receptors, such as GPR40, GPR43, and GPR120, are highly expressed in K-cells and might be involved in GIP secretion after high-fat diet ingestion.

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Temporal profiles of hormone expression in mouse enteroendocrine cells

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Background and aims: Hormones from intestinal endocrine cells coordinate a complex array of metabolic effects including the regulation of intestinal motility, insulin secretion and appetite, with the two incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) being therapeutic candidates for treating type 2 diabetes. In a recent study using reporter mice in which a yellow fluorescent protein Venus is driven by the promoter for either GIP or GLP-1, we showed that the respectively labelled K and L cell populations have a more plurihormonal nature than previously thought. Cholecystokinin (CCK), for example, traditionally considered a product of I-cells, was co-produced by the majority of small intestinal K and L cells. This study examined whether enteroendocrine cells express different hormones during the course of their lifespan.
Materials and methods: We used mouse models in which cre-recombinase was expressed under the control of promoters for either GIP (GIP-Cre) or proglucagon (GLU-Cre), which when crossed into a floxed tandem red fluorescent protein (tdRFP) reporter strain resulted in permanent labelling of the cells after first expression of either GLP-1 or GIP. Fixed tissue samples from GIP- or GLU-Cre x tdRFP mice were immunostained with antibodies against different hormones. Percentages of cells producing different hormones were assessed by immunostaining of cell suspensions and flow cytometric (FACS) analysis. Confocal microscopy of immunostained fixed tissue was performed to analyse hormonal co-localisation in intracellular compartments.
Results: By immuno-FACS analysis, 94±1% of RFP+ve cells from GIP-Cre x tdRFP mice stained for GIP, and 71±2% of colonic RFP+ve cells from GLU-Cre mice stained for GLP-1. In these same mouse models, 60±3% of GIP positive cells and 72±2% of colonic GLP-1 positive cells contained tdRFP. The high percentage of RFP+ve cells staining for GIP in GIP-Cre x tdRFP mice indicates that once a cell has started expressing GIP in the small intestine, it continues to produce the hormone at the protein level during its entire lifespan. In these mice with labelled K-cells, the percentages of RFP positive cells that were immunostained for GLP-1 (4±0.5%), CCK (94±1%) and somatostatin (8±1%) were similar to the proportions revealed previously using the model in which Venus was driven directly by the GIP promoter. In the small intestine of GLU-Cre x tdRFP mice, 80±6% of RFP+ve cells stained for CCK and 16±2% stained for GIP, proportions similar to our previous observations in GLU-Venus mice (90% and 15%, respectively). By confocal microscopy of triple-stained small intestinal tissue, a subset of cells was found to contain GLP-1, Peptide YY (PYY) and CCK, and the hormones were found located in the same intracellular compartments.
Conclusion: Our data suggest that individual enteroendocrine cells can produce a number of hormones, and that under stable dietary conditions the proportions of co-expressed hormones do not vary markedly throughout the lifetime of a single K or L-cell. Whether the percentage overlaps are altered by diet or metabolic disease will be an interesting area for future studies.
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Loss of GIP responsiveness in human adipocytes from an obesity context: potential therapeutic implications

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Background and aims: Incretins has emerged as important players in the glucose homeostasis since both glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) stimulates glucose-dependent insulin secretion. In fact, new pharmacological agents based on incretin action have emerged as useful tools fighting insulin resistance and type 2 diabetes (T2D). Contrary to GLP-1, GIP has been discarded for diabetes treatment because a low insulin secretory property has been detected in T2D patients. However the extra-pancreatic effects of these molecules deservers further investigations and adipose tissue effect of incretins is far from clear. Interestingly, genome-wide association studies for identifying loci correlated with body mass index (BMI) have shown that GIP receptor (GIPR) is a new loci associated with body BMI.
Materials and methods: GIPR expression was analysed in subcutaneous (SAT) and visceral (VAT) human adipose tissue sample from lean and obese subjects and replicated in three different cohorts. A series of lipogenic, lipolytic and GPCRs signaling gens were also screened in adipose tissue. In vitro studies were performed to evaluate the effect of GIP on insulin sensitivity and inflammation. GIP responsiveness was evaluated in human adipocyte cell lines in normoxic and hypoxic environment, as well as in adipose-derived stem cells obtained from lean and obese patients.
Results: GIPR expression was consistently down-regulated in adipose tissue from obese patients, both at mRNA and protein expression levels. Body mass index, adrenergic receptor kinase 1 (GRK2) and adipose triglyceride lipase (ATGL) genes were the main determinants of the GIP mRNA expression in SAT depot (ß coefficients: -0,199; 0,78 and 0,28; p: 0,03; <0,001 and 0,003 respectively). Study of glucose uptake and insulin signalling in human subcutaneous/visceral adipocytes revealed GIP as an insulin sensitizer incretine. Long-term treatment with GIP not only increases insulin sensitivity on glucose uptake but also attenuated insulin resistance induced by IFN-γ in human subcutaneous adipocytes. Analysis of IL-6 and MCP-1 mRNA expression also revealed an anti-inflammatory effect of GIP. These effects of GIP observed under normoxia were not detected in human fat cells cultured in mild hypoxia environment. In agreement, GIP increases insulin sensitivity in human adipose-derived stem cells from lean but not obese patients.
Conclusion: Beneficial effects of GIP in human adipocytes might be compromised in an obesity context. Normalizing GIP function on insulin sensivitiy and inflammatory state in human adipocytes might represent a potential therapeutic approach in the treatment of obesity-associated metabolic disorders.
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Glucose-dependent insulinotropic polypeptide: blood glucose stabilising effects around fasting glycaemia in patients with type 2 diabetes

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Background and aims: We have previously shown in healthy subjects that the gut-derived hormone glucose-dependent insulinotropic polypeptide (GIP) has inverse effects on glucagon and insulin release, highly dependent on the plasma glucose levels. In addition, disturbances in GIP signalling have been causally linked to the inappropriate insulin and glucagon levels characterising patients with type 2 diabetes. Consequently, we aimed to determine the glucose-dependency of GIP effects in patients with type 2 diabetes.
Materials and methods: Twelve male subjects with type 2 diabetes (age: 62±1 years (mean±SEM); BMI: 29±1 kg/m^2; HbA_1c: 6.5±0.1%) were each studied on six different occasions. In a randomised order, the effects of intravenously physiological doses of GIP or placebo (saline) were compared at three glycaemic levels i.e. fasting glycaemia, insulin-induced hypoglycaemia, or hyperglycaemia (1.5×fasting plasma glucose).
Results: During *fasting glycaemia* (plasma glucose ~8 mmol/l), GIP elicited significant increments in both insulin and glucagon levels, resulting in neutral effects on plasma glucose. During insulin-induced *hypoglycaemia* (plasma glucose ~3 mmol/l), GIP elicited a minor first phase insulin response and a significant glucagon response during the initial 30 minutes, resulting in less glucose needed to be infused to maintain the clamp (29±8 vs. 49±12 mg×kg^{-1}, p<0.03). During *hyperglycaemia* (plasma glucose ~12 mmol/l), GIP augmented both first and second phase insulin secretion, with similar or slightly less glucagon suppression compared to saline, resulting in more glucose needed to maintain the clamp during GIP infusions (265±21 vs. 213±13 mg×kg^{-1}, p<0.001).
Conclusion: In patients with type 2 diabetes, GIP counteracts insulin-induced hypoglycaemia conceivably through a predominant effect on glucagon

release and subsequent enhancement in endogenous glucose production. In contrast, during hyperglycaemia, GIP increases glucose disposal through predominant effects on insulin release.
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GLP-1 response after oral glucose overload test in patients with a history of gestational diabetes
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Background and aims: Assessing levels of GLP-1 before and after an oral glucose overload a year after the delivery in two groups of women who developed gestational diabetes mellitus (GDM) during pregnancy: healthy and pathological according to oral glucose overload.
Materials and methods: We have studied 41 women with prior GDM at 12.20 ± 1.36 months postpartum with a mean age of 32.88 ± 3.58 years and a BMI of 27.5 ± 5.23 kg/m2. Levels of glucose, insulin, C-peptide and GLP-1 were analysed before and 2 hours after an oral load of 75 g glucose. Women were classified as healthy and unhealthy according to oral glucose load test results.
Results: Levels of glucose (95.52 ± 9.45 mg / dl vs. 11.76 ± 38.72 mg / dl), C-peptide (2.44 ± 0.95 ng / ml vs. 10.41 ± 3.16 ng / ml), insulin (10.83 ± 6.68 mU / ml vs. 72.84 ± 43.2 mU / ml) and GLP-1 (2.27 ± 1.51 vs. 2.43 ± 1.92 ng / ml) were significantly increased after oral glucose overload. After dividing the patients according to healthy and pathological oral glucose tolerance test, we found that baseline levels of insulin (9.55 ± 5.82 mU / ml vs. 13.48 ± 7.66 mU / ml), glucose (91, 79 ± 5.70 mg / dl vs. 103.85 ± 11.0 mg / dl) and C-peptide (2.22 ± 0.82 ng / ml vs. 2.88 ± 1.07 ng / ml) were increased in pathological women compared to healthy ones, whereas GLP-1 levels (2.49 ± 1.72 ng / ml vs. 1.79 ± 0.71 ng / ml) were decreased. After loading, the levels of insulin and C-peptide increased significantly in both groups. Glucose levels increased significantly only in the group of pathological women and by contrast, GLP-1 levels increased significantly in the group of healthy women.
Conclusion: Women with a pathological response to the oral glucose overloading test showed a dysfunction in the production of GLP-1 after oral glucose overloading test compared with healthy women that showed a significant increase.

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Time course of the incretin effect across the spectrum of glucose tolerance assessed by modelling analysis
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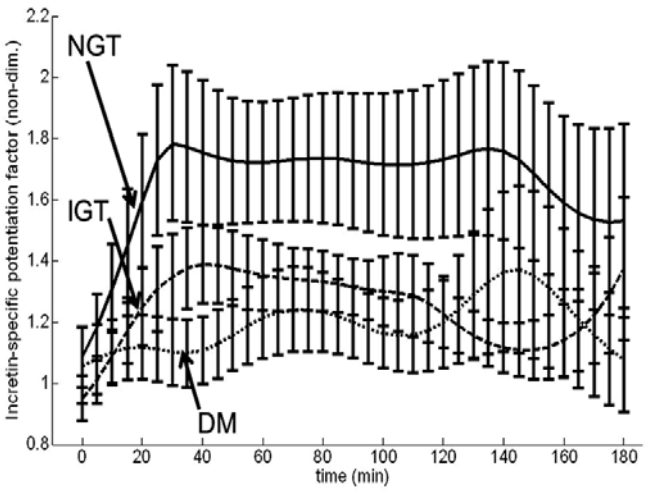
Background and aims: An oral glucose tolerance test (OGTT) determines a higher insulin response than an isoglycemic intravenous glucose infusion (IIGI), due to the incretin effect. The incretin effect is typically evaluated as the relative increase in insulin secretion (ISR) in the OGTT compared to the IIGI test. This integral index does not reveal how the action of incretins develops over time. We aimed at evaluating the time course of the incretin effect and its impairment in glucose intolerant states using paired OGTTs and IIGIs and a mathematical model.
Materials and methods: Modeling analysis was performed in 50 subjects; 23 had normal glucose tolerance (NGT), 17 had impaired glucose tolerance (IGT) and 10 had type 2 diabetes (DM). The subjects underwent a 3-hr OGTT (75 g) and an IIGI test, with measurement of glucose, insulin, C-peptide, GLP-1 and GIP. The model represents ISR during IIGI as the sum of two components. The first term describes the dependence of ISR on glucose concentration through a dose-response function (slope = glucose sensitivity), modulated by a glucose-dependent potentiation factor. In the second term, early ISR is proportional to the glucose rate of change with time; its characteristic parameter is *rate sensitivity*. During the OGTT, ISR is represented in an analogous way, but using an OGTT-specific rate sensitivity and multiplying the IIGI dose-response component by a time varying term representing *incretin potentiation* (P_{INCR}).
Results: During IIGI, β-cell glucose sensitivity was progressively impaired from NGT to DM (85.4±8.6, 58.4±8.4, 36.3±5.7 pmol min^{-1} m^{-2} mM^{-1}; NGT, IGT, DM), as was rate sensitivity (673±158, 571±126, 80±36 pmol m^{-2} mM^{-1}). The incretin effect on rate sensitivity (OGTT: 1733±269, 1410±210, 912±172 pmol m^{-2} mM^{-1}) was clearly present in all glucose tolerance groups (P<0.002), but was impaired in DM compared to NGT (P<0.038). In NGT, incretin potentiation P_{INCR} (Figure) rapidly increased and remained sustained during the whole OGTT (mean P_{INCR}>1 both in the period 30-90 min and 120-180 min, P<0.009). In IGT, the increase was transient (P_{INCR}>1 only in the period 30-90 min, P<0.007), while in DM there was no significant increase (P>0.2). After adjustment for BMI, a factor known to influence the incretin effect, a significant difference in P_{INCR} AUC between NGT and DM was observed (P=0.049). In the whole group, mean P_{INCR} was significantly but loosely correlated with GLP-1 AUC (r=0.49, P<0.006), while the relationship was not significant for GIP. The individual minute-by-minute correlation between P_{INCR} and GLP-1 or GIP were significant only in 20% of the subjects.
Conclusion: We have quantified the time course of the incretin effect by modeling analysis in NGT, IGT and DM. The incretin effect on early ISR is present in all groups, although blunted in DM. The onset of the incretin effect is rapid and sustained in NGT, transient in IGT and virtually absent in DM. The profiles of the incretin effect are poorly related to those of the incretin hormones.



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High glycaemic index (GI) diet induced hepatic fat accumulation and insulin resistance is associated with high glucose-dependent insulinotropic polypeptide (GIP) secretion
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Background and aims: A high GI diet promotes the development of non-alcoholic fatty liver disease and insulin resistance. Also, high GI diet strongly stimulates GIP secretion. Here, the aim was to investigate GIP secretion in response to diets with different GIs and its contribution to hepatic fat accumulation upon long term intervention and related molecular mechanisms.
Material and methods: Weight matched, male C57Bl/6J mice were fed a high or low GI diet identical in macro and micronutrient composition, 40% Kcal from fat, 40% Kcal from carbohydrate, using sucrose or an identical glucose-fructose dimer which is cleaved slowly but resorbed completely resulting in a bypass of the upper GI tract. Body weight and food intake were assessed weekly. Body composition was measured by nuclear magnetic resonance spectroscopy. GTT was performed by injecting 2mg/g glucose intraperitoneally (IPGTT). Liver triglyceride content (TG) was determined from livers in fasted mice. Studies were repeated in GIP receptor knockout (Gipr-/-) and their Wild Type (WT) littermates. Transcriptome analysis was performed using Illumina Mouse Ref-8 v2.0 microarrays and data was interpreted using the Ingenuity pathway software.
Results: After 22 weeks of consuming isocaloric high fat diets, either containing high or low GI carbohydrates, body weight, body composition and food

intake did not differ between groups. Postprandial plasma GIP level was 2.3 - fold higher compared to the low GI group (AUC 49640 ± 3770 vs. 21730 ± 3380, p<0.001), while GLP-1 level was unchanged. Hepatic TG content was increased 2- fold in the high GI group (3.1 ± 0.4 vs. 1.6 ± 0.3 mg/mg, p<0.01). During an IPGTT, the high GI group showed an increased glucose and insulin excursion (AUC 3787 ± 310 vs. 2873 ± 175 and 113360 ± 18530 vs. 66273 ± 7003, p<0.05; respectively) compared to the low GI diet group, indicative of aggravated insulin resistance in the high GI group. Deletion of GIP receptor in Gipr-/- mice completely abolished the metabolic derangements compared to WT mice on a high GI diet. Microarray analysis of WT animals resulted in 136 probes with fold changes>1.3x, which were significant (p<0.01) differentially expressed between high GI and low GI groups. Of these, 63% were upregulated and 37% were downregulated by high GI diet. Notably, upregulated genes were involved in the NAD biosynthesis, Type I Diabetes Mellitus and Maturity Onset Diabetes of Young (MODY) signaling pathways. Downregulated genes were related to inflammation and immune responses. Suppressor of cytokine signaling-2 (Socs2) was upregulated 2.3-fold in high compared to low GI fed mice and is known to be involved in the JAK2/STAT5 signaling pathway.
Conclusion: Together, these data suggest that high GI feeding leads to increased GIP levels, which contributes to elevated liver fat and insulin resistance even under isocaloric conditions. Our molecular analysis suggests that Socs2 could be a potential biomarker for high GI induced fatty liver without differences in body weight and composition.
Supported by: DFG

PS 045 Insulin secretion and action: using animal models

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The non-hematopoietic erythropoietin analogue, ARA290, improves glucose tolerance by stimulating insulin secretion in spontaneously type 2 diabetic Goto-Kakizaki rats

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Background and aims: Beside its well-known function in hematopoiesis, erythropoietin (EPO) has been shown to exert tissue protective effects in many animal models including in the diabetic db/db mouse. However, use of EPO as tissue protection is limited due to increased hematopoietic activity and associated adverse effects. We have studied effects of ARA290, a non-hematopoietic EPO analogue, in vivo on glucose homeostasis and in vitro on β-cell function in diabetic Goto-Kakizaki (GK) rats and non-diabetic Wistar (W) rats.
Materials and methods: GK and W rats were treated during 4 weeks with ARA290 (daily s.c. injection, 30μg/kg body weight) or PBS as placebo. Non-fasting plasma glucose (PG) levels were measured every week. Intraperitoneal glucose tolerance test (IPGTT) was performed prior to treatment and after 4 weeks. Insulin release was assessed by radioimmunoassay.
Results: In ARA290-treated GK rats, PG levels were significantly lower after 4 weeks of treatment (8.0±0.2 vs. 9.3±0.2 mmol/l, p<0.05) and the IPGTT was significantly improved compared to placebo-treated GK rats (AUC being 1348±146 vs.1873±156 mmol/l per 240 min), while PG levels and IPGTT were similar in ARA290- and placebo-treated W rats. ARA290 improved β-cell function: 1) in vivo, insulin response during IPGTT (from 0 min to 30 min) after 4 weeks treatment tended to be augmented in ARA290 treated-GK rats compared to placebo (13.8±1.0 to 20.8±3.5 mU/l, p=0.067, and 15.5±3.4 to 18.1±2.5 mU/l, p=0.2, respectively), 2) in isolated islets from ARA290 treated-GK rats, glucose induced-insulin secretion was two-fold higher than control (p<0.05), 3) acute treatment with ARA290 (10ng/ml for 1 h) improved glucose-stimulated insulin release from GK rat islets compared to control GK islets (42.75±4.9 μU/islet/h vs. 12.38±2.4 μU/islet/h, p<0.001). Furthermore, islets from ARA290 treated-GK rats showed significantly higher intracellular Ca^{2+} concentrations in response to several β-cell secretagogues (p<0.02). GK islets treated with ARA290 and a Ca^{2+} channel blocker, nimodipine, showed significantly reduced insulin secretion compared to ARA290-treated islets (6.18±1.76 vs. 29.05±5.06 μU/islet/h, p<0.001). ARA290-induced insulin secretion in GK islets was significantly inhibited by diazoxide (K^+-ATP channel opener) (25.59±5.76 vs. 2.05±0.2, p<0.001) but in islets treated with ARA290 and combination of diazoxide/KCl insulin release is significantly increased compared to diazoxide/KCl alone (157.6±21.4 vs. 89.6±11.35 μU/islet/h, p=0.005), suggesting an effect of ARA290 on insulin secretion amplification pathways. PKA inhibitor H89 significantly inhibited ARA290-induced insulin secretion (17.31±1.76 vs. 35.97±4.74μU/islet/h, p<0.002) but not the PKC inhibitor Calphostin C (31.55±6.5 vs. 30.82±4.23 μU/islet/h).
Conclusion: In summary, our results suggest that ARA290 could improve glucose tolerance in diabetic GK rats, most likely due to stimulating effect on insulin secretion via K^+-ATP channel closure, calcium influx and insulin exocytosis.
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Effects of exogenous DLK1-Fc treatment on insulin secretion and insulin sensitivity in an animal model of diet-induced obesity

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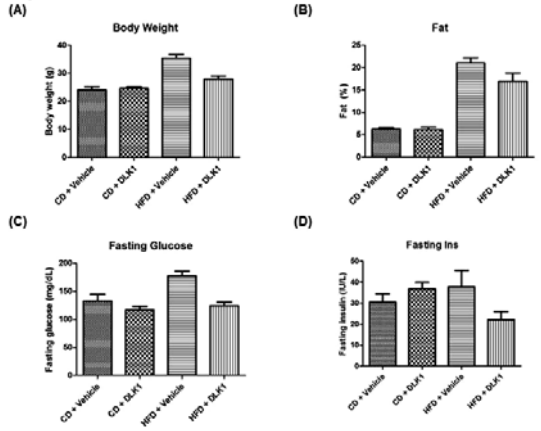
Background and aims: Delta-like 1homolog (DLK1) is an imprinted gene encoding a transmembrane protein known to inhibit adipogenesis and to

be associated with muscle hypertrophy in animal models. We wanted to investigate the metabolic effects of exogenous DLK1-Fc treatment on skeletal muscle mass and total fat mass in a diet-induced obesity animal model and if any further protective effect against obesity and insulin resistance exists.
Materials and methods: Male C57BL/6 mice were separated into four groups: (1) fed with chow diet and vehicle treated; (2) fed with chow diet and DLK1-Fc treated;(3) fed with high-fat diet and vehicle treated; (4) fed with high-fat diet and DLK1-Fc treated for a period of 8 weeks. DLK1-Fc (15mg/kg) was injected intraperitoneally twice weekly. Body weight and food intake were measured weekly and fasting glucose was checked every 2 weeks. After 8 weeks, total body composition was checked by nuclear magnetic resonance (NMR) and an oral glucose tolerance test (OGTT) was done.
Results: In mice fed a standard chow diet, body weight, fat weight, muscle weight, and body composition were similar in both groups. However, the pancreas weight/tibia length ratio in DLK1-Fc treated group was higher than that of control group. Moreover, overnight fasting glucose was lower and fasting insulin and HOMA-b were higher in DLK1-Fc treated mice compared to the control group. In mice fed a high-fat diet, DLK1-Fc treated animals showed significantly decrease in body weight, especially in fat weight and fat mass analyzed by NMR, fasting glucose, postprandial glucose, AUC for glucose in OGTT, fasting insulin, and HOMA-IR. HOMA-b was higher in mice treated with DLK1-Fc. There was no difference in pancreas weight and muscle weight between the two groups.
Conclusion: These results show that exogenous DLK1-Fc regulates the insulin secretion positively and can improve insulin sensitivity by decreasing fat mass especially in an animal fed with a high-fat diet. In addition, this effect may be related to therapeutic strategies of anti-obesity and/or anti-diabetic drugs by enhancing both insulin secretion and insulin sensitivity simultaneously.

Figure 1. Effects of 8 weeks exogenous DLK1-Fc treatment or vehicle on (a) body weight, (b) fat mass, (c) overnight fasting glucose, and (d) fasting insulin level. Data are mean±s.e.m. CD, chow diet; HF, high-fat diet.



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Favourable effects of PERK reduction on insulin secretion in autophagy-deficient pancreatic beta cells

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Background and aims: Autophagy was reported to have a function in maintaining β cell mass and function. Autophagy has a close relation to endoplasmic reticulum (ER) stress and unfolded protein response (UPR). ER stress can activate autophagy,and autophagy can remove dysfunctional ER. In this study, we evaluated the effects of PERK deletion, a constituent of UPR, on the β cell mass and function in β cell-specific autophagy deficient mice.
Materials and methods: β cell-specific *Atg7* knockout mice ($Atg7^{\Delta\beta\text{-cell}}$) were use as autophagy deficient model. They were crossed with $Perk^{+/-}$ mice to generate double knockout (KO) mice. Blood glucose levels and body weight were monitored. Intraperitoneal glucose tolerance test was carried out at 20 weeks of age, and then pancreas tissue was harvested to observe the morphologic changes, to measure the pancreatic hormone contents and to isolate islets for RT-PCR.
Results: Heterozygous deletion of *Perk* improved glucose intolerance and glucose-stimulated serum insulin secretion in $Atg7^{\Delta\beta\text{-cell}}$ mice. Pancreatic proinsulin content and prohormone convertase 1/3 activity in the islets were decreased in the $Atg7^{\Delta\beta\text{-cell}}$ mice, which suggested dysfunctional insulin synthesis. It was not impaired in the double KO mice. Increased pancreatic insulin content and suppressed insulin secretion on glucose stimulation in the $Atg7^{\Delta\beta\text{-cell}}$ mice suggested that there was impairment in insulin exocytosis and resultant insulin accumulation in the islets. This phenomenon was somewhat relieved in the double KO mice. Vacuolated change and accumulation of ubiquitin aggregates and p62 inthe islets of $Atg7^{\Delta\beta\text{-cell}}$ mice on immunohistochemical staining were markers for impaired autophagic activity. As these were less observed in the islets of double KO mice, we could speculate that the favourable effects of PERK reduction might be related to recovery of autophagic activity. RT-PCR with isolated islets revealed that some UPR markers were suppressed in the $Atg7^{\Delta\beta\text{-cell}}$ mice but not in the double KO mice. Accordingly, distended ER was found much more on electron microscopy of β cells from the $Atg7^{\Delta\beta\text{-cell}}$ mice compared to those from the double KO mice.
Conclusion: We demonstrated that partial suppression of *Perk* improved the impairment of structure and function of islets in the $Atg7^{\Delta\beta\text{-cell}}$ mice, leading to a recovery of insulin synthesis and secretion. As a result, glucose intolerance of the β cell-specific autophagy deficient mice was relieved by PERK reduction. The mechanisms could be regaining of autophagic activity and recovery of adequate UPR.

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Beta cell CART overexpression in mice increases insulin secretion, enhances beta cell survival and attenuates insulin sensitivity

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Background and aims: Cocaine- and amphetamine-regulated transcript (CART) is a regulator of pancreatic islet hormone secretion, enhancing insulin secretion and lowering glucagon secretion, thereby fine-tuning blood glucose levels. CART also protects beta cells against glucotoxicity-induced apoptosis. Furthermore, beta cell CART expression in rat islets is regulated by glucose and CART is up-regulated in beta cells of type 2 diabetes (T2D) patients and rodent models of T2D. The aim of this study was to examine the impact of up-regulated beta cell CART on islet function, beta cell survival and whole body metabolism.
Materials and methods: We generated transgenic mice that express CART under the control of the PDX1 promotor resulting in overexpression of CART in the pancreatic beta cells (CARTtg). Mice fed either standard diet or a high fat diet were subjected to oral glucose tolerance test (OGTT), intravenous glucose tolerance test (IVGTT) and insulin tolerance test (ITT). After 4 months, insulin secretion from isolated islets, as well as islet morphology was assessed. Furthermore, glycerol release from adipocytes and metabolomic plasma profiling using GS/TOF-MS were assessed. In addition, mice were subjected to streptozotocin (STZ)-treatment to induce diabetes, followed by glucose monitoring and beta cell mass quantification.
Results: On a standard diet CARTtg had normal in vitro and in vivo insulin secretion, as well as beta cell mass. However, when fed a high fat diet, CARTtg displayed elevated insulin secretion (insulin AUC: wt 674 vs CARTtg 908, p=0.01), but impaired glucose elimination during OGTT (Kg: wt 1.1 vs CARTtg 0.65, p=0.02), increased glucose levels during IVGTT (glucose 5 min (mM): wt 20.0 vs CARTtg 22.0, p=0.005) and decreased insulin sensitivity during ITT (AUC glucose: wt 703 vs CARTtg 754, p=0.02). Metabolomic profiling of plasma revealed that CARTtg had decreased levels of 5 out of 8 analyzed free fatty acids (FFA) (CARTtg: 16-56% reduction compared to wt, p<0.05). In addition, CARTtg had reduced basal glycerol release from isolated adipocytes on both diets (normal diet: CARTtg 42% reduction compared to wt, p=0.004, high fat diet: CARTtg 68% reduction compared to wt, p=0.004) and visceral fat mass was increased in CARTtg fed a high fat diet (wt 2.35g vs CARTtg 3.23g, p=0.03). Furthermore, after STZ-treatment CARTtg mice displayed improved glucose homeostasis (glucose increase (mM) 1 week after STZ-treatment, wt 3.18 vs CARTtg 1.32, p=0.01), and higher beta cell mass compared to wt.

Conclusion: CARTtg mice display normal insulin secretion capacity, beta cell mass and islet morphology when fed a standard diet. Plasma levels of FFA are reduced in the fasting state and lipolysis is decreased. However, on a high fat diet, CARTtg mice become insulin resistant and have more visceral fat. If this is a consequence of elevated levels of circulating insulin, CART or other islet hormones remains to be elucidated. Beta cells in CARTtg mice are protected against STZ-treatment, resulting in an improved glucose tolerance. In the view of CART being up-regulated in islets of T2D patients, these findings emphasize the importance of beta cell CART during T2D progression.
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Impaired islet function and structure in mice with hereditary predisposition to high fat diet-induced glucose intolerance
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Background and aims: Several animal models are currently used in the research field of type 2 diabetes. However, they could not fully explain the hereditary predisposition to diet-induced diabetes. Recently, we established two lines of mice with different susceptibilities (prone and resistant) to high fat diet (HFD)-induced glucose intolerance by selective breeding (designated SDG-P and SDG-R, respectively). After a 5-week HFD feeding, SDG-P mice showed evidently higher blood glucose levels relative to SDG-R mice. Here, we analyzed islet function and structure in these new polygenic model mice.
Materials and methods: Male mice of each strain fed a HFD (32% energy as fat) for 5 weeks (from 5 to 10 weeks of age). Blood glucose levels (0, 15, 30, 60, 120 min) and serum insulin levels (0, 15, 30 min) were measured in OGTT. Histological examination of pancreas and morphometric analysis of islet cells were performed under a microscope. Glucose-induced insulin secretion (GSIS) was assessed by batch incubation of isolated islets in medium containing 2.8 or 16.7 mmol/l glucose. Gene expression levels in the isolated islets were measured by real-time quantitative PCR.
Results: Before the HFD feeding (at 5 week-old under normal chow feeding), SDG-P mice showed slightly higher postchallenge blood glucose levels in OGTT as compared with SDG-R mice. Although serum insulin levels were higher in SDG-P mice under fasting condition (p=0.0042), no significant differences were observed in postchallenge insulin levels between the two lines. The in vivo indices of β-cell function (insulin secretion ratio and insulinogenic index; 15 min/0 min in OGTT) were significantly lower in SDG-P mice as compared with SDG-R mice (p=0.0026 and p=0.018, respectively). Correspondingly, isolated islets of SDG-P mice showed significantly lower GSIS than those of SDG-R mice (p=0.024). Gene expression levels of GLUT-2 (p=0.024), SNAP25 (p=0.014), and PDX-1 (p=0.023) were significantly lower, whereas FAT/CD36 was significantly higher (p=0.0064), in SDG-P islets relative to SDG-R ones. Although no significant differences were seen in islet mass and structure before the HFD feeding, SDG-P mice showed two-fold greater β-cell mass (p=0.024) as compared to SDG-R mice after the 5-week HFD feeding. Meanwhile, GSIS in SDG-P islets was still impaired after the HFD feeding (p=0.0010 vs SDG-R).
Conclusion: SDG-P mice showed spontaneous impairments in insulin response to glucose in vivo and lower GSIS in isolated islets. Reduced gene expressions of glucose sensing, exocytosis and transcriptional factors may contribute to the impaired insulin secretion and insufficient β-cell morphological adaptation in SDG-P islets. In addition, increased expression of FAT/CD36, a transmembrane fatty acid transporter, in SDG-P mice may participate in the acceleration of hyperglycemia by HFD feeding. Taken together, these hereditary impairments in pancreatic islets may determine the susceptibility to HFD-induced glucose intolerance.
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Evaluation of the effect of liraglutide on beta cell fate in alloxan diabetic mice by tamoxifen-inducible Cre/loxP system
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Background and aims: Although GLP-1 receptor agonists have been shown to exhibit beneficial effects on not only beta-cell function but also beta-cell mass, the precise mechanism of the effect of the drugs on beta-cell mass is still unclear. The objective of this study is to determine whether liraglutide, a long-acting GLP-1 analog, would favor regeneration of beta cell mass in alloxan-induced diabetic mice. By using tamoxifen-inducible Cre/loxP system, in which beta-cell neogenesis can be distinguished from self-replication, we evaluate the effect of liraglutide on beta-cell fate.
Materials and methods: We crossbred Ins2-CreER knock-in mice with R26R-YFP mice to generate Ins2-CreER/R26R-YFP double knock-in mice, in which pancreatic beta-cells can be labeled specifically and permanently upon injection of tamoxifen, and traced the beta-cells by pulse and chase experiment. Pancreatic beta-cells were labeled by 5 consecutive injection of tamoxifen (4 mg/head/injection) to the male mice at 6 weeks of age. Ten days after the last injection, diabetes was induced by a single dose of alloxan (60 mg/kg, i.p.). On the next day, mice with blood glucose concentration above 300 mg/dL were used for the study. Liraglutide (200 μg/kg s.c.) or vehicle (saline) was administered once daily from the next day (day 0) of alloxan injection to day 30. Food intake and body weight were measured everyday and blood glucose levels were measured twice a week just before administration of drugs. Blood samples were collected to measure serum insulin levels. Beta-cell mass was estimated by immunofluorescent staining of pancreas sections.
Results: By alloxan treatment, pancreatic beta-cell mass was significantly decreased to about 20% of that in normal mice, and blood glucose levels were significantly increased. There was no significant difference in body weight between vehicle- and liraglutide-treated groups after 30 days. Food intake tended to be decreased in the liraglutide-treated group. While blood glucose levels of mice in the vehicle group continued to be increased gradually after alloxan injection, liraglutide treatment significantly suppressed the increment. Serum insulin levels in the liraglutide-treated group were significantly higher than those in the vehicle group. In addition, beta-cell mass in the liraglutide-treated group was two-fold higher than that in the vehicle group, which is correlated with the increment of serum insulin levels. We found that the frequency of insulin-positive cells labeled with YFP was not reduced by treatment of liraglutide for 30 days.
Conclusion: Chronic treatment of liraglutide expands beta-cell mass in alloxan-induced diabetic mice. Our results suggest that contribution of neogenesis is limited, if any, to the increment of beta cell mass by liraglutide treatment.
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A role for kisspeptin in islet function during pregnancy
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Background and aims: Kisspeptin stimulates insulin release, but the physiological role of endogenous kisspeptin in islets remains unclear. The extremely high levels of kisspeptin released by the placenta suggest a possible role in modulating islet function during pregnancy. This study therefore determined the effects of kisspeptin on islet function in both pregnant and non-pregnant mice.
Materials and methods: Control or pregnant mice were implanted with subcutaneous osmotic minipumps containing either saline, kisspeptin (50nmol/day) or the kisspeptin antagonist kisspeptin-234 (200nmol/day). Intraperitoneal (i.p.) glucose tolerance tests and insulin tolerance tests were performed at mid and late pregnancy (10-12 days, and 16-18 days respectively). Glucose and insulin tolerance tests were performed following a single i.p. administration of glucose (2g/kg body weight) or insulin (0.75U/kg body weight), respectively at mid and late pregnancy (10-12 days, and 16-18 days respectively). Plasma samples were also taken during glucose tolerance tests for the measurement of plasma insulin levels using an ultra-sensitive ELISA.
Results: Chronic administration of kisspeptin-234 in pregnant mice impaired glucose tolerance during both mid-pregnancy (pregnant: 15.5±0.4mmol/L, kisspeptin-234 treated: 18.2±0.7mmol/L plasma glucose 30min post glucose administration, $p<0.05$, n=6) and late-pregnancy (pregnant: 13.1±0.8mmol/L,

kisspeptin-234 treated: 16.4±0.7mmol/L plasma glucose 30min post glucose administration, p<0.05, n=6). Kisspeptin-234 also reduced insulin release in response to glucose (mid-pregnant: 166±18%, kisspeptin-234 treated mid-pregnant: 115±11%; late-pregnant: 199±16%, kisspeptin-234 treated late-pregnant: 142±13% increase in plasma insulin relative to baseline, p<0.05, n=6). Similarly, chronic administration of kisspeptin in non-pregnant mice improved glucose tolerance (control: 17.4±1.2mmol/L, kisspeptin treated: 13.3±0.9mmol/L plasma glucose 15min post glucose administration, p<0.05, n=6) and increased insulin release in response to glucose (control: 157±18%, kisspeptin treated: 202±15% increase in plasma insulin relative to baseline, p<0.05, n=6). Whilst pregnant mice had reduced insulin sensitivity compared to non-pregnant controls in insulin tolerance tests, neither kisspeptin nor kisspeptin-234 had any significant effect on insulin sensitivity in either pregnant mice or non-pregnant controls.
Conclusion: These data support a role for placental kisspeptin in the regulation of islet function during pregnancy, indicating that both endogenous kisspeptin during pregnancy and kisspeptin administration in non-pregnant animals improve glucose tolerance. Furthermore, this effect of kisspeptin is due to increased insulin release in response to glucose rather than an effect on insulin sensitivity.
Supported by: DRWF

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Maternal high fat diet induced insulin resistance and deterioration of pancreatic beta cell function with a gender difference in mature offspring
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Background and aims: Intrauterine environment may have a significant influence on the health of postnatal offspring. However, the mechanism of developmental origins of health and disease is yet to be clarified. In addition, the role of maternal diet on fetal overnutrition has been less studied than its relationship with fetal undernutrition. Here, we examined whether maternal high fat diet (HFD) may have an effect on glucose metabolism and pancreatic β cell function in mature offspring in mice.
Materials and methods: Female and male C57/BL6J mice were mating and fed either a control diet (CD) or HFD from conception to weaning, and offsprings were fed CD or HFD from 6 to 20 weeks age, resulting in four groups: CD-CD, CD-HFD, HFD-CD and HFD-HFD. Body weight was assessed at birth and once a week for 4 - 20 weeks. At 20 weeks, intraperitoneal glucose tolerance tests (IPGTT) and insulin tolerance test (ITT) were performed, and we evaluated insulin secretion at IPGTT, insulin contents and mRNA levels of *PDX-1*, NADPH oxidase components in isolated islets. In addition, oxidative stress in islets was evaluated by immunostaining of 8-hydroxy-2'-deoxyguansosine (8-OHdG). Liver triacylglycerol content, fat pad weight, average area of adipocyte and mRNA levels of *TNF-α*, *IL-6*, *MCP-1*, *F4/80* and *CD11* in white adipose tissue (WAT) were also evaluated.
Results: Body weight in maternal HFD was greater at birth and from 4 to 6 weeks, while there was a significant difference only between HFD-HFD and CD-HFD in female from 6 to 20 weeks. At 20 weeks, IPGTT and ITT showed that maternal HFD induced glucose intolerance and insulin resistance in offspring. Additionally, WAT mass and mRNA levels of *TNF-α*, *IL-6*, *MCP-1*, *F4/80* and *CD11* in WAT and liver triacylglycerol content were increased in maternal HFD. In contrast, plasma insulin secretion at IPGTT, insulin contents and mRNA levels of *PDX-1* in isolated islets were higher in maternal HFD in female, while they were lower in maternal HFD in male. To investigate the mechanism underlying a gender difference in pancreatic β cell function, oxidative stress in islets was evaluated by immunostaining of 8-OHdG. Staining intensities of 8-OHdG were increased in maternal HFD only in male. In addition, mRNA levels of *gp91phox*, a major source of superoxide production in islets, were increased in maternal HFD only in male.
Conclusion: Maternal HFD during gestation and lactation induced insulin resistance and deterioration of β cell function with a gender difference in mature offspring, accompanied by the progression of adipose tissue inflammation and liver steatosis.
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Attenuated hyperinsulinaemia protects female mice with reduced Insulin2 gene dosage from diet-induced obesity
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Background and aims: Hyperinsulinemia can precede obesity, but the causal relationship between these two phenomena has remained controversial. The most widely held paradigm posits that obesity leads to peripheral insulin resistance, which subsequently causes compensatory insulin hypersecretion. We recently demonstrated that prevention of hyperinsulinemia in male mice with reduced *Insulin* (*Ins*) *1* gene dosage ($Ins1^{+/-}$:$Ins2^{-/-}$) was sufficient to prevent diet-induced obesity, which occurred normally in hyperinsulinemic $Ins1^{+/+}$:$Ins2^{-/-}$ control mice. However, *Ins1* is the rodent-specific result of a duplication-transposition event of *Ins2*, and it is unclear whether these genes have distinct functional roles. In the present study, we sought to examine the effect of reduced *Ins2*, which is analogous to the ancestral gene found in humans, on the development of diet-induced hyperinsulinemia, obesity, insulin resistance, and glucose intolerance, in female and male mice.
Materials and methods: Male and female $Ins1^{-/-}$:$Ins2^{+/-}$ mice and $Ins1^{-/-}$:$Ins2^{+/+}$ controls were placed on a high fat diet (HFD) or chow diet (CD) from weaning. Body mass, insulin secretion, and blood glucose response to intraperitoneal injections of glucose or insulin were assessed over a 1-year period.
Results: Female mice with deleted *Ins1* and reduced expression of the ancestral insulin gene ($Ins1^{-/-}$:$Ins2^{+/-}$) had lower basal circulating insulin as early as eight weeks of age, compared to $Ins1^{-/-}$:$Ins2^{+/+}$ controls ($p < 0.05$). Hyperinsulinemia in HFD-fed $Ins1^{-/-}$:$Ins2^{+/-}$ females (at 15 weeks, HFD: 0.6 ± 0.1 ng/mL, CD: 0.4 ± 0.0 ng/mL; $p < 0.05$) preceded the onset of obesity. At 27 weeks, all HFD-fed females had elevated basal and glucose-stimulated insulin levels, but this was reduced by over 50% in $Ins1^{-/-}$:$Ins2^{+/-}$ females ($p < 0.05$). Diminished hyperinsulinemia corresponded with attenuated diet-induced obesity (on HFD at 52 weeks, $Ins1^{-/-}$:$Ins2^{+/+}$: 36.1 ± 1.2 g, $Ins1^{-/-}$:$Ins2^{+/-}$: 31.0 ± 1.5 g; $p < 0.05$). A progressive worsening of glucose tolerance and insulin sensitivity coincided with the development of obesity in all HFD-fed mice, despite the decreased weight gain in $Ins1^{-/-}$:$Ins2^{+/-}$ females. Thus, diet-induced obesity could be selectively attenuated in female mice with reduced *Ins2* gene dosage. Surprisingly, male $Ins1^{-/-}$:$Ins2^{+/-}$ mice did not show the expected reduction in insulin levels with reduced *Ins2* gene dosage, and all HFD-fed males in our study became comparably hyperinsulinemic (*e.g.* basal insulin on HFD at 27 weeks, $Ins1^{-/-}$:$Ins2^{+/+}$: 2.1 ± 0.6 ng/mL, $Ins1^{-/-}$:$Ins2^{+/-}$: 1.9 ± 0.7 ng/mL; $p = 0.9$). Consistent with a role for hyperinsulinemia in weight gain, all HFD-fed male groups also reached a similar degree of diet-induced obesity (at 52 weeks, $Ins1^{-/-}$:$Ins2^{+/+}$: 43.1 ± 1.7 g, $Ins1^{-/-}$:$Ins2^{+/-}$: 43.4 ± 2.0 g; $p = 0.9$). $Ins1^{-/-}$:$Ins2^{+/-}$ males did exhibit minor glucose intolerance as early as six weeks, and unlike $Ins1^{-/-}$:$Ins2^{+/-}$ females, it was not transient. However, $Ins1^{-/-}$:$Ins2^{+/-}$ males did not appear otherwise distinct from $Ins1^{-/-}$:$Ins2^{+/+}$ controls, suggesting that a single allele of *Ins2* is mostly sufficient to meet the insulin secretory demands of male mice under these conditions.
Conclusion: Our results point to sex-specific differences in requirement for two alleles of *Ins2* to sustain hyperinsulinemia and weight gain. Collectively, these experiments strongly support the concept that protection from hyperinsulinemia leads to attenuated obesity in high fat-fed mammals.
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Characterisation of glucose homeostasis in a novel rat model of leptin deficiency
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Background and aims: The *ob/ob* mouse has a spontaneous mutation of the gene encoding leptin, resulting in the development of obesity and impaired glucose homeostasis. It has been the only animal model of leptin deficiency to date. However, through the use of zinc finger nuclease technology, a rat model of leptin deficiency was created by deletion of 151 bp of the leptin gene. Here we sought to characterize the metabolic phenotype and glucose handling of leptin deficient rats to determine if they are comparable to *ob/ob* mice.
Material and methods: Rats heterozygous for the mutated leptin gene were bred to produce wildtype (WT) and knockout (KO) littermates. Blood glu-

cose, plasma insulin and insulin sensitivity were compared between WT and KO rats after a 4-hour fast. Glucose tolerance was measured via oral gavage of glucose (1.5 g/kg body weight) after an overnight fast. These measures were then evaluated in the presence of leptin therapy (100 μg/day via mini osmotic pump) to determine if leptin replacement in KO rats would reverse the phenotype. Alpha and beta cell area and pancreas morphology were assessed by immunostaining for insulin, glucagon and GLUT2.
Results: Differences in body weight between WT and KO rats were evident by 6 weeks of age and continued to increase so that by 36 weeks of age male KO rats weighed 1.8-fold more than WT littermates (KO=1048.27±29.67 g *vs*. WT=567.13±28.01 g; p<0.05). A similar trend was observed in females (581.53±28.56 g *vs.* 347.63±20.85 g; p<0.05). Total fat mass was 2-fold and 2.8-fold higher in male and female KO compared to WT rats, respectively. By 6 weeks of age, fasting insulin levels were 22-fold higher in male KO compared to WT (12.59±1.82 ng/ml *vs.* 0.57±0.12 ng/ml, p<0.05) and 54-fold higher in female KO compared to WT (37.38±0.84 ng/ml *vs.* 0.69±0.02 ng/ml, p<0.05). Fasting insulin remained elevated at 36 weeks of age. KO had larger islets and the proportion of beta cell area per pancreas section was 3-fold higher in KO compared to WT (1.7±0.9 % *vs*. 0.5±0.1%; p<0.05). No differences in alpha cell area were evident (p=0.625). Despite larger islet size, KO rats had reduced GLUT2 expression, impaired glucose stimulated insulin secretion, and were glucose intolerant by 7 weeks of age. The same trend of glucose intolerance and impaired glucose stimulated insulin secretion was observed in female KO rats. Leptin therapy (100 μg/day) resulted in an attenuation of weight gain, improved fasting insulin levels (4.83±1.32 ng/ml compared to 12.74±3.05 ng/ml pre-leptin) and glucose tolerance that was comparable to WT (KO AUC=249.5±46.5 *vs*. WT AUC=178.9±41.3; p>0.05).
Conclusion: The absence of functional leptin in rats results in a phenotype similar to the leptin-deficient *ob/ob* mouse, marked by obesity, fasting hyperinsulinemia, and defective *in vivo* glucose stimulated insulin secretion. Furthermore, defects in pancreas morphology and function that have been observed in *ob/ob* mice were also evident in this rat model, including hypersecretion of insulin, increased islet size and reduced GLUT2 expression. These data provide compelling evidence of the conserved effects of leptin on glucose homeostasis across species and indicate that the leptin KO rat will be a useful model to further study the mechanisms of action of leptin as well as therapies for obesity and glucose intolerance.
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Hypothalamic nitric oxide regulates peripheral insulin bioavailability
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Background and aims: The bioavailability of insulin for peripheral tissues is defined by the rates of insulin secretion and hepatic insulin clearance. Human studies demonstrated that NO is an important regulator of insulin clearance. Whether the control of insulin clearance is solely dependent on hepatic mechanisms or also relies on hypothalamic regulation is uncertain. Hypothalamus is a critical regulator of energy metabolism and endocrine functions. Therefore, we hypothesized that NO production by central/hypothalamic axis regulates insulin clearance and therefore peripheral insulin bioavailability.
Materials and methods: Male Wistar rats were submitted to brain surgery using a stereotaxic apparatus to the implantation of the simple, for intracerebroventricular (i.c.v.), or the double cannulas, for nucleus specific infusion. After the bregma localization the following coordinates were used: Lat: 1.2mm, AP: 1.0mm for lateral ventricle (i.c.v.), AP: -1.8mm, Lat: +/- 0.4mm, DV: -8.0mm for paraventricular nucleus (PVN) and AP: -2.52mm, Lat: +/- 0.6mm, DV: -9.2mm for ventromedial hypothalamus (VMH). Bolus infusions of 250ug/2uL of L-NAME (or 2uL of saline in the control animals) were performed in each side of the brain. An oral glucose tolerance test (OGTT) (2g/kg) was performed 45min after L-NAME bolus infusion. Glycemia was monitored and blood samples were collected. Insulin and c-peptide levels in the plasma were quantified. Insulin clearance was evaluated by the ratio between plasma c-peptide and insulin areas levels across the OGTT.
Results: Acute L-Name infusion did not affect glycaemia either basal or upon the OGTT (AUC Ctrl vs. LN: i.c.v.: 949.8 ± 36.8 vs. 921.0 ± 18.2, n.s.; PVN: 960.5 ± 30.3 vs. 944.4 ± 11.0, n.s.; VMH: 1015.0 ± 68.9 vs. 994.4 ± 44.2, n.s.). After the glucose bolus, both i.c.v. and PVN L-NAME treated animals demonstrated a decrease in plasma insulin levels (AUC Ctrl vs. LN: i.c.v.: 14.9 ± 0.8 vs. 10.1 ± 1.4, p<0.5; PVN: 14.8 ± 2.3 vs. 10.3 ± 0.9, p<0.5; VMH: 9.3 ± 1.4 vs. 9.6 ± 1.6, n.s.) with no alterations in c-peptide levels (AUC Ctrl vs. LN: i.c.v.: 13155 ± 984 vs. 13482 ± 1445, n.s.; PVN: 10167 ± 756 vs. 10596 ± 922, n.s.; VMH: 9744 ± 1550 vs. 10010 ± 1039, n.s.). Insulin clearance was calculated and both i.c.v. and PVN but not VMH nitric oxide synthesis suppression resulted in an increase in insulin clearance after the glucose bolus (AUC Ctrl vs. LN: i.c.v.: 8574 ± 534 vs. 11510 ± 632, p<0.5; PVN: 6514 ± 338 vs. 8044 ± 498, p<0.5; VMH: 8118 ± 597 vs. 8518 ± 1234, n.s.).
Conclusion: Together these results reveal that after a glucose bolus, and not in the fasting state, increased levels of nitric oxide in the hypothalamic/PVN region lead to decreased insulin clearance supporting the hypothesis that hypothalamic function is a regulator of peripheral insulin bioavailability.
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PS 046 Gut and gut hormones

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Continuous glucose monitoring (CGM) discloses high glycaemic variability in patients with "late dumping" after RYGB

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Background and aims: Weight loss after Roux-en-Y gastric bypass surgery (RYGB) has been repeatedly associated with at least transient regression of type 2 diabetes in obese persons. The dark side of improved glycaemic control is postbariatric noninsulinoma pancreatogenous hypoglycaemia syndrome (NIPHS). NIPHS prevalence is largely unknown with published rates ranging from 0.2% to more than 30%. Both figures however may not represent real life: The former originates from retrospective data from the SOS-study counting hospitalisation for diagnoses associated with post-gastric bypass hypoglycaemia. The latter results from measuring reactive hypoglycemia after an oral glucose challenge, a procedure which is not validated and may not be reasonable after RYGB. We therefore examined RYGB patients under daily life conditions by combining continuous tissue glucose monitoring (CGM) with simultaneous assessment of nutrition, physical activity and symptoms of hypoglycaemia.
Materials and methods: 27 consecutive patients (22 women, 5men; mean age 43 y) with type 2 diabetes who had undergone RYGB for obesity, were examined 6-14 months after bariatric surgery because they reported symptoms that were compatible with dumping syndrome. During 7 days of continuous measurement of interstitial glucose (IG; Dexcom seven$^+$, lower alert limit: 70 mg/dl), nutrition and physical activity were recorded in detail. In addition, autonomous and neuroglucopenic symptoms of hypoglycaemia were assessed semiquantitatively according to the Edinburgh Hypoglycaemia Scale. Glycaemic variability (mean absolute glucose; MAG) was calculated using EasyGV software.
Results: As a consequence of weight loss (BMI pre-OP 46.9±1.5 kg/m^2 / post-OP 35.6±2.5 kg/m^2) most of the subjects had reduced or stopped their antidiabetic medication. Average HbA1c was 5.9±0.3 % (pre-OP 6.5±0.3%). Average IG was 116 mg/dl, however 25 (93%) of the patients had at least one episode with IG<70 mg/dl. The typical IG-pattern was a steep prandial glucose peak already after small amounts of carbohydrates and consecutive postprandial nadir into the hypoglycaemic range and a high glucose variability likewise (MAG 3.0±0.2 mg/dl/h; normal range 0.5-2.2). Autonomous and neuroglucopenic symptoms showed high interindividual variability and invariably occurred after the sensor alert and thus were not helpful for early recognition of low IG. Percentage of time spent at IG concentrations greater than 180 mg/dl and less than 70 mg/dl was on average 10.4 and 10.5 %, respectively.
Conclusion: Using CGM, both hypo- and hyperglycaemic excursions are detectable more frequently than expected and perceived. Thus 93% of the RYGB-patients had reactive low IG after marked prandial glucose peaks and a high overall glycaemic variability. The popular perception of postbariatric subjects with type 2 diabetes focusing on successful weight loss, improved fasting plasma glucose and normalized glycated hemoglobin obviously does not consider the complex postoperative situation of many patients. In particular, high glucose variability as well as the surprisingly high percentage of time spent above the renal threshold of glucose and below the threshold of neuroglucopenia should give rise to closer glucose monitoring and more intense nutrition counseling.

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Mechanisms of diabetes remission after bariatric surgery: role of the gut

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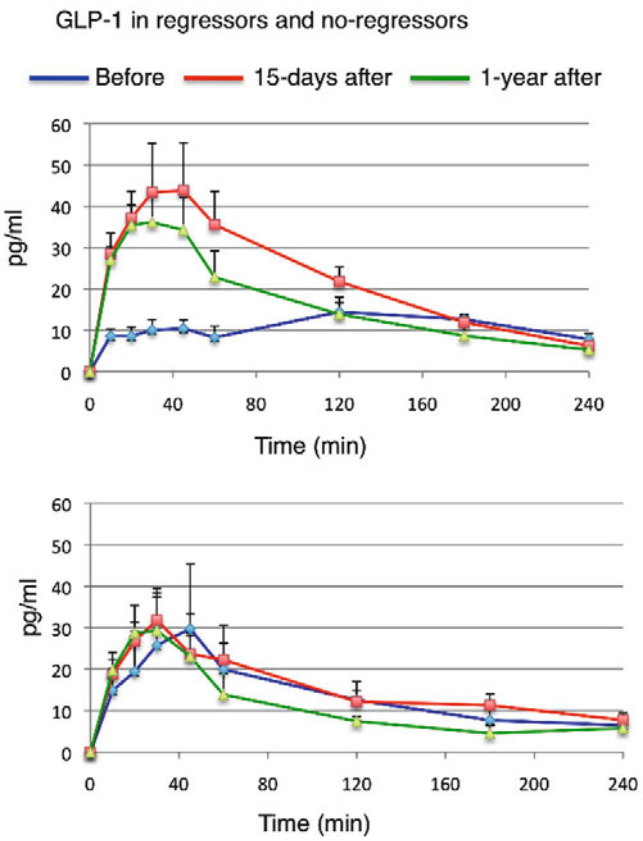
Background and aims: In morbidly obese patients with type 2 diabetes (T2DM), Roux-en-Y-gastric-bypass (RYGB) and sleeve gastrectomy (SLG) similarly restore euglycaemia, but the mechanisms are not completely understood. We investigated the mechanisms of diabetes remission in patients undergoing either surgery.
Materials and methods: 35 T2DM, 23 treated with RYGB and 12 with SLG, received an MTT before, 15 days and 1 year after surgery. An OGTT was performed at 1 year. Insulin sensitivity was assessed by Matsuda index and ß-cell function by modelling analysis of the C-peptide response to meal. Plasma amylin, ghrelin, PYY, pancreatic polypeptide (PP), glucagon, and GLP-1 concentrations were measured during MMT.
Results: Based on fasting and 2-h post-OGTT glycaemia, at 1 year diabetes resolved in 20 of 35 patients (13 RYGB and 7 LSG); in the remaining 15 patients, diabetes was improved but not in remission. Age, BMI and kind of surgery did not differentiate regressors (R) from non-regressors (NR). Diabetes duration (6 ± 4 vs 12 ± 5 years, p<0.0001, R vs NR), baseline HbA1c, (7.5 ± 1.4 vs 9.2 ± 2.4%, p=0.002), and higher baseline mean plasma glucose levels separated R from NR. Before surgery, time to glucose peak during MTT was earlier in R than NR (70 ± 33 vs 97 ± 36 min, p=0.003). At baseline, ß-cell glucose sensitivity was 3 times higher in R than NR (p<0.003). Furthermore, while all metabolic parameters showed the expected improvement post-surgery, the extent of the improvement was significantly less in NR as compared to R for glycaemic control, insulin sensitivity and ß-cell glucose sensitivity (p<0.05 or less for all). Before surgery, fasting amylin concentrations were lower, and fasting GLP-1 levels were higher, in R than NR (both p<0.05). Fasting GLP-1, PYY, PP, ghrelin, glucagon and amylin showed similar changes following surgery in R and NR. Before surgery, R and NR differed only for their GLP-1 response to MTT, which was blunted in R group (p=0.04). After surgery, meal-stimulated GLP-1 concentrations increased in R, but did not change in NR, while postprandial glucagon decreased more in NR than R. The responses of the other hormones to MTT did not differ between R and NR. In a logistic regression analysis, remission of diabetes was independently predicted by diabetes duration, ß-cell glucose sensitivity and MTT-stimulated GLP-1.
Conclusion: In morbidly obese T2DM patients, RYGB and LSG have a similar impact on remission of diabetes. Baseline ß-cell glucose sensitivity, duration of diabetes and GLP-1 concentrations are predictors of diabetes remission.



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Intestinal lipogenesis in morbidly obese subjects is higher in those with high insulin resistance

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Background and aims: Intestine is a significant site of triglyceride production in the body. Both the fructose-fed hamster and the sand rat models of insulin resistance exhibit upregulated *de novo* lipogenesis in the intestine. However, little is known about *de novo* lipogenesis in human intestine. In this study we investigate the association between insulin resistance and lipogenesis in the intestine of morbidly obese subjects with low and high insulin resistance.
Material and methods: The study was undertaken in 30 morbidly obese subjects, 15 with low and 15 with high insulin resistance. The HOMA-IR was calculated to determine insulin resistance. Jejunum samples were obtained during gastric bypass to analyze the mRNA expression of different lipogenic genes.
Results: The morbidly obese persons with high insulin resistance had low mRNA expression of ACC1 (179.9±45.0 vs. 117.5±46.4, p=0.011), and a higher mRNA expression of apolipoprotein AIV (32.2±31.1 vs. 82.1±46.9, p=0.016), PEPCK (26.8±16.2 vs. 47.3±17.1, p=0.049), and SREBP1c (31.6±41.0 vs. 88.5±38.5, p=0.019). No significant differences were found in the mRNA expression of MTP, DGAT and SCD1. The apolipoprotein AIV mRNA expression correlated positively with body weight (r=0.467, P=0.033), insulin (r=0.729, P<0.0001) and HOMA-IR (r=0.653, P=0.001), and negatively with serum adiponectin (r=-0.692, P=0.013). The PEPCK mRNA expression correlated positively with insulin (r=0.494, P=0.017) and HOMA-IR (r=0.465, P=0.025). The SREBP1c mRNA expression correlated positively with insulin (r=0.669, P<0.0001) and HOMA-IR (r=0.616, P=0.008). The DGAT mRNA expression correlated positively with body weight (r=0.587, P=0.005), BMI (r=0.483 P=0.027), insulin (r=0.503, P=0.020) and HOMA-IR (r=0.445, P=0.043).
Conclusion: Increased lipogenesis (PEPCK and SREBP1c) and synthesis of intestinal lipoproteins (apolipoprotein AIV) has been found in human intestine from morbidly obese subjects with high insulin resistance.
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Eradication of gut bacteria: effect on postprandial glucose metabolism

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Background and aims: Accumulating data suggest that intestinal microbiota contributes to glucose metabolism, but only few human studies have investigated the causality between alterations in gut microbiota and glucose metabolism. We investigated the impact of eradication of intestinal bacteria on postprandial glucose metabolism, gastric emptying and gallbladder emptying in healthy young men.
Materials and methods: Twelve healthy young men (age (mean±SEM): 23±2 years, BMI: 23±1 kg/m2; HbA1c: 5.1±0.1%) received a 4-day oral antibiotic eradication treatment (500 mg vancomycin, 40 mg of gentamicin and 500 mg meropenem once-daily). Eradication was confirmed by culture of faeces. Before, immediately after, and 42 days after eradication, 4-hour meal tests with 1.5 g paracetamol (for evaluation of gastric emptying) with determination of plasma levels of glucose, insulin and paracetamol were made. Postprandial gallbladder emptying was determined using ultrasonography.
Results: We found a significant decrease in both total AUC and incremental AUC for postprandial plasma glucose from before (day 0) to immediately after eradication (day 4) (total AUC: 1314±23 vs. 1290±24 mmol/l×min, p=0.02; incremental AUC: 91±18 vs. 63±20 mmol/l×min, p=0.04). Postprandial insulin and C-peptide responses decreased from day 0 to day 4, although these changes did not reach statistical significance. From day 4 to day 42, postprandial plasma glucose excursions and insulin and C-peptide responses increased to levels not statistically different from baseline. No differences in gallbladder emptying or gastric emptying between day 0, 4 and 42 were observed. Complete gut bacteria eradication (no growth after cultivation of faeces) was obtained after antibiotic treatment (day 4) in six out of the 12 subjects. The remaining six subjects had minimal growth with severely reduced species diversity and bacterial counts. In all 12 subjects yeast could be grown from day 4 faecal samples.
Conclusion: Gut bacteria eradication was associated with a brief, apparently reversible change in postprandial glucose metabolism. These results support the evidence that intestinal bacteria exert important roles in human glucose homeostasis.
Clinical Trial Registration Number: NCT01633762
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Evidence for a leaky gut in type 2 diabetes

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Background and aims: There is increasing evidence for a role of the intestine and its microbiota in the pathogenesis of metabolic disease. In animal models of obesity and diabetes, high-fat, high-energy diets alter the composition of the microbiota, favouring the colonisation of gram-negative bacteria with increased concentrations of lipopolysaccharide (LPS). Permeability of the intestine is increased due to impairment of tight junction proteins, with increased absorption of LPS which binds to macrophages in adipose tissue resulting in increased release of proinflammatory cytokines and, ultimately, to insulin resistance.
Materials and methods: Intestinal permeability was measured using urinary recovery of ^{51}Cr-EDTA following an oral dose in twenty well-controlled male subjects with T2DM, and in a control group matched for age, gender, BMI and habitual fat intake.
Results: Urinary recovery of ^{51}Cr-EDTA was significantly increased in T2DM compared to matched controls: 0-6 hour recovery 1.74 ± 0.18 vs 1.01 ± 0.09% (P=0.002); 6-24 hour recovery 2.02 ± 0.29 vs 1.34 ± 0.12% (P=0.043) and total 24h recovery 3.76 ± 0.35 vs 2.35 ± 0.17% (P=0.001) indicative of paracellular permeability of the small intestine, large intestine and total gut respectively. Levels in the subjects with diabetes were similar to those which have been reported in patients with coeliac disease and with some subjects showing levels associated with inflammatory bowel disease. Within the diabetic cohort, measured small intestinal permeability correlated significantly with serum levels of CRP (r=0.694, P=0.001), IL-6 (r=0.548, P=0.012) and TNFα (r=0.564, P=0.01). There was no association with renal function or urine albumin excretion, or with markers of glycaemic control (HOMA-IR, HbA1c, fasting glucose or insulin), BMI or fat intake, but there was a trend towards a relationship between increased permeability and both protein intake (r=0.436, P=0.055) and time since diagnosis (r= -0.442, P=0.051).
Conclusion: Raised levels of inflammatory markers are a key feature of metabolic syndrome. Our findings raise the intriguing possibility that diet-related changes in gut microbiota and intestinal permeability may be key factors in the initiation of inflammation in both visceral and hepatic adipose tissue, and, furthermore, that toxic products of proteolysis such as ammonia, amides, phenols and sulphides may be important mediators of this process.
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Postprandial plasma glycine-conjugated deoxycholic acid levels correspond with plasma insulin levels in healthy subjects

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Background and aims: Bile acids (BAs) are released into the gut after a meal. A fraction escapes reuptake from the portal circulation to enter the systemic

circulation in postprandial peaks. BAs are important in metabolic signalling through their receptors TGR5 and FXR. The physiological role of postprandial BA peaks in the postabsorptive state is not fully known. In this study in healthy volunteers, we investigated postprandial BA levels in relation to insulin levels in different nutritional conditions.
Materials and methods: Seven healthy volunteers underwent a mixed meal test (MMT, Nutridrink, Nutricia; 25% of calculated daily energy requirements) after a 36 hour fast (F), 3 days of hypercaloric feeding (H) or after an overnight fast (control, C). Hypercaloric feeding consisted of 0,5L of cream per day, containing 1680 kcal (fat 88%, carbohydrates 7%, protein 5%) added to the habitual diet. All subjects underwent all interventions in random order, separated by a 4-week period. Serum BA, insulin and glucose levels were measured at 0, 30, 60, 90, 120, 180 and 240 minutes. Individual BAs were measured with HPLC MS; we report on total BA levels and conjugated deocycholic acid (DCA) and chenodeoxycholic acid (CDCA).
Results: Postprandial glucose excursions were not significantly different between interventions. Insulin AUC was higher after fasting (C 28 ± 10 pmol/L vs F 51 ± 19 pmol/L; p = 0.04) but not after hypercaloric feeding (H 26 ± 9 pmol/L; p = 0.82). Total BA as well as individual BA species levels in plasma showed a postprandial rise with postprandial peaks of glycine-conjugated CDCA (gCDCA) and DCA (gDCA) but there were no differences between the interventions. Insulin levels at 0 and 30 minutes after a meal did not correlate to BA levels. However, in groups C and H, there were strong positive correlations between insulin at 60 minutes and gDCA levels at 60 minutes (C r=0.89, p<0.01; H r=0.99, p<0,01), 90 minutes (C r=0.89, p<0.01; H r =0.82, p<0,05), and 120 minutes (C r =0.82, p<0.05; H r =0.82, p<0.05). In contrast, fasting abrogated the correlations between insulin and gDCA levels. gCDCA levels did not correlate with insulin levels.
Conclusion: Postprandial levels of gDCA associate strongly with postprandial insulin levels in healthy humans in control and high fat feeding conditions. Interestingly, our data might imply, if proven in causal studies, that gDCA may sustain the second phase of the biphasic insulin response. There was a strong positive correlation between gDCA levels and insulin levels one hour after a meal. This correlation was not detectable for other BA species, which might suggest that gDCA specifically may modulate the postprandial insulin response. In accordance with this notion, DCA is a strong agonist of the BA transmembrane receptor TGR5, which stimulates insulin release directly through TGR5 on β cells and indirectly via GLP-1 release from enteroendocrine cells. Interestingly, peak postprandial gDCA levels in this study were well above the EC50 for TGR5, supporting a potential physiological, TGR5-mediated effect on insulin secretion.
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Activation of renal GLP-1 receptors located in the afferent arteriole causes an increase in renal blood flow
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Background and aims: It has become evident that glucagon-like peptide-1 (GLP-1) has a range of extra pancreatic effects such as renal effects. It is unknown how these effects are mediated, but the GLP-1 receptor has been identified in numerous tissues outside the pancreas including the kidney. Therefore, it is possible that the extra pancreatic effects are mediated through the GLP-1 receptor. However, the exact cellular localization of the receptor in these extra pancreatic tissues is poorly described. The aim of the present study was to investigate the localization of a renal GLP-1 receptor and to describe possible renal effects of activation of this receptor.
Materials and methods: *In vivo* autoradiography studies in both mice and rats with ^{125}I-GLP-1 were carried out in order to localize specific GLP-1 binding and thereby identify the localization of the GLP-1 receptor. In rats, changes in blood pressure, renal blood flow and diuresis were investigated when GLP-1 was administered through a catheter directly into the renal artery. Furthermore, the dependency of primary endothelial cell mediated smooth muscle cell relaxants nitric oxide synthase (NOS) and cyclooxygenase (COX), was examined by inhibiting these mediators using L-NAME and Indomethacin.
Results: In both mice and rats, binding of ^{125}I-GLP-1 to the smooth muscle cells of the afferent arteriole was observed. Other vascular structures including the glomeruli were negative. This binding could be completely inhibited by simultaneous administration of excess non-radioactive GLP-1. Infusion of GLP-1 in rats mediated an increase in renal blood flow, from a basal level of 9.3±0.5 ml/min to 10.6±0.5 ml/min during GLP-1 infusion (p<0.01). GLP-1 caused a 2.5 fold increase in urine excretion. No changes were observed in blood pressure. The increase in both renal blood flow and diuresis was independent of NOS and COX.
Conclusion: The renal GLP-1 receptor is localized to the smooth muscle cells of the afferent arterioles. GLP-1 infusion mediates an increase in renal blood flow, due to vasodilatation of the renal artery or pre-glomerular vessels, along with an increase in diuresis. The vasodilatation may be caused by specific binding of GLP-1 to GLP-1 receptors on smooth muscle cells in the afferent arterioles.

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Up-regulated insulin secretion during insulin resistance involves enhanced GLP-1 response and signalling
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Background and aims: Insulin secretion is compensatively up-regulated in insulin resistance to maintain near normal glycemia. The mechanism of this up-regulation has not been fully established. We previously showed in the insulin resistant high-fat diet fed mouse model that the beta-cell expression of the GLP-1 receptor (GLP-1R) is increased which could suggest that GLP-1 signalling contributes to insulin up-regulation in insulin resistance. The validity of this hypothesis depends, however, also on the potential changes in circulating GLP-1 levels in insulin resistance. In particular, it is not known whether insulin resistance is accompanied by a co-increase in GLP-1 levels. Therefore, we have in this study examined whether insulin resistant vs. control mice exhibit differences in mixed meal-induced GLP-1 levels.
Materials and methods: Female C57BL/6J mice were fed a control (CD, 10% fat) or a high-fat (HFD, 60% fat) diet for eight weeks. Then, anesthetized mice were orally given a mixed meal (0.285 kcal; 60/20/20 glucose, protein and fat) with serial blood sampling for determination of glucose (glucose oxidase), insulin and intact GLP-1 (both with ELISA). Areas under the concentration curves (AUC) were calculated for glucose and insulin until 60 min and for intact GLP-1 until 20 min. Baseline (QUICKI) and dynamic (ISI) insulin sensitivity were determined by modelling of glucose and insulin data and beta-cell function was estimated by AUC insulin/AUC glucose until 30 min.
Results: HFD mice (n=8) had lower baseline (0.27±0.01 vs. 0.31±0.01, P<0.001) and dynamic (0.28±0.03 vs. 0.76±0.07, P<0.001) insulin sensitivity than CD mice (n=9), as well as excessive weight (36±1.1 vs. 23±0.3 g, P<0.001). There was no difference in AUC glucose after mixed meal intake between groups in the presence of a significant increase in AUC insulin (109±20 vs. 52±8 pM x min, P=0.026) showing a complete up-regulation of insulin secretion to maintain normal glycemia. Mixed meal-induced GLP-1 responses were significantly increased by three fold in insulin resistant mice. Thus, AUC intact GLP-1 was 66±11 pg/ml x min in high-fat fed mice versus 22±4 pg/ml x min in control mice (P=0.006). This was associated with an improved beta-cell function (257±34 vs. 158±25 pM/mM, P=0.037).
Conclusion: Insulin resistance in mice leads to increased intact GLP-1 levels after mixed meal ingestion in association with a compensatory increase in beta-cell function and insulin secretion. Based on our findings, we suggest that the increased level of intact GLP-1 after meal intake, together with the previously documented increased islet GLP-1R expression, facilitates the beta-cell adaptation to insulin resistance. Sufficient increase in the GLP-1 response after meal intake may therefore be of importance in insulin resistant individuals that do not develop diabetes.

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Exendin-4 and rosiglitazone reduced beta cell degeneration and islet inflammation in male Zucker diabetic fatty rats
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Background and aims: The Zucker diabetic fatty rat (ZDF) is a well established rodent diabetes model, where the development of hyperglycemia is associated with impaired beta cell function and loss of beta cell mass. The aim of this study was to compare the effects of two different anti-diabetic drugs; the GLP-1 receptor agonist, exendin-4, acting directly on the islets, and the

PPARgamma agonist, rosiglitazone, improving peripheral insulin sensitivity, on blood glucose control and beta cell function.
Materials and methods: Male ZDF rats (7 weeks old) were treated with exendin-4 (70µg/kg/day) or rosiglitazone (1mg/kg/day) for three weeks (n=10/group). Exendin-4 was given subcutaneously twice daily, while rosiglitazone was given orally once daily and control ZDF rats were given vehicle by either route of administration. Lean rats were used as healthy controls. Body weight, food intake and blood glucose and insulin levels were measured regularly. At termination, HbA1c levels were analyzed and pancreases were fixed and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and assessed microscopically.
Results: Random fed blood glucose was reduced by both drugs from 11.8±0.4mM, before start of treatment, to 8.1±0.2mM with exendin-4 and 8.4±0.2mM with rosiglitazone ($p<0.01$), while in vehicle treated ZDF rats, blood glucose was 27.9±0.4 mM ($p<0.001$ compared to treatment groups) at termination. The HbA1c was 5.4±0.1% and 5.2±0.1% in both treatment groups, being similar to lean control rats (4.7±0.04%), while in vehicle groups, HbA1c was 8.2±0.2% ($p<0.001$) at termination. Insulin levels were 10-fold higher in ZDF rats compared to lean controls at the start of the experiment (10.9±0.4 vs. 1.3±0.1 ng/ml, $p<0.001$). During diabetic progression in vehicle treated ZDF rats, insulin levels were reduced, while in exendin-4 and in rosiglitazone treated rats, insulin levels were only marginally reduced. Proinsulin levels were high in ZDF rats (277±27 pmol/l), but significantly reduced with both exendin-4 (125±18 pmol/l;$p<0.001$) and rosiglitazone (27±2pmol/l; $p<0.001$). Rosiglitazone treatment increased food intake and body weight gain. In contrast, food intake was slightly reduced in the rats treated with exendin-4, but with no effect on body weight. Control ZDF islets showed degeneration with associated inflammation/ fibrosis/haemorrhage. Islet morphology was improved in rats treated with either exendin-4 or rosiglitazone. Both drugs showed tangible evidence of increased beta cell hyperplasia and beta cell clustering and reduced beta cell degeneration and islet inflammation.
Conclusion: This study demonstrates that exendin-4 and rosiglitazone were equally potent in maintaining normoglycemia in male ZDF rats. However, the different mechanisms of action were obvious in that the insulin and proinsulin levels were significantly reduced with rosiglitazone compared to exendin-4. However, beta cell function was maintained with both treatments, as demonstrated by a significant reduction in insulin/proinsulin ratio. In summary, both drugs demonstrated protective effects on beta cell function with improved islet morphology in male ZDF rats.

PS 047 Exercise: physiology and clinical benefits

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Effects of a training programme at the crossover point on the metabolic abnormalities and cardiovascular risk factors in obese women with metabolic syndrome

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Background and aims: The crossover point of substrates utilization (COP) is defined as the exercise intensity at which energy from carbohydrate-derived fuels predominates over energy from lipids. COP is considered by some authors as the optimal exercise intensity in patients with metabolic abnormalities. However, to our knowledge, no study has evaluated the effects of training program at COP on the abnormalities (i.e., central obesity, hypertriglyceridemia, reduced high density lipoprotein cholesterol, hyperglycemia, and arterial hypertension) defining the metabolic syndrome (MetS). Thus, the purpose of the present study was to examine the effects of training program at COP on metabolic abnormalities and cardiovascular risk factors in obese women with MetS.
Materials and methods: Eighteen obese women with MetS (age: 54.8 ± 8.4 yrs, height: 160 ± 6 cm) volunteered to take part in this study. MetS was diagnosed according to the criteria proposed by the National Cholesterol Education Program. All participants followed a training program over 12 weeks. This training program consisted of three 45-minute sessions each week on cycle ergometer. The intensity imposed during the training sessions corresponded to COP. Before and after the training program, anthropometrical (i.e., body mass, fat mass, body mass index: BMI, waist circumference), biological (i.e., triglyceridemia, total cholesterol, high and light density lipoprotein cholesterols, glycosylated hemoglobin, fasting plasma glucose, quantitative insulin-sensitivity check index: QUICKI) data and (systolic and diastolic) blood pressures were measured, then compared.
Results: After the training program, body mass (88.4 ± 12.3 vs 85.7 ± 11.1 kg), fat mass (43.2 ± 4.8 vs 41.8 ± 4.8% body mass), BMI (34.3 ± 3.9 vs 33.2 ± 3.6 kg.m-2) and waist circumference (105 ± 10 vs 100 ± 9 cm) were significantly lower (p 0.05). A significant reduction of systolic blood pressure was also noticed (141 ± 15 vs 129 ± 11 mmHg; $p = 0.02$). After the training program, the number of patients with fasting plasma hyperglycemia and with an arterial hypertension were significant decreased of 54.4% and 44.4%, respectively. Moreover, the number of patients with MetS was not significantly reduced of 22.2% ($p = 0.10$).
Conclusion: The present study reveals that a training program at COP has beneficial effects on body mass, fat mass, BMI, and waist circumference, fasting plasma glucose, QUICKI and systolic blood pressure in obese women with MetS. These improvements permit to reduce non-significantly the patients' number with MetS after the training program (decrease of 22.2%), which is probably due to the significant reduction of patients' number with arterial hypertension (reduction of 44.4%) and/or a significant decrease of 54.5% of patients' number with hyperglycemia.
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Exercise training improves insulin sensitivity in abdominal adipose tissue but not in brown adipose tissue in healthy, sedentary, middle aged men

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Background and aims: Recently it has been suggested that exercise stimulates the secretion of a polypeptide hormone irisin. Irisin has been shown to

induce browning of white adipocytes by increasing brown-fat-like development, resulting in an increase in adipose tissue thermogenesis, basal energy expenditure, reduced body fat mass and in rodents enhanced glucose homeostasis. We studied the effects of two different modes of exercise training on insulin-stimulated glucose uptake in white and brown adipose tissue (BAT). We further investigated the effects of exercise on irisin and catecholamine levels and their relation to insulin-stimulated BAT glucose uptake.

Materials and methods: Twenty six healthy, sedentary, middle aged men (age: 48 ± 5 years; BMI: 26.1 ± 2.4 kg·m^{-2}; VO_{2max}: 34.2 ± 4.1 ml·kg^{-1}·min^{-1}) were randomized either to high intensity interval training (HIT) or traditional endurance training (TET) group. The groups were studied before and 48 - 96 hours after two weeks and six sessions of HIT (4-6 x 30 s all out sprints on cycle ergometer with 4 minutes of recovery) or TET training (40-60 min cycling with ergometer at 60 % of VO_{2max}). Insulin-stimulated glucose uptake in BAT, abdominal subcutaneous and visceral fat and skeletal muscle were measured using 18F-FDG and PET-CT. In addition, abdominal MRI, OGTT, plasma irisin and catecholamine levels were determined.

Results: Following intervention, VO_{2max} increased by 4.5 ± 5.9 % (p = 0.001), whole body fat percentage (- 4.0 ± 4.9 %, p = 0.002), abdominal subcutaneous (- 1.6 ± 4.1 %, p = 0.04) and visceral fat (-5.0 ± 8.5 %, p = 0.045) and total cholesterol level (4.9 ± 1.0 to 4.4 ± 0.7 mmol·l^{-1}, p = 0.003) decreased without group differences. As only thirteen out of eighteen subjects (4 HIT/9 TET) showed active BAT, the groups were combined. In the whole study group, no change was observed in insulin-stimulated BAT glucose uptake post exercise (3.1 ± 1.4 vs 2.8 ± 0.7.0 µmol·100 g^{-1}·min^{-1}, p = 0.3). In addition to the exercising skeletal muscles, exercise improved insulin-stimulated glucose uptake in abdominal subcutaneous and visceral fat (p = 0.03 and p = 0.048, respectively). After the intervention, BAT glucose uptake correlated positively with VO_{2max}, HDL cholesterol and inversely with BMI, waist circumference, body fat percentage and abdominal fat masses, all p < 0.05. The results of irisin and catecholamine will be presented in the congress.

Conclusion: Abdominal adipose tissue seems to be more sensitive to exercise-induced changes in insulin-stimulated glucose uptake compared to brown adipose tissue.

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Eccentric endurance exercise significantly improves both fasting and postchallenge metabolism in overweight and obese individuals

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Background and aims: Eccentric endurance exercise (e.g. hiking downwards) is less strenuous than concentric exercise (e.g. hiking upwards) but data on its potential to reduce cardiovascular risk are scarce.

Materials and methods: We allocated 68 overweight and obese sedentary individuals to an exercise intervention program, consisting of hiking downwards the same route over two months. For the opposite way, a cable car was used where compliance was recorded electronically. The difference in altitude was 540 meters; the distance was covered three to five times a week. A matched group of 12 individuals served as a control group. Fasting and postprandial metabolic profiles were obtained at baseline and after the two months period.

Results: Compared with baseline, eccentric endurance exercise significantly lowered fasting glucose (99±17 vs. 96±13 mg/dl; p=0.036) as well as glucose tolerance following the oral intake of 75g glucose (250±49 vs. 228±54 mg*dl^{-1} h; p<0.001), whereas these parameters remained unchanged in the control group (p=0.495 and p=0.182, respectively). Furthermore, eccentric endurance exercise significantly improved triglyceride tolerance in a standardized oral fat challenge test (2121±1398 vs. 1744±1143 mg*dl^{-1} h; p <0.001), whereas triglyceride tolerance did not change significantly in the control group (p=0.695). Body mass index was slightly but significantly lowered in the eccentric endurance exercise group (29.6±3.1 vs. 29.2±3.3 kg/m^2; p=0.004) but not in the control group (p=0.237).

Conclusion: Eccentric endurance exercise is a promising new exercise modality with favorable effects on both fasting and postchallenge metabolism.

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Glucose requirements for prevention of hypoglycaemia during exercise in individuals with type 1 diabetes mellitus

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Background and aims: More information is needed to improve guidelines for the prevention of exercise-induced hypoglycaemia in individuals with type 1 diabetes mellitus (T1DM). The aim of our study was to determine the relationship between exercise intensity and the amount of exogenous glucose required to prevent hypoglycaemia during and after exercise at basal insulin levels.

Material and methods: Nine individuals with T1DM (6F, 3M, aged 21.5±4.0 years, BMI of 25.4±5.5 kg/m^2, HbA_{1c} 7.9±0.8%; mean±SD) were subjected to a euglycaemic clamp, whereby stable blood glucose levels between 4.5-6.0 mmol/L were achieved by infusing insulin at a rate that required no glucose infusion (basal insulin infusion rate). Then, while insulin infusion rate remained unchanged, participants performed 40 minutes of exercise at four different exercise intensities (35%, 50%, 65% and 80% VO_2 peak) on four different days. Glucose was infused to maintain euglycaemia during and for two hours after exercise. All 4 treatments were administered following a randomized counterbalanced study design.

Results: The glucose infusion rate (GIR) to maintain euglycaemia during exercise at 35%, 50% and 65% VO_2 peak ranged from 0 to 15g/h. The GIR at each of these three intensities was higher (p<0.05) than at 80% intensity where no glucose was required. The GIR to maintain euglycaemia during recovery ranged from 0 to 8g/h for all exercise intensities.

Conclusions: No glucose is required to maintain euglycaemia during high intensity exercise in individuals with T1DM at basal insulin levels. The amount of glucose needed to maintain euglycaemia during exercise and recovery is less than 15g/h irrespective of the exercise intensity. Glucose requirements to prevent hypoglycaemia during exercise and early recovery vary between individuals and exercise intensities.

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Preventing hypoglycaemia by heavily reducing pre- and post-exercise rapid-acting insulin dose may cause hyperglycaemia, but not hyperketonaemia in type 1 diabetes patients

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Background and aims: Type 1 diabetes patients are encouraged to reduce their pre-exercise, prandially-administered, rapid-acting insulin dose to prevent hypoglycaemia *during* exercise. To combat hypoglycaemia *after* exercise, current opinion advocates a reduction in post-exercise meal-time rapid-acting insulin dose. However, there is little empirical evidence to support this recommendation, and no information pertaining to the glycaemic and metabolic consequences of adopting such a strategy. Therefore, the aim of this study was to examine the glycaemic, metabolic and hormonal effects of large reductions in pre- and post-exercise rapid-acting insulin dose in male type 1 diabetes patients.

Materials and methods: Following written consent, 11 male patients (24±2 years, HbA1c 7.7±0.3% / 61±3 mmol.l^{-1}) attended the laboratory on three mornings in a random and counterbalanced fashion. Firstly, patients self-administered a 25% rapid-acting insulin dose immediately before consuming a standardised breakfast (1g carbohydrate.kg^{-1}BM; 380±10 kcal). After 60 minutes of rest patients performed 45 minutes of treadmill running at 72.5±0.9% VO_{2peak}. At 60 minutes post-exercise, patients ingested a second meal (1g carbohydrate.kg^{-1}BM; 660±21 kcal) administering a Full, 75%, or 50% reduced rapid-acting insulin dose. Blood glucose and lactate, serum insulin, cortisol,

non-esterified-fatty-acid, β-Hydroxybutyrate, and plasma adrenaline and noradrenaline concentrations were measured for 3 h post-meal. Statistical analysis was performed on all data using repeated measures ANOVA, with significance accepted at $p \leq 0.05$.
Results: No conditional differences in glycaemia, or metabolic or hormonal responses were observed before, during or for 60 minutes post-exercise. Following the post-exercise meal, serum insulin concentrations were greatest under Full, and lowest under 50% ($p<0.05$). Declines in blood glucose were evident under Full and 75%, but glycaemia was preserved under 50%. As a result, all patients under 50% were protected from hypoglycaemia (blood glucose ≤3.9 mmol.l^{-1}; 50% n=0, 75% n= 2, Full n=5), but were more exposed to hyperglycaemia (blood glucose ≥8.0 mmol.l^{-1} 50% n=9, 75% n=5, Full n=4). During the post-meal period, β-Hydroxybutyrate concentrations under all conditions were lower than those deemed hyperketonaemic (>1.0 mmol.l^{-1}). All counter-regulatory hormone and other metabolite concentrations were similar between conditions ($p>0.05$), with concentrations similar or lower than resting measures ($p>0.05$).
Conclusion: Heavily reducing rapid-acting insulin dose administered with the meal before and also after running exercise preserves glycaemia and prevents hypoglycaemia in male type 1 diabetes patients. Although some patients may be exposed to periods of hyperglycaemia following the post-exercise meal, this strategy does not augment ketonaemia or cause other metabolic disturbances.
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The role of interleukin 6 in regulation of liver metabolism during exercise
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Background and aims: During exercise the skeletal muscle glucose uptake increases creating an increased demand for hepatic glucose production. Gluconeogenesis in the liver has been shown to be regulated both through increases enzyme activity of phophorenolpyruvat carboxy kinase (PEPCK), increased transcription of gluconeogenetic enzymes such as PEPCK, glucose 6 phosphatase (G6Pase) and genes involved in regulating liver substrate choice for example pyruvate dehydrogenase kinase 4 (PDK4). The systemic regulation of such gene expression during exercise is poorly described. Interestingly it has been shown that infusion of the myokine interleukine-6 (IL-6) during low intensity exercise increases the glucose rate of appearance. Liver PEPCK expression has been suggested to be regulated by CCAAT/enhancer binding protein β (C/EBPβ), an IL-6 regulated transcription factor, and a number of studies have reported that injections of IL-6 increased the mRNA content of PEPCK in the liver. However an exercise-induced PEPCK mRNA was absent in both wild type (WT) and IL-6 knockout (KO) mice leaving the role of IL-6 in liver PEPCK regulation unresolved. In addition it has been suggested that liver PDK4 also could be regulated by C/EBPβ, indicating that IL-6 may influence liver substrate choice during exercise through regulation of PDK4. The aim of the present study was to investigate the effect of IL-6 on PDK4 mRNA content in the liver during exercise.
Materials and methods: This was investigated by acute intraperitoneal injection of 3 ng/g IL-6 in C57BL6 WT mice and by a single 1h treadmill exercise bout in C57BL6 WT and IL-6 KO mice. The mice were euthanized by cervical dislocation and liver samples obtained 1 h after the injection and before, immediately after and 4 h after the exercise bout.
Results: The injection of IL-6 increased ($p<0.05$) the PDK4 mRNA content in the liver 2 fold but there was no effect on either PEPCK or G6Pase mRNA content. The exercise bout increased ($p<0.05$) PDK4 mRNA 7 fold in the WT, but this effect was abolished in the IL-6 KO mice. In addition exercise increased PEPCK ($0.5<p<0.1$) and G6Pase ($p<0.05$) mRNA in both WT and IL-6 KO mice.
Conclusion: These data suggest that exercise-induced PDK4 mRNA increases in the liver is dependent on IL-6 signaling, and thus that IL-6 may be involved in the regulation of substrate choice in the liver during exercise.
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Resistance exercise induces increases in circulating interleukin-6 in type 1 diabetes individuals
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Background and aims: Research has demonstrated a role for interleukin-6 (IL-6) in glucose regulation. Physical exercise has been shown to increase intramuscular release of IL-6 but the magnitude of this rise seems dependent on the degree of hyperglycaemia in individuals with type 1 diabetes (T1DM). 'Weights training' or resistance exercise (RE) can increase IL-6 concentrations in non-diabetic individuals and is recommended for those with T1DM, but can evoke post-exercise hyperglycaemia. Thus, the role of RE in inducing changes in IL-6 and its relationship to blood glucose regulation in T1DM is unclear. The aim of this study was to explore the changes in IL-6 and blood glycaemia during recovery from acute RE sessions of different volume in T1DM.
Materials and methods: After a preliminary session to determine maximal strength, 8 T1DM (7 male: 1 female; age 38±6years, BMI 26.9±1.5kg/m^2, HbA_{1C} 8.7±1% / 71.3±10.6mmol/mol) attended the laboratory fasted having taken their usual basal insulin but omitted rapid-acting insulin, on four separate occasions. Participants completed a **REST** trial, or **1SET (15-min), 2SET (30-min),** or **3SET (45-min)** of RE (8-exercises x 10-repetitions) at 67±3% of 1-RM followed by 60-min recovery. Plasma IL-6 was sampled at rest and for an hour post-exercise. Venous blood pH, HCO_3^-, B_{ecf} (base-extracellular fluid) and blood glucose (BG) were measured before, during and after exercise. Data (mean±SEM) were analysed using repeated-measures ANOVA.
Results: Resting IL-6 concentrations were similar across trials (**1SET** 2.32 ± 1.14, **2SET** 2.16 ± 0.94, **3SET** 2.61 ± 1.11 pg/ml, $p > 0.05$); with 58% of the variance in resting IL-6 accounted for by duration of T1DM ($p < 0.05$). Whereas IL-6 remained similar to rest throughout recovery under **1SET** ($p > 0.05$), IL-6 was greater than rest at 60 min post-exercise under **2SET** (2.94 ± 0.94 pg/ml, $p < 0.05$) and at both 30 (4.01 ± 1.00 pg/ml, $p < 0.05$) and 60 (4.28 ± 1.25 pg/ml, $p = 0.084$) min post-exercise under **3SET**. Under **3SET**, the rise in IL-6 from rest was associated with the magnitude of acid-base disturbance [as reflected in *nadir*; pH r = -0.85, B_{ecf} r= -0.76, HCO_3^- r = -0.81, $p < 0.05$]. Resting BG concentrations were similar between trials (**1SET** 11.7 ± 1.1, **2SET** 11.8 ± 2.0, **3SET** 12.2 ± 1.6 mmol/l, $p > 0.05$). Exercise-induced rises in BG after 30 and 60 min of recovery were similar across exercise trials ($p > 0.05$). Peak BG concentrations during recovery were similar between exercise trials (**1SET** 14.0 ± 1.5, **2SET** 14.9 ± 2.1, **3SET** 13.9 ± 1.9 mmol/l, $p > 0.05$).
Conclusion: The magnitude of rise in IL-6 over an hour after RE was dependent on the number of exercise sets and the corresponding acid-base disturbance in T1DM. In contrast, the number of RE sets did not influence the magnitude of post-exercise hyperglycaemia. The findings demonstrate a link between RE volume and circulating IL-6 in T1DM but its importance in glucose regulation following RE in comparison to other counter-regulatory factors is unclear.
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Altered follistatin secretion profile during exercise in type 2 diabetes
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Background and aims: Follistatin is known as an endogenous negative regulator of myostatin and activin A. In different animal models, it has been shown to have a large effect on both skeletal muscle mass and glucose homeostasis. It has therefore been proposed as a potential target to improve skeletal muscle regeneration and glucose homeostasis. We have previously demonstrated that plasma follistatin is secreted during an acute bout of exercise in young healthy men and that the origin most likely is the liver. In addition, we have recently demonstrated that plasma follistatin is elevated in patients with type 2 diabetes mellitus (T2DM) and that it correlates with markers of insulin

resistance. The aim of the present study was to investigate plasma follistatin in response to exercise in patients with and without T2DM.
Materials and methods: Subjects with (n=7) and without (n=8) T2DM were recruited to the project matched for age and BMI. They were initially screened with a medical examination, an OGTT, an incremental exercise test (VO_2max test) on a bicycle ergometer, a DXA scan and baseline blood tests. All anti-diabetic treatment was paused one week prior to the experimental day. At the experimental day, after an initial 2 hour rest they performed one hour of bicycle exercise at an intensity of 50% of individual VO_2 max. This was followed by a three hours recovery period of supine rest. Blood samples were obtained during both exercise and recovery.
Results: Subjects with and without T2DM were matched for age 58 vs. 56 years, resp. (p=0.49) and BMI 27.4 vs. 25.1 kg/m^2, resp. (p=0.14). Subjects with T2DM had higher fasting glucose 7.7 vs. 5.1 mM, resp. (p=0.004), HbA_{1c} 6.8 vs. 5.6 %, resp. (p=0.003) and 2h glucose during OGTT 15.7 vs. 5.0 mM, resp. (p<0.001) compared with controls. Subjects with T2DM had lower lean mass 56.0 vs. 65.7 kg, resp. (p=0.009) but no difference in fat mass compared with controls. Subjects with T2DM had similar VO_2max 32.0 vs. 37.0 L/kg/min (p=0.07) compared with controls during the exercise test. At the experimental day, the two groups performed the same relative work 51.3 vs. 54.4% of VO_2max (T2DM vs. controls, resp., p=0.25). During the one hour exercise bout, plasma follistatin was unaffected in both groups. However, two hours into recovery plasma was increased by 45% in controls whereas only by 20% in subjects with T2DM (p=0.002) and three hours into recovery plasma follistatin was increased by 60% in controls but only by 30% in subjects with T2DM (p=0.0007). Subjects with T2DM had elevated plasma follistatin compared to controls (group difference in ANOVA analysis, p<0.05).
Conclusion: In the present study, we demonstrate that exercise-induced follistatin secretion is impaired in subjects with T2DM compared with controls. We also show that plasma follistatin is elevated in subjects with T2DM. These data show that the follistatin secretion profile is altered in T2DM which could indicate an impaired exercise response in these subjects. These findings could have implications for both glucose metabolism and skeletal muscle regeneration in relation to exercise in patients with T2DM.

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Physical activity, blood glucose and C-peptide in healthy school children: a substudy of the prospective longitudinal ABIS cohort
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Background and aims: The incidence of type 1 diabetes increased rapidly during the 90-ies and early 2000 parallel to a rapid increase in weight of the general children population. This fits with the beta cell stress hypothesis suggesting that increased insulin demand might contribute to type 1 diabetes. As also physical activity might have decreased we wanted to investigate if lower physical activity further increases beta cell stress already in children.
Materials and methods: A subgroup of school children from the prospective cohort ABIS (All Babies in Southeast Sweden) were asked to participate in some additional tests. 200 children (100 girls, 100 boys) in two communities participated at age 8 and 107 children from one of the communities participated in a follow-up at age 12 (51 girls and 56 boys). Physical activity was **o**bjectively measured as average daily steps (by pedometers), subjectively estimated by questionnaires. We used anthropometric data and measured HbA1c, C-peptide, f-glucose, HOMA-IR and HOMA-B.
Results: Low physical activity was related not only to anthropometric measures (r=.328; p=0.001 for average daily steps *vs* waist circumference at age 12) but also to insulin resistance and beta cell stress (r=-,404; p<0.001 for average daily steps *vs* HOMA-IR at age 12). The connection was especially pronounced in boys, who were more physically active than girls at both time points (p<0.001).
Conclusion: Already in young children low physical activity is related not only to body measures eg increased waist circumference but also to insulin sensitivity, increasing the load on the beta cells. Facilitating physical activity in children might decrease the risk of diabetes, both type 1 and Type 2.
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PS 048 Liver metabolism

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GLP-1-derived nonapeptide GLP-1(28-36)amide represses hepatic gluconeogenesis and improves pyruvate tolerance in high fat diet fed mice
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Background and aims: The incretin hormone glucagon-like peptide-1 [GLP-1/GLP-1(7-36)amide] is secreted by intestinal endocrine L cells in response to feeding and exerts beneficial effects on glucose homeostasis. While GLP-1 is quickly degraded in the circulation, it has recently been suggested that certain degradation products of GLP-1 have biological functions. GLP-1 is cleaved by the neutral endopeptidase, NEP 24.11, to produce the nonapeptide GLP-1(28-36)amide. Recently, GLP-1(28-36)amide was shown to improve glucose disposal and attenuate the development of diabetes and hepatic steatosis in high fat diet (HFD) fed mice. However, the mechanisms underlying these effects are unclear. The aim of this study was to examine the biological actions of GLP-1(28-36)amide in the liver and determine whether GLP-1(28-36)amide affects hepatic glucose metabolism.
Materials and methods: C57BL/6 mice (8 weeks old) were subjected to HFD feeding (60% calories from fat) for 13 weeks followed by administration of GLP-1(28-36)amide (18.5 nmol/kg/day) via daily i.p. injection for 6 weeks while maintaining HFD feeding. We measured body weight and food intake, performed i.p. glucose and pyruvate tolerance tests, and examined hepatic gene expression and protein phosphorylation. In addition, we examined the effects of GLP-1(28-36)amide (100 nM) on glucose production, gene expression, and signaling events in isolated mouse primary hepatocytes.
Results: No significant change in body weight gain in response to HFD feeding was observed upon GLP-1(28-36)amide administration. Although GLP-1(28-36)amide intervention slightly improved glucose disposal during i.p. glucose tolerance test, a more profound improvement was observed in the i.p. pyruvate tolerance test, associated with reduced hepatic expression of PEPCK and glucose-6-phosphatase (G6P), two rate-limiting enzymes of gluconeogenesis, suggesting that GLP-1(28-36)amide may reduce hepatic glucose production in vivo. In mouse primary hepatocytes, GLP-1(28-36)amide treatment reduced glucose production and the mRNA levels of PEPCK, G6P, as well as the gluconeogenic transcriptional co-activator PGC-1α, associated with increased CREB Ser133 and β-catenin Ser675 phosphorylation. These effects were partially blocked by the PKA inhibitor H89. Finally, GLP-1(28-36)amide increased cytoplasmic cAMP levels.
Conclusion: Our observations suggest that the beneficial effect of GLP-1(28-36)amide in repressing glucose production in the livers of diet-induced obese animals involves the activation of cAMP/PKA signaling and its downstream targets including CREB and β-catenin. The repression of glucose production along with the activation of CREB by GLP-1(28-36)amide are contradictory to the well-characterized signaling cascade of glucagon involving PKA/CREB in stimulating glucose production in response to starvation. Our observations, however, are analogous to the actions of glucagon and GLP-1 in similarly activating cAMP/PKA signaling, yet resulting in opposite effects in regulating glucose homeostasis. Additional mechanistic study and the identification of the receptor that mediates the effect of GLP-1(28-36)amide in hepatocytes will further the understanding of the therapeutic potential of GLP-1 metabolites in regulating glucose homeostasis and attenuating diabetes.
Supported by: CIHR

PS 048 Liver metabolism

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Reduction of hepatic glucagon receptor expression with an antisense drug (ISIS-GCGR$_{RX}$) increases total GLP-1 levels without affecting cholesterol or BP in normal subjects

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Background and aims: We have previously reported that antisense reduction of hepatic glucagon receptor expression normalized blood glucose levels in rodent T2DM models and produced pharmacological activity in monkeys. In the current double-blind, placebo-controlled dose escalation study, we evaluated the safety and tolerability; and also included plasma biomarkers for PD of ISIS-GCGR$_{Rx}$ after administration of multiple doses to normal subjects.
Materials and methods: This was a randomized, double-blind, placebo-controlled dose escalation study. Subjects were assigned to a dose level (50, 100, 200 or 300 mg/wk) and received 6 doses of the study drug over a 4-week period (3 doses during the first week on Days 1, 3, 5 and once weekly for an additional 3 weeks).
Results: ISIS-GCGR$_{RX}$ treatment was well tolerated and did not cause clinically significant changes in vital signs, triglycerides, total cholesterol, LDL-cholesterol, BP, hepatic or renal function as compared to placebo treated subjects. Also, no hypoglycemia was observed during the treatment period. In addition to demonstrating an acceptable safety profile, ISIS-GCGR$_{RX}$ increased total GLP-1 levels in treated subjects. Total GLP-1 levels increased significantly in all dosing groups starting on Day 15 and continued to increase until Day 36, two weeks post- treatment. By Day 36, mean change from baseline in total GLP-1 levels in the 200 mg and 300 mg dosing groups was 38 ±51 pg/mL (*p=0.0012) and 34 ±15 pg/mL (*p=0.0012) as compared to the placebo treated subjects (0.29 ± 5 pg/mL). Increases in active GLP-1 levels were observed in some groups; however, variability in data precluded a firm conclusion to be made.
Conclusion: Taken together, the safety, tolerability and PD data support further development of ISIS-GCGR$_{RX}$ for T2DM patients who are severely diabetic and uncontrolled with existing therapies.

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Liver fructokinase: a new target for GLP-1 receptor agonists

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Background and aims: Normal rats fed with a fructose rich diet (FRD) portrait an increase in liver triglyceride (TG) and glycogen content, together with an increase in glucokinase (GK) activity. Since fructose metabolism in the liver starts with its phosphorylation by fructokinase (FK), the aim of this study was to evaluate the effect of co-administration of exendin-4 (GLP-1R agonist) or the DPP-IV inhibitor sitagliptin upon this enzyme as well as on liver TG content and GK activity *in vivo* and *in vitro*.
Materials and methods: Adult male Wistar rats received (3 weeks) a standard commercial diet, without (C) or with 10% fructose (w/v) in the drinking water (F), with or without sitagliptin (115 mg/day per rat) (CS and FS) or exendin-4 (0.35 nmol/kg of bw, ip) (CE and FE). Human HepG2 cells were incubated for 72 with fructose 1.5 mM alone, plus exendin-4 (5 nM), exendin-9 (100 nM) or both. After the treatments, we measured in rats: 1) Serum glucose (G) (enzymatic method), insulin (I) (RIA) and TG (enzymatic method) levels; 2) GK activity in cytosolic (CF) and nuclear (DNF) fractions (enzyme-coupled photometric assay), 3) GK and PFK-2 protein expression (Western blot [Wb]), 4) liver TG content and 5) FK activity. In HepG2 cells we measured TG content, GK and FK activity.
Results: Blood parameters: similar G values were recorded in all groups but F animals evinced a higher level of plasma TG (F: 1.2±0.12 *vs.* C: 0.5±0.01 mM, P <0.05), and I (F: 1.1±0.28 *vs.* C: 0.3±0.02 ng/ml, P<0.05). Co-administration of exendin-4 or sitagliptin prevented the above mentioned changes (TG: FE 0.7±0.09 and 0.7±0.05 P<0.05 vs. F and I: FE 0.3±0.05 and FS 0.29±0.04 P<0.05 vs. F)

Liver results:

	C	CE	CS	F	FE	FS
TG (µg/100 mg tissue)	179±12	181±13	174±12	465±30*	374±30**	301±16**
GK activity (mU/mg protein)	2.7±0.1	2.3±0.1	2.2±0.0	6.8±0.2*	3.3±0.2**	3.7±0.2**
FK activity (mU/mg protein)	3.4±0.1	2.9±0.2	3.7±0.2	5.5±0.1*	3.8±0.1**	3.4±0.1**
PFK-2 protein level (AU)	99.8±2	117±2	76±2	185±8*	72±17**	84±16**

Values are means ± SEM (n =20). * F *vs.* C; ** FE and FS *vs.* F, P <0.05. No changes were observed in GK protein concentration. *In vitro results*: Similar increment in TG content, GK and FK activity were recorded in HepG2 cells incubated with F and again prevented by addition of exendin-4 to the incubation medium. The effect of the later was blunted in the presence of a specific receptor blocker exendin-9.
Conclusions: Sitagliptin and exendin-4 prevented the liver metabolic changes induced by a FRD by blocking the activity of FK, thus preventing fructose metabolism. This effect would be mediated throughout GLP-1 receptor.
Supported by: MSD

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Metformin reverses FOXO3-induced hyperactivation of hepatic gluconeogenesis and catabolic pathways

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Members of the FoxO family of Forkhead transcription factors represent the key downstream targets of insulin and growth factors regulating cell survival, energy metabolism, and longevity. Under caloric restriction and cellular stress, FoxOs translocate to the nucleus to activate gene expression, while at postprandial stage, they are inactivated through insulin/PI3K/Akt signalling. Mammals harbor four FoxO family members, i.e. FoxO1, FoxO3, FoxO4, and FoxO6. Evidence that FoxO1 is a critical regulator in insulin signalling for metabolism, the interplay between different FoxO family members is far from being understood. Especially the role of FoxO3 in metabolism has not been well investigated, although the FOXO3 genotype is associated with insulin sensitivity phenotypes and longevity in human. To understand the importance of FoxO3 in the liver in a time- and cell-type-specific manner, we generated transgenic mice that allow the conditional expression of a constitutively active FoxO3 allele. We crossed transgenic mice carrying the tetracycline-regulated transactivator (tTA) under the control of the liver activator protein promotor with transgenic mice constitutively active form of the FoxO3 allele (FoxO3CA) under the control of a tTA-responsive promotor. Double-transgenic mice displayed tetracycline-regulated FoxO3CA expression in hepatocytes. Activation of transgene expression at five weeks of age led to progressive hepatic atrophy in the absence of significant inflammation. In addition, up-regulation of gluconeogenesis-associated genes, complete loss of hepatocyte glycogen and activation of lipid catabolic pathways in the liver were noted. Within two weeks, the animals showed elevated blood glucose and insulin levels as well as impaired insulin sensitivity. The liver phenotype (i.e. hepatic atrophy, increased gluconeogenesis, and hyperglycaemic phenotype) was completely abolished and the global change in metabolic gene expression was normalised after treatment with metformin, an oral antidiabetic drug suppressing the hepatic glucose production. Our findings identify FoxO3 as an essential mediator of hepatic gluconeogenesis and a potential hepatic target of metformin.
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Cyclin-dependent kinase 4 inhibits hepatic gluconeogenesis

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Background and aims: Cyclin-dependent kinase 4 (Cdk4) is a major cell cycle regulator but is also involved in the regulation of metabolism in non-proliferative conditions. For instance it positively regulates adipogenesis as well as insulin secretion in pancreatic β-cells. Data from our laboratory indicate that Cdk4 activity is increased in response to insulin in liver. Here we study the role of Cdk4 in the control of hepatic gluconeogenesis, a metabolic pathway that is absolutely required for survival during prolonged fasting or starvation, but is inappropriately activated in diabetes mellitus.
Materials and methods: Wild type mice were treated for five days with a 50mg/ kg dose of Cdk4 inhibitor (PD-0332991) or vehicle (DMSO). Gluconeogenesis in vivo was assessed by a pyruvate tolerance test (PTT) after over night fasting. PTT was also performed in fasted R24C mice (constitutively active Cdk4) compared to wild type littermates. Primary hepatocytes were isolated from male mice following portal vein collagenase perfusion. AML12 and HepG2 hepatocytes were cultured and treated for 24 hours with PD-0332991. Hepatocytes were infected with Cdk4 adenovirus for 48 hours. Glucose output in the medium was measured by colorimetric methods. For gene expression analyses, RNA was isolated, reverse transcribed into cDNA and quantitative RT-PCR was performed.
Results: Chemical inhibition of Cdk4 in wild type mice enhances gluconeogenesis in vivo (PTT, AUC 1.23 fold $p = 0.0007$). Cdk4 inhibition increases the expression of gluconeogenic genes (Glucose-6-phosphatase and PEPCK mRNAs) and enhances glucose production in AML12 hepatocytes (2.5 fold, $p < 0.05$). Accordingly, Cdk4 KO primary hepatocytes present increased glucose production compared to wild type hepatocytes (1.5 fold, $p < 0.005$). Inhibition of Cdk4 increases Glucose-6-phosphatase promoter activity in transfected HepG2 hepatocytes under basal conditions (2 fold, $p < 0.05$) and under forskolin stimulation (2.5 fold, $p < 0.05$). In contrast, constitutive activation of Cdk4 (R24C mice) decreases gluconeogenesis in vivo (PTT, AUC 0.8 folds, $p < 0.005$) and impairs glucose production in R24C primary hepatocytes (0.7 fold, $p < 0.05$). Accordingly, Cdk4 adenoviral transduction strongly decreases Glucose-6-phosphatase gene expression (0.2 fold $p < 0.01$) in hepatocytes.
Conclusion: Taken together, our results indicate that Cdk4 inhibits hepatic gluconeogenesis possibly acting as an effector of insulin action in liver metabolism.

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AICAR regulates G6Pase independently of SRC-2/RORα

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Background and aims: Hepatic glucose output is important for sustained circulating glucose levels during fasting and this is determined by degradation of glycogen (glycogenolysis) and *de novo* production (gluconeogenesis). The final step in both processes is the conversion of glucose-6-phosphate to glucose by the rate-limiting enzyme glucose-6-phosphatase (G6Pase), which is regulated at transcriptional level. Subjects with type 2 diabetes mellitus (T2DM) exhibit increased hepatic glucose output and thereby elevated fasting glucose levels. Metformin, a synthetic biguanide drug, is the preferred drug for reducing hyperglycaemia in T2DM. Its ability to suppress hepatic gluconeogenesis is attributed to activation of the energy sensing AMP-activated protein kinase (AMPK) pathway, and this has been shown to reduce transcription of G6Pase. Previously, it was demonstrated in mice that the transcriptional coregulator SRC-2 coactivates the nuclear receptor RORα and thus promotes transcription of G6Pase. The aim of this study was to investigate whether AMPK-dependent reduction of G6Pase transcription is partly due to an inhibitory effect on the SRC-2/RORα complex.
Materials and methods: Transactivation assays using either a GAL4-based luciferase system or a reporter construct harbouring the proximal wild type mouse G6Pase promoter (pGL3-G6Pase-Luc -231/+52) were performed in the human hepatoma cell line HepG2. Endogenous expression levels of G6Pase, SRC-2 and RORα in the rat hepatoma cell line FaO were determined by qPCR. Cells were treated with either vehicle (H_2O) or 1mM AICAR.
Results: We employed a GAL4-based luciferase system in which GAL4-RORα (aa 272-523) and SRC-2 were overexpressed in HepG2 cells. Addition of 1mM AICAR significantly reduced luciferase activity ($p<0.001$). We then transfected HepG2 cells with a reporter construct harbouring the proximal wild type mouse G6Pase promoter, and overexpressed full length RORα and SRC-2. In accordance with a previous report, we found that overexpression of SRC-2 and RORα had a strong synergistic effect on reporter activity. Whereas treatment with AICAR reduced the basal activity of the reporter construct, we observed no inhibiting effect when RORα and SRC-2 were overexpressed. Incubation of FaO cells with 1mM AICAR significantly reduced G6Pase expression, whereas mRNA levels of SRC-2 and RORα remained unchanged.
Conclusion: Although it has been shown that SRC-2 co-activates RORα at the proximal G6Pase promoter, upstream regulation of this interaction remains undefined. Our data suggest that treatment with the AMPK-activating agent AICAR reduces cellular G6Pase expression independently of the SRC-2/RORα complex.
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Significance of hepatic AMPK in metabolic regulation by sympathetic nervous system

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Background and aims: 5'AMP-Activated Protein Kinase (AMPK) is a key metabolic regulator which increases catabolic processes such as lipid oxidation and decreases anabolic processes such as lipogenesis. An anti-obesity adipocytokine, leptin is a potent stimulator of lipid and carbohydrate metabolism. Leptin is reported to activate AMPK in skeletal muscles, which is widely accepted as a major mechanism of its metabolic action. Recently we reported that AMPK activity and its downstream signals are impaired in the fatty liver from A-ZIP/F-1 transgenic mice (A-ZIP), a well-established model of generalized lipodystrophy which lacks adipocytokines. Leptin strikingly ameliorates metabolic disorders including fatty liver in lipodystrophy and is clinically in use. We also found that leptin activates hepatic AMPK via central nervous system and α1-adrenergic effects of sympathetic nervous system, which is followed by reduction of ectopic fat in the liver in vivo. Therefore we hypothesized that hepatic AMPK should play a significant role in metabolic regulation by sympathetic nervous system. The purpose of this study is to elucidate functions of hepatic AMPK in the sympathetic metabolic regulation.
Materials and methods: The effects of AMPK activation were determined in A-ZIP using an AMPK activator, A769662. The effects of an α1-agonist, phenylephrine on AMPK activities, triglyceride (TG) content and glucose production in the liver were studied in isolated primary hepatocytes, normal and high fat diet (HFD)-fed mice.
Results: A769662 activated hepatic AMPK and ameliorated hyperglycemia and fatty liver in A-ZIP analogously to leptin but without affecting body weight or food intake. Phenylephrine also activated AMPK in isolated primary hepatocytes and liver from normal mice in vivo in a dose-dependent manner. In the hepatocytes, AMPK inhibition by a specific inhibitor, BML-275, enhanced glucose production induced by phenylephrine stimulation. AICAR, phenylephrine or a β-agonist, isoproterenol, decreased TG content in the hepatocytes. The AICAR- and phenylephrine-induced TG reduction were completely inhibited by BML-275 while the effect of isoproterenol was not affected by AMPK inhibition. Phenylephrine also activated hepatic AMPK even in the liver from leptin-resistant HFD-fed mice. Continuous administration of phenylephrine for a week ameliorated glucose tolerance, insulin resistance and fatty liver in HFD-fed mice with activating hepatic AMPK.
Conclusion: We found that the α1-adrenoreceptor stimulation causes AMPK activation in hepatocytes. The hepatic AMPK activation mediates reduction of TG content by α-adrenergic stimulation, while it counteracts the hepatic glucose production. In addition, α-adrenergic stimulation as well as the AMPK activator improved fatty liver, insulin resistance and hyperglycemia in diet-induced insulin resistant mice, which is supposed to be mediated by hepatic AMPK activation at least in part. In conclusion, hepatic AMPK plays significant roles in glucose and lipid metabolism regulated by sympathetic nervous system.

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Redox as a master regulator of liver function

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Background and aims: The redox state is the ratio of reduced NAD(P)H or glutathione (GSH) to their oxidized forms $NAD(P)^+$ or GSSG. Redox controls tissue metabolic function by regulating nutrient oxidation, mitochondrial function and handling of reactive oxygen species (ROS). Redox molecules do not pass in and out of the cells, but are in equilibrium with circulating metabolites that freely move across membranes: lactate (L), pyruvate (P), β-hydroxybutyrate (βOHB) and acetoacetate (Acoc). Thus, the ratio of these circulating metabolites is expected to reflect the intracellular redox state and concomitantly, cell metabolism. We hypothesize that all tissues communicate through a master circulating regulatory system that consists of extracellular metabolites that reflect the intracellular redox state: L/P, βOHB/Acoc or cysteine (CyS)/cystine (CySS). The aim of this project was to determine if manipulation of the extracellular redox state affects function of one important metabolic cell type, hepatocytes.
Materials and methods: Primary mouse hepatocytes were treated with extracellular redox metabolites at physiological ratios. The hepatocytes were assessed for production of ROS by fluorescence techniques, glucose production by direct assay and mitochondrial respiration by XF24 Seahorse Analyzer in 3-5 independent experiments and cultures. All of the reported changes were significant at $p \leq 0.05$ (student's t-test).
Results: Endogenous glucose production increased 2.74-fold (± 0.35) after reduction with βOHB, and decreased 0.59-fold (± 0.07) after oxidation with Acoc, compared to the control. In order to understand the mechanism by which these extracellular redox metabolites modulated glucose production, we assessed mitochondrial function by respirometry, as energy is required for gluconeogenesis. Maximal mitochondrial respiratory capacity in primary hepatocytes increased by 0.34-fold (± 0.04) with βOHB and decreased by 0.79-fold (± 0.04) with Acoc. Many reports support a causal relationship between redox changes and ROS production. When primary hepatocytes were treated with ratios of βOHB/Acoc, CyS/CySS and GSH/GSSG that were relatively oxidized in the physiological context, ROS generation was induced by 0.10-fold (± 0.03), 2.0-fold (± 0.5) and 1.75-fold (± 0.15), respectively, thereby corroborating the relationship between redox state and ROS production.
Conclusion: Changes in extracellular ratios of redox metabolites modulated liver gluconeogenesis through a mechanism likely involving changes in ROS levels and mitochondrial respiration. To our knowledge, these experiments show for the first time that the alteration of the extracellular redox environment, which could occur by modulating physiological circulating redox metabolites, can impact liver function. Such changes in circulating redox have been reported in human in response to high fat feeding. Validation of these findings could lead to the establishment of a novel regulatory mechanism constituted by the extracellular redox state acting as a master metabolic regulatory system, which can affect all organs in the body through the bloodstream. Furthermore, future study of extracellular redox pairs in liver and other metabolically-relevant tissues such as adipose tissue or pancreas may provide new approaches for the early detection and/or treatment of metabolic diseases such as diabetes and obesity.
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ISIS-$GCCR_{RX}$, a novel glucocorticoid receptor antisense drug reduces CHOL,TG, attenuates dexamethasone induced hepatic IR without systemic GC antagonism in normal subjects

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Background and aims: Reduction of excessive glucocorticoid (GC) action has been reported to alleviate hyperglycemia in rodents and humans. However, systemic GC inhibition results in adrenal insufficiency and stimulation of the HPA axis. We previously demonstrated that liver selective glucocorticoid receptor (CR) antagonism with antisense drugs improved hyperglycemia and hyperlipidemia in preclinical models without systemic GC antagonism. This current Phase 1 study evaluated the safety, tolerability, and PD of ISIS-$GCCR_{Rx}$ after administration of multiple doses in normal subjects.
Materials and methods: This was a randomized, double-blind, placebo-controlled, Phase 1 study in which subjects were assigned to a dose level (60, 120, 210 or 420 mg/wk) and received 8 doses of the study drug over a 6-week period (3 doses during the first week on Days 1, 3, 5 and once weekly for an additional 5 weeks). A dexamethasone (DEXA) challenge (8 mg for 2 days) was administered in the 420 mg MD cohort before and after 6 weeks of treatment.
Results: DEXA induced hepatic insulin resistance and compensatory hyperinsulinemia as well as increased HOMA-IR. ISIS-$GCCR_{Rx}$ attenuated DEXA induced hepatic insulin resistance as reflected by a 10% and 11% reduction in mean fasting insulin levels and HOMA IR, respectively. DEXA induced lymphopenia was not affected with $GCCR_{Rx}$ treatment indicating no systemic CR antagonism. Even in subjects with high-normal cholesterol (mean baseline cholesterol 202 mg/dL and triglycerides 126 mg/dL), reductions in mean total cholesterol (8.4 mg/dL), triglycerides (17%), VLDL (19%) and ApoCIII (16%) were observed. No clinically significant changes in blood pressure (including no orthostatic hypotension), hepatic, renal and standard safety assessments were reported. Importantly, no hypoglycemia or evidence of HPA axis stimulation (no ACTH increases) was observed.
Conclusion: In conclusion, this study demonstrated that liver specific CR antagonism with ISIS-$GCCR_{Rx}$ was well tolerated, improved lipid profiles, and attenuated DEXA induced hepatic changes without affecting the HPA axis, supporting further evaluation in patients with T2DM.

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Effect of resveratrol in hepatic and renal expression of the glucose transporters GLUT2 and SGLT2 in diabetic rats

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Background and aims: Sirtuins (SIRT) comprise an important family of proteins that possess histone deacetylase activity. Resveratrol, a natural polyphenol, has been reported to be an activator of NAD+-dependent deacetylase sirtuin 1 (SIRT1). Healthful effects of this polyphenol comprises among others, protection against insulin resistance and type 2 diabetes. Increased hepatic output and renal glucose reabsorption contribute to impair glycemic homeostasis in diabetes mellitus. For that, increased expression of Slc2a2 (liver and kidney) and Slc5a2 (kidney) plays a key role. Additionally, increased expression of these glucose transporters in kidney (out cortex tubular cells) has been also related to the development and/or progression of diabetic nephropathy; and increased expression of gluconeogenic enzymes in liver is fundamental to enhance hepatic glucose outflow. Several studies have reported that resveratrol increases insulin sensitivity in muscle and adipose tissue, but the effect of this polyphenol on genes related to glucose production and efflux in liver and kidney and is unknown. The aims of this study are to investigate in diabetic rats treated with saline (DP), insulin (DI) or resveratrol plus insulin (DIP): 1) the expression of glucose-6-phosphatase (G6Ps), phosphoenolpyruvate carboxikinase (Pepck) and Slc2a2 mRNA in liver; and 2) the Slc2a2 and Slc5a2 mRNA expression in kidney.
Materials and methods: Male Wistar rats were rendered diabetic at 75 days of age by single intravenous injection of alloxan (38 mg/kg). After 20 days, diabetic rats were treated during 30 days with saline (DP), insulin NPH (DI) or insulin plus resveratrol (DIR). Insulin was given subcutaneously (4 U/day) and resveratrol intraperitoneally (10 mg/day). In these animals, glycemia, glycosuria, body weight, liver glycogen content, liver Pepck, G6pase and Slc2a2 mRNA content, and renal Slc2a2 and Slc5a2 mRNA content were analyzed.
Results: Plasma glucose concentration and 24-hour urinary glucose decreased with insulin treatment ($p<0.001$ vs DP). Besides, plasma glucose concentration was also further decreased by resveratrol treatment ($p<0.01$ vs DI). The use of the insulin (DI) and insulin+resveratrol (DIR) increased the body weight of rats ($p<0.001$ vs DP), pointing out that resveratrol treatment induced the highest body weight gain ($p<0.01$ vs DI). In liver, insulin treatment increased glycogen content (45%, $p<0.01$ vs DP), and addition of resveratrol induced a further increasing (35%, $p<0.05$ vs DI). Similarly, insulin decreased Pepck mRNA in liver (50%, $p<0.001$ vs DP), and addition of resveratrol induced a further decreasing (33%, $p<0.05$ vs DI). Insulin treatment decreased Slc2a2 mRNA in both liver and kidney (~50%, $p<0.001$, vs DP), as well as the Slc5a2 mRNA in kindey (25%; $p<0.05$ vs DP). Neither the Slc2a2 nor theSlc5a2 expression was altered by addition of resveratrol to the insulin treatment.
Conclusion: The results indicate that addition of resveratrol to insulin treatment increased hepatic insulin sensitivity, which by reducing glucose output, may contributing to improve glycemic control. However, the addition of resveratrol to insulin treatment did not alter the expression of glucose transporters in out cortex renal tubule, suggesting it was not able to alter the nephropathy development.

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PS 049 Mitochondria, ROS and metabolism

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Cytochrome C oxidase activity in islets and lymphocytes is directly correlated to glucose stimulated insulin secretion in Cohen diabetes rats fed different diets

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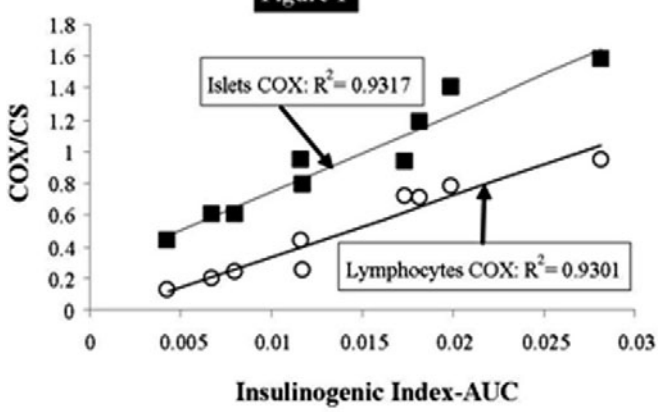
Background and aims: Pancreatic β-cells couple glucose stimulated insulin secretion with oxidative phosphorylation via cytochrome c oxidase (COX) a copper-dependent, mitochondrial respiratory chain enzyme. The Cohen diabetic sensitive (CDs) rat exhibit hyperglycaemia when fed a high-sucrose-low-copper-diet (HSD) but maintain normoglycaemia on regular-diet. The fact that β-cell dysfunction could be restored in hyperglycaemic-CDs rats when they were fed HSD supplemented with copper lead us to investigate the relation between COX activity and glucose stimulated insulin secretion (GSIS) in isolated pancreatic islets. We also investigated the possibility that COX activity in lymphocytes could serve as an additional biomarker for the prediction of β-cells dysfunction in diabetes.
Materials and methods: CDs-rats were fed 4, 11, 20 and 30 days HSD. Hyperglycaemic-CDs rats were fed 7, 11 and 20 days HSD-copper-supplemented (HSD+Cu). Blood glucose and insulin concentrations were measured before and during oral glucose tolerance test (OGTT) performed at the different periods on HSD or HSD+Cu. COX activity in islets and lymphocytes was determined by spectrophotometry and normalized to citrate-synthase (CS , a mitochondrial matrix control enzyme).
Results: The glucose area under the curve (AUC) of CDs-rats fed HSD increased gradually (1115±47, 1463±85, 1832±76, 1975±37 mmol/l/120min) while GSIS AUC and COX activity decreased (insulin- 61±1.7, 54±2.4, 41±2.4, 26±3.9 ηmol/l/120min and COX/CS- 0.9±0.5, 0.8±0.01, 0.6±0.1, 0.4±0.06) for 4, 11, 20 and 30 days HSD respectively. When fed HSD+Cu, glucose AUC of hyperglycaemic-CDs rats decreased (1583±91, 1026±40, 986±81 mmol/l/120min) while GSIS and islets-COX-activity increased (insulin- 40±4, 56±4, 69±5 ηmol/l/120min and COX/CS- 0.6±0.01, 0.9±0.01 1.4±0.03) for 7, 11 and 20 days HSD+Cu respectively. Reduced COX activity and insulin secretion were detected already after 4 days and partial recovery of COX activity and insulin secretion was initiated after 4 days of HSD+Cu. The insulinogenic index AUC highly correlated with both islets and lymphocytes COX activity (R^2 = 0.9317 and 0.9301 respectively (figure 1).
Conclusion: Our study implies a direct link between dietary low-copper concentrations, reduced COX activity and impaired glucose tolerance in CDs rats. The correlation between COX activity in islets and lymphocytes supports the possibility of lymphocyte COX activity as a biomarker for possible prediction of type 2 diabetes in humans.

Figure 1



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Study of key proteins in the newly identified mitochondrial target of thiazolidinediones (mTOT): a new target for insulin sensitisers

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Background and aims: Insulin sensitization is an attractive mode of action to interrupt and prevent the course of diabetes; however, drugs interacting with the only known molecular target to produce this pharmacology, the nuclear receptor PPARγ, have dose-limiting side effects that limit their use. Here we describe the study of key proteins in a newly identified mitochondrial target, mTOT, for compounds shown to be insulin sensitizers in both animal models and in the clinic. Two proteins of this complex, Mpc2 and Mpc1, are involved in the transport of pyruvate into mitochondria. Studies were undertaken to obtain a greater understanding of this target and its potential role in mediating the differentiation of brown adipose cells.
Materials and methods: C-terminal fusions with either a 6xHis-tag or GFP extension on Mpc1 and Mpc2 were expressed in HEK 293 cells in order to study their interaction and to further define the mTOT complex. Mpc2, the protein identified by drug-analog photoaffinity crosslinking was expressed as both the full length and as an N-terminal truncated protein. The activity of mTOT modulators and the complex were examined in primary dedicated progenitor cells from the murine interscapular brown adipose pad.
Results: All fusion proteins were exclusively located in the mitochondrial membrane. Immunoprecipitation of the C-terminal tagged proteins included Mpc2, Mpc1, and the transcription factor prohibitin. Western blots from brown adipose tissue (BAT) cells differentiated in vitro by mTOT modulator compounds also showed an increase in these three components of the mitochondrial membrane complex. Early events in drug action include increased phosphorylation of AKT and changes in acetylation of some lysine residues. Longer term effects include increases in the expression of the mitochondrial deacetylase SIRT3.
Conclusion: In addition to the proteins, Mpc2 and Mpc1, the mTOT complex contains the potential transcription factor prohibitin. Signal transduction may involve connecting changes in mitochondrial metabolism to posttranslational modification of other signaling molecules including transcription factors.

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In situ detection of mitochondria-endoplasmic reticulum interactions by proximity ligation assay: defective organelle interactions cause hepatic insulin resistance

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Background and aims: Mitochondria and endoplasmic reticulum (ER) are interconnected organelles. Their close contacts, known as mitochondria-associated membranes (MAM), play a pivotal role in Ca^{2+} signalling and energy metabolism. Particularly, Ca^{2+} transfer from the ER to mitochondria involves the VDAC1/Grp75/IP3R1 complex. Recently, we identified cyclophylin D (cypD) as a mitochondrial protein interacting with the VDAC1/Grp75/IP3R1 complex at MAM interface and demonstrated that loss of cypD altered Ca^{2+} transfer from ER to mitochondria and induced hepatic insulin resistance, suggesting a potential role of organelle coupling in the control of hepatic insulin signalling.
Materials and methods: Here we described an approach based on *in situ* proximity ligation assay (PLA) to detect and quantify the proximity between ER and mitochondrial proteins within the VDAC/Grp75/IP3R1 complex in fixed cells, and investigated a general role of defective MAM in hepatic insulin resistance.
Results: We demonstrated that the close proximity of both VDAC1/IP3R1 and Grp75/IP3R1 could be visualized by *in situ* PLA in fixed HuH7 cells, confirming their interaction at MAM interface. Down-regulation of VDAC1 and Grp75 expression using specific siRNA (25Nm, 48h), reduced both VDAC1/IP3R1 (-100% and -50%, respectively, $p<0,05$) and Grp75/IP3R1 interactions (-40% and -60%, respectively, $p<0,05$), whereas transient overexpression of

Grp75 (2µg, 48h) increased them in HuH7 cells (VDAC1/IP3R1 :+63% and Grp75/IP3R1:+77%, p<0,05), as well as with the over-expression of mitofusin 2 (mfn2) (VDAC1/IP3R1 :+58% and Grp75/IP3R1:+65%, p<0,05) validating that *in situ* PLA is useful for monitoring perturbations of ER-mitochondria interactions. Using this technique, we demonstrated that both genetic and pharmacological inhibition of cypD reduced ER-mitochondria interactions and altered insulin signalling in both mouse and human primary hepatocytes (-31%, p<0,05). Furthermore, we observed a severe down-regulation of ER-mitochondria interactions in primary hepatocytes of diet-induced diabetic mice (VDAC1/IP3R1: -40%, p<0,05), in parallel of alterations of insulin signalling. In contrast, improvement of organelle contacts, by overexpressing cypD (VDAC/IP3R1: +12,3% p<0,05) or mfn2 using adenovirus (VDAC/IP3R1: +144% p<0,05), enhanced insulin action in hepatocytes of diet-induced diabetic mice.

Conclusion: Collectively, our data indicate that *in situ* PLA is an efficient method to visualize and quantify ER-mitochondria interactions in fixed cells and reveal a new role of altered organelle juxtaposition in hepatic insulin resistance, providing a new pharmacological target for the modulation of insulin signalling.

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An mtDNA mutation in the cytochrome-c oxidase impacts age-related processes in liver, muscle and brain

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Background and aims: mtDNA mutations were recently discussed to contribute to the development of type 2 diabetes mellitus. In previous studies we described an age dependent increase of glucose-induced insulin secretion of pancreatic islets in a conplastic mouse strain carrying an mtDNA mutation in the cytochrome-c oxidase of the respiratory chain. Therefore the aim of this study was to investigate the capacity of liver, muscle and brain metabolism in C57BL/6NTac-mtNOD/AtJ mice.

Materials and methods: Liver, brain and muscle tissue, and primary hepatocytes were isolated from C57BL/6NTac-mtNOD/AtJ (mtNOD) and C57BL/6NTac (control) mice. Mitochondrial morphology and membrane potential were determined by MitoTracker and TMRE staining. Dendra2 was used to study mitochondrial dynamics. Reactive oxygen species (ROS) were detected by MitoSOX. A luminescence assay was used for ATP measurements. The mtDNA copy number was determined by a mitochondrial to nuclear DNA ratio kit.

Results: In control mice a continuous loss of the homogeneous mitochondrial network structure in liver was observed with ageing. In mtNOD mice the variation in the mitochondrial network started significantly earlier. Thus, the mitochondrial network structure of 6 months old mtNOD mice showed a fragmentation level comparable to 12 months old control mice. In addition a significantly higher amount of circular structures was detected in hepatocytes of mtNOD mice. Studies of mitochondrial dynamics showed that those structures are not participating in fission-fusion processes. In early lifetime significantly fewer mitochondria in hepatocytes from mtNOD mice had a high membrane potential compared to controls. However, this amount increased stepwise in mtNOD mice between the age of 6 and 12 months, whilst it remained unchanged in controls. Likewise the ATP content in hepatocytes of control mice kept unchanged with ageing. In contrast, the ATP content in mtNOD mice was significantly lower at the age of 3 months. At the age of 6 and 9 months the ATP content was increased in mtNOD mice and comparable to control. However, at the age of 12 month again a lower ATP content was observed. At this lifetime the mtDNA copy number of mtNOD mice was significantly higher than in controls. In addition ROS production was significantly elevated compared to control and to earlier points of life of mtNOD mice. Notably, in brain and muscle of mtNOD mice ROS production was significantly higher compared to control at all investigated time points.

Conclusion: Our results indicated that an mtDNA mutation in the cytochrome-c oxidase significantly affects the mitochondrial network structure in liver. In addition we detected an adaptive process in mitochondrial function between the age of 6 and 9 month. The higher ROS production in liver, brain and muscle suggest cellular dysfuntion. Thus, the C57BL/6NTac-mtNOD/AtJ mouse strain represents an interesting model to study trigger effects of age-dependent mitochondrial phenotypes in the pathogenesis of type 2 diabetes mellitus.

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Liver mitochondrial function in Zucker diabetic fatty rats during the early stages of the diabetes disease

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Background and aims: Zucker Diabetic Fatty fa/fa (ZDF) rats show signs of diabetes by 8 weeks of age that could be related to impairments of skeletal muscle mitochondrial function. Despite its central role in the whole body metabolism, the liver hasn't been extensively studied. Our purpose was to characterize the early alterations of the liver mitochondrial function in ZDF (fa/fa) rats that develop diabetes compared to that of their lean counter-parts ZDF fa/+.
Materials and methods: Liver mitochondrial function was examined in 11- and 14-week-old ZDF (fa/fa) and fa/+ lean control rats (n=6 per group). Oxygen consumption, H2O2 release, calcium retention capacity (CRC) and membrane potential were measured on isolated mitochondria under various conditions of substrates. Membrane fluidity and fatty acid composition of mitochondria were analyzed by electron paramagnetic resonance and RMN. Moreover oxidative stress parameters were performed on liver.
Results: Results show that state 3 (ADP phosphorylating condition) oxygen consumption with palmitoyl-coA as a substrate increases between 11 and 14 weeks of age in lean but not in diabetic animals. These changes are not seen with other substrates (glutamate/malate (G/M) or succinate) suggesting that the use of fatty acids is impaired by the diabetic rats. Moreover, we report an increase with age of the uncoupled respiration (in presence of DNP) in control but not in diabetic animals suggesting a lack of adaption of the respiratory chain in the latter. Using G/M as a substrate, H2O2 release by the respiratory chain was lower in 14-week-old ZDF (fa/fa) diabetic rats at compared to 11-week-old ZDF (fa/fa) and to lean ZDF (fa/+) controls in the presence of rotenone (inhibitor of complex I) or antimycine A (inhibitor of complex III). These changes were not associated with a different in enzymatic activity of the respiratory complexes suggesting regulatory mechanisms independent of their expression levels. Membrane fluidity and composition analyses shown only slight effect linked to diabetes progression but calcium retention capacity analyses show a decrease of CsA effect suggesting mPTP function deregulation.
Conclusion: Diabetes appears with time in ZDF (fa/fa) rats by about 8 weeks of age for the earlier signs. Our data suggest some mitochondrial alterations. Since the age of 11 weeks liver mitochondria present a decreased effect of CsA on CRC. Other alterations occur between 11 and 14 weeks of age, starting by reduced H2O2 release and absence in of O2 consumption increase as seen in lean control animals, suggesting lack of adaptive mechanisms at the mitochondrial level that need to be further characterized.

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Role of peroxiredoxin 6 in the pathogenesis of diabetes mellitus

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Background and aims: High levels of insulin resistance (IR) can lead to increased fasting glucose levels, due to augmented hepatic glucose production and reduced peripheral glucose uptake. Furthermore, Type 2 Diabetes usually develops when IR is associated to a defect of glucose mediated insulin secretion. Previous studies demonstrated the relationship between ROS production and increased levels of IR, highlighting the important role of antioxidants enzymes. Peroxiredoxin 6 (PRDX-6) is a peroxidase belonging to a relatively newly discovered class of antioxidant enzymes; it is capable to neutralize peroxides and phospholipid hydroperoxides. Based on this evidence, we investigated PRDX-6 in the pathophysiology of Diabetes Mellitus (DM), using PRDX6 knock-out (KO) and wild type (WT) mice.
Materials and methods: C57BL6/J mice and PRDX-6 KO on C57BL6/J background were used as experimental model. Intraperitoneal Glucose Tolerance Test (IPGTT), Insulin Tolerance Test (ITT), ELISA and hyperinsulinemic-euglycemic clamp were accomplished to evaluate the IR level of our mice model. Mice were stimulated with an infusion of insulin 1 UI/kg into portal vein. Western blot analysis and immunoprecipitation assay of protein extracts from skeletal muscle and liver were performed to analyzed the insulin signaling pathway. Immunohistological analysis was accomplished on liver and pancreas of untreated mice.
Results: Our data revealed that PRDX-6 KO mice developed DM due to reduce glucose dependent insulin secretion and increased levels of IR. We observed a significant decrease in AKT1/2 signaling (respectively $p<0.001$ and $p<0.005$) with subsequent reduction of glucose uptake ($p<0.005$). However, the same analysis on liver protein extracts didn't show any significant modification in insulin signaling activation. Altered lipid metabolism (increased levels of serum triglycerides $p<0.05$ and serum VLDL $p<0.05$) in PRDX-6 KO mice might be associated to hepatic ultra-structurul changes, as defined by NASH score ($p<0.005$). Moreover, in PRDX-6 KO mice immunohistological analysis highlighted significant changes in islets of Langerhans number and morphology ($p<0.05$).
Conclusion: We demonstrated, for the first time, the potential role of PRDX-6 in the pathophysiology of DM, opening interesting perspectives in finding new therapeutics options for DM care.

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Effect of association between insulin and PPAR agonists on renal cortex glucose transporters and gluconeogenic enzymes expression in diabetic rats

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Background and aims: GLUT2 and SGLT2 in renal cortex can be markers of nephropathy development. Insulin suppresses gluconeogenesis through the Foxo-1 phosphorylation by Akt which leads to Foxo-1 nuclear exclusion and transcriptional inhibition of Pepck and G6pase genes. Although not equate to liver, kidney is a important gluconeogenic organ and contributes to the excessive glucose release in diabetes. Both PPAR alpha and gamma are expressed in the kidney and studies have demonstrated, in addition to hypolipidaemic and anti-diabetic effects, the healthful effects of PPAR agonists due to their metabolic, anti-inflamatory, anti-fibrotic and anti-proliferative properties. These data strongly suggest a potential benefit of these drugs on diabetic nephropathy. We investigate the effect of association between insulin and PPAR agonists (PPAR gamma and alpha, pioglitazone and fenofibrate, respectively) on glucose transporters and gluconeogenic enzymes expression in renal cortex of diabetic rats.
Materials and methods: Diabetic rats were treated with saline (DS), insulin (DI-4U/day), insulin plus pioglitazone (DIP - 20mg/Kg bw), insulin plus fenofibrate (DIF-100mg/Kg bw) or insulin plus pioglitazone and fenofibrate (DIPF) during 5 weeks. Non diabetic rats (ND) were studied as a control group. We evaluated in these groups after treatment: 1) glycosuria and proteinuria; 2) glycemia; 3) SGLT2 and GLUT2 protein expression; 4) nuclear content of Foxo-1 protein; and 5) mRNA content of Pepck and Glucose-6-phosphatase.
Results: DS showed hyperglycemia, glycosuria, proteinuria, increased expression of renal cortex glucose transporters (GLUT2, ~26%, and SGLT2, ~55%, $P<0.05$), increased expression of nuclear Foxo-1 (~39%, $P<0.01$) and PEPCK mRNA content (~84%, $P<0.001$) in comparison to ND. Glycemia of diabetic rats was reduced in DI, DIP, DIF and DIPF (DI~69%, DIP~82%, DIF~53% and DIPF~29%, $P<0.05$ vs DS) and the most prominent reduction was found in DIP ($P<0,05$ vs. all studied groups). DI, DIP, DIF and DIPF presented lower glycosuria (DI~73%, DIP~80%, DIF 80% and DIPF~88%, $P<0.01$ vs DS) and proteinuria (DI~48%, DIP~49%, DIF~54%, DIPF~61%, $P<0.01$vs DS) than diabetic rats. GLUT2 expression was reduced in DI and DIP (DI~19%, DIP~23%, $P<0.05$ vs DS) but increased in DIF and DIPF (DIF~19%, DIPF~21%, $P<0.05$ vs DS). SGLT2 protein was decreased in DI, DIP and DIF (DI~52%, DIP~63%, DIF~41%, $P<0.01$ vs DS), but in DIPF SGLT2 expression back to DS value. Nuclear Foxo-1 protein was decreased in DI and DIP (DI~39%, DIP~29%, $P<0.05$ vs DS) but increased in DIF and DIPF (~60%$P<0.001$ vs DI). G6pase mRNA content was reduced in DI, DIP and DIF in comparison to DS (~38%, $P<0.05$) but increased in DIPF (~27%, $P<0.01$ vs DS). Pepck mRNA content was decreased in DI and DIP (~49%, $P<0.01$ vs DS), unaltered in DIF but increased in DIPF (~45%, $P<0.01$ vs DS).

Conclusion: Pioglitazone promoted additional improvement to the beneficial effect of insulin treatment on glycemic control. However, pioglitazone had no additional effect on insulin-induced reduction of nuclear Foxo-1 and expression of gluconeogenic enzymes and glucose transporters GLUT2 and SGLT2 expression. Finally, the addition of fenofibrate to the insulin/pioglitazone treatment worsened the effects of this association on glycemic control and expression of the nephropathy markers.
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A new mutation in SLC5A2 causing familial renal glycosuria in individuals with impaired glucose control
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Background and aims: Renal glycosuria is a condition of glucose excretion in the urine despite normal blood glucose levels. Familial renal glycosuria is in a majority of cases caused by deleterious mutations in SLC5A2, a gene coding for the SGLT2 transporter responsible for 90% of the glucose reuptake in the kidney. The aim of the present study is to characterize the underlying genetic cause of glycosuria in a family with renal glycosuria and impaired fasting glucose/impaired glucose tolerance (IGT/IGT) or diabetes and to explore whether glycosuria protects from deterioration of glucose tolerance.
Materials and methods: We performed exome sequencing of 23 family members, 8 with renal glycosuria. Of the 8 affected individuals, 5 were diagnosed with IFG/IGT or diabetes. Change in glucose tolerance was tested as change in area under the glucose curve (AUCOGTT). Glucosuria was estimated during the OGTT.
Results: In total, 5 variants were found in the sequenced region of SLC5A2, 4 SNPs and one 6 bp deletion. The snpEff and SIFT programs ranked the deletion as highly detrimental. The 6 bp deletion causes a frame shift and splice site mutation in the third exon with a high probability of functional consequences on the protein. The deletion was seen in 9 individuals (6 with glycosuria and 3 without), and significantly associated with glycosuria (p=0.023). Of the nine carriers of the deletion, five had IFG/IGT/diabetes; 3 of them also had glycosuria. There was no significant difference in change in AUCOGTT over time between carriers and non-carriers of the deletion.
Conclusion: Glycosuria in this family is most likely caused by a deletionin the SLC5A2 gene. The data do not provide any support for the view that increasing glucose excretion in the urine would protect from diabetes.
Supported by: VR, ERC

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The impact of reduced kidney mass on adipose tissue metabolism and whole-body glucose homeostasis in mice
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Background and aims: Reduced kidney function deteriorates insulin sensitivity in children and adults. However, the underlying mechanisms are poorly understood. Activation of the renin-angiotensin system (RAS)/angiotensin receptors (ATR) impairs insulin signalling in adipose tissue, skeletal muscle and liver and its prevention by ATR blockade (pharmaceutically or genetically) improves glucose homeostasis. We therefore hypothesise that reduced kidney mass impairs glucose metabolism via activation of the RAS.
Materials and methods: Seven-week-old C57Bl6/J mice underwent uninephrectomy (UniNx) or sham operation. After operation, animals were fed either a chow (standard) or a high fat diet (HFD) and glucose homeostasis was assessed 2, 8, and 20 weeks after surgical intervention.
Results: No significant differences were observed in glucose tolerance in chow-fed animals. However, in HFD-fed animals glucose tolerance was further impaired in UniNx mice after 8 and 20 weeks when compared to sham-operated mice. Moreover, skeletal muscle insulin resistance was significantly deteriorated (IS-GDR 22.4 ± 2.8 mg/kg*min in sham vs. 10.6 ± 1.2 mg/kg*min in UniNx; $p < 0.01$) and adiposity was increased in UniNx mice after 20 weeks of HFD. In contrast, hepatic steatosis was decreased and hepatic insulin sensitivity was improved in UniNx mice (insulin-mediated inhibition of EGP 50.2 ± 7.0 % in sham vs. 99.9 ± 18.9 % in UniNx; $p < 0.01$). Plasma angiotensin I concentration was elevated in UniNx compared to sham-operated mice under both chow and HFD 2, 8 and 20 weeks after surgical intervention. Surprisingly, treatment with the ATR blocker telmisartan improved glucose tolerance in HFD-fed sham-operated but not UniNx mice.
Conclusion: Uninephrectomy further impairs obesity-induced skeletal muscle insulin sensitivity but protects from obesity-induced hepatic insulin resistance and steatosis.
Supported by: Swiss National Science Foundation

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Early insulin treatment preserved beta cell function by alleviating local oxidative stress in db/db mice
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Background and aims: It was shown that local oxidative stress was involved in β-cell dysfunction, meanwhile it was demonstrated that early intensive insulin therapy could improve β-cell function in several clinical trials. In this study, we explored the potential pathophysiological mechanisms of early insulin treatment in db/db mice.
Materials and methods: High-fat diet fed diabetic db/db mice were divided to control, early insulin treatment (EI) and late insulin treatment (LI) groups. EI or LI mice were injected subcutaneously with glargine from the onset of diabetes or two weeks later, respectively. Introperitoneal glucose tolerance test was performed, and fasting C-peptide (FCP) were tested. The levels of local superoxide dismutase (SOD), glutathione perioxidase (GPX) and reactive oxygen species (ROS) in pancreas were measured.
Results:Early insulin treatment caused a significant decrease in the AUC of glucose (EI 43.56 ± 5.35 vs LI 54.69 ± 4.72 mmol/L*min, $P<0.01$ and control 67.38 ± 3.14 mmol/L*min, $P<0.001$), and a significant rise in FCP (EI 982.45 ± 107.38 vs LI 571.66 ± 83.45 pM, $P<0.001$ and control 339.63 ± 134.81 pM, $P<0.001$). There was a significant elevation in the activity of SOD in pancreas (EI 103.71 ± 21.35 vs LI 87.48 ± 16.92, $P<0.001$ and control 69.55 ± 24.61, $P<0.001$). So did in the inhibition of ROS (EI 648.37 ± 54.29 vs LI 451.64 ± 71.30, $P<0.001$ and control 316.52 ± 16.92, $P<0.001$). There had no change in the activity of GPX between EI, LI and control.
Conclusion: Early insulin treatment increases the activity of antioxidants SOD, and reduces local ROS that were involved in the amelioration of glucose tolerance and β-cell secretion in high-fat diet fed diabetic db/db mice.
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PS 050 Gender: difference and sex hormones in metabolism

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Withdrawn

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Hyperandrogenaemia in morbidly obese women is associated with increased risk of metabolic syndrome, dysglycaemia and dyslipidaemia

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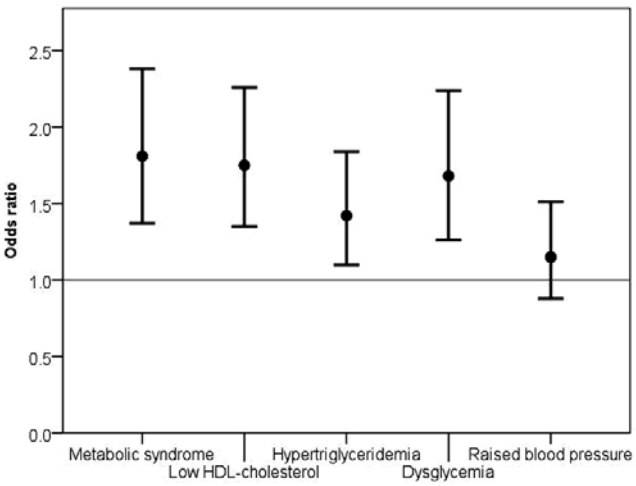
Background and aims: Obesity and the polycystic ovarian syndrome (PCOS) are associated with insulin resistance, hyperandrogenemia and metabolic syndrome (MS). Insulin resistance and compensatory hyperinsulinemia may promote hyperandrogenemia, an important component of PCOS. We aimed to test the hypothesis that morbidly obese women with hyperandrogenemia had higher risk of MS and its components than women without hyperandrogenemia (control group).
Methods: A total of 1698 morbidly obese women under 50 years of age consulted our clinic during the period from November 28th 2005 until January 17th 2013 and were consecutively included in the study. We defined MS if waist circumference was ≥ 80 cm combined with minimum two out of four criteria present: 1) low HDL-cholesterol; HDL cholesterol <1.3 mmol/L, 2) hypertriglyceridemia; triglycerides ≥1.7 mmol/L, 3) dysglycemia; fasting serum glucose ≥5.6 mmol/L or diabetes mellitus, and 4) raised blood pressure; systolic blodpressure ≥130 mmHg or diastolic bloodpressure ≥85 mmHg or use of blood pressure lowering medication. We defined PCOS as known PCOS or a free testosterone index (FTI) >0.6 combined with clinical signs of hyperandrogenemia (irregular bleeding or hirsutism). Hyperandrogenemia was defined by free testosterone index (FTI) >0.6. Continuous and categorical variables were compared using student t-test and χ^2 test as appropriate. Backward stepwise logistic regression analysis was used to assess the association between hyperandrogenemia and MS and its components.
Results: A total of 969 (57%), 273 (16%), and 636 (37%) morbidly obese women had MS, PCOS, and hyperandrogenemia, respectively. Compared with the control-group, women with hyperandrogenemia had a significantly higher prevalence of MS (53% vs. 63%, p<0.001), low HDL cholesterol (49% vs. 68%, p<0.001) and hypertriglyceridemia (42% vs. 49%, p=0.004), but a lower prevalence of raised blood pressure (59% vs. 54%, p=0.031). Multivariable adjustments for well known risk factors for MS showed that hyperandrogenemia was independently associated with MS (OR 1.81 [95% CI 1.37-2.38]), low HDL cholesterol (1.75 [1.35-2.26]), hypertriglyceridemia (1.42 [1.10-1.84]), and dysglycemia (1.68 [1.26-2.24]). Conversely, hyperandrogenemia was not associated with increased risk of raised blood pressure (1.15 [0.88-1.51]).
Conclusion: We document a high prevalence of both MS and hyperandrogenemia in a large population of treatments seeking morbidly obese women under 50 years of age. Hyperandrogenemia was associated with 81% increased odds of MS, mainly explained by significant associations between hyperandrogenemia and the lipid and glucose components of the MS.



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Testosterone replacement in 181 obese, hypogonadal men leads to continuous weight loss and over 5 years and improved glucose homeostasis

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Background and aims: Obesity can cause testosterone deficiency and vice versa. Weight loss has been shown to increase testosterone levels. This study analysed effects of normalization of testosterone in obese hypogonadal men.
Materials and methods: Open-label, single-center, cumulative, prospective registry study of 181 men (mean age 59.11 ± 6.06 years) with testosterone levels ≤ 12.1 nmol/l were treated with testosterone undecanoate 1000 mg/12 weeks after an initial 6-week interval.
Results: After 5 years the following changes occurred: weight (kg) decreased from 114.71 ± 11.59 to 93.24 ± 8.49. The statistical significance was p<0.0001 vs baseline and vs the previous year over 5 years indicating a continuous weight loss. Waist circumference (cm) declined from 111.2 ± 7.54 to 100.47 ± 7.11 (p<0.0001 vs baseline and vs the previous year over 5 years). Body mass index (BMI, m/kg^2) declined from 36.72 ± 3.72 to 30.22 ± 2.6 (p<0.0001 vs baseline and vs the previous year over 5 years). The mean per cent weight loss after 1 year was 5.2 ± 0.24%, after 2 years 9.11 ± 0.25%, after 3 years 11.58 ± 0.27%, after 4 years 13.78 ± 0.28% and after 5 years 16.41 ± 0.3%. 71 (39%) of our patients had known type 2 diabetes at baseline which was treated by their family physician. In these men, waist circumference decreased by 10.06 cm, weight by 18.29 kg (15.71%). Fasting glucose declined from 6.61 ± 0.77 mmol/L (119.07 ± 13.89 mg/dl) to 5.42 ± 0.16 mmol/L (97.63 ± 2.83 mg/dl), HbA_{1c} from 8.33 ± 0.78 to 5.88 ± 0.4%.
Conclusion: Normalising testosterone produced progressive loss of weight, waist circumference and BMI over the full 5 years of the study. In the diabetic subgroup, both fasting glucose and HbA_{1c} improved progressively over the full 5 years of the study.
Supported by: GD received payment from Bayer AG for statistical analyses

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Weight loss and beneficial effects on the metabolic syndrome as a result of testosterone treatment for up to 16 years in 381 hypogonadal men

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Background and aims: Testosterone treatment in men diagnosed with hypogonadism is considered standard therapy, particularly in younger men with congenital forms of hypogonadism. We analysed long-term treatment with

long-acting intramuscular injections of testosterone undecanoate at 3-month intervals after an initial 6-week interval.
Materials and methods: 381 patients (169 with primary, 113 with secondary and 99 with "late-onset hypogonadism") aged 15 to 72 years (mean 42 ± 13 years) received intramuscular injections of 1000 mg of TU during a maximal treatment time of 16 years, corresponding to 8,514 injections. Patients were entered into a registry database once they had received at least 4 injections. Hypogonadism was defined as total testosterone below 12 nmol/L and occurrence of symptoms.
Results: Serum T concentrations rose from 5.3 ± 2.1 to 12.7 ± 2.7 nmol/L within the first year of treatment, 15.6 ± 4.1 nmol/L after the second year, and remained stable thereafter. Body weight decreased from 106.8 ± 16.4 kg at baseline to 86.5 ± 12.7 kg at the end of the observation period. Body mass index declined from 32.6 ± 5.5 to 26.4 ± 3.3 kg/m^2. Waist circumference declined from 117.0 ± 11.1 to 94.2 ± 8.5 cm. Systolic blood pressure decreased from 150.0 ± 14.0 to 131.0 ± 11.06 mmHg, diastolic blood pressure from 99.0 ± 11.0 to 82.0 ± 10.0 mmHg. Heart rate declined from 90.0 ± 9.5 to 76.0 ± 8.1 beats per minute. Total cholesterol improved from 239 ± 35 to 172 ± 22 mg/dl, LDL cholesterol from 161 ± 30 to 111 ± 19 mg/dl, triglycerides from 201 ± 35 to 148 ± 20 mg/dl, and HDL cholesterol from 36.3 ± 9.4 to 52.7 ± 11.6 mg/dl. Fasting glucose decreased from 108.9 ± 29.6 to 94.7 ± 15.3 mg/dl.
Conclusion: Intramuscular injections of testosterone undecanoate lead to improvements in body composition and other features of the metabolic syndrome. These changes are maintained over a long-term follow-up period.

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Increased free testosterone levels following bariatric surgery are related to weight loss and glycaemic control in men with type 2 diabetes: analysis from a RCT

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Background and aims: Obesity and type 2 diabetes are commonly associated with male hypogonadism. We hypothesized that bariatric surgery would increase male sex hormones.
Materials and methods: Forty two men (age 49 ± 8y) with moderate obesity (BMI = 37±3 kg/m2) and poorly controlled DM (HbA1c =9.2± 1.4%) were randomized to medical therapy alone (MT; N =14), gastric bypass (RYGB; N =17) or sleeve gastrectomy (SG; N =11) plus medical therapy. Total and free testosterone (ultrafiltration), sex hormone binding globulin (SHBG) and luteinizing hormone (LH) levels were determined at randomization and 12 month follow up and correlated to metabolic parameters and adiposity measures (DXA, leptin in a subset of N=19). No subjects were on testosterone supplementation during this time.
Results: At 12 months, total body weight decreased by 5% in the medical group, 26% in gastric bypass and 27% in SG. HbA1c levels reduced after all interventions; -1.6% in medical, -2.6% in SG and -3.2% in RYGB. Median levels of total testosterone increased similarly in RYGB (468 vs. 243 ng/dL, P < 0.001) and SG (418 vs 287 ng/dL, P < 0.01), but did not change in MT (265 vs 254 ng/dL). Median percent change and absolute change in free testosterone levels were greater in RYGB vs. MT (49% vs. 6%, P = 0.05) and (32.8 vs. 4.2 pg/mL, P = 0.05) respectively, but were not different between SG and RYGB (59% vs. 49%, P = NS). LH levels were similar at baseline and did not change at 12 m among the three groups. The median percent increase in SHBG levels was 71% in SG, 110% in RYGB vs 14% in MT. The increase in free testosterone levels strongly related to the decrease in body weight (r = -0.36, P = 0.02), HBA1c (-0.32, P = 0.04), leptin (r = -0.52, P = 0.02), and truncal fat (-0.57, P = 0.009), but not with CRP levels (r = -0.23, P = 0.3).SHBG rise strongly associated with the decrease in BMI (r = -0.60, P < 0.0001), leptin (r = -0.60, P = 0.006) and insulin resistance measured by HOMA IR (r = -0.58, P = .006).
Conclusion: In summary, surgically induced weight loss improves male hypogonadism in moderately obese males with type 2 diabetes to a greater extent than medical anti-diabetic therapy.The improvment in free testosterone are linked to better glucose control and loss of truncal fat. Mechanistic studies to investigate these linkages are warrented.
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Comparison of carotid intima-medium thickness, endothelial function and C-reactive protein in male patients with type 2 diabetes with normal and low testosterone levels

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Background and aims: Type 2 Diabetes (T2D) and low serum testosterone are associated with increased risk of atherosclerotic complications. The magnitude of such association; however, in middle-aged patients with T2D has not been determined. We evaluated atherosclerotic markers in patients with T2D with normal and low total testosterone.
Materials and methods: We report preliminary analysis of 115 male patients < 70 years without previous cardiovascular events with normal (≥ 3,5 ng⁄ml, n=79) and low (<3,5 ng⁄ml, n=36) total testosterone (TT). We measured serum C reactive protein (CRP), carotid artery intima-media thickness (IMT), presence carotid atherosclerotic plaque (Plaque) by high-resolution B-mode ultrasound, and endothelial dysfunction (ED) by brachial artery flow meditad dilación. Data were analyzed using simple calculation, Spearman correlation coefficient, Mann Whitney U tests and x2 test. Odds ratio [OR] and 95% confidence intervals (CI) were calculated using simple and multiple logistic regression.
Results: There were no difference in age 56.68±76,7, DM duration: 6.67±3 yrs, BMI 30.1±3.6 kg⁄m2, LDL 119.3±23 mg⁄dl, HDL 39.9±7 mg⁄dl, HbA1c 6.9±0.6%, between normal and low TT groups, p= NS. Frequencies of Low TT was 31.3% (n=36); 53.91% (n=62) and plaque, ED 52.17% (n=60). Mean IMT (0,100±0,014 mm) was significantly correlated with TT, r: -0.39 (p <0.0001) Compared to patients with normal T, those with low T have higher IMT ≥0.100 (80% vs 39%, odds ratio (OR) 6.41(CI95%: 2.5-16.4), p<0,001 and atherosclerotic plaques 68.5% vs. 45%, OR 2.60 (1.12-6.03), p<0.0001; ED (80.5 vs 42.5%, OR 5.77 (2.77-14.77), p <0001, and higher CRP (2.74±582 vs 0.89±0.88 mg⁄L, p<0.0001). Using multiple logistic regression analyses adjusted for age, diabetes duration, HbA1c, lipids, treatment effect, and BMI, we found that TT levels were associated with greater IMT [OR: 8.43 (2.5-25.8)] and ED [OR: 4.98 (1.72-14.37)], but not with the presence of atherosclerotic plaques (p=NS).
Conclusion: Male T2D patients with low total testosterone have greater IMT, ED and CRP compared to diabetic patients with normal TT. Low testosterone in middle age T2D is associated with more advanced atherosclerotic markers (IMT, CRP and endothelial dysfunction).

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Association of glypican-4 with body fat distribution, insulin resistance and non-alcoholic fatty liver disease

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Background and aims: Because visceral adipose tissue is more pathogenic than subcutaneous adipose tissue, the site of fat accumulation plays a pivotal role in metabolic disorders. However, little is known about the factors that determine sites of fat accumulation. Recently, glypican-4 was identified as a novel adipokine capable of enhancing insulin signaling and modulating adipocyte differentiation. Furthermore, glypican-4 is differentially expressed in visceral and subcutaneous adipose tissue, and that its expression in human white adipose tissue is highly correlated with body mass index (BMI) and waist-to-hip ratio (WHR). Therefore, we explored the associations between circulating glypican-4 levels and body fat distribution, insulin resistance, and non-alcoholic fatty liver disease (NAFLD) in non-diabetic Asian subjects.
Materials and methods: We analyzed baseline cross-sectional data from the Korean Sarcopenic Obesity Study (KSOS), an ongoing prospective cohort study. NAFLD was diagnosed by unenhanced computed tomography (CT) using the liver attenuation index. First, we compared circulating glypican-4 levels between subjects with NAFLD and age- and gender-matched control groups. Second, we evaluated the relationships between serum glypican-4 levels and various cardiometabolic risk factors including insulin resistance, arterial stiffness (represented by brachial ankle pulse wave velocity; baPWV), and the ratio of visceral to subcutaneous fat area (VFA/SFA) measured by abdominal CT. Finally, we investigated the effect of a 3-month combined

aerobic and resistance exercise program on changes in circulating glypican-4 levels in obese women.
Results: Circulating glypican-4 levels were higher in men than in women (1.83 [1.19, 2.78] ng/ml vs. 1.17 [0.66, 2.00] ng/ml, P <0.001) and had a significant positive relationship with the waist-to-hip ratio (WHR) (r = 0.20, P = 0.014) and the ratio of visceral to subcutaneous fat area (VFA/SFA) (r = 0.30, P <0.001). Women with NAFLD had significantly higher plasma glypican-4 levels than subjects without NAFLD (1.58 [1.01, 2.62] ng/ml vs. 0.90 [0.59, 1.28] ng/ml, P = 0.001), whereas men did not show any significant difference in glypican-4 levels based on NAFLD (1.97 [1.17, 3.25] ng/ml vs. 1.71 [1.33, 2.71] ng/ml, P = 0.496). Furthermore, glypican-4 levels in women were positively correlated with AST (r = 0.32, P = 0.005), triglyceride (r = 0.27, P = 0.020), glucose (r = 0.31, P = 0.006), and HOMA-IR (r = 0.31, P = 0.006) levels, baPWV (r = 0.23, P = 0.048), and VFA/SFA ratio (r = 0.30, P = 0.009) and were independently associated with NAFLD by multiple logistic regression analysis (P = 0.017, R^2 = 0.33). However, there were no significant correlations between plasma glypican-4 levels and various metabolic risk profiles in men. The 3-month combined exercise training program significantly improved several cardiometabolic parameters. Changes in glypican-4 levels after the exercise program were significantly different between subjects with an increased WHR compared with those with a decreased WHR (P = 0.034).
Conclusion: A gender-based difference in circulating glypican-4 levels was apparent as these were increased in women with NAFLD and related to body fat distribution, insulin resistance, and arterial stiffness.
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Gender differences in the HPA axis are abolished in type 2 diabetes
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Background and aims: Dysfunction of the HPA axis has been implicated in the pathogenesis of type 2 diabetes (T2D). The aim of this study was to compare the regulation of the HPA axis between subjects with and without T2D, and secondarily to study the influence of gender.
Materials and methods: 1 μg synthetic ACTH, a low-dose short Synacthen test, was administered to 21 subjects with T2D (median age 62 [range 54-70] years, 11 men/10 women, HbA_{1c} 49 ± 2 mmol/mol, treated with diet or oral antidiabetic agents) and 39 healthy controls (age 58 [41-67] years, 20 men/18 women). Fasting B-glucose, serum cortisol, insulin and C-peptide were measured at baseline and 30, 60 and 90 minutes after ACTH injection. At a later date, patients took 0.25 mg dexamethasone at 10-11 p.m., and returned the next morning for measurement of serum cortisol.
Results: Patients with T2D had higher baseline glucose (p <0.001), insulin (p = 0.012), C-peptide (p = 0.006) and WHR (p = 0.008) but not BMI vs healthy subjects. Despite similar baseline cortisol, T2D subjects had higher peak cortisol after ACTH injection (693 ± 31 vs 624 ± 18 nmol/L, p = 0.043). The men with T2D had higher peak cortisol vs healthy men (691 ± 42 vs 582 ± 21 nmol/L, p=0.024), while peak levels did not differ between healthy women and women with T2D. Among healthy subjects, women had higher peak cortisol vs men (675 ± 26 vs 582 ± 21 nmol/L, p=0.014). This gender difference did not exist among subjects with T2D. Multiple linear regression showed that gender (β = 0.417 ± 0.178, p = 0.023) and BMI (β = -0.277 ± 0.137, p = 0.048) affected peak cortisol, whereas the fasting insulin level and WHR did not. The morning serum cortisol after dexamethasone was similar between the groups (283 ± 25 in T2D, 261 ± 15 in healthy subjects; no gender differences).
Conclusion: In this sample of T2D patients with good metabolic control, adrenal sensitivity to ACTH was increased compared with healthy controls whereas sensitivity to feedback inhibition of the HPA axis remained intact. Increased cortisol production may contribute to insulin resistance and reduced β-cell function in T2D. The absence of gender differences in HPA axis reactivity in T2D, which are present in healthy subjects, suggests that gender differences in cortisol secretion may play a role in the pathogenesis of T2D.
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Exenatide improved sperm quality and promoted testosterone metabolism in high-fat-diet induced obese C57BL/6J mice
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Background and aims: Previous study in animal experiments and population showed that obesity impaired male reproductive ability and reduced serum testosterone levels. Weight loss or lipid improving could reverse sperm function and restore serum testosterone levels. Exenatide was reported that it could induce weight loss by suppressing appetite and increasing satiety, and improve lipid metabolism, but few studies have evaluated the effects of this drug on sperm quality and testosterone levels. This study aimed to investigate the effects of exenatide on sperm quality and testosterone metabolism in obese mice fed on high-fat diet.
Materials and methods: Eight-week old male C57BL/6J mice were randomly assigned into two groups for 12 weeks: chow diet (NC, 5% fat w/w) and high-fat-diet (HFD, 37% fat w/w). After the diet induction, the mice on HFD were further randomly allocated to the following interventions for anthoer 8 weeks: HFD+saline (HFD), HFD+Exenatide (HFD+EX). Quantitative parameters of sperm motility were determined by computer assisted sperm analysis. Sperm mitochondrial membrane potential (MMP) were measured on flow cytometer. Sperm DNA damage was assessed by sperm chromatin dispersion test. Serum testosterone levels were measured by ELISA. Testis and liver tissues were collected to test mRNA expression of inflammatory factors and protein expression of the enzymes involved in testosterone metabolism.
Results: Testis and seminal vesicle weight and sperm density had no significant difference among three groups. Sperm motility and viability, percent of sperm positive for high MMP was lower in HFD group than those in NC group. Compared with NC group, percent of sperm with DNA damage in HFD group increased significantly. Exenatide improved sperm motility (63.70±3.32% vs 52.14±3.22%, p<0.05) and percent of sperm positive with high MMP (45.97±2.42% vs 31.16±1.41%, p<0.05), reduced sperm DNA damage (7.17±1.89% vs 13.67±2.14%, p<0.05). The levels of serum testosterone in HFD group were significantly lower than that of NC group (1.02±0.22ng/ml vs 1.83±0.15ng/ml, p<0.05). The levels of serum testosterone in HFD+EX group were comparable with those in HFD group (1.16±0.23 vs 1.02±0.22ng/ml, p>0.05). The mRNA and protein expression of STAR and 17b-HSD were statistically lower in HFD group, which could be reversed by exenatide. In testis tissues of mice, IL-1, TNFa, MCP-1 and F4/80 mRNA expression was significantly higher in HFD group than in NC group. However, these inflammatory cytokines mRNA levels were compared between HFD group and HFD+EX group. The expression of SRD5A1 in liver tissues, an enzyme that reduces testosterone, was significantly higher in HFD+EX group than that in the other two groups.
Conclusion: Our study demonstrated that diet-induced obesity leads to impairment of fertility and leydig cell function in male mice. It also indicated that exenatide treatment improved sperm motility and mitochondrial function of sperm, reduced sperm DNA damage, increased metabolism of testosterone in diet-induced obese C57BL/6J mice.

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Impaired GLUT4-mediated glucose uptake in skeletal muscle plays a key role in the development of glucose intolerance induced by testosterone deficiency
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Background and aims: Low serum testosterone concentrations are associated with glucose metabolism disorders such as impaired glucose tolerance and type 2 diabetes. However, the precise mechanism of this association is unclear. On the other hand, a high-fat diet (HFD) induces insulin resistance and glucose intolerance in part through impaired insulin signaling and decreased glucose uptake in skeletal muscle. In the present study, we sought to determine whether the mechanism by which testosterone deficiency causes glucose metabolism disorders is through impaired glucose uptake in the skeletal muscle.

Materials and methods: Male C57BL/6J mice aged 7 weeks were either orchiectomized (ORX) or sham-operated (SHAM), and then were fed HFD (507.6 kcal/100 g, fat kcal 56.7%) or standard diet (346.8 kcal/100 g, fat kcal 10%) for 12 weeks starting at 8 weeks of age. Serum testosterone concentrations were analyzed by liquid chromatography-tandem mass spectrometry. Glucose tolerance was assessed by an intraperitoneal glucose tolerance test (IPGTT, 2 g/kg body weight). Gene expression involved in skeletal muscle glucose metabolism was analyzed by quantitative real-time RT-PCR.
Results: Serum testosterone concentrations were not detected in ORX mice. In mice fed standard diet, testosterone deficiency did not affect all metabolic parameters. As expected, HFD increased body weight, subcutaneous and visceral fat mass, fasting blood glucose and serum insulin concentrations in both SHAM and ORX mice. Fasting blood glucose concentrations were higher in ORX mice than SHAM mice (ORX 10.00 ± 1.19 vs SHAM 6.57 ± 0.97 mmol/L, ANOVA $p < 0.0001$, n = 7). In the IPGTT, moreover, blood glucose concentrations at 30, 60 and 120 min after glucose load were significantly higher in ORX mice than SHAM mice (120 min: ORX 30.20 ± 4.54 vs SHAM 25.63 ± 3.95 mmol/L, $p < 0.05$, n =7). Area under the curve for glucose (0 - 120 min) was also higher in ORX mice than SHAM mice ($p < 0.0001$). The expression of GLUT4 was decreased to 1.4-fold by HFD, and further decreased to 2.3-fold by testosterone deficiency. Similarly, the expression of myocyte enhancer factor 2A (MEF2A) and p38 mitogen-activated protein kinase (MAPK) was down-regulated 1.9-fold and 2.2-fold, respectively, both by HFD and by testosterone deficiency. In addition, the expression of TC10, which is a positive regulator of GLUT4 translocation from intracellular site to plasma membrane, was down-regulated in ORX mice fed HFD. These perturbations in ORX mice fed HFD were normalized to the levels of SHAM mice fed HFD by testosterone supplementation. These results suggest that the effect of testosterone is mediated by the p38 MAPK/MEF2 axis, which is a strong inducer of GLUT4 expression.
Conclusion: Decreased GLUT4-mediated glucose uptake in the skeletal muscle due to impaired p38 MAPK signaling pathway may play an important role in the development of glucose intolerance induced by testosterone deficiency. A high-fat diet could be an initiating factor for those changes.

PS 051 Bariatric surgery

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Laparoscopic sleeve gastrectomy ameliorates mRNA expression of inflammation-related genes in subcutaneous adipose tissue but not in peripheral monocytes of obese patients

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Background and aims: The aim of this two-year prospective interventional study was to determine the effects of laparoscopic sleeve gastrectomy (LSG) on systemic inflammatory markers and the expression profile of genes involved in inflammatory pathways in subcutaneous adipose tissue (SCAT) and peripheral monocytes (PM) in subjects with obesity.
Materials and methods: Thirteen non-diabetic obese females (OB group) and 18 healthy lean sex-matched control subjects (C group) were included into the study. Anthropometric, biochemical and hormonal measurements and adipose tissue sampling were performed at baseline and 6, 12, and 24 months after LSG. mRNA expression of 45 genes involved in inflammatory processes was estimated in SCAT and PM using quantitative real-time PCR.
Results: At baseline, OB group had significantly higher body weight, BMI (22.4 ± 0.5 vs. 42.3 ± 1.1 kg/m^2, p<0.05) and increased serum concentrations of hsCRP (0.42 ± 0.16 vs. 1.45 ± 0.24 mg/l; p<0.05), insulin and proinflammatory cytokines as compared to C group. mRNA expression of macrophage antigen CD68, cytokines (IL-10, IL-18), chemokines (CCL-3, -17, -22) and chemokine receptor CCR1 was increased, whereas the expression of adiponectin receptor 2 (ADIPOR2), JUN proto-oncogene, NFκB2 and vascular endothelial growth factor A (VEGFA) was reduced in SCAT of obese females relative to controls. In PM mRNA expression of CD68, chemokine receptors (CCR-1, CCR-2, CCR-3) and other proinflammatory receptors (TLR-2, TLR-4, TNFRSF1A), macrophage migration inhibitory factor (MIF), ICAM-1, PPARδ and ADIPOR2 were significantly increased in OB relative to C group. After 24 months LSG decreased body weight (BMI 33.0 ± 2.1 vs. 42.3 ± 1.1 kg/m^2; p<0.05) and improved lipid profile (LDL cholesterol 2.37 ± 0.39 vs. 2.76 ± 0.30 mmol/l; p<0.05; TAG 0.95 ± 0.21 vs. 1.71 ± 0.24 mmol/l; p<0.05) without significantly affecting blood glucose (5.18 ± 0.29 vs. 4.99 ± 0.22 mmol/l; p<0.05) or parameters of insulin resistance (HOMA-IR 7.24 ± 1.76 vs. 8.51 ± 1.43; p<0.05). In SCAT, LSG reduced mRNA expression of almost all up-regulated chemokines, chemokine receptors and proinflammatory factors (CCL-3, CCL-17, CCL-22, CCR-1, CCR-4, CD68, IL-1B, IL-18) with maximum changes at month 12 and 24 after the surgery. In contrast, only minimal effect on the proinflammatory expression profile in PM could be seen throughout the whole study period with a persistent increase in mRNA expression of most of the factors including MIF, TLR-2, TLR-4 and other inflammation-related receptors.
Conclusion: In the present study we demonstrate that obese patients have increased mRNA expression of chemotactic and proinflammatory factors in both SCAT and PM. LSG improves this profile in SCAT but not in PM. We suggest that activation of the TLR stress-pathway and increased expression of MIF in PM may contribute to limited effects of LSG on the improvement of insulin resistance. The sustained proinflammatory and chemotactic expression profile in PM even 2 years after LSG may contribute to the persistence and/or possible future re-manifestation of metabolic complications in obese patients after metabolic surgery.
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Laparoscopic greater curvature plication improves metabolic control and modulates adipose tissue inflammation in obese type 2 diabetic patients during a 6-month follow-up

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Background and aims: Laparoscopic greater curvature plication (LGCP) is a recently introduced restrictive bariatric procedure reducing gastric volume without the need of resection by invaginating the greater curvature into

the stomach. The aim of our study was to prospectively assess the effects of LGCP on body weight, metabolic parameters and adipose tissue inflammation in obese subjects with type 2 diabetes mellitus (T2DM) during the first 6 months after the operation.
Materials and methods: Eleven T2DM patients undergoing LGCP (T2DM group) and 13 age-matched healthy lean controls (C group) were included into the study. Anthropometric, biochemical and hormonal parameters were measured at baseline, at 1 month and at 6 months after LGCP. Subcutaneous adipose tissue (SCAT) samples were obtained from abdominal region using needle aspiration biopsy. mRNA expression of 44 genes involved in inflammation, immune cell recruitment and tissue remodeling was assessed using quantitative real-time PCR.
Results: After 6 months LGCP substantially decreased body weight (BMI 42.3 ± 2.0 vs. 36.6 ± 2.0 kg/m2, p<0.05), blood glucose (9.88 ± 1.30 vs. 6.96 ± 0.53 mmol/l, p<0.05), HbA1C (63.8 ± 4.7 vs. 56.3 ± 4.2 mmol/mol, p<0.05), lipid profile (total cholesterol 5.48 ± 0.29 vs. 4.61 ± 0.24 mmol/l, p<0.05; triglycerides 3.05 ± 0.72 vs. 1.88 ± 0.28 mmol/l, p<0.05) and low-grade inflammation (hsCRP 4.11 ± 0.76 vs. 2.55 ± 0.90 mg/l, p<0.05). Compared to healthy controls SCAT of T2DM group at baseline showed increased mRNA expression of macrophage markers (CD68, CD11c), proinflammatory cytokines (TNFα), chemokines (CCL-3, -8, -22, CXCL-10), corresponding chemokine receptors (CCR-1,-5, CXCR-3) and tissue remodeling markers (MMP-9, TIMP), whereas the expression of monocyte marker CD14, chemerin and fractalkine (CX3CL1) was decreased. For most factors this expression profile was even more enhanced 1 month after LGCP, while dropping again after 6 months, though only mRNA expression of macrophage antigen CD68, M1 macrophage marker CD11c and M2 marker LYVE1 changed significantly compared to previous values.
Conclusion: During the first 6 months LGCP markedly decreased body weight and improved glucose control, metabolic profile and systemic low-grade inflammation in obese patients with type 2 diabetes mellitus. The increase in SCAT proinflammatory expression profile after 1 month suggests that LGCP-induced weight reduction is initially associated with local adipose tissue stress reaction which is not directly related to systemic parameters and which might be most probably caused by changes in local immune cell content and their phenotype.
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Bile acids: key actors of metabolic improvement after gastric bypass?
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Background and aims: Gastric bypass (GBP) makes rapid ameliorations in glucose and energy homeostasis before any weight loss in obese and diabetic patients. The increase in intestinal gluconeogenesis (GNG) has been reported to be a causal factor in these metabolic improvements in a murine model of GBP. Indeed, intestinal GNG causes satiety and potentiates insulin sensitivity. This intestinal glucose production has opposite effects compared to hepatic production, which is associated with deleterious effects such as insulin resistance. Another consequence of GBP is the absence of bile in the alimentary limb, with in parallel an increase in plasma bile acids. The latter are potent inhibitors of GNG. We tested the hypothesis that plasma bile acids might inhibit hepatic GNG, while their absence in the intestinal lumen might lead to an induction of intestinal GNG.
Materials and methods: Bile diversions are performed from bile duct to a mesenteric vein (MV), the mid-jejunum (MJ) or the mid-ileum (MI), in order to mimic different GBP, in standard-diet fed rats. A sham-operated group, which just undergo a laparotomy, is studied in parallel. In order to evaluate the rapid metabolic improvements potentially caused by bile diversions, insulin sensitivity and glucose tolerance tests are performed eight and eleven days after surgeries. The expression of gluconeogenic genes is analysed in both the liver and intestine eight days after surgeries.
Results: All the diversions induce an increase in plasma bile acids (156.5 ± 25.3 µmol/L for MV, 218.8 ± 74.6 µmol/L for MJ, 231.7 ± 69.4 µmol/L for MI vs 19.1 ± 6.5 µmol/L for sham-operated). This high plasma bile acid concentration is associated to a drastic inhibition of hepatic GNG. Indeed, hepatic glucose-6-phosphatase (Glc6Pase) activity is strongly reduced (38.6 ± 3.3 U/g protein for MV, 41.6 ± 4.3 U/g for MJ, 47.5 ± 6.6 U/g pour MI vs 76.2 ± 2.7 U/g for sham), as is its protein expression (about 70% fold decrease for the three diversions). Besides, hepatic mRNA levels of Glc6Pase and phosphoenolpyruvate carboxykinase-c are reduced by about 80 and 50%, respectively, for all diversions. In parallel, the absence of bile in the intestinal lumen leads to an increase in intestinal GNG. For the MV diversion, Glc6Pase activity shows a 1.5 to 2.5-fold increase in the entire intestine. It is induced upstream of the site of bile reinsertion (45.1 ± 6.1 U/g for MJ, 37.9 ± 4.9 U/g for MI vs 24.4 ± 2.2 U/g for sham) and decreased downstream (1.1 ± 0.5 U/g for MJ, 0.1 ± 0.1 U/g for MI vs 4.7 ± 1.7 U/g for sham). Finally, insulin sensitivity and glucose tolerance are markedly improved in mid-jejunum diverted rats.
Conclusion: The only modification of bile routing reproduces the amelioration of glucose metabolism observed after GBP. This improvement is associated with a decrease in hepatic GNG in parallel with an increase in intestinal GNG, in standard-diet fed rats. These results suggest that the regulation of endogenous glucose production by bile acids is a key factor in the metabolic improvements after GBP.
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Improvements in insulin sensitivity after gastric bypass is not due to changes in physical fitness
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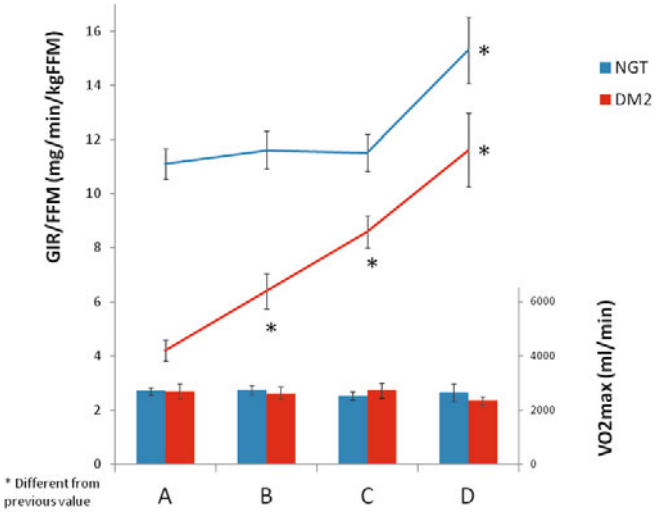
Background and aims: Gastric bypass surgery (GBP) is known to bring 75-80% of type 2 diabetes patients into remission shortly after their operation. We studied insulin sensitivity, body composition and aerobic fitness before and up to 1½ yr after GBP in morbidly obese patients with type 2 diabetes (DM2) and patients with normal glucose tolerance (NGT). The aim was to test if changes in insulin sensitivity were correlated to changes in aerobic fitness. Furthermore, we determined if insulin sensitivity is affected differently by a diet induced or by a GBP induced weight loss.
Materials and methods: 27 subjects (17F/10M, 16NGT/11DM2) were recruited. Subjects were studied 4 times each after a 12h overnight fast: At baseline (A), after 10-12% weight loss (before surgery) obtained by diet induced weight loss (B), 4 months after surgery (C) and 18 months after surgery (D). Each time body composition was measured by Dual X-ray Energy Absorption (DXA) scan followed by measurement of aerobic fitness (maximal oxygen uptake (VO_2max) on a stationary ergometer bike (75W warm-up period, increase 25W/1 min)). On a different test day during the same week, we measured whole-body insulin sensitivity by a hyperinsulinemic (80mU/m²/min) euglycemic clamp.
Results: At baseline body mass index (BMI) and age did not differ between groups. Fasting glucose was significantly (p<0.05) higher (8.6±0.7 vs. 5.4±0.3 mM) and VO_2max (2691±263 vs. 2701±129 ml O_2/min) similar in DM2 vs. NGT, respectively. Baseline (A) Glucose Infusion Rates (GIR) per fat free mass (FFM) were lower in the DM2 vs. NGT (4.2±0.4 vs. 11.1±0.6 mg/min/kg FFM), respectively. Weight loss in NGT vs. DM2 from A-B (6.1±0.6 vs. 5.2±0.8 kg), B-C (22.1±1.1 vs. 26.4±2.6 kg) and C-D (12.6±2.4 vs. 10.0±1.7 kg) was similar in the two groups. In the NGT there was no difference in GIR/FFM from A-B and B-C (p>0.05) - see figure. Remarkably, a weight loss of 28.2±1.3 kg from A to C did not result in increased GIR/FFM in the NGT group. However, after 1½ yr GIR/FFM increased (p<0.05) in NGT. In the patients with DM2 both diet induced weight loss (A-B) and GBP induced weight loss (B-C and C-D) resulted in significantly (p<0.05) increasing GIR/FFM. In contrast to the marked changes in GIR/FFM, aerobic fitness did not differ between the groups at any time (p>0.05) and no significant change was seen at any time A through D (p>0.05).
Conclusion: GBP does not improve GIR/FFM in NGT during the first 4 months postoperatively, even in spite of a marked weight loss. Most likely this is due to an initially relatively well preserved insulin sensitivity. In DM2 the insulin sensitivity improves even by a small weight loss (A-B) and the improvement continues as weight loss continues. However DM2 do not reach same levels of insulin sensitivity as NGT even 18 months after surgery. The improvement in GIR/FFM is not due to increases in peripheral insulin sensitivity resulting from exercise training, as the patients have unchanged VO_2max, indicating that they did not take up exercise after GBP.



Clinical Trial Registration Number: H-C-2009-050
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GLP-1 Antagonism alters the rate of gastrointestinal transit but not the rate of meal appearance after Roux-en-Y Gastric Bypass in diabetic and non-diabetic subjects

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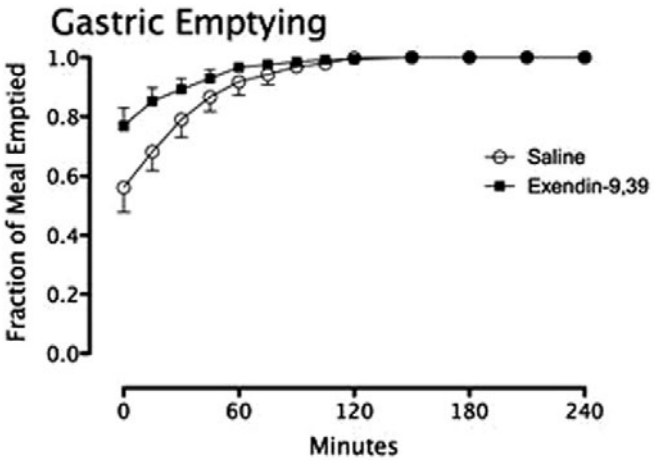
Background and aims: The contribution of elevated incretin hormones to postprandial glucose metabolism after Roux-en-Y Gastric Bypass (RYGB) has been the subject of some uncertainty. Exendin-9,39, (Exe-9,39) a competitive antagonist of GLP-1 enables examination of its effects on islet hormone secretion and gastrointestinal transit. As part of a study examining the contribution of GLP-1 to the remission of type 2 diabetes after RYGB we studied subjects > 12 months after RYGB together with age and weight-matched controls in the presence or absence of Exe-9,39 infused at 300pmol/kg/min. In addition we studied subjects before and 4 weeks after RYGB randomized to Exe-9,39 or saline.
Materials and methods: Subjects were studied using a standardized triple-tracer mixed meal consisting of 15g of bacon, 1 scrambled egg and 35g of Jell-O labeled with [1-^{13}C]-glucose. [6-^{3}H] glucose was infused intravenously to measure the systemic rate of meal appearance (Meal Ra). Infused [6,6-$^{2}H_2$] glucose enabled measurement of endogenous glucose production (EGP) and glucose disappearance (Rd). The meal was also labeled with ^{111}In-DTPA to enable measurement of gastrointestinal transit. Insulin action (Si) and β-cell responsivity indices (ϕ) were estimated using the oral minimal model.
Results: RYGB resulted in decreased postprandial glucose within 4 weeks of surgery (3.5±0.5 vs. 2.8±0.3 Mol per 6hr, *P*=0.05; 3.0±0.5 vs. 2.5±0.4 Mol per 6hr, *P*=0.02; Exe-9,39 and saline respectively). The net change in glucose concentrations did not differ between groups (*P*=0.39). In contrast, in subjects >1 year after RYGB, Exe-9,39 was associated with increased glycaemic excursion (3.6±0.5 vs. 2.0±0.4 Mol per 6hr, *P*<0.01) due to impaired insulin secretion (13.3±2.5 vs. 19.9±4.2 nmol per 6hr, *P*=0.01). While gastrointestinal transit in controls was unaffected by Exe-9,39, post-RYGB, the % of stomach content emptied at 30 minutes increased (89±4 vs. 79±1%, *P*<0.01) implying accelerated transit by Exe-9,39. Intriguingly, peak MRa was unchanged post-RYGB (157±19 vs. 143±17μmol/kg/min, *P*=0.60) and in control subjects (79±12 vs. 58±12μmol/kg/min, *P*=0.19).
Conclusion: The acceleration of gastrointestinal transit post-RYGB by Exendin-9,39 implies an effect of GLP-1 on gastric emptying even after bypass. However, despite this alteration in transit, together with decreased insulin secretion, no effect on Meal Ra was observed implying differential regulation of meal appearance.



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Insulin secretion before and after gastric bypass operation in morbidly obese subjects with and without type 2 diabetes

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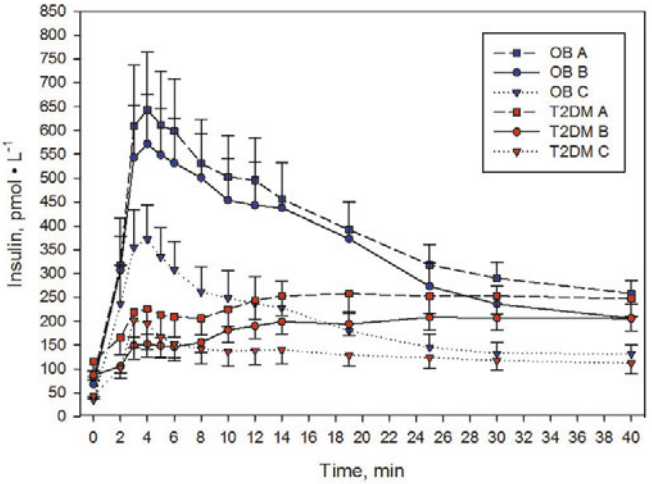
Background and aims: Roux-En-Y gastric bypass (RYGB) may cure 70 % of patients suffering from type 2 diabetes (T2DM) by inducing increased insulin secretion, primarily due to a higher postprandial incretin release, and decreased peripheral insulin resistance due to weight loss. It is likely that the benefit of the operation depend on the remnant β-cell function. Thus patients with little ability to secrete insulin might benefit less than those with a larger secretory capacity. Our aim was to compare insulin secretion after a diet and a RYGB induced weight loss in morbidly obese patients with or without (OB) type 2 diabetes.
Materials and methods: 20 morbidly obese individuals (6M/14F, 10 T2DM/10 OB) were recruited. Subjects reported to the lab thrice: At baseline when assigning for surgery (A), immediately prior to operation after a diet induced weight loss (B) and 4 mo. after RYGB (C). At each visit a 25 g intravenous glucose tolerance test (IVGTT) was performed. Blood samples for determination of insulin were drawn at baseline and frequently for 40 minutes after the injection. First phase insulin response (AIRg) was determined by applying the MINMOD millennium model and total area under the curve (AUC) followed the trapezoidal rule. Two way repeated measures analysis of variance was used to test for significant effect.
Results: Initial body weight (133 ± 6 vs. 123 ± 6 Kg), BMI (44 ± 2 vs. 41 ± 1 Kg/m2) and weight loss (A-B: OB 7 ± 1 and T2DM 6 ± 1 Kg, and B-C: OB 23 ± 1 and T2DM 21 ± 2 Kg) were similar in OB and T2DM, respectively. OB were younger than T2DM (34 ± 3 vs. 44 ± 2 yrs, P < 0.05) had lower fasting insulin at A (P < 0.05) (A: 115 ± 12, B: 94 ± 10, C: 56 ± 6 vs. A: 155 ± 30, B: 115 ± 18, C: 62 ± 8 pM), but similar at test B and C. AIRg decreased significantly postoperatively, but were always higher in in OB, compared to T2DM in whom AIRg did not change throughout the study (A: 4359 ± 861, B: 4029 ± 756, C: 2307 ± 566 vs. A: 774 ± 312, B: 527 ± 166, C: 888 ± 272 pmol/L • 10 min, P < 0.05). Insulin AUC (A: 15350 ± 2251, B: 13670 ± 2397, C: 7641 ± 1553 vs. A: 8774 ± 1205, B: 7078 ± 730, C: 4603 ± 810 pmol/L • 40 min, P < 0.05) was higher in OB than T2DM and decreased postoperatively in both groups.
Conclusion: Insulin secretion patterns prior to and after RYGB were different in OB and T2DM. In OB the markedly decrease in AIRg and AUC clearly is the result of a continuous process starting when weight loss is begun prior to RYGB, i.e. A-B (Figure 1), most likely due to increased peripheral insulin sensitivity. While AUC decreased in T2DM, AIRg was unchanged with weight loss both by diet and by RYGB. Thus in spite of the fact that 8/10 T2DM in the study had remission of their diabetes 4 mo. post-RYGB (e.g. no antidiabetic medicine, normal range fasting glucose and HbA1c) their β-cell dysfunction persisted.

Figure 1. Insulin secretion in morbidly obese subjects with or without type 2 diabetes at baseline (A), after a diet induced weight loss (B) and 4 mo. after gastric bypass surgery.



Supported by: Danish strategic research council

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Implantation of an endoscopically deployed Duodenal-Jejunal Bypass Liner in obese type 2 diabetes subjects: 8 months follow up of ten subjects

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Background and aims: Purpose of this follow up of obese type 2 diabetes mellitus subjects was to evaluate the effect of the duodenal-jejunal bypass liner (DJBL, EndoBarrier®), a 60-cm, impermeable polymer liner anchored in the duodenum to create a duodenal-jejunal bypass, on metabolic parameters in obese subjects with type 2 diabetes.
Materials and methods: Ten subjects with type 2 diabetes (3F, 7M; age 55.5 years (IQR 44.0-59.5); diabetes duration 8.0 years (IQR 6.5-11.3)) and a mean body mass index of 47.1 kg/m2 (IQR 40.6-53.8) were enrolled in a 14 months, prospective, open label study and followed regularly. Endoscopic device implantation was performed under general anesthesia, and the subjects are examined periodically during the next 14 months. This data evaluation presents data from the first eight months follow up.
Results: Implantation procedures were without complications, abdominal pain and nausea were commonly described side effects on the day of implantation. Antidiabetic medication was reduced upon implantation (IM), HbA1c level remained constant during the follow up of 8 months (8M) (IM: 7.65% IQR 6.80-9.15; 8M: 7.70% IQR 6.43-8.87). BMI was lowered significantly (-2.6 kg/m2) (8M: 44.5 kg/m2 IQR 38.4-51.9), body composition changed (fat loss: 3.5%, gain of lean mass: 3.5% (p=ns)). Laboratory values of cholesterol did not change significantly, but liver parameters improved significantly during the follow up: ASAT: IM: 25.0U/l IQR 21.5-41.0; 8M: 22.5U/l IQR 17.0-25.3; p=0.04; ALAT: IM: 34.5U/l IQR 26.8-48.5; 8M: 21.5U/l IQR 11.8-25.8; p=0.04; γGT: IM: 44.0U/l IQR 27.0-76.0; 8M: 25.0U/l IQR 18.3-41.0; p=0.006. Eight subjects took part in the standardized mixed-meal-tolerance tests (500 kcal) and these data were analyzed with focus on GLP-1, Leptin and Ghrelin levels during the evaluation using appropriate ELISA-tests. MMTT took place before implantation, one week later (1W), after 4 months (4M) and after 8 months. Area under the curve analysis was performed and results were compared by repeated measurement ANOVA. Regarding GLP-1 an increase was detectable in the cohort after implantation which was not statistically significant: IM: 293 pmol/l (IQR 170-326), 1W: 319 pmol/l (IQR 286-426), 4M: 285 pmol/l (IQR 134-451), 8M: 328 pmol/l (IQR 290-568). Leptin levels decreased significantly (p<0.05) after 1 week; IM: 1,914 ng/ml (IQR 1,494-6,318), 1W: 1,165 ng/ml (IQR 723-4,558), 4M: 1,323 ng/ml (IQR 1,114-5,377), 8M: 2,137 ng/ml (IQR 1,416-4,228). Ghrelin levels increased non-significantly after implantation: IM: 59,700 pg/ml (IQR 51,350-91,230), 1W: 65,830 pg/ml (IQR 52,230-124,000), 61,160 pg/ml (IQR 56,630-67,150), 8M: 54,700 pg/ml (IQR 33,080-90,750).
Conclusion: The DJBL improves status in obese subjects with diabetes and therefore represents a non-surgical, reversible alternative to bariatric surgery. However, response to the device with regard to weight loss and improvement in diabetes is highly individual. Information from trials including more patients is needed to evaluate the effects in more detail.

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Influence of severe obesity on circulating endothelial progenitor cells levels and effects of bariatric surgery

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Background and aims: Endothelial damage and dysfunction are major mechanisms for cardiovascular (CV) morbidity and mortality risk in morbid obesity. The number of circulating endothelial progenitor cells (EPCs), which play an important role in vascular repair and maintenance of the integrity of the endothelial layer, is reduced in obese subjects. Weight loss is associated with reduced inflammation, improved endothelial function and decreased CV risk. Literature on the effect of weight loss on endothelial function and EPCs, however, is scantier. Therefore, we have determined the number of circulating EPCs in subjects with class II-III obesity undergoing bariatric surgery (BS).
Materials and methods: So far, we have enrolled 62 obese subjects (age 51±13 years, range 23-75; BMI 44.0±7.4 kg/m2; 37% class II and 63% class III obesity; M/F 37/63%; 68% DM+ and 32% DM-). Thirty-one healthy subjects (BMI 24.5±2.5 kg/m2) matched for age and gender were evaluated as controls. All subjects are currently regularly followed up after BS and data at 1 (n=20), 6 (n=17), 12 (n=15), and 18 months (n=10) after BS are reported here. Peripheral blood samples were collected and CD34+/KDR+/CD133+ EPCs measured by fluorescence activated cell sorting (FACS) analysis along with metabolic and inflammatory parameters including plasma glucose, insulin, HbA1c, triglycerides, total cholesterol, HDL-C, LDL-C, hs-CRP, fibrinogen, adiponectin, leptin, PAI-1 and IL-6.
Results: At baseline, EPCs were significantly reduced in obese subjects (250±30 cells/ml, M±SE) as compared to controls (484±56 cells/ml, p<0.0001). In the whole cohort, circulating EPCs levels were inversely related to age (r=-0.334; p=0.011), BMI (r=-302; p=0.022), and HbA1c (r=-0.317; p=0.020). Within the obese group, there was no difference in EPCs between males and females (234±39 vs. 260±41, p=0.682), DM+ and DM- (232±32 vs. 289±83, p=0.372), obesity class II and III (220±54 vs. 253±46, p=0.657). As compared to controls, obese subjects had worse metabolic and inflammatory profile. One month after BS (15 DM+ and 5 DM-) BMI and waist circumference were reduced by -6.0±2.1 kg/m2 (p=0.016) and -7.3±2.7 cm (p=0.054), respectively along with improvement in metabolic and inflammatory parameters. On the contrary, EPCs were reduced, thought not significantly, as compared to baseline (168±35 cells/ml; p=0.092). Such a reduction persisted 6 months after BS (216±40 cells/ml) in spite of further reduction in BMI (-13.5±3.3 kg/m2 from baseline; p<0.001) and waist (-17.2±4.1 cm from baseline; p=0.005). However, after 12 months, EPCs increased (343±86 cells/ml, p=0.067; p=0.024 vs. month 1) and remained steady at 18 months (325±58 cells/ml).
Conclusion: Our data confirm that circulating EPCs levels are reduced in morbidly obese subjects with no differences between class II and class III adiposity, and irrespective of the coexistence of diabetes. Body weight loss following BS causes a dual response with an initial reduction followed by a longer-term increase in the number of circulating EPCs. The mechanisms and, more importantly, the clinical implications of these observations require further analysis.

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Effects of gastric bypass surgery on glucose homeostasis, pancreatic islet and gut morphology, and metabolite profile in a porcine model

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Background and aims: Gastric bypass (GBP) is currently the most common surgical treatment of obesity. Interestingly, the majority of Type 2 diabetes (T2D) patients display remission of the disease after GBP. The underlying mechanisms behind this remission are not known. We used a porcine model to study how hormonal and metabolite profiles are affected by GBP. In addition we studied the impact of GBP on endocrine cell populations in the gut and pancreatic islets.
Materials and methods: Swedish Landrace x Yorkshire x Hampshire (26±2.5 kg) were operated through an upper midline incision. The gastric pouch (12-15 ml volume) was constructed with staplers using 3-4 cm of the upper stomach. The pouch was carefully completely separated from the remaining main stomach not to interfere with neuromuscular bundles. The jejunal end of the Roux limb (alimentary limb) was brought up and anastomosed to the lowest part of the gastric pouch. GBP-pigs were subjected to oral (OGTT) and intravenous (IVGTT) glucose tolerance tests before and after surgery. Sham-operated, pair-fed pigs served as controls.
Results: During IVGTT GBP-pigs displayed lower glucose (basal levels 2-fold lower) and 2-fold higher insulin levels compared to controls. During OGTT, GBP-pigs displayed 1.5-fold higher glucose and a more rapid and 2.5-fold higher insulin response than controls. In line with this, GBP-pigs had 2-fold higher beta cell mass and, as a sign of beta cell neogenesis, 2-fold higher density of extra-islet beta cells. In addition, insulin and glucagon mRNA was 3-fold higher in GBP-pigs. Further, during OGTT GBP-pigs displayed 3-fold higher GIP levels, whereas GLP-1 levels were unchanged. Furthermore, GBP-pigs displayed elevated density of GIP-producing K-cells, but reduced density of GLP-1-producing L-cells in the gut. Metabolomic analyses revealed a difference in the metabolite pattern between the two groups, mainly explained by the fact that GBP provoked 3.5-fold lower levels of free fatty acids (FFA) and 1.5-3-fold higher levels of branch-chain amino acids (BCAA).
Conclusion: GBP in pigs provokes 1) enhanced insulin secretion and increased beta cell mass. 2) enhanced GIP, but not GLP-1 secretion. 3) increased number of K-cells, but reduced number of L-cells . 4) reduced FFA- and elevated BCAA plasma levels. These responses are all concomitant with improved glycemia.
Supported by: ALF, ERC Advanced Grant and Påhlsson´s Foundation

PS 052 Brain metabolism

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Variation in the obesity risk gene FTO determines the postprandial cerebral processing of food stimuli

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Background and aims: Variation in the FTO locus is the strongest known genetic determinant of body weight. FTO is strongly expressed in the CNS. In humans, there is evidence that FTO influences food intake and nutrient-specific food preference. How this gene affects brains' processing of food-related stimuli is unknown. We recently described specific effects of glucose ingestion on the processing of food stimuli. We now investigated how FTO genotype determines these postprandial responses.
Materials and methods: We studied 24 healthy subjects genotyped for FTO SNP rs8050136. Age, gender and BMI were comparable in risk and non-risk-allele carriers (all p≥0.5). Participants were examined at three time points (0, 30, and 120 minutes) after ingestion of 75g glucose solution or water on two days in a randomized order using functional magnetic resonance imaging (fMRI) during visual food picture presentation.
Results: We detected significant differences between FTO genotypes in the prefrontal cortex 30 minutes post-glucose load to food pictures (p=0.0017), while no differences were detected after water ingestion. Non-risk allele carriers responded with a slight increase to glucose intake, while obesity-risk allele carriers showed a significant reduction. Because we previously found this specific area to be insulin responsive, we analyzed possible interactions between FTO genotype and changes in insulin levels after glucose intake. Interestingly, none such interactions were present (ANCOVA p=0.6). Accordingly, prefrontal response differed between FTO genotypes independent of insulin levels (p=0.0003).
Conclusion: Since the prefrontal cortex plays a central role in the inhibitory control of eating, we propose that reduced post-load activity in risk allele carriers contributes to overeating and their risk for obesity. Our findings help to understand how genetic variation influences body weight via the brain.
Supported by: BMBF to DZD e.V.

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Six weeks of hypercaloric high-fat-high-sugar snacking decreases diencephalic serotonin transporters, but not dopamine transporters, in lean men

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Background and aims: Brain dopamine and serotonin systems are affected in obese subjects. We recently found a decrease in serotonin transporter (SERT) immunoreactivity in hypothalamic post-mortem tissue of overweight compared to lean subjects which is in line with a previously described negative correlation between cerebral SERT and BMI. We furthermore found a decrease in striatal dopamine D2/3 receptor availability in morbidly obese women, also in line with a previously described negative correlation between brain dopamine and BMI. Both dopamine and serotonin are involved in food intake regulation, but whether changes in serotonin and dopamine in obesity are related to eating behaviour or absolute body weight is unclear. We hypothesized that brain serotonin and dopamine systems are altered by both macronutrient content and eating pattern during a hypercaloric diet.
Materials and methods: Twenty five lean male subjects (age: 22.1yrs±2.5; BMI: 22.5±1.3 kg/m2) followed a hypercaloric diet with 40% caloric surplus on top of their regular diet for 6 weeks. Subjects were randomised into a high-fat-high-sugar (HFHS) diet or a high-sugar (HS) diet group. Within the HFHS- and the HS-group, subjects consumed the caloric surplus with meals (increase in meal size) or in between meals (increase in meal frequency). We also included a control group (N=5) who did not follow a diet. At baseline and after 6 weeks subjects underwent SPECT imaging using the well-validated radiotracer 123I-FP-CIT to measure SERT and dopamine transporter (DAT)

availability in the diencephalon ((hypo)thalamus) and striatum, respectively. Specific-to-nonspecific binding ratios (SNS-BR) were calculated and used as the outcome measure.
Results: Weight gain was significantly increased to the same degree in all diet groups, yet BMI remained within normal ranges (19-25 kg/m2) in almost all subjects. SERT SNS-BR in the diencephalon decreased significantly only in the HFHS-group with increased meal frequency. Striatal DAT SNS-BR was not altered by any of the hypercaloric diets. None of the measurements were changed in the control group.
Conclusion: The reduction in SERT was only found in the HFHS group with increased meal frequency. A low SERT was previously shown to be associated with obesity. Our finding suggests that specifically snacking high-fat-high-sugar products induces changes in the human brain and may predispose to a disturbed food intake resulting in obesity.
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Increasing HOMA-IR modulates brain responses to meal ingestion in insulin sensitive men: a continuous arterial spin labelling functional magnetic resonance imaging study
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Background and aims: Functional neuroimaging has demonstrated altered brain responses to food and food cues in type 2 diabetes and obesity, which may persist despite weight loss. Dysregulation of the neural networks that control appetite may therefore play an aetiological role in these insulin resistant conditions. We explored the impact of early systemic resistance to insulin on brain responses during meal ingestion.
Materials and methods: Twenty healthy right-handed non-obese men (mean age 33.6±7.7 years, BMI 24.5±1.9 kg/m^2), with no family history of diabetes, with normal glucose tolerance and without formal insulin resistance (HOMA-IR<2, median 0.88, range 0.35-1.81) were studied twice in random order. Regional cerebral blood flow (rCBF), a surrogate marker of brain activation, was measured using continuous arterial spin labelling magnetic resonance imaging (cASL MRI) in a 1.5T MRI scanner, immediately before (-14 to -8 minutes) and after (0 to +6, +8 to +14, and +34 to +40 minutes) consuming 50ml water or 630 kcal mixed meal following an overnight fast. Subjective measures of appetite were collected using 100-point visual analogue scale with concurrent blood sampling before and after each cASL scan, and net incremental area under curves (iAUC) calculated. ANOVA and multiple regression analysis to calculate whole brain statistical parametric maps of rCBF were performed using SPM-8.
Results: The meal significantly reduced hunger (mean net iAUC, water vs meal: +296.7±85.6 vs -843.6±188.6 point•min, p<0.001) and increased fullness scores (-17.6±58.6 vs 711.2±159.0 point•min, p<0.001), with no correlation with HOMA-IR. The change in rCBF after the meal was of significantly greater magnitude than that after water ingestion in orbitofrontal cortex (OFC, Brodmann area [BA] 10), right dorsolateral prefrontal cortex (DLPFC, BA9), thalamus, pre-central gyrus and precuneus (main effect of meal, voxel wise p<0.001 uncorrected). Multiple regression analysis, correcting for age and BMI, showed no correlation between HOMA-IR and any change in rCBF after water ingestion. However, HOMA-IR was positively associated with change in rCBF between +34 to +40 minutes after meal ingestion, when insulin concentration was at its peak (mean insulin, water vs meal: 4.1±0.5 vs 20.8±3.3 mU/L, p<0.01 corrected), in the left primary gustatory cortex (operculum), superior frontal gyrus (BA9) and OFC (BA11), supplementary motor area (BA6) and bilateral middle occipital gyrus (BA19) (voxel wise p<0.005 uncorrected).
Conclusion: In healthy men, meal ingestion elicits significant changes in the activity of brain networks that relay somatosensory and taste signals (thalamus) to regions involved in the representation of the hedonic experience of food (OFC) and inhibitory control of eating (DLPFC). Yet minor changes in systemic insulin resistance, short of any pathology, may also independently influence meal-induced activity in brain regions involved in processing taste perception and interoception (primary gustatory cortex) and hedonic experience of food (OFC) in ways that may promote continued eating after a meal and subsequent weight gain.
Clinical Trial Registration Number: ISRCTN18732138
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The brain's response to food ingestion in insulin sensitive and insulin resistant obesity: a [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) study
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Background and aims: Insulin resistance is associated with obesity. We hypothesised that differences in the brain's response to food ingestion in obesity might be associated with insulin resistance rather than obesity itself. Aim: To investigate the effects of obesity with and without insulin resistance on the brain's response to food ingestion using FDG-PET functional neuroimaging.
Materials and methods: Three groups have been studied: 12 normal weight insulin sensitive people (NWIS, age 32.3±9.3 years, BMI 22.3±1.4kg/m^2, HOMA2IR 0.54±0.16); 9 obese insulin sensitive (ObIS, age 32.7±10.1 years, BMI 33.8±2.6kg/m^2, HOMA2IR 0.56±0.11) and 12 obese insulin resistant (ObIR, age 29.8±11.2 years, BMI 34.3±2.7kg/m^2, HOMA2IR 2.03±0.59). Individuals underwent FDG-PET brain imaging on 2 occasions in random order after an overnight fast: once FED (400kcal meal 15mins prior to FDG injection) and once FASTED (no calorie intake). Brain FDG uptake, a marker of neuronal activation, was compared using Statistical Parametric Mapping. Satiety was assessed using visual analogue scales and a post-scan *ad-libitum* meal.
Results: Across all groups, the FED state was associated with increased fullness scores at 10mins (p<0.001) and reduced food consumption in the post scan *ad-libitum meal* (-180±37kcal, p<0.001) with no significant difference between groups. In NWIS the FED, compared to FASTED, state was associated with increased (cluster-level corrected p<0.05) FDG uptake in the hypothalamus, regions involved in reward (ventral striatum) and interoception (anterior inferior insula) and the cerebellum and decreased FDG uptake in regions involved in inhibitory control (anterior medial frontal cortex, MFC, and dorsolateral frontal cortex, DLFC), interoception, sensory integration and reward (superior insula and lateral orbitofrontal cortex, OFC) and resting state network (precuneus and angular gyrus). There were differences between the three groups in the brain's response to food ingestion (rmANOVA p<0.015, cluster size>50 voxels) in regions involved in reward (globus pallidus, lateral OFC), interoception (anterior inferior insula/frontal operculum), inhibitory control (anterior MFC, posterior DLFC), and resting state network (precuneus, angular gyrus). Both obese groups, compared to NWIS, showed attenuated deactivation in response to food ingestion in inhibitory control regions (anterior MFC and posterior DLFC) (p<0.005, cluster size>50 voxels). ObIR, compared to NWIS, also showed increased activation in the insula and attenuated deactivation in lateral OFC and resting state network that was not seen in ObIS. ObIR showed increased activation in globus pallidus and insula compared to ObIS.
Conclusion: Obesity is associated with attenuated responses to food ingestion in regions involved inhibitory control. However, only insulin resistant obesity is associated with differences in the brain's response to food ingestion in regions involved in interoception and reward. Insulin resistance-associated differences in appetite control may contribute to the obesity of the metabolic syndrome.
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Early Alzheimer-type dementia and type 2 diabetes: analysis using Voxel-based specific regional analysis system for Alzheimer's disease (VSRAD)
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Background and aims: Diabetes is known to be a risk factor for Alzheimer's disease (AD).Hyperglycemia itself and insulin resistance are suggested to be the underlying mechanisms. Voxel-based specific regional analysis system for Alzheimer's disease (VSRAD) is the software of automatic image processing which enables statistical analysis of the degree of parahippocampal cortex atrophy and is widely used for early diagnosis of AD in Japan. The purpose of this study is to assess the factor related with the development of early AD in patients with diabetes using VSRAD.

Materials and methods: We studied 121 out-patients with type 2 diabetes over 60 years old (Age 71.1±8.8 years, male 58/female 63, HbA1c 6.9±1.0 %) before and after 5 years of ordinal diabetic treatment. The patients were divided into four groups according to the degree of atrophy with VSRAD, 0-1: little, 1-2: moderate, 2-3: considerable, 3-: marked atrophy. BMI, insulin resistance, glucose/lipid metabolism, and diabetic complications were also assessed in the patients.
Results: The degree of atrophy advanced from 1.59±1.22 to 1.79±1.29 during a 5-year study period. Compared with patients with little atrophy, 32 patients with more than 0.2 advanced degree showed significantly older age (70.8±8.8 vs 70.0±8.8 years old, p=0.012), higher HbA1c (7.0±0.9 vs 6.6±0.7%, p=0.038), and higher 2-hour-postprandial plasma glucose (224±71 vs 190±53 mg/dl, p=0.018). No significant difference was seen between the two groups in the duration of diabetes, fasting plasma glucose, blood pressure, BMI, LDL, HDL, triglyceride, creatinine, or uric acid. Patients in the groups of more severe atrophy had significantly higher incidence of hypoglycemia (more than three times / month, p=0.033) and higher prevalence of visceral obesity (p=0.013). No significant relation was seen between the atrophy and the presence of diabetic retinopathy / nephropathy / neuropathy, cerebrovascular disease, coronary artery disease, arteriosclerosis obliterans, hypertension, dyslipidemia, or the smoking.
Conclusion: Hypoglycemia and postprandial plasma glucose, and HbA1c were suggested to be related with the development of early AD in patients with type 2 diabetes.

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Does sleep loss induce diabetes?
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Background and aims: Sleep/wake regulatory system and energy metabolism are intertwined each other. Strong associations of diabetes with sleep impairment have been reported. For example, it has been known that an attenuation of slow-wave activity (SWA, power density of the EEG delta band between 0.5 and 2.0 Hz in human), a parameter of sleep pressure, during non-rapid eye movement (NREM) sleep can decrease insulin sensitivity and glucose tolerance in humans. However, the mechanisms for the interaction of sleep loss with diabetes are still unknown.
Materials and methods: In the present study, we investigated whether sleep loss by rearing on different environment would induce diabetes in mice. Male ICR mice (8-week-old) were randomly assigned to two groups to be reared on either normal sawdust (control) or wire net. After 2 weeks of rearing in a different environment, sleep recording, glucose tolerance test (GTT, 2 g/kg) and insulin tolerance test (ITT, 1 U/kg) were performed.
Results: Mice reared on wire net showed a decreased amount of NREM sleep in the dark period and an attenuated SWA (0.5 - 4.0 Hz) during NREM sleep in the light period compared with that of control mice. These sleep-disrupted mice showed an impaired glucose tolerance measured by GTT, although the response to ITT was similar to that of control mice. Plasma insulin response to glucose (2 g/kg) was also disrupted in mice reared on wire net. There was no significant difference in plasma concentration of corticosterone between the two groups. Interestingly, rearing on wire net for 1 week did not induce an impaired glucose tolerance, while it already attenuated SWA during NREM sleep.
Conclusion: These results suggest that sleep loss may induce diabetes via abnormality of insulin secretion in mice. Our data indicate that sleep quality is important for energy metabolism.

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Effect of feeding rhythm on sleep/wake regulation
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Background and aims: Disturbance of feeding rhythm has been understood as a risk factor for development of insulin resistance, however, it is not elucidated the detailed mechanism for the effect on sleep/wake regulation. We thus investigated whether different feeding rhythm only during dark period affects sleep/wake regulation without a significant change in amount of food intake.
Materials and methods: Three groups of C57BL/6J mice were given lab chows freely during dark period (ZT12-24, Control group), first half of dark period (ZT12-18;Morning group), or last half of dark period (ZT18-24, Evening group) for 2 weeks respectively. A telemetric device for recordings of body temperature and locomotor activity was implanted in the peritoneal cavity of mice. Two stainless steel miniature screw electrodes were implanted in the skull to record the electroencephalogram (EEG). Teflon-coated stainless steel wires were implanted in the neck muscles on both sides to record the electromyogram (EMG). Flexible cables connected the mice to a polygraph and computer-assisted data acquisition system. Off-line sleep scoring was done on the computer screen by visual assessment of the EEG and EMG activities, thereby distinguishing phases of wake, rapid eye movement (REM) and non-REM (NREM) sleep. The EEG delta and theta frequency band was set at 0.5-4.0Hz and 4.0-7.8Hz, respectively. In this study, power density of the EEG delta (ratio of delta to theta) during NREM sleep was used as a parameter of sleep pressure (slow-wave activity, SWA).
Results: Mice in Evening group showed lower SWA than that of other 2 groups. On the other hand, an amount of NREM, REM and wake in 3 groups did not change. We observed higher monoamine concentrations (serotonin, dopamine and its metabolites), which activates wake system, in cerebral cortex in Evening group. We also found increased mRNA expression of orexin in hypothalamus in Evening group. Body weight in Evening group increased similar with Control group whereas body weight in Morning group did not change during 2 weeks. There were no differences in food intake, locomotor activity and body temperature during 24 hours among 3 groups.
Conclusion: These results indicate that feeding only in the last half of dark period alters sleep homeostasis. This effect may be partly involved in increase of orexin expression in hypothalamus and elevated monoamine concentration in cerebral cortex.

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The effects of brain insulin action on hepatic glucose metabolism, glucagon secretion and lipolysis in the absence of somatostatin infusion
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Background and aims: Previous studies suggest that insulin action in the brain of rodents can acutely inhibit hepatic glucose production, glucagon secretion, and lipolysis. In contrast, increasing brain insulin action in dogs did not inhibit glucose production. In those studies, we used the pancreatic clamp technique, which employs somatostatin infusion, and portal vein hormone replacement to control hormones. This raises the possibility that somatostatin may have impaired the impact of brain insulin action on the liver. In this study we used the dog to examine brain insulin effects in the absence of somatostatin.
Materials and methods: Each animal underwent liver catheterization (femoral artery; portal and hepatic veins) and cannulation of the 3rd ventricle (ICV) two weeks prior to study. After a basal sampling period (-30 to 0 min), artificial cerebrospinal fluid (aCSF; n=5) or the PI3K inhibitor LY294004 (to block brain insulin action; LY; n=6) was infused ICV (0-300 min) in fasted conscious dogs. After 60 min, insulin was infused into the hepatic portal vein (1.8 mU/kg/min; ~6-fold basal), while euglycemia was maintained using glucose infusion.
Results: Intraportal insulin infusion elevated hepatic and non-hepatic (including the brain) insulin levels 6-fold in both groups. The decreases in hepatic sinusoidal plasma glucagon levels did not differ in the presence or absence of increased brain insulin signaling (Δ 79±18 vs 58±11%, p=0.32, aCSF vs LY, respectively). The equal suppression in blood glycerol (Δ 50±9 vs 52±6%; p=0.90) and plasma NEFA (Δ 86±4 vs 88±3%; p=0.64) indicate that brain insulin action did not affect lipolysis or fatty acid reesterification. Likewise, insulin in the brain did not affect the time course or magnitude of insulin's effects on glucose turnover or GIR. The suppression of endogenous glucose Ra (Δ 65±9 vs 57±4%; p=0.50), the increase in whole body Rd (Δ 3.8±0.7 vs 4.7±0.5 fold; p=0.33), and the rate of net hepatic glucose uptake (0.36±0.23 vs 0.49±0.11 mg/kg/min; p=0.60) did not differ whether or not brain-liver insulin action was blocked. At the end of the study there was no difference in insulin signaling at the liver in the two groups (hepatic pAkt / total Akt ratios increased equally; p=0.41). On the other hand, brain-liver insulin signaling was blocked by inhibition of PI3K in the brain as indicated by abolition of the insulin induced increase in the ratios of hypothalamic pAkt

/ total Akt (1.7±0.1 vs 1.0±0.1 fold relative to a basal insulin control group; p<0.01) and hepatic pSTAT3 / total STAT3 (2.0±0.5 vs 0.7±0.5 fold; p<0.01) in the LY group.
Conclusion: A 6-fold rise in brain insulin action had no impact on insulin's ability to inhibit hepatic glucose metabolism, glucagon secretion, or lipolysis. Therefore, the presence of somatostatin does not explain the lack of brain insulin effect on hepatic glucose production seen in our earlier studies. The inability of brain insulin action to acutely modify glucose production in the dog is in contrast to its ability to do so in the rodent. This suggests that neural control of the liver is enhanced in rodents or it may reflect the constraints put on experimental design in the rodent because of its size.
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Alx3 deficient mice exhibit hypothalamic dysfunction and altered energy expenditure
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Background and aims: Although glucose-stimulated insulin release from pancreatic islets as well as insulin-induced glucose clearance by the liver, muscles and adipose tissue play a central role in the control of glucose homeostasis, it has long been recognized that glucose- and hormone-sensitive neurons located within hypothalamic nuclei also participate in this process operating as a metabolic hub to coordinate the regulation of glucose homeostasis, feeding and energy expenditure. Despite the functional importance of these neurons, the molecular mechanisms by which they control glucose and energy homeostasis are poorly understood. Previous work in our laboratory demonstrated that the aristaless-type homeodomain transcription factor Alx3 is important for the systemic control of glucose homeostasis. Although many phenotypic features of Alx3 deficient mice can be largely correlated with its absence from pancreatic islets, where it is normally expressed, alterations due to defects in extrapancreatic Alx3-expressing cells cannot be ruled out. In the present study, we investigated whether Alx3-deficient mice show phenotypic features that can be correlated with altered hypothalamic function.
Materials and methods: Alx3 knock out and control mice of similar age and body weight were used. Indirect calorimetry was determined using calorimetric cages (Phenomaster, TSE Systems GmbH, Bad Homburg, Germany). Body mass composition was analyzed by MRI. PET was used to monitor the uptake of 18F-Fludeoxyglucose (~300 μCi/mouse i.p.) in the brain. Hypothalamic activity following fasting or feeding periods was evaluated through changes in the ADC (Apparent Diffusion Coefficient) values by functional diffusion weighted magnetic resonance imaging. Expression of Alx3 in the central nervous system was examined by RT-PCR, western blot and immunohistochemistry. Statistical significance was considered for p<0.05.
Results: We confirmed that fasting glucose levels were significantly higher in mutant than in control mice. Alx3-deficient mice exhibited reduced whole energy expenditure and oxygen consumption, and their respiratory exchange rate was also lower than that of control mice. In addition, food and water intake was reduced in Alx3-null animals. No differences were found in locomotor activity. No differences in the uptake of 18F-Fludeoxyglucose were found in any of the brain regions tested, except in the hypothalamus, where tracer uptake was significantly reduced. ADC values in Alx3-deficient mice were significantly lower that those found in control animals both in the fasting and the feeding state. Fasting (16 h) induced significant ADC changes in the arcuate (ARC) and dorsomedial (DMN) hypothalamic nuclei in wild type animals consistent with neuronal activation. No effects were detected in the ventromedial nucleus (VMN). In contrast, in Alx3-deficient mice, no differences were found in ARC whereas similar changes were observed in DMN.
Conclusion: Our data support the notion that Alx3 expressed in the hypothalamus contributes to the regulation of glucose homeostasis and to the control of energy expenditure.
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Insulin action in the brain depends on whole-body insulin sensitivity
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Background and aims: Insulin action in the brain determines food intake, physical activity and finally glucose homeostasis. In particular, insulin improved brain activity and promoted locomotion in lean humans and mice while obese individuals are characterized by impaired insulin action in the brain and physical inactivity. As insulin enters the brain in a receptor-mediated fashion, we hypothesized that whole body insulin resistance might affect the transport of insulin into the brain with impacts on brain activity and locomotion.
Materials and methods: In mice, insulin was applied for four consecutive days by subcutaneous injection (1 unit/kg body weight), and assessment of cortical activity and locomotion in whole-body insulin-sensitive and -resistant C57BL/6 mice were performed by radiotelemetry recordings. Moreover, twenty-two healthy human subjects participated in an oral glucose tolerance testing (oGTT) to determine whole body insulin sensitivity and underwent lumbar puncture to measure insulin and glucose concentrations in the cerebrospinal fluid (CSF).
Results: Subcutaneous insulin injection on four consecutive days did not affect blood glucose or body weight. In insulin-sensitive mice, brain activity was significantly increased in the theta, alpha and beta frequency bands (theta: by 10.0±2.8%; alpha: by 12.3±2.0%; beta: by 11.7±1.4%; P<0.05; n=10/group) while this was not present in insulin-resistant animals. In addition, locomotor activity was significantly increased by 32.4±3.7% (P<0.001) during the insulin treatment period in insulin-sensitive mice but not in those with insulin resistance. Interestingly, when insulin was intracerebroventricularly applied into insulin-resistant animals it increased brain activity in abovementioned frequency bands (alpha: by 18.9±3.5%; beta: by 11.1±2.0%; P<0.005; n=8/group). In humans, blood and CSF concentrations of glucose and insulin were significantly correlated (glucose: P=0.008; insulin: P=0.021). The CSF/serum ratio for insulin was significantly associated with whole body insulin sensitivity (P=0.01, r2=0.4) with reduced insulin transported into the CSF in insulin-resistant subjects, while this was not true for glucose (P=0.3).
Conclusion: Physiological doses of insulin improve brain activity and locomotion in insulin-sensitive mice. However, impaired transport of insulin across the blood-brain-barrier (BBB) contributes to the absent response in insulin-resistant animals which can be in part overcome by an intracerebroventricular injection. This is well in line with our human data where insulin transport into the CSF was impaired in whole-body insulin-resistant subjects. Together, our data point toward a major contribution of insulin resistance at the BBB in the pathophysiology of brain insulin resistance. This underlines the need for sensitizing measures accompanying insulin therapy in insulin resistant patients.
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PS 053 Inflammation in obesity and type 2 diabetes

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The interleukin-1 receptor antagonist anakinra improves beta cell function in subjects with impaired glucose tolerance

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Background and aims: A defect in insulin secretion underlies type 2 diabetes, and is progressive over the course of the disease. The underlying mechanisms are not completely elucidated, but inflammation at the level of the β-cell seems to be involved. The inflammatory response may be fuelled by lipotoxicity or hyperglycaemia and strong evidence has emerged for the involvement of interleukin (IL)-1 β in this process. Blocking the effects of IL-1 in patients with type 2 diabetes mellitus by anakinra, a recombinant human IL-1 receptor antagonist, has been shown to improve glycaemic control by enhanced β-cell function. However, subsequent studies have not confirmed a beneficial effect of other interventions aiming at blocking IL-1 β. We hypothesised that IL-receptor blockade by anakinra would be capable to improve β-cell function in subjects with impaired glucose tolerance.
Materials and methods: The study population consisted of 16 first-degree relatives of patients with type 2 diabetes mellitus who had abnormal glucose tolerance according to ADA criteria including impaired fasting glucose (IFG), impaired glucose tolerance (IGT) assessed by 75 g oral glucose tolerance test, or HbA1c levels of 5.7-6.4%. In a randomised, double-blind, placebo-controlled crossover study subjects were treated with anakinra 150 mg subcutaneously once daily for 4 consecutive weeks or placebo sc once daily for 4 consecutive weeks. After a wash-out period of 4 weeks subjects crossed over to the other treatment arm. At the end of each treatment period β-cell function was assessed by hyperglycaemic clamp and an oral glucose tolerance test was performed.
Results: Eighteen of the 37 initially screened subjects underwent randomization. Of these 18 one subject withdrew before start of the study medication. One participant withdrew in the first treatment period due to discomfort of injection site reaction during the use of anakinra. A total of 16 participants, 6 females and 10 males, completed the trial. Anakinra treatment siginificantly reduced CRP levels, leukocyte and neutrophil counts. Fasting fasting glucose levels (anakinra 5.48±0.17 vs placebo 5.48±0.25 mmol/l) and HbA1c levels (anakinra 5.60±0.11 % vs placebo 5.74±0.11%, P = 0.068) were not significantly different during anakinra. During the hyperglycaemic clamp the glucose levels were nearly identical in both treatment periods (anakinra 9.98± 0.04 mmol/l vs placebo 10.02±0.04 mmol/l respectively, P = 0.43) and mean CV of the hyperglycaemic clamp was below 4 % in both treatment periods. First phase insulin secretion improved after anakinra treatment compared to placebo, 148±20 vs 123±14 mU/l respectively (P = 0.03). In line with these results, the insulin disposition index derived from the oral glucose tolerance tests was lower during anakinra. Second phase insulin secretion, insulin response after arginine stimulus and maximal insulin secretion, did not differ between the two treatment arms. Anakinra had no effect on insulin sensitivity index.
Conclusion: Four weeks treatment with anakinra improves β-cell function in first degree relatives of patients with type 2 diabetes who have IFG and/or IGT supporting the concept of involvement of IL-1β in the (progressive) decrease of insulin secretion capacity associated with type 2 diabetes.
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PS 053 Inflammation in obesity and type 2 diabetes

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Activation of the inflammasome in glomerular cells aggravates experimental diabetic nephropathy

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Background and aims: Apoptosis and inflammation are both associated with the progression of diabetic nephropathy (dNP) and both can be triggered by mitochondrial dysfunction or ROS-generation, key culprits diabetic complications. NLRP3-inflammasome-triggered inflammation leads to pyroptosis which can be technically difficult to distinguish from apoptosis, leaving the question open, whether apoptosis or pyroptosis has a prevailing role during dNP. To evaluate this question we inhibited apoptosis or inflammation/pyroptosis and directly compared the functional consequences in vivo. .
Materials and methods: The role of apoptosis and the inflammasome in dNP was analyzed in murine models of type 2 (db/db) and type 1 (streptozotocin [STZ] with or without uninephrectomy) diabetes. Diabetic nephropathy was quantified based on albuminuria, histological changes (fractional mesangial area, FMA), and biochemical markers of dNP. Expression levels and proteolytical processing of caspase-1, IL-18 and IL1b was determined by immunoblotting. A subset of mice was injected with anakinra, an IL-1 receptor antagonist, a caspase inhibitor (Casp-3,6,7,8,9), or minocycline, a tetracycline derivate known to stabilize mitochondria. To determine the role of the inflammasome in bone-marrow derived versus tissue resident cells we transplanted bone marrow from Nlrp3-/- mice into db/db or STZ DM. In addition, diabetic mice deficient for the mitochondrial RedOx-enzyme p66shc were analyzed. In vitro NLRP3-WT and mutated NLRP3 (Q705K), a constitutive active NLRP3 mutant, were retrovirally transduced into human endothelial cells or podocytes, followed by the assessment of IL-1b and IL-18 levels by immunoblotting.
Results: In renal cortex extracts of diabetic mice, but not of control mice, an increase of Nlrp3 expression, caspase-1 activation, and maturation of IL-1b and IL-18 was observed. NLRP3-/- or caspase1-/- mice as well as anakinra and minocycline (but not caspase inhibitor) treated mice were protected against dNP. Double/triple confocal immunofluorescence for podocyte/endothelial markers revealed that inflammasome activation (Nlrp3 expression, caspase-1 / IL-1b maturation) is enhanced in glomerular cells (podocytes and endothelial cells) of diabetic mice. The severity of dNP was unchanged in bone marrow chimeras (Nlrp3-/- à db/db or wt à db/db), demonstrating that inflammasome activation in tissue resident cells is sufficient to promote dNP. In diabetic p66Shc-/- mice ROS-makers and inflammasome activation were reduced and these mice were protected against diabetic nephropathy. In vitro studies demonstrated activation of the Nlrp3 /caspase-1/ pro-IL-1b inflammasome pathway upon glucose stimulation in glomerular cells, which was suppressed by minocycline or a caspase-1 inhibitor. This suppressive effect was abolished in cells transduced with the constitutive active NLRP3 mutant (Q705K).
Conclusion: Taken together, these results strongly support that activation of the NLRP3-inflammasome in residential glomerular cells contributes to inflammation/pyroptosis and that this is sufficient to promote diabetic nephropathy. Therefore, the modulation of the inflammasome is a potential therapeutic target in diabetic nephropathy.

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Protection of beta cell function by therapy with interleukin-1beta antibody in the Cohen diabetes-sensitive rat

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Background and aims: Cytokines are mediators of β-cell dysfunction in diabetes. Cohen-diabetes-sensitive-rats (CDs) fed a diabetogenic diet develop blunted glucose-stimulated-insulin-secretion (GSIS) and hyperglycemia associated with peri-islet-infiltration of fat and macrophages expressing IL1 β.

We examined, in vivo, the role of IL-1β in the β-cell dysfunction induced by the diabetogenic-diet, by blockading the IL1-β receptor with Anakinra, or by neutralizing IL1-β using an anti-IL-1-β antibody (IL1-β-Ab) in parallel with the diabetogenic-diet feeding.
Materials and methods: CDs rats fed 15 days a diabetogenic-diet were in parallel injected subcutaneously with Anakinra (50 mg twice daily), rat specific IL1-β Ab (0.5 mg/kg) or vehicle. On day 16, rats were weighed, blood glucose and insulin concentrations were assessed before and during the 120 min of an oral glucose tolerance tests (OGTT). Thereafter, rats were killed and pancreases were dissected, weighed and fixed. Pancreatic morphology, macrophage-infiltration (ED1) and IL1-β expression were evaluated in pancreatic sections using electron-microscopy and immunohistochemistry.
Results: 15-days Anakinra-treated CDs rat fed a diabetogenic-diet exhibited a high glucose area under the curve (AUC) that was comparable to the vehicle-treated CDs-rats (1325±43 vs 1351±53 mmol/l/120 min, respectively). Pancreatic fat, macrophage infiltration and IL1-β expression also remained high in these rats. Thus, Anakinra did not protect the CDs-rats from the deleterious effect of the diabetogenic-diet**.** In contrast, a 15-days treatment with IL1 β-Ab protected the CDs-rats from the harmful outcome of the diabetogenic-diet. IL1-β-Ab treated-CDs-rats exhibited a significant reduction in glucose-AUC compared to saline-treated rats (999±88 vs 1351±53 mmol/l/120min, p<0.01 respectively). Accordingly, peak insulin secretion 30 min after glucose administration increased in IL1 β-Ab-treated CDs-rats compared to controls (361±26 vs 270±22pmol/l p<0.05, respectively) and the value of AUC-insulinogenic-index was significantly higher (12±2 IL1-β-Ab vs 6.5±1 ηmol/l/120min p<0.05, respectively) indicating increased insulin secretory capacity of IL1-β-Ab treated CDs-rats. The pancreatic sections of CDs rats treated with IL-1β Ab demonstrated a 2/3 reduction in the number of infiltrating macrophages (6.6±1.3 for IL1-β-Ab vs 19.5±5.1 for saline per mm^2 pancreas, p<0.05) and the IL-1β expression was markedly reduced in the remaining infiltrating macrophages (3.5±0.9 for IL1-β-Ab vs 16.2±3.7 for saline per mm^2 pancreas, p<0.01). In parallel the adipose tissue in the pancreatic tissue was 50 % reduced. All animal groups had a similar body weight (223±15 g for Anakinra vs 230±8 g for IL1-β-Ab vs 240±7 g saline).
Conclusion: Our study provide proof-of-principle evidence that blocking of the IL-1 β action by a neutralizing anti-IL-1 β Ab could improve β-cell function and glucose tolerance and confirms the major role for IL-1β in the development of diabetes in our rat model.
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Pivotal role of TNFalpha in mediating ET-1 vasocostrictive effects in small arteries of obese patients
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Background and aims: Obesity is characterized by vascular low-grade inflammation which reduces NO availability, with an augmented endogenous endothelin (ET)-1-mediated vasoconstriction (VC) and a blunted tonic nitric oxide (NO)-mediated relaxation (VD). We evaluated how TNFα localized in the vascular wall and in perivascular adipocytes (PVA) contributes to the VC induced by endogenous ET-1 and whether this action is indirectly mediated by an effect on tonic NO release in small resistance arteries from obese patients (Ob) and controls (Ctrl).
Materials and methods: Sample of visceral fat were obtained in 14 Ob and 14 Ctrl, matched for metabolic profile and blood pressure values, undergoing a surgical laparoscopic procedure. Small arteries were investigated on a pressurized micromyograph. Endogenous ET-1 effect was assessed in pre-contracted arteries by vascular response to ET_A receptor blockade BQ-123 (1μM). TNFα and NO responses were tested at baseline and after anti-TNFα antibody Infliximab (IFX, 100 μM), and L-NAME (100 μM), respectively. Results are given as maximal response reached at steady state (30' infusion). Gene and protein expression of TNFα, ET-1, ET_A and ET_B receptors were determined by RT-PCR (gene/reference) and IHC (AU) on arterial wall and in PVA.
Results: Ob showed higher TNFα plasma levels (15.3±4.2 *vs* 8.1±2.5 pg/ml, $p<0.05$). In Ctrl, L-NAME-induced VC (15.5±0.6%) was not affected by IFX (15.1±0.4%). In contrast, Ob showed a blunted L-NAME-induced VC (6.0±0.7; $p<0.01$ vs Ctrl), which was potentiated ($p<0.01$) by IFX (12.5±0.8%). Ob were also characterized by a significantly higher TNFα expression, either at the level of arterial wall (24.9±19.6 *vs* 2.8±2.5 AU, $p<0.001$) or in PVA (2.9±1.8 *vs* 1.2±0.7, $p<0.005$); arterial TNFα receptor 1 was also more represented in Ob than in Ctrl (18.4±8.4 *vs* 5.7±3.4, $p<0.001$). In Ob, the VD to BQ-123 (47.0±1.5%) was attenuated ($p<0.01$ by IFX (29.1±2.4%) but not affected by L-NAME (43,3±0.6%). However, when co-infused with IFX, L-NAME further reduced the VD to BQ-123 (19.4±3.0%; $p<0.01$ vs BQ-123+IFX). In Ctrl, VD to BQ-123 was blunted (26.3±1.3%; $p<0.01$ vs Ob) and not affected by IFX (24.1±0.6%). L-NAME significantly reduced the VD to BQ-123 (12.3±1.1%), independently of IFX co-infusion (12.1±0.9%). These results were paralleled by a higher arterial expression of ET-1 (45.8±10.3 *vs* 24.3±15.0, $p<0.001$), ET_A (69.4±6.0 *vs* 9.6±2.8, $p<0.001$) and ET_B receptors (49.5±27.0 *vs* 9.2±2.4, $p<0.001$) in Ob respect to Ctrl. Looking for the intracellular signaling potentially mediating these responses, both p38 and JNK pathways were involved, with IFX significantly reducing JNK phosphorylation in both groups, and p38 phosphorylation only in Ob.
Conclusion: Resistance small arteries of obese patients show an enhanced ET_A-mediated VC and a blunted NO-mediated VD. An excess of vascular and perivascular TNFα, coupled with an increased expression of ET-1 and ET_A receptor in the vasculature of these patients, contribute to the enhanced ET-1-mediated contracting tone by a mechanism which involves an impairment of tonic NO release.

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Plasma levels of N^{ε}-(Carboxymethyl)lysine are inversely associated with BMI and inflammation, and explain a part of the obesity-related increases in inflammation
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Background and aims: Dysregulated production of adipokines is a characteristic of obesity and plays an important role in the development of insulin resistance and type 2 diabetes. We have previously shown that levels of the advanced lipoxidation endproduct, N^{ε}-(Carboxymethyl)lysine(CML) in plasma were decreased in obese subjects due to trapping of CML in adipose tissue, where it may contribute to the dysregulation of inflammatory cytokines. In this study, we investigate the relation between BMI and inflammation, and the role of plasma CML in this association.
Materials and methods: We studied 36 obese and 36 control subjects (case-control study), and 513 participants from a large cohort study (Cohort study of Diabetes and Atherosclerosis Maastricht (CODAM study)) with a wide range of BMI, in whom CML plasma levels and levels of circulating inflammatory markers CRP, SAA, sICAM-1, sVCAM-1, and IL-6 were measured. These markers were compiled into a total inflammation score by averaging the respective z-scores (standard deviations of difference from the population mean). Associations between BMI, inflammation and plasma CML levels were analysed with linear regression models, with adjustments for age, sex, smoking status and other metabolic risk factors. To analyse the role of CML plasma levels in the association between BMI and inflammation, additional adjustment for CML plasma levels was performed.
Results: After adjustment for age, sex, smoking and other risk factors, increasing levels of BMI (per 10 kg/m^2) were associated with higher levels of inflammation score [regression coefficient (β)=0.34SD (95% CI: 0.20; 0.48)] in the case-control study, and [β=0.35SD (95% CI: 0.21; 0.50)] in the cohort study. In addition, BMI is associated with lower plasma levels of CML: [β per 10kg/m^2 increase in BMI=-0.31μmol/L (95%CI: -0.44; -0.17) in the case-control study, and [β=-0.27μmol/L (95% CI: -0.38; -0.16)] in the cohort study. Plasma levels of CML were inversely associated with the inflammation score in both the case-control [β=-0.50SD (95%CI: -0.74; -0.26)] and the cohort [β=-0.21SD (95% CI: -0.32; -0.10)] studies. In addition, further adjustment for CML attenuated the associations between BMI and the inflammation score from [β=0.34SD (95% CI: 0.20; 0.48)] to [β=0.25SD (95% CI: 0.09; 0.41)], in the case-control study, and from [β=0.35SD (95% CI: 0.21; 0.50)] to [β=0.30SD (95% CI: 0.15; 0.44)] in the cohort study, thus explaining a significant portion of the association between BMI and inflammation (26 and 14%, respectively).
Conclusion: Obesity is characterized by higher levels of inflammatory markers but by lower levels of CML in plasma, the latter significantly explaining a portion of the positive association between obesity and inflammation. This attenuation suggests that, in the context of obesity, lower levels of CML in plasma, most likely as a result of CML trapping in the adipose tissue, influence the expression of pro-inflammatory cytokines.

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Increased leukotriene production is associated with metabolic syndrome in obese subjects

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Background and aims: Obesity is a major risk factor for insulin resistance and type-2 diabetes. A wealth of evidence indicates that these metabolic comorbidities are associated with presence of low-grade chronic inflammation. In addition to adipokines, adipose tissue inflammation may also be driven by activation of classical proinflammatory pathways such as arachidonate lipoxygenases, leading to the formation of leukotrienes. Indeed, results from animal's studies are in favor of a role of the leukotriene (LT) pathway in obesity induced-insulin resistance. However, there is a paucity of data regarding this association in human obesity. Therefore, the aim of this study was to investigate whether LT production was associated with insulin resistance and with metabolic syndrome parameters in a cohort of obese subjects.
Materials and methods: Subjects admitted for assessment of obesity (BMI≥30 kg/m2) were included and classified according to the presence or not of a metabolic syndrome. Exclusion criteria were treatment with anti-inflammatory medications, asthma, known diabetes mellitus and hs-CRP above 10 mg/l. Laboratory procedures included FPG, HbA1c, Total cholesterol, triglycerides (TG) high and low-density lipoprotein, Hs-CRP, evaluation of insulinresistance by HOMA-IR. Urinary leukotriens (U-LTE4) was measured using enzyme immune assay kit. Correlations between the urinary-LTE4 and clinical /biological parameters parameters were established by Spearman correlation. A multiple stepwise linear regression was performed to evaluate the impact of the components of the metabolic syndrome on U-LTE4. $p<0.05$ was considered significant.
Results: 46 subjects (70% females) were included, mean age 44 years (16-80) and mean BMI 36,8kg/m2 (30-51). Sixteen subjects fulfilled at least 3 of the criteria for metabolic syndrome. There was a positive association of U-LTE4 with metabolic syndrome in a multivariable analysis adjusted for age and gender (β-coefficient 0.192; P=0.023). To assess what part within the metabolic syndrome U-LTE4 correlated with, a stratified comparison was performed for each factor Whereas the levels of U-LTE4 did not differ in groups with and without hypertension, and not between high and low HDL and TG, U-LTE4 was significantly higher in subjects with a high waist to hip ratio and in subjects with high fasting plasma glucose. An age- and gender-adjusted multivariate analysis including the waist to hip ratio, BMI, LDL level, HDL and TG level, blood pressure, HOMA-IR, and Hb1Ac revealed that the waist to hip ratio remained the only parameter significantly correlated with U-LTE4 (P=0.015).
Conclusion: Taken together, our results confirm the previously established notion of chronic low grade inflammation in obesity and further described a novel inflammatory pathway, i.e. the LT pathway that could be involved during obesity-induced insulin resistance. Since this is an observational study, no definite conclusion of causality can be drawn from the present results. One possible mechanism is that increased LT production from visceral fat may be involved in the development of insulin resistance. This notion has received support from animal models. Further studies are needed to investigate the precise mechanisms involved in LT-induced metabolic effects in human disease.
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Dipeptidyl peptidase 4 (DPP4) induces inflammation and proliferation of human smooth muscle cells by activation of the ERK and NF-kB signalling pathway

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Background and aims: DPP4 is a ubiquitously expressed cell-surface protease and a novel adipokine, as shown by us. Due to DPP4-mediated degradation of the incretin hormone glucagon-like peptide (GLP)-1, inhibition of DPP4 is now widely used as a therapeutic approach for type 2 diabetes mellitus treatment. In addition, DPP4 inhibitors display beneficial cardiovascular effects that are not only GLP-1 mediated. However, nothing is known about direct cellular effects of soluble DPP4, the circulating form of this enzyme. The aim of this study was to investigate the effects of DPP4 on primary human smooth muscle cells (hSMC) and to identify responsible signaling pathways.
Materials and methods: Primary human vascular smooth muscle cells (VSMC) from three distinct donors were exposed to DPP4 for 6 and 24h, and examined for the activation of different signaling pathways by Western blot analysis. To investigate the expression of different cytokines VSMC were treated with DPP4 for 24h and mRNA were measured by real-time PCR. Effects on proliferation and secretion were detected by ELISA. The specific DPP4 Inhibitor K579 was used in a concentration of 100nM and was added concomitant with DPP4.
Results: Using supraphysiological and physiological concentrations of DPP4 (2-500ng/ml), we could observe a concentration-dependent activation of ERK1/2 (3-fold) after 6h, which remained stable for up to 24h. Additionally, DPP4 treatment induced a 1.5-fold phosphorylation of the NF-κB subunit p65. The increased ERK1/2 phosphorylation as well as the NF-κB phosphorylation could be totally abrogated by the specific DPP4 inhibitor. In accordance with DPP4-induced stress and inflammatory signaling, DPP4 also stimulates hSMC proliferation, which could be completely inhibited by the specific ERK1/2-Inhibitor PD98059 as well as by the DPP4 inhibitor. Downstream of ERK and NF-κB, DPP4 induces iNOS in a concentration-dependent manner. Furthermore we could observe an increased expression and secretion of proinflammatory cytokines like IL-6, IL-8 and MCP-1 (2.5-, 2.4- and 1.5-fold, respectively) by the DPP4 treatment. Both, expression and secretion of these cytokines could be completely blocked by the DPP4 inhibitor.
Conclusion: In conclusion, we show for the first time that soluble DPP4 activates the MAPK and NF-κB signaling cascade resulting in the induction of inflammation and proliferation of hSMC. Our data might therefore partially explain GLP-1 independent cardiovascular effects of DPP4 inhibitors. Due to increased circulating levels of DPP4 in obesity, DPP4 may play a role in linking obesity to cardiovascular disease.

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ITCH deficiency protects from diet induced obesity

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Background and aims: Obesity is associated to increased CD8+ T cell infiltrations and a defective regulation of M2 macrophage polarization state in the adipose tissue that predispose to a chronic state of low-grade inflammation, and in turn insulin resistance, type 2 diabetes and hepatic steatosis. ITCH Knockout (KO) mice show a Th2 polarization; in T cell, ITCH down regulation causes aberrant expression of JunB, IL-4 overproduction and Th2 differentiation. We tested whether the Th2 bias of ITCH KO mice might counteract the defective Th2 /M2 polarization state in obesity-related inflammation and insulin resistance.
Materials and methods: We compared C57BL/10 WT and ITCH KO mice in a context of diet-induced obesity (High fat diet, HFD). Animals were subjected to metabolic characterization: weight and blood glucose monitoring, glucose tolerance tests and indirect calorimetric measurement. At the end of 12 weeks of treatment, WT and KO mice were sacrificed and metabolically active organs were collected for molecular and histological studies. Fasting and feeding sera were collected for assessment of the amount of insulin.
Results: During HFD treatment, ITCH KO mice compared to WT littermates showed lower weight ($p<0.001$), characterized by reduced adiposity associated with decreased fat pad mass and adipocyte size ($P<0.001$), significantly lower levels of blood glucose and HOMA IR index ($p<0.001$). Calorimetric analysis showed an increased O2 consumption and CO2 production in KO mice compared to WT ($p<0.001$), suggesting an accelerated metabolism. In muscle from ITCH KO mice compared to WT we found increased phosphorylation of Ser473 AKT ($p<0.05$). Gene expression analysis of epididimal adipose tissue of ITCH KO mice revealed significantly reduced level of F4/80 ($p<0.05$), increased levels of IL4, IL13 ($p<0.01$), and M2 markers such as YM1, Arg1 and Mgl2 ($p<0.01$), when compared to WT. This suggested a high presence of alternatively activated macrophages M2. We found also significantly increased expression of genes involved in adipocytes differentiation, fatty acid oxidation and mitochondrial function: PPARs, C/EBPs, SREBP1, PGC1 α/β, ACOX1, Tfam, NRF1, SIRT1 ($p<0.01$). After HFD treatment histological analysis of livers from ITCH KO mice showed no signs of macrovesicular steatosis that instead occurred in WT mice. These results suggested that ITCH

deficiency protect mice from obesity-related NAFLD. Finally improved insulin sensitivity of ITCH KO livers was suggested by increased phosphorylation of AKT (Ser473) ($p<0.01$).
Conclusion: When subjected to HFD ITCH KO mice did not show increase in body weight, insulin resistance and hepatosteatosis. The molecular analysis of metabolically active tissues revealed increased levels of M2 markers and fatty acid oxidation. Taken together, our data indicate that ITCH KO mice are partially protected from the metabolic injury caused by HFD.
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Parasitic nematode *Trichinella* infection improves obesity-induced diabetes
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Background and aims: Insulin resistance associated with obesity, particularly visceral obesity, is involved in the pathogenesis of lifestyle-related diseases such as type 2 diabetes. In addition, inflammation caused by chronic bacterial infection such as periodontal disease is known to deteriorate glucose tolerance and insulin sensitivity. However, the effects of parasite infection on glucose tolerance have remained unknown. In addition, that chronic low grade inflammation in adipose tissue is currently considered as a main cause of insulin resistance associated with obesity. Interestingly, a parasitic helminth is known to secrete an immunosuppressive agent and anti-inflammatory materials to suppress an attack from a host. We investigated the effects of nematode parasite, *Trichinella spiraris*, infection on glucose tolerance and macrophage status in adipose tissue.
Materials and methods: Ob/ob mice and C57/BL mice fed with normal or high fat diet (HF) were divided into two groups (10 mice each), and then infected orally with (infected group) or without (un-infected group) *Trichinella*. Four weeks later, body weight, fat weight, fasting plasma glucose and insulin levels were measured. To evaluate glucose tolerance, intraperitoneal glucose tolerance test (ipGTT, glucose: 2g/kg) was performed. To determine the expression levels of adipocyte specific genes (PPARγ and adiponectin) in adipocytes isolated from epididymal fat, M1 macrophage markers (CD11c Nos and IL6) and M2 macrophage marker (CD206, arginase 1 and IL10) in stromal vascular fraction (SVF) and peritoneal lavage (PT), real time PCR was performed. Immunostaining of paraffin sections of the fat tissues was performed using anti-CD11c antibody and anti-CD206 antibody.
Results: Although *Trichinella* infection for four weeks reduced fasting plasma glucose level, it had no influence on the plasma insulin level in C57 /BL and ob /ob mice. These results implied that *Trichinella* infection improves insulin sensitivity. *Trichinella* infection did not affect body weight and fat weight. *Trichinella* infection suppressed plasma glucose levels during GTT in ob/ob mice and HF fed mice, but not lean C57 /BL mice. The expression of M1 markers were suppressed in PT and SVF isolated from infected mice. In contrast, M2 marker, mRNA levels were elevated in SVF and PT isolated from infected mice. These results indicated that *Trichinella* infection shifted macrophage polarization from M1 to M2 in SVF and PT. On the other hand, *Trichinella* infection did not influence mRNA levels of PPARγ, adiponectin, IL-6, IL-10 and MCP-1 in adipocytes. Double immunostaining for CD11c, and for CD206 revealed that more abundant expression of CD206 in the infected group was detected.
Conclusion: *Trichinella* infection increased the ratio of M2 /M1 macrophage, which results in a pro-inflammatory state in adipose tissue and amelioration of glucose tolerance in obese mice.

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Obesity and inflammation: molecular pathways involved in CCL5 chemokine regulation in adipocytes
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Background and aim: Obesity is a chronic, multifactorial disorder that has reached epidemic proportions globally. Derangement of glucose and lipid metabolism are frequently observed in obese individuals. In obesity, adipose tissue is characterized by a chronic low-grade inflammation with secretion of inflammatory cytokines and chemokines that recruit immune cells. Among chemokines, RANTES/CCL5 participates in mediating leukocyte infiltration of adipose tissue. Moreover circulating CCL5 concentrations are elevated in obesity, impaired glucose tolerance (IGT) and type 2 diabetes. We have investigated the effect of metabolic alterations on the control of CCL5 expression and secretion in adipocytes.
Materials and methods: 3T3-L1 preadipocytes were cultured up to confluence and then differentiated into adipocytes. Thereafter adipocytes were incubated in the presence of different concentrations of glucose (5mM; 15mM; 25mM), fatty acids (Oleate 10uM; Palmitate 100uM) or insulin (100 nM). In order to investigate CCL5 secretion and gene expression, ELISA and real time RT-PCR assays were used, respectively. Activation of specific intracellular pathways was assessed by Western Blot analysis. Chromatin Immunoprecipitation assays were performed to evaluate NFkB binding to CCL5 promoter.
Results: We have obtained evidence that, in differentiated 3T3-L1 adipocytes, both glucose and fatty acids (oleate and palmitate) induced CCL5 secretion in a dose dependent manner. At variance, insulin reduced CCL5 secretion. However, CCL5 mRNA levels did not significantly change when 3T3-L1 adipocytes were cultured in the presence of 5mM or 25 mM glucose, while both oleate and palmitate enhanced CCL5 mRNA levels. Again, insulin exerted an inhibitory effect on CCL5 mRNA and prevented fatty acid-induced stimulation. Fatty acid effect on CCL5 expression was paralleled by increased JNK activity. Interestingly, treatment of the cells with SP600125, a JNK inhibitor, significantly reduced the stimulatory effect of oleate and palmitate on CCL5 mRNA. At the opposite, both LY294002 and PD98059, inhibitors of PI3K and MAPK, respectively, increased CCL5 expression levels and prevented insulin inhibitory effect. Finally, both oleate and palmitate increased NFkB binding to CCL5 promoter. Consistently, insulin exposure reduced NFkB recruitment onto CCL5 promoter, and almost completely prevented fatty acid effect.
Conclusion: Oleate and palmitate, while not glucose, induce CCL5 mRNA, possibly via JNK and NFkB pathways. Fatty acid effect on CCL5 is largely prevented by insulin and may depend on PI3K/Akt and MAPK pathways.

PS 054 Fatty acids and triglyceride storage: impact on inflammation and glucose metabolism

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Plasma acylcarnitine levels have limited predictive value for metabolic characteristics as insulin sensitivity and energy expenditure

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Background and aims: Acylcarnitines are fatty acid oxidation (FAO) intermediates and have been implicated in diet-induced insulin resistance and type 2 diabetes mellitus. Moreover plasma acylcarnitines have been associated with multiple clinical parameters associated to glucose metabolism such as fasting glucose levels and HbA1c. We investigated plasma acylcarnitines in relation to energy metabolism (including energy expenditure (EE)) and insulin sensitivity measurements in obese subjects before and after weight loss. Since acylcarnitines reflect FAO profiles at the mitochondrial level, we expected plasma acylcarnitines either to be predictive for energy expenditure as measured by indirect calorimetry or the amount of weight loss during a weight loss intervention.
Materials and methods: 60 obese subjects were recruited to take part in an outpatient study and were randomized to one of three 12-week interventions: 1- diet (-600 kcal) alone, 2- diet with moderate exercise, and 3- diet with sibutramine. Subjects visited the clinical unit at 0, 4 and 12 weeks for anthropometry by DXA, indirect calorimetry and blood sampling (e.g. plasma acylcarnitine, glucose, insulin and leptin levels). Plasma acylcarnitine profiles were analyzed by tandem mass spectrometry (MS). Statistical analysis (including multivariate regression analysis and subgroup analyses for diet only, diet and exercise and sibutramine) was performed using SPSS. Here, we report on whole group analyses.
Results: We included 60 obese subjects; average age 40.1 ± 8.6(SD) years; 23 males and 37 females. After 12 weeks there was a significant reduction in body weight (pre 100.9 ± 12.6kg vs. post 96.5 ± 13.1, $p < 0.05$) due to a significant decrease in body fat accompanied by significant reductions in HOMA-IR (pre 3.7 ± 2.9 vs. post 2.9 ± 1.4, $p < 0.05$) as well as EE (pre 5.2 ± 0.83 kJ/min vs. post 4.9 ± 0.8 kJ/min, $p < 0.05$) and plasma leptin levels (pre 33.8 ± 22 ng/ml vs. post 23.3 ± 13.4 ng/ml, $p < 0.05$). In general plasma acylcarnitines were significantly higher after 12 weeks: (e.g. free carnitine (CN) pre 33 ± 7.8 umol/l vs. post 36.1 ± 6.7 umol/L; C2-CN pre 4.9 ± 1.7 umol/L vs. post 5.821 ± 2.4 umol/L; C4-3-OH-CN pre 0.026 ± 0.02 umol/L vs. post 0.037 ± 0.04 umol/L and C18-1-CN pre 0.087 ± 0.03 vs. post 0.104 ± 0.03 umol/L; all $p < 0.05$). Multivariate regression analysis did not attribute significant predictive value to the analysed acylcarnitines in relation to our major outcome parameters such as HOMA-IR or EE. There was no relation between attained weight loss and any of the plasma acylcarnitines at baseline.
Conclusion: Previous studies suggested that elevated plasma acylcarnitines play a role in the induction of insulin resistance or reflect insulin resistance. In contrast, despite the amelioration of HOMA-IR, plasma acylcarnitines levels increased during weight loss. This is most probably attributable to higher lipid oxidation due to the caloric deficit. Also EE, RER and body composition were not related to plasma acylcarnitines. Our data warrant the cautious interpretation of plasma acylcarnitines in human metabolic studies.

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Plasma acylcarnitines poorly reflect tissue acylcarnitine metabolism

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Background and aims: Acylcarnitines (ACs) are fatty acid oxidation (FAO) derived metabolites which have been implicated in the induction of insulin resistance. Several studies, both in animals and humans, relate elevated ACs to obesity and type 2 diabetes mellitus. The majority of these studies focus on plasma measurements of ACs. Since insulin resistance occurs inside tissues, it is relevant to know whether plasma AC profiles accurately reflect AC profiles in any of the tissues implicated in insulin resistance. The aim of this study was to investigate if AC profiles in plasma correlate with AC profiles in specific tissues, in both fed and fasted BALB/cJ- and more insulin resistant C57BL/6N (Bl6) mice.
Materials and methods: Mice were housed under standard conditions and fed a chow diet. Both the BALB/cJ and Bl6 groups were split in two, of which one group was fed ad libitum, whereas the other half was fasted overnight to increase fatty acid oxidation and induce insulin resistance. Then, animals were anesthesized with a pentobarbital solution for venous blood sampling and subsequent dissection and harvesting of organs. AC profiles were analyzed by tandem mass spectrometry in plasma and several tissues (eg. liver, adipose tissue and muscle). Metaboanalyst 2.0 was used to determine correlation patterns followed by individual Spearman's rank correlation coefficients for specific metabolites and compartments.
Results: Fasting significantly induced ketosis in BALB/cJ (fasted β-OH-butyrate 1.77 mmol/l vs fed 0.12 mmol/l, $p < 0.001$) and Bl6 mice (fasted 1.06 mmol/l vs fed 0.07 mmol/l, $p < 0.001$). Fasting ketosis was higher in BALB/cJ compared to Bl6 mice ($p < 0.05$). Fasting free carnitine levels decreased whereas in general all other AC levels increased in both mouse strains ($p < 0.05$). Overall, no correlations were found between plasma AC species and their tissue counterparts in either mouse strain. Plasma free carnitine showed no correlations with free carnitine in tissues in all mice. No other short chain species showed correlations, with the exception of plasma C4-3OH-CN (i.e. a ketone body derived AC), which in fed Bl6 mice only correlated with quadriceps femoris muscle (r^2 0.622, p 0.031). However in fasted BALB/cJ mice, which exhibited higher ketosis compared to Bl6 mice, plasma C4-3OH-CN correlated with muscle (quadriceps femoris muscle (r^2 0.627, p 0.039), gastrocnemius muscle (r^2 0.791, p 0.004) and soleus muscle (r^2 0.624, p 0.040)), heart (r^2 0.673, p 0.023) and negatively with BAT (r^2 -0.664, p 0.026). In general all medium and long chain AC species did not correlate in any metabolic state in both BALB/cJ and Bl6 mice.
Conclusion: These data show that plasma AC levels poorly reflect tissue AC levels with the exception of C4-3OH-CN under fasted conditions. A probable explanation for this lack of correlation is that the plasma pool represents only a small percentage of the total AC pool and that the turnover rates of plasma ACs are larger than that of tissue ACs. Therefore, plasma AC levels should be interpreted with caution and a focus on tissue AC levels is warranted in metabolic studies.
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γ-Butyrobetaine impairs glucose tolerance in mice on a high carbohydrate diet

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Background and aims: Acylcarnitines (ACs) are fatty acid oxidation (FAO) intermediates and have been implicated in diet-induced insulin resistance and type 2 diabetes mellitus. Elevated AC levels coincide with lower free carnitine and may reflect impaired FAO. Moreover, carnitine acetyltransferase (CrAT) is suggested to play a pivotal role in substrate switching and glucose tolerance via converting acetyl-CoA into acetylcarnitine and regulating mitochondrial and intracellular carbon trafficking. Modulating carnitine availability may facilitate FAO, thereby improving insulin sensitivity. Since the carnitine precursor γ-butyrobetaine (γBB) is most effective in increasing carnitine levels, we compared the effects of γBB administration on glucose metabolism in C57BL/6N (Bl6) mice on chow versus high fat diet (HFD).
Materials and methods: Mice were fed a high fat or chow diet with or without γBB treatment via the drinking water (n=10/group). After 8 weeks of diet, indirect calorimetry, intra peritoneal glucose tolerance test (IPGTT) and intra peritoneal insulin sensitivity test (IPIST) were performed. Then animals were euthanized for blood sampling and dissection. Plasma AC profiles and carnitine biosynthesis intermediates were analyzed by tandem mass spectrometry (MS) and liquid chromatography (LC) MS MS. Statistical analysis was performed using SPSS.
Results: Treatment with γBB did not effect body weight gain (HFD +/- γBB 48.7g vs 49.4g; chow +/- γBB 25.1g vs 25.6g respectively). Whole blood free carnitine levels were lower after HFD, which was restored to chow diet levels upon γBB treatment ($p < 0.01$). Interestingly γBB treatment did not increase whole blood carnitine in chow-fed mice. Treatment with γBB caused a gen-

eral increase of short- (including acetylcarnitine), medium- and long-chain ACs in HFD and chow mice. Mice fed a HFD showed lower RER compared to chow fed mice (mean RER 0.82 vs 0.97 respectively, $p < 0.001$), indicating higher FAO. RER did not change by γBB treatment in both groups. Glucose tolerance was lower in HFD mice, which was not improved by γBB treatment (AUC HFD +γBB 230.6 vs HFD -γBB 199.1, p 0.38). In contrast, γBB treatment of chow fed mice reduced glucose tolerance (AUC Chow +γBB 354.2 vs Chow -γBB 222.5, $p<0.001$). HFD fed mice were insulin resistant compared to chow fed mice as revealed by IPIST, but there was no effect of γBB treatment (AUC HFD +γBB -12.3 vs HFD -γBB -10.2, p 0.53, AUC Chow +γBB -21.3 vs HFD -γBB -19.9, p 0.55).

Conclusion: Although γBB supplementation restored free carnitine levels in HFD fed mice, insulin resistance was not changed. Unexpectedly, in chow-fed mice, γBB treatment impaired glucose tolerance with an unaffected insulin tolerance. This suggests that elevating intracellular carnitine levels via γBB treatment reduces metabolic flexibility in chow animals with regard to glucose tolerance. This is intriguing since substrate availability (i.e. carbohydrates) and oxidation (RER) were equal in the chow groups. Ongoing tissue analyses may help to elucidate the mechanism at hand. We speculate that γBB treatment increases mitochondrial fatty acid uptake and oxidation leading to increased levels of acetyl-CoA as reflected by higher acetylcarnitine levels. Elevated acetyl-CoA inhibits the pyruvate dehydrogenase complex thereby decreasing glucose oxidation with possible negative effects on glucose tolerance.

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Relationship between inflammatory phenotype of human macrophages and the lipid storage in lipid droplets

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Background and aims: Macrophages play a major role in atherosclerosis growing into foam cells while absorbing lipids in atherosclerotic plaques. Infiltrating macrophage polarity (pro/anti-inflammatory balance) in human atherosclerotic plaques has been reported to be associated with increased vulnerability and clinical incidence of stroke. Plaques in patients with symptomatic stroke were more unstable and had a greater infiltration of pro-inflammatory M1 macrophages compared to plaques found in patients with asymptomatic stroke which contained less macrophages, but most of them anti-inflammatory M2 phenotype. Lipid droplets in macrophages and foam cells are covered with Perilipin2 (PLIN2, ADRP), a lipid droplet protein. In adipose tissue, immature lipid droplets are coated with PLIN2, and replaced by Perilipin1 (PLIN1) during maturation. PLIN1 regulates proteins involved in the storage of triglycerides in lipid droplets and thereby prevents lipotoxicity by sequestering harmful lipids in stable lipid droplets. In macrophages, however, the expression and function of PLIN1 and the connection with the macrophage polarity and the function of PLIN proteins in human specimens are poorly understood.

Materials and methods: Monocytes were isolated from healthy human peripheral blood mononuclear cells and differentiated into macrophages with M-CSF. M2 macrophages were obtained by stimulating with low concentrations of recombinant human IL-4 for 6 days. M1 macrophages were obtained by differentiating in the presence of TNFα. Macrophages were cultured for 24 hours or 7 days in the presence of oxidized low density lipoprotein (oxLDL) or very low density lipoprotein (VLDL), and collected for protein analysis, or fixed in formaldehyde for morphological analysis.

Results: In cultured macrophages, both PLIN1 and PLIN2 appeared on the surface of lipid droplets. Small lipid droplets were covered with PLIN2, while large droplets were coated with PLIN1 and contained mainly triglyceride. oxLDL increased macrophage number and the expression of PLIN2 protein. VLDL dramatically increased the expression of PLIN2 during the first 24 hours followed by enhanced expression of PLIN1 and decreased expression of PLIN2. Cholesterol was added, the expression of CD11c, a pro-inflammatory macrophage marker, was increased and the expression of the anti inflammatory marker CD163 rapidly disappeared in M1 macrophages. PLIN2 was expressed after 24 hours while PLIN1 expression was observed only on day 7th. In M2 macrophages, there was no expression of CD11c in the whole period and the expression of CD163 decreased slowly. PLIN2 was slightly expressed after 24 hours, and there was also PLIN1.

Conclusion: Lipid droplets proteins in human macrophages may keep stability of atherosclerotic lesion by storing lipid efficiently, through PLIN1 switched from PLIN2. On the other hand, large amount of lipids, not fully absorbed in macrophages, have increased pro-inflammatory character. Inflammation has a negative effect on plaque stability, and might leads to lipotoxicity to surrounding environments and to rupture of atherosclerotic plaque, rapidly inhibiting PLIN proteins switching.

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Coordinated effects of macrophages and free fatty acids on the inflammatory gene expression in pancreatic islets

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Background and aims: The numbers of macrophages in the pancreatic islets of patients with type 2 diabetes were reportedly increased. Yet, the pathophysiological significance of macrophages infiltration into islet is unclear. To address this question, we investigated the interaction of islets with macrophages and free fatty acids.

Materials and methods: Isolated mouse pancreatic islets were co-cultured with unstimulated peritoneal macrophages in the presence of lipopolysaccharide (LPS), palmitate, oleate, or adipocytes derived from epididymal fat, in vitro. The mRNA expressions of co-cultured islets were analyzed by real-time PCR. Glucose-stimulated insulin secretion (GSIS) in the co-cultured islets was analyzed. Effects of glucose concentration (5.6 mM/22.2 mM) and TLR4-inhibitory peptide (IMG-2011A) on the gene expression in the co-cultured islets were also assessed.

Results: In the presence of macrophages, the expressions of IL-1β, TNF-α, IL-6, CCL2, S100A8, and S100A9 in islets were increased (2.3-fold**, 2.9-fold**, 7.3-fold**, 9.6-fold**, 13.3-fold** and 72.7-fold**, respectively). LPS enhanced the macrophages-induced expression of IL-1β, TNF-α, IL-6 and CCL2 in islets (1.5-fold; p=0.12, 1.7-fold*, 2.4-fold** and 2.3-fold**), whereas palmitate augmented the expression of S100A8 and S100A9 under the same conditions (4.8-fold** and 3.6-fold**). Oleate unaffected those gene expression levels in islets. Neither LPS nor palmitate was sufficient to facilitate these cytokine expressions in islets in the absence of macrophage. When islets were co-cultured with adipocytes, the expressions of IL-1β, TNF-α, IL-6 and CCL2, S100A8 and S100A9 in islets were tended to elevated (2.4-fold n.s., 2.1-fold*, 2.4-fold n.s., 3.3-fold n.s, 6.0-fold n.s. and 35.6-fold n.s.). The induction of S100A8 and S100A9 expressions by adipocytes in islets were up-regulated by the addition of macrophages (3.0-fold n.s., and 4.3 fold**). Co-culture with palmitate increased glucose-induced insulin secretion from isolated islets (3.1-fold, n.s.), and this insulin secretion was further incremented in the presence of both palmitate and macrophages (4.8-fold*). The expression levels of S100A8 and S100A9 were potentiated at high glucose concentration compared with those at low glucose concentration in the presence of palmitate (7.3-fold** and 4.6-fold**). By blocking TLR4 signaling with IMG-2011A, the rises of S100A8 and S100A9 expression in islet were cancelled even in the presence of macrophages and palmitate (0.08-fold** and 0.09-fold**). On the other hand, with IMG-2011A, the expressions of IL-1β, TNF-α, IL-6, and CCL2 in islets were modestly increased in the presence of both macrophages and palmitate (2.9-fold n.s., 1.2-fold n.s., 2.9-fold* and 1.9-fold*). (n.s.: not significant, †$p<0.1$, *$p<0.05$, **$p<0.01$)

Conclusion: These results suggest that infiltrating macrophages evoke inflammatory gene expressions in islets by the mutual interaction of islets with macrophages and free fatty acids derived from adipocytes via TLR4-mediated signaling especially under hyperglycemic situation, namely, diabetes. However, these factors did not impair insulin secretion from pancreatic islets at this stage.

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Early over-nutrition and dietary fat induce beta cell failure in Swiss Webster mice

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Background and aims: Childhood obesity and rapid early growth increase the risk for type 2 diabetes. Such early over-nutrition can be modelled in mice by reducing litter size, resulting in rapid weight gain, hyperinsulinemia and hyperleptinemia in early life. This chronic postnatal overnutrition (CPO) in Swiss Webster mice significantly increases susceptibility to diabetes (75% incidence vs. 36% in controls, CTR), characterised by profound hyperglycaemia followed by beta-cell death (typical onset ranges from 2 to 6 months of age).

We examined islets in young, normoglycaemic mice to determine whether beta-cell dysfunction precedes the development of hyperglycaemia.
Materials and methods: Litters from Swiss Webster mice were adjusted to 3 (CPO) or 10 (CTR) pups shortly after birth and maintained on a moderate fat (25 kcal%) diet. Insulin and glucose tolerance tests were performed in 2 to 3 month old normoglycaemic mice, and insulin regulation was assessed in the pancreas. To determine the role of dietary fat content in diabetes development, plasma triglyceride levels were measured, and a separate cohort of mice was maintained on either low-fat diet (LFD; 10 kcal% fat) or high-fat diet (HFD; 45 kcal% fat) throughout life.
Results: Normoglycaemic CPO mice exhibited significantly heavier body weight and higher circulating triglyceride levels but normal insulin and glucose tolerance. In size-matched islets, CPO mice exhibited reduced insulin and proinsulin content ($p<0.05$) but no difference in insulin secretion from islets perifused with 20 mM glucose. In situ hybridisation revealed strong Ins2 expression in islets of 4/4 CTR mice but only 1/4 CPO mice. The remaining 3 CPO mice had very low Ins2 expression, corresponding with reduced insulin and proinsulin immunoreactivity. However, CPO mice had equivalent total pancreatic insulin content, plasma insulin and proinsulin levels compared to CTR mice, likely due to their 1.8-fold greater islet number. Electron microscopy revealed that 2/3 CPO mice and 1/3 CTR mice examined had drastic reductions in insulin granules together with swollen mitochondria and increased autophagy, suggesting beta-cell failure. HFD exacerbated diabetes even further, with 83% of CPO and 50% of CTR mice developing diabetes as early as 7 weeks of age. In contrast, LFD-fed CPO mice were entirely diabetes free, maintaining normal glucose and insulin levels to 1 yr of age, despite a higher body weight than LFD-fed CTR mice.
Conclusion: These findings suggest that Swiss Webster mice have a susceptibility to diabetes that is exacerbated by early overnutrition and increased dietary fat content, whereas a low-fat diet is protective. While a higher islet number in CPO mice may compensate for their reduced insulin expression in the short term, prolonged metabolic stress eventually results in beta-cell failure.
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Fatty acid composition in adipose triglyceride in high-fat fed rats treated with pioglitazone

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Background and aims: Pioglitazone improves insulin resistance and increases small adipocytes. We elucidated that the fatty acid (FA) composition in adipose triglyceride is closely related to insulin resistance induced with high-fat diet in rats. In addition, it was reported that adipocyte size is potentially relevant to a part of FA content. In the present study, we administered pioglitazone to insulin-resistant, high-fat fed rats to increase small adipocytes, and compared the FA composition to that in high-fat fed, control rats.
Materials and methods: Male Wistar rats were fed a high-fat diet from 6 weeks of age, and were separated into 30 mg/kg/day pioglitazone-treated (PIO(+)) and non-treated (PIO(-)) groups (n=6, respectively) at 11 weeks of age. Rats were killed at 15 weeks of age, and blood glucose (BG), plasma insulin (PI), triglyceride (TG), and free fatty acid (FFA) levels were measured. FA contents (μmol/g tissue) in triglyceride fraction in epididymal (EPI), mesenteric (MES), retroperitoneal (RET), and subcutaneous (SUB) adipose tissues were analyzed by a gas chromatography. Also, adipocyte diameters (μm) in a part of each adipose tissues were evaluated under a microscope (n=4, respectively).
Results: PI, TG, and FFA in PIO(+) group were significantly lower ($p<0.05$) than those in PIO(-) group, respectively, whereas no difference was detected in BG. In saturated (SFA) and monounsaturated fatty acids, 14:0, 16:0, and 18:1n-9 contents in SUB in PIO(+) group were lower ($p<0.05$) than those in PIO(-) group, respectively (14:0: 26.9 ± 8.3 vs. 37.1 ± 6.8; 16:0: 346 ± 86 vs. 454 ± 69; 18:1n-9: 938 ± 167 vs. 1220 ± 177). In n-3 polyunsaturated fatty acid (PUFA) content, 22:5n-3 and 22:6n-3 in all the tissues in PIO(+) group were higher ($p<0.01$) than those in PIO(-) group, respectively (22:5n-3: EPI= 0.359 ± 0.164 vs. 0.036 ± 0.059, MES= 0.282 ± 0.124 vs. 0.014 ± 0.033, RET= 0.249 ± 0.119 vs. 0.000 ± 0.000, SUB= 0.484 ± 0.166 vs. 0.052 ± 0.127; 22:6n-3: EPI= 0.745 ± 0.452 vs. 0.103 ± 0.126, MES= 0.771 ± 0.450 vs. 0.029 ± 0.045, RET= 0.590 ± 0.253 vs. 0.000 ± 0.000, SUB= 1.07 ± 0.41 vs. 0.113 ± 0.150). In n-6 PUFA, 18:3n-6 in MES in PIO(+) group was lower ($p<0.05$) than that in PIO(-) group (1.06 ± 0.20 vs. 1.47 ± 0.32) whereas 20:3n-6 in EPI and 22:4n-6 in EPI, MES, and SUB in PIO(+) group were higher ($p<0.05$), respectively (20:3n-6: 0.936 ± 0.370 vs. 0.437 ± 0.289; 22:4n-6: EPI= 0.975 ± 0.749 vs. 0.163 ± 0.118, MES= 1.11 ± 0.79 vs. 0.136 ± 0.119, SUB= 0.609 ± 0.295 vs. 0.198 ± 0.300). Average adipocyte diameter in PIO(+) group tended to be smaller than those in PIO(-) group (EPI= 98 ± 9 vs. 104 ± 7, MES= 83 ± 5 vs. 95 ± 9, RET= 89 ± 11 vs. 116 ± 13 ($p<0.05$), SUB= 84 ± 4 vs. 92 ± 11).
Conclusion: Pioglitazone reduced PI, TG, and FFA levels, and tended to increase small adipocytes. These effects of pioglitazone led to decrease in 14:0, 16:0, 18:1n-9, and 18:3n-6 contents but to increase 22:5n-3, 22:6n-3, 20:3n-6, and 22:4n-6 contents in adipose tissues. High SFAs and 18:1n-9 are usually in insulin resistant state. High contents in several C20 and C22 PUFAs in PIO(+) group, probably increased due elongation process, may have improved the insulin resistance. On the other hand, in PIO(-) group, the elongation and/or desaturation of longer than 18:3n-6 may be reduced.

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Intramuscular triglyceride synthesis rate influences skeletal muscle DAG localisation in humans

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Background and aims: We recently reported that diacylglycerol (DAG) localisation influences insulin resistance in humans, with only DAG in membranes related to insulin resistance and PKC activation. It is currently unknown what influences DAG localization in skeletal muscle. We sought to evaluate if the basal rate of intramuscular triglyceride (IMTG) synthesis was related to DAG localisation in humans.
Materials and methods: Six obese sedentary controls (Con: 2W, 4M, Age: 39.5±2.3 yrs, BMI: 33.3±1.4 kg/m2, Si: 3.2±0.4 10-4/μU/ml), five individuals with type 2 diabetes (T2D: 0W, 5M, Age: 44±1.8 yrs, BMI: 30.1±2.3 kg/m2, Si: 2.4±0.6 10-4/μU/ml), and ten endurance trained athletes (Ath: 2W, 8M, Age: 35.4±3.1 yrs, BMI: 23.3±0.8 kg/m2, Si: 12.6±1.7 10-4/μU/ml) were studied. Insulin sensitivity was determined using an IVGTT, and basal IMTG synthesis rate (FSR) measured using a 4 hour infusion of U-13C palmitate after an overnight fast. Muscle biopsies were taken and a portion used to isolate triglyceride enrichment which was measured using GC/C/IRMS. Another portion was fractionated using ultracentrifugation, and DAG species measured using LC/MS/MS.
Results: Endurance trained athletes had significantly greater insulin sensitivity (Obese: 3.15±0.3 min-1/uU/ml, T2D: 2.58±0.7 min-1/uU/ml, Ath: 8.65±2.2 min-1/uU/ml, $p<0.0001$), and IMTG FSR compared to the other two groups (Obese: 0.51±0.18 %/hr, T2D: 0.48±0.17 %/hr, Ath: 1.58±0.15 %/hr, $p<0.0001$). The percent of DAG in the membrane fraction was significantly lower in athletes compared to the other two groups ($p=0.01$). Further, there was a significant inverse relationship between IMTG FSR and % membrane DAG ($r=-0.71$, $p=0.0004$) and total membrane DAG ($r=-0.20$, $p=0.007$), and a positive relationship between IMTG FSR and cytosolic DAG content ($r=0.48$, $p=0.002$).
Conclusion: These data suggest that the synthesis rate of IMTG in skeletal muscle promotes DAG accumulation in the cytosolic compartment, preventing membrane DAG accumulation. Therefore, the turnover rate of the intramuscular triglyceride pool appears to influence skeletal muscle diacylglycerol localisation.
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Involvement of the endocrine factor FGF21 in the beneficial effects of dietary long-chain n-3 polyunsaturated fatty acids

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Background and aims: Diets rich in long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) protect against insulin resistance and obesity in rodent mod-

els of metabolic disease. Fibroblast growth factor-21 (FGF21) is a hormonal factor released mainly by the liver, strongly regulated by PPARalpha, and having powerful anti-diabetic effects. We tested whether the healthy metabolic effects of n-3 PUFA involve FGF21

Materials and methods: C57BL/6J mice were exposed to an obesogenic corn-oil-based high-fat diet (cHF) or a diet in which vegetable oils were replaced by a fish-oil extract highly enriched in long-chain n-3 PUFA (cHF+F). Control mice were treated with a convential high-carbohydrate diet (HC). Food intake, body weight, systemic metabolites (glucose, triglycerides) were measured using spectrophotometric methods, hormones and cytokines using a Multiplex system (Millipore), and plasma FGF21 using ELISA (Biovendor). The expression of FGF21gene, molecular actors of cellular FGF21 responsiveness (FGF receptors 1 and 4, and β-Klotho), and marker genes of lipid metabolism (CPTII, carnitine palmitoyl transferase II; MCAD, medium chain acyl-CoA dehydrogenase; ACO, acyl-CoA oxidase) pathways in liver, white and brown adipose tissues, were evaluated by quantitative real-time PCR using standard TaqMan probes and procedures (Applied Biosystems.

Results: The cHF+F diet prevented body weight increase and reduced insulinemia and triglyceridemia, relative to cHF. The treatment of mice with cHF-F did not modify glycemia but resulted in a significant reduction in blood triglyceride levels (P = 0.008) as well as a significant reduction in insulin levels (P = 0.006) relative to cHF, indicating enhanced systemic insulin sensitivity as a consequence of L-n3 PUFA enrichment in the high-fat diet. Plasma FGF21 levels were markedly increased (1.8-fold, P = 0.03) in cHF-treated mice relative con HC but much less in cHF+F-treated mice (1.3-fold, P=0.07). Hepatic FGF21 gene expression was strongly induced by cHF (7.3-fold, P = 0.002) and only slightly increased by cHF+F (2.3-fold, P=0.04), relative to HC. In contrast, the hepatic expression of typical PPARalpha target genes (CPTII, MCAD, ACO) was much more strongly induced by cHF+F than by the cHF diets. FGF21 gene expression was unaltered by the diet conditions in white and brown adipose tissues. Expression of FGFR1, FGFR4 and β-Klotho were also unaltered by diet conditions in hepatic and adipose tissues.

Conclusion: Healthy metabolic effects caused by n-3 PUFA are not associated with enhanced levels and expression of FGF21, but, conversely, FGF21 levels are preferentially induced by an obesogenic standard high fat. Enhanced FGF21 levels appear not to be the mechanisms by which long chain n-3 PUFA prevents obesity and ameliorates the metabollic profile. Moreover, a pathway of regulation of hepatic FGF21 expression different from PPARalpha appears to take place in response to high-fat diets.

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Effect of carnitine palmitoyltransferase (CPT) 1A activity regulation on the control of the food intake

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Background and aims: Hypothalamus is a key regulator of food consumption mainly through melanocortin system modulation. Despite the ongoing effort to unveil the role of lipid metabolim in the mechanism, the exact gimmick has not been fully elucidated. Levels of malonyl-CoA, which is the first intermediate in lipogenesis, and levels of long chain fatty acyl-CoA (LCFA-CoA) have been proposed to act as hypothalamic satiety molecular signals. Carnitine Palmitoyltransferase 1A (CPT1A) catalyses the first reaction in the LCFA-CoA transport from cytoplasm to mitochondria and it is directly inhibited by malonyl-CoA. Thus CPT1A is the rate-limiting step in mitochondrial fatty acid oxidation (FAO), and a LCFA-CoA modulator and a malonyl-CoA sensor. The aim of this work is the study of the effect on the control of food intake with CPT1A activity modulation; to do so, we used two experimental approaches: long-term over-expression of a malonyl-CoA insensitive isoform of CPT1A (CPT1AM) in ventromedial hypothalamus (VMH); and pharmacological inhibition of CPT1A in hypothalamus.

Materials and methods: Long-term adeno-associated virus 1 (AAV1)-mediated over-expression of a mutated malonyl-CoA insensitive isoform of CPT1A (CPT1AM) was perfomed in VMH. Viral particles were delivered with a stereotactic injection to VMH. Control animals were injected with AAV1-GFP particles, which were used to track the correct diffusion mainly into VMH. Body weight and food intake were checked weekly and mRNA levels were checked after dead in qRT-PCR assays. C75 enantiomers were sinthesized separately and injected icv to check their effect on food intake and body weight after injection. CPT1 activity was checked with a radiometric assay.

Results: On the one hand, the overexpression of CPT1AM in the hypothalamus produced hyperphagia and overweight in rats. It also up-regulated the hypothalamic mRNA levels of the transcription factor BSX, FoxO1 and CREB described to control the expression of the orexigenic neuropeptides, and of the NPY receptor (NPY1R). On the other hand, the central administration of the compound C75 decreases hypothalamic CPT1 activity, food intake and body weight. Noteworthy, we proved that only the enantiomer (+)-C75, but not (-)-C75, reduces food intake and CPT1 activity in the hypothalamus, suggesting that central inhibition of this enzyme is essential for C75-mediated anorexia.

Conclusion: The data presented demonstrate the fundamental role of hypothalamic CPT1 in the control of food intake and highlights this enzyme as a possible therapeutic target for new drugs designed to limit appetite.

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PS 055 Exercise, muscle and lipid metabolism

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Serum irisin after moderate exercise test in obese and non-obese adolescent boys

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Background and aims: Skeletal muscle was recently identified as an active secretory organ and irisin has been found to be one of its hormones. Irisin secretion is stimulated by physical activity and seems involved in the browning of the white fat tissue in mice, increased energy expenditure and reducing obesity. The aim of the study was to determine whether, and to what extent, moderate physical activity, usually recommended to overweight/obese patients, affects serum irisin levels in obese and non-obese adolescent boys.
Materials and methods: The study comprised 22 obese (BMI ≥2SDS) and 11 non-obese (BMI <2SDS) boys, aged 15.0±1.6 and 15.3±1.4 years respectively. Their body composition was evaluated by bioelectrical impedance (Body Composition Analyzer MC-980 MA). Irisin and leptin levels were estimated by means of commercial ELISA assays. Additional serum analyses comprised glucose, insulin, cortisol and lactate measurements. Patients were assessed in different metabolic conditions: after overnight fast, 2hrs after the normalised mixed meal (350kcal), and then directly after and 1 hr after 30-minutes aerobic exercise (a walk on a treadmill Precore 9.33 at 3% incline). Mean distance of the walk was 2.2 km (approx. 3.5 Metabolic Equivalent Tasks/hr). Data were presented as mean ±SD. Two-tailed p-values<0.05 were considered significant.
Results: No significant difference in baseline serum irisin (ng/ml) was found between the two studied groups (110.3±73.3 in obese individuals *vs.* 143.8±72.3 in controls, p=0.224), and irisin levels were not affected by the food ingestion (115.2±80.1 and 145.9±139.6, respectively). However, after the exercise irisin concentrations significantly decreased in obese subjects (to 76.5±65.6, p=0.007 compared to baseline) whereas the control group displayed similar levels to the baseline results (137.3±91.0; p=0.035 compared to obese subjects). After one-hour post-walk rest irisin levels were 87.9±64.5 in obese and 129.1±92.4 in the non-obese group (p=0.150). Fasting serum irisin was correlated with fat-free mass in kg (r=0.628; p=0.039) and muscle mass in kg (r=0.628; p=0.038) among non-obese adolescents, while these correlations were not significant among their obese peers (both p>0.05). No correlation was found between fasting glucose or insulin levels and irisin concentrations in any of the studied cohorts. Irisin was positively correlated with glucose level after exercise (r=0.720; p=0.013) in healthy patients but not in obese boys. Serum irisin levels were not correlated with leptin, cortisol or lactate.
Conclusion: Declining serum irisin concentration, which was found in obese boys immediately after moderate physical activity may indicate that working muscles limit the secretion of this myokine in obese status. This suppressive effect was not observed in non-obese patients who displayed irisin level correlation with muscle mass and fat-free mass. Studies suggest that obesity can affect the regulation of irisin release as well as diminish its potential metabolic benefits.

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Serum FGF21 relation to nutritional status and physical activity in adolescent boys

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Background and aims: Fibroblast Growth Factor 21 (FGF21) is a liver hormone involved in fat burning and peripheral glucose utilization. It seems to be implicated in obesity, non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes. The study was designed to investigate changes in serum FGF21 at different metabolic states: fasting, fed and after a moderate exercise in obese and non-obese adolescent boys. These circumstances mimicked frequent recommendations for obese persons.
Materials and methods: 22 obese (BMI-SDS ≥2) and 11 non-obese (BMI-SDS &lt2) were evaluated by anthropometric parameters, including body mass composition assessed using bioelectrical impedance (Tanita Analyzer MC-980 MA). Liver ultrasound was performed to detect signs of non-alcoholic fatty liver disease (NAFLD). Serum measurements of glucose, insulin, lactate, ketones, triglycerides, free fatty acids and uric acid, leptin and FGF21 (the latter two by commercial ELISA assays) were performed after overnight fast, 2h after a mixed meal (350kcal), and subsequently at the end and 1h after a 30-minute aerobic exercise (a moderate walk on Precore 9.33 treadmill at 3% incline). Mean distance of the walk was 2.2 km (approx. 3.5 Metabolic Equivalent Tasks/hr). Data are presented as mean ±SD. Two-tailed p-values<0.05 were considered significant.
Results: Fasting serum FGF21 levels (pg/ml) were significantly higher in obese subjects compared to non-obese controls (240.8±130.6 vs. 145.7±76.3; p=0.026). Two hours after mixed meal FGF21 levels were significantly decreased to 103.4±59.0 and 69.4±55.5, respectively, and statistical significance between both studied groups was lost (p=0.105). Moderate exercise induced further reduction of serum FGF21 levels in both cohorts to 82.4±46.8 in obese and 49.5±42.5 in non-obese subjects (p=0.049). An hour later FGF21 was still in decline: 54.9±37.5 and 17.8±22.4, respectively (p=0.001). FGF21 levels did not differ between subjects with moderately elevated aminotransferase activity or ultrasound features of NAFLD and those with intact liver (p>0.05) however, none of the studied individuals presented serious hepatic impairment. FGF21 was negatively correlated with age (r=-0.397, p=0.017) but not with BMI-SDS and body fat mass. A positive correlation was found between FGF21 and leptin levels (r=0.347, p=0.038), which was lost after 30min exercise, but reappeared one hour later (r=0.459, p=0.005). In obese patients, a borderline correlation was detected between FGF21 and lactate levels after physical activity (r=0.405, p=0.050). Serum FGF21 levels displayed no correlation with glucose and insulin levels, ketones, triglycerides, free fatty acids and uric acid (p>0.05).
Conclusion: Higher serum FGF21 levels in obese compared to non-obese boys at all investigated metabolic states may reflect functional resistance to FGF21. Fast promotes FGF21 secretion, while food ingestion and physical activity can decline its serum levels in obese and non-obese male adolescents. Further studies are warranted to recognize if particularly elevated FGF21 concentrations in obese subjects might be stably diminished by moderate exercise, and to explain the distinct effect of different nutrients, particularly with regard to therapeutic diet recommendations in obesity.

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Skeletal muscle mitochondrial uncoupling induces a metabolic rescue cycle involving FGF21 as a myokine

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Ectopic expression of uncoupling protein 1 (UCP1) in skeletal muscle (SM) improves whole body energy metabolism and promotes longevity in transgenic mice (UCP1-TG). However, exact physiological mechanisms underlying this metabolic improvement have not yet been resolved. Here, we show that uncoupling in SM induces Fgf21 expression using UCP1-TG mice, resulting in >5 fold elevated circulating FGF21 levels. UCP1-TG mice display maintained muscle function and morphology but reduced muscle mass. In muscle, the autophagic machinery is activated, which is reflected by increased intracellular eIF2alpha/Atf4. Moreover, ER-stress markers such as the Chop, Atf5, Atf6 and Gadd34 are induced. Furthermore, amino acid stress pathways are induced, as shown by increased amino acid response elements (AAREs), which correlate with Fgf21 expression. The serine/glycine biosynthesis pathway was strongly up-regulated in SM of UCP1-TG mice. In addition, gene expression of glucose transporter 1 (Glut1) as well as basal glucose uptake are increased in transgenic mice SM. Accelerated flux through this alternative glycolysis pathway may help SM to adapt to chronic metabolic stress. Strikingly, β-klotho, a required co-factor for global FGF21 action, is increased in skeletal muscle, liver and white adipose tissue (WAT) of UCP1-TG mice, leading to increased futile cycle (increased lipogenesis/lipolysis) in WAT depots and increased hepatic gluconeogenesis. We conclude that remote metabolic improvements caused by mitochondrial uncoupling in SM are mediated by a metabolic rescue cycle involving Fgf21 as a protective myokine to

prevent muscle wasting and linked to starvation-like response mechanisms. The results provide new insights into FGF21 function as a mediator of muscle induced regulation of whole body substrate metabolism and partitioning.

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Specific changes in plasma lipidome in obesity and type 2 diabetes are associated with physical inactivity and muscle mitochondrial function

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Background and aims: Type 2 diabetes associates with decreased muscle mitochondrial content&function and changes in plasma lipid composition, both of which might be related to physical inactivity, leading to obesity-related metabolic disease. We compared physical activity, skeletal muscle mitochondrial content/function and plasma lipidome of lean healthy (n=28; 23.2±2.2 $kg.m^{-2}$; body fat 18.4±4.7%), obese/overweight (n=29; 30.4±2.8 $kg.m^{-2}$; body fat 28.6±4.6%), prediabetic (n=24; 31.3±3.2 $kg.m^{-2}$; body fat 30.5±3.3%), and type 2 diabetic men (n=15; 31.4±3.9 $kg.m^{-2}$; body fat 30.9±4.1%).
Materials and methods: Complex metabolic phenotyping included euglycemic hyperinsulinemic clamp (assessment of insulin sensitivity), oral glucose tolerance test, magnetic resonance imaging (adipose tissue content and distribution) and ^{1}H-MR spectroscopy (hepatic-, intramyocellular lipids). Physical activity was monitored with accelerometers and "Beacke"-activity questionnaire. Samples of *vastus lateralis* were obtained by needle biopsy. The expression of peroxisome proliferator activated receptor coactivator 1 alpha (PGC1-α) and mtDNA content were determined by qRT-PCR. The activity of cytochrome C oxidase (COX) was measured by oximetry in permeabilised muscle fibers. Plasma lipidomics were analyzed by mass spectroscopy.
Results: Physical activity, activity of COX, mtDNA copy number and expression of PGC1-α were decreased in T2D. Decreased physical activity was associated with worsened metabolic phenotype and decreased PGC1-α expression. Lipids in plasma (phosphatidylcholine, cholesterin, phosphatidylinositol) were increased in prediabetes and diabetes, or only in diabetes (phosphatidylethanolamine) and were negatively correlated with physical activity and PGC1-α expression. Lysophosphadidylcholine unsaturated was decreased in obesity, prediabetes and diabetes and positively associated with sport and leisure-time indexes and COX activity in muscle.
Conclusion: T2D and physical inactivity is accompanied with changes in plasma lipidome, which could be connected to changes in mitochondrial parameters in muscle.
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Effects of resveratrol and exercise training on HFD induced changes in white adipose tissue; role of muscle PGC-1α

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Background and aim: Obesity is a consequence of long term imbalance between calorie intake and output and is characterized by excess deposition of triglycerides as well as molecular changes in the adipose tissue such as low grade inflammation. Exercise training and resveratrol (RSV) have been shown to prevent the negative effects of excess calorie intake and have also been reported to increase the expression and/or activity of muscle PGC-1α. Whether this upregulation of muscle PGC-1α affects the white adipose tissue is unknown. Aim: To test the hypothesis that muscle PGC-1α is required for mediating the beneficial effects of exercise training and RSV on the molecular and morphological changes in white adipose tissue caused by HFD.
Materials and method: 12 weeks old muscle specific PGC-1α knockout mice (MKO) and littermate wildtypes (WT) completed 16 weeks of intervention grouped as follows: Chow, high fat diet (HFD), HFD with resveratrol (HFD+RSV), HFD with exercise training (HFD+EX) and HFD with both resveratrol and exercise training (HFD+RSV+EX). Subcutaneous (S-AT) and visceral (V-AT) adipose tissue were obtained.
Results: HFD increased S-AT and V-AT mass compared with chow ($p<0.05$) and this was rescued by RSV in S-AT for both genotypes and in V-AT in WT ($p<0.05$). The PDK4 mRNA level decreased with HFD ($p<0.05$) but this was rescued by RSV in WT only. UCP-1 mRNA levels were decreased in HFD+RSV, HFD+EX and HFD+RSV+EX ($p<0.05$) compared with HFD in both genotypes. TNFα mRNA levels were unaffected by the interventions.
Conclusion: HFD did not induce inflammation, while RSV prevented HFD induced increases in fat mass and for VAT this required muscle PGC-1α. RSV prevented a HFD induced decrease in PDK4 mRNA in WT indicating that RSV maintains glyceroneogenesis in mice on HFD and that PGC-1α is required for this effect. The lowered UCP-1 mRNA levels with EX and/or RSV may suggest reduced oxidative stress.
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Effects of a combined aerobic and resistance exercise programme on C1q/TNF-related protein-3(CTRP3) and CTRP-5 levels

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Background and aims: Adiponectin is a well-known adipokine which has anti-inflammatory and anti-diabetic effects. Recently, the C1q/TNF-related protein (CTRP) family has been reported to have structural homologies to adiponectin. To date, 15 CTRP family members have been found that might play major roles in metabolism and inflammation. CTRP-3 is a potent anti-inflammatory adipokine that inhibits pro-inflammatory pathways induced by fatty acids, lipopolysaccharides (LPS), and toll-like receptor (TLR) ligands in adipocytes and monocytes. CTRP-5 induces the phosphorylation of AMPK and enhances glucose uptake by glucose transporter 4 (GLUT4). However, due to the unavailability of valid laboratory methods to measure circulating CTRP-3 and CTRP-5 levels, their clinical implications in humans have not been elucidated.
Materials and methods: We examined the gender-difference regarding CTRP-3 and CTRP-5 levels, and explored their relationship with cardiometabolic risk variables in 453 non-diabetic Asian subjects using newly-developed ELISA. In addition, we evaluated the impact of a three-month combined exercise program on CTRP-3 and CTRP-5 levels in 76 obese women. The exercise program consisted of 45 minutes of aerobic exercise at an intensity of 60-75% of the age-predicted maximum heart rate (300 kcal/session) and 20 minutes of resistance training (100 kcal/session) five times a week.
Results: Women had significantly higher CTRP-3 levels compared to men (429.9 [344.6-526.2] vs. 351.8 [279.2-421.9] ng/mL, P <0.001). CTRP-5 levels were also slightly higher in women than in men (50.4 [40.4-67.0] vs. 47.4 [36.4-59.8], P = 0.030). In a stepwise multiple regression analysis, log-transformed CTRP-3 levels were independently associated with age, gender, triglycerides, LDL cholesterol, adiponectin and RBP4 levels (R^2 = 0.174). In contrast, log-transformed CTRP-5 values were not significantly associated with other metabolic parameters (data not shown). When obesity was defined as a BMI of 25 kg/m^2 or higher according to the criteria recommended by Korean Society for the Study of Obesity (KSSO) (14), CTRP-3 and CTRP-5 levels were not significantly different between subjects with and without obesity (P = 0.130 and P = 0.335, respectively). After three months of a combined aerobic and resistance exercise program, CTRP-3 levels also decreased significantly (444.3 [373.8, 535.0] to 374.4 [297.2, 435.9], P <0.001) while CTRP-5 levels increased slightly (34.1 [28.6-44.3] to 38.4 [29.8-55.1], P = 0.048)
Conclusion: Similar to adiponectin, both circulating CTRP-3 and CTRP-5 concentrations showed sexual dimorphism, which is higher in women compared to men. A three-month combined aerobic and resistance exercise program significantly decreased CTRP-3 levels and modestly increased CTRP-5 levels, accompanied by improvements of cardiometabolic risk factors in obese Korean women.
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Effect of exercise and rosiglitazone on neprilysin protein expression in db/db diabetic mice

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Background and aim: Diabetic nephropathy (DN) is one among the main microvascular complications of uncontrolled diabetes, which eventually lead to end stage renal disease. Alteration in renin-angiotensin system (RAS) is considered to be the primary cause underlying the disease process. Hyperglycemia activates RAS and causes an increase in the level of Angiotensin II (Ang II). Emerging evidence suggests that the deleterious effects of Ang II could be opposed by the formation of Ang (1-7), partly generated by the actions of Angiotensin converting enzyme 2 (ACE2) and neprilysin (NEP). NEP is a neutral endopeptidase, a member of zinc-containing metallopeptidase group, which plays a crucial role in the formation of Ang (1-7) from Ang I. Our previous studies reported that renal NEP is decreased in diabetic animal models. We tested the hypothesis that exercise training or rosiglitazone treatment improve glucose homeostasis, up regulate renal NEP protein expression and improve albuminuria in diabetic *db/db* mice. We also hypothesized that changes in renal NEP could be detected in the urine and be used as an index for intrarenal status and progression of chronic kidney disease.
Materials and methods: Seven weeks old lean and *db/db* male mice were subjected either to exercise training or rosiglitazone treatment (20 mg/kg/day) for 10 weeks. Exercise groups were run on a mouse forced exercise walking wheel system at a speed of 8 m/min for 1 hour a day, 7 days a week. Weekly monitoring included 24-hr urinary volume, albumin, creatinine and blood glucose.
Results: *db/db* mice demonstrated hyperglycemia ($p<0.0001$ Vs lean controls), albuminuria ($p<0.0001$ Vs lean controls) and decreased NEP levels in the kidney. Exercise training or rosiglitazone treatment significantly attenuated hyperglycemia ($p<0.0001$ Vs untreated *db/db* mice) and reduced urinary albumin excretion ($p<0.001$ Vs untreated *db/db* mice). Improvements were seen as early as 2 weeks after the initiation of treatment. In addition, *db/db* mice subjected to exercise or rosiglitazone treatment demonstrated a significant increase in renal and urinary NEP protein expression compared to untreated *db*/*db* mice.
Conclusion: Exercise training and rosiglitazone treatment normalized hyperglycemia and up regulated neprilysin levels in the kidney of *db*/*db* mice. Augmentation of renal neprilysin could have a pivotal role in the pathogenesis of diabetic nephropathy. The data suggest that urinary NEP reflects intrarenal RAS status in chronic kidney disease and may be used as an early marker of diabetic nephropathy.
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Maturation of AMP-activated protein kinase in skeletal muscle in pre-weaning mice

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Background and aims: AMP-activated protein kinase (AMPK) is a multisubunit protein, which plays a key role in control of skeletal muscle metabolism. Namely, lipid and glucose metabolism are modulated at both gene and protein levels. Surprisingly little is known about changes in AMPK subunits expression and AMPK activity in skeletal muscle during the early postnatal development, between birth and weaning. The aims of the present study were to (i) characterize the activity of AMPKα1 and AMPKα2 isoforms and expression of the genes encoding its catalytic subunits in murine skeletal muscle during postnatal development, namely between birth and weaning; and (ii) assess possible roles of both gender and genetic background of the mice.
Materials and methods: Male (M) and female (F) pups of the obesity prone mice C57BL/6 (B/6) and obesity resistant mice A/J were born and maintained at temperature closed to thermoneutrality (30 °C), mothers were fed Chow, and mice were weaned at 28 days (D) of age. Two animals from litter containing 4 - 6 pups were anesthetized by ketamin/xylazin and gastrocnemius muscle was collected by freeze-clamping. Activity of AMPKα1 or AMPKα2 was assessed in 10, 15, 20 and 28 D old pups (n=6). AMPK was immunoprecipitated from tissue extracts and the activity was determined using a peptide substrate. Immunoprecipitates were mixed with AMP, [γ-32P]ATP and AMARA peptide. Gene expression was assessed by real-time quantitative RT-PCR in muscle from 5, 10, 15, 20 and 28 old pups (n=5). Evaluation of data was performed by ANOVA.
Results: At 10D, the activity of AMPKα1 was significantly higher in comparison with AMPKα2 in all tested groups (A/J F ~1.9-fold; A/J M ~3.3-fold; B/6 F ~2.6-fold; B/6 M ~3.7-fold). Between 10D and 28D, the activity of AMPKα1 decreased in mice of both strains except for A/J F (A/J M ~2-fold; B/6 M ~2.6-fold; B/6 F ~3.7-fold). In A/J mice at 28D, activity of AMPKα2 was higher than that of AMPKα1 (A/J F ~1.4-fold; A/J M ~1.6-fold). Total activity of AMPK (α1+α2) in B/6 mice decreased significantly between 10D and 28D (B/6 F ~1.9-fold; B6 M ~ 1.5-fold) but it stayed constant in A/J mice. Expression of AMPKα1 gene was constant in both A/J and B/6 mice between 10D and 28D. Expression of AMPKα2 gene increased between 5D and 28D in both strains (A/J F 5D 3.10±0.96 vs. 28D 12.04±0.80 AU; A/J M 5D 3.54±0.47 vs. 28D 16.00±3.21 AU; B6 F 5D 2.24±0.28 vs. 28D 12.85±1.10 AU; B6 M 5D 2.00±0.08 vs. 28D 9.66±0.80 AU).
Conclusion: During lactation, i.e., the period of the switch from high- to low-fat intake, strain-specific changes in AMPK activity in murine skeletal muscle were observed. While in the obesity-resistant A/J mice the activity stayed constant, it declined in the obesity-prone B/6 mice. The developmental change in AMPK activity reflected the activity of AMPKα1 isoform, but not expression of the genes for the catalytic subunits of the enzyme. Changes in AMPK activity in skeletal muscle during early postnatal development may affect propensity to obesity in adulthood, depending on the genetic background of the mice.
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Different feeding rhythm affects hypothalamic regulation of fatty acid metabolism in skeletal muscle

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Background and aims: Disturbance of feeding rhythm has been understood as a risk factor for development of insulin resistance, however, it is not elucidated the detailed mechanism. Here we show a possibility that feeding only in the evening impairs insulin sensitivity because of lipid accumulation in skeletal muscle via hypothalamic regulation.
Materials and methods: In this study, three groups of C57BL/6J mice were given lab chows freely during dark phase (ZT12-24, Control group), first 4-hour in dark phase (ZT12-16; Morning group), or last 4-hour in dark phase (ZT20-24, Evening group) for 8 weeks respectively.
Results: There were no differences in spontaneous motor activity and body temperature during 24-hours among 3 groups. Mice in Evening group showed impaired whole body insulin sensitivity by insulin tolerance test (1 unit/kg) despite mice in the group ingested the smaller food intake than that of Control group, while mice in Morning group showed normal insulin sensitivity. We observed higher triglyceride (TG) content, increased gene expression of fatty acid synthase (FAS) and impaired insulin signals in skeletal muscle in Evening group compared to other group. These effects were not observed in liver. On the other hand, mRNA expression of agouti-related protein (AgRP), an endogenous antagonist for melanocortin receptor, was increased in hypothalamus in Evening group while mRNA expression of POMC, a precursor of endogenous agonist for melanocortin receptor, was decreased. Noradrenaline turnover in skeletal muscle was reduced by ICV-injection of AgRP. In addition, acute and/or chronic ICV-injection of AgRP increased FAS expression and TG content in skeletal muscle. Moreover, inhibition of central AgRP expression by antisense oligo improved insulin resistance in Evening group.
Conclusion: These results indicate that feeding rhythm like as ingestion only in the evening impairs insulin sensitivity. This phenomenon may be partly mediated by hypothalamic melanocortin system, reduced sympathetic nerve activity and TG accumulation in skeletal muscle.
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PS 056 Adipose tissue: expandability

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Bip/GRP78 inhibits adipogenesis through Wnt/beta catenin signalling

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Background and aims: Bip/GRP78 is one of major molecular chaperons, accounting for protein assembly and folding in endoplasmic reticulum (ER) lumen. Adipocytes act as endocrine cells to secret larger amounts of cytokines and lipid mediators. The process requires abundant protein chaperons for the ER homeostasis and functional maintenance. The characteristic elicits an importance of chaperons in the regulation of adipocyte metabolism. In this study, we try to investigate the role of Bip/GRP78 in adipocyte biology.
Materials and methods: We over-expressed Bip/GRP78 via adenovirus-mediated gene transfer in pre- and differentiated 3T3-L1 CAR adipocyte. Morphology of adipocytes was monitored and lipid accumulation was determined by Oil Red O staining. Functional analysis was performed by real-time PCR and immunoblotting.
Results: Bip/GRP78 was first over-expressed in differentiated adipocytes. Gene related with lipid metabolism was detected, no significant change was found. Insulin signaling was then determined by AKT phosphorylation. However, Insulin-stimulated p-AKT level was not altered by Bip/GRP78, either. Subsequently, in order to investigate whether Bip/GRP78 exerts potential role in the chaperon-deficient situation, FFA was used to induce deprivation of chaperons in adipocyte, which was determined by increased ER stress and insulin resistance. Unexpectedly, over-expression of Bip/GRP78 failed to rescue cells from FFA-induced stresses. The results suggest a limited effect of Bip/GRP78 in mature fat cell. In contrast, when we over-expressed Bip/GRP78 in pre-adipocytes, inhibition of adipogenesis was found. Lipid accumulation was decreased by 40% at 6 days post-induction in Bip/GRP78 over-expressed pre-adipocyte, accompanied with decreased genes expression of lipid synthesis (PPARγ, ap2, LPL, LXRα, SREBP1c) and mature adipocytes markers (Adiponectin, Leptin). The further molecular mechanism analysis determined that wnt/β-catenin signaling was activated in Bip/GRP78 over-expressed pre-adipocytes, and inhibition of β/cateinin activity with its inhibitor restored Bip/GRP78-mediated adipogenesis inhibition.
Conclusion: The study suggests BIP plays a major role in the development of adipocyte rather than regulation of the physiology of mature adipocytes. Accordingly, Bip/Grp78 might be a potential target therapeutics therapy for obesity.
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WISP1 (Wnt1 inducible signalling pathway protein 1) is a novel marker of adipose tissue differentiation and obesity

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Background and aims: WISP1 (Wnt1 inducible signalling pathway protein 1) belongs to CCN family of extracellular matrix-associated signalling proteins, which were key signalling molecules involved in the processes such as proliferation, adhesion, apoptosis and differentiation. WISP1 acts via the induction of the Wnt / beta-catenin signalling pathway and plays a leading role in the pathogenesis of fibrosis and diabetes complications. Recently study suggested involvement of CCN family proteins in adipogenesis. Here we tested hypothesis that WISP1 gene expression may be regulated by weight changes and insulin. Moreover, we examined WISP1 gene expression in subjects with nonalcoholic fatty liver (NAFL), as an example of ectopic fat accumulation.
Materials and methods: In a subcohort of "Diet, Obesity, and Genes" (DiOGenes) study (n = 45), weight loss of at least 8% of baseline body weight was achieved using a low-calorie diet. Subcutaneous fat biopsies were obtained before and after weight loss. Monocytes were isolated from the whole blood and differentiated with GM-CSF and M-CSF into macrophages. Human mesenchymal stem cells were differentiated in vitro into adipocytes. On 1st, 5th and 12th day of differentiation RNA was isolated. Differentiated adipocytes were incubated with 100nM insulin for 4 hours. In a cross-sectional study (n=29), samples of liver tissue was taken intrasurgically and graded histologically by an expert pathologist according to the NAFLD activity score. The mRNA expression of WISP1 was measured with RT-PCR in subcutaneous adipose tissue biopsy, in differentiated adipocytes, monocytes and macrophages as well as in liver samples.
Results: Weight loss of 10.9 ± 3.6 kg was achieved after low-calorie diet. Gene expression of WISP1 fell significantly after weight reduction (mean ± SEM, 0.66 ± 0.08 vs. 0.52 ± 0.06; P <0.05). A strong and significant increase in WISP1 gene expression during the differentiation of adipocytes on 5th and on 12th day and after stimulation with 100nM insulin was observed. In monocytes as well as in M-and GM-macrophages we couldn't identify WISP1 at mRNA level. We found no correlation between NAFLD activity score as well as liver fat content and WISP1 gene expression in NAFL patients.
Conclusion: Weight loss decreased WISP1 expression in subcutaneous adipose tissue in vivo, referred only to gene expression in adipocytes. Adipocytes differentiation is associated with increase of WISP1 expression and is positively regulated by insulin. WISP-1 expression was not altered in the NAFL. Thus, WISP1 plays an important role in adipogenesis and can be used as a marker of adipose tissue differentiation in obesity and lipodystrophy, but not as marker of ectopic fat accumulation.
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The potential role of iron and endogenous transferrin biosynthesis in human and 3T3-L1 adipocyte differentiation

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Background and aims: Cross-sectionally, iron metabolism-related protein gene expression has been found to be altered in adipose tissue in association with obesity and insulin sensitivity. Transferrin (*TF*) gene expression decreased in association with obesity and insulin resistance, whereas ferritin light chain (*FTL*) followed an opposite pattern. We aim to investigate the effects of iron overload and/or depletion in adipocyte differentiation.
Materials and methods: Human and 3T3-L1 preadipocytes were incubated with fresh media (control), fresh media containing $FeSO_4$(3 and 30 μg/ml), deferoxamine (DFO, 20 and 100 μM), and several $FeSO_4$and DFO combinations during human and 3T3-L1 adipocyte differentiation. Permanent silencing was also performed using Tf- and Ftl-targeted and control shRNA lentiviral particles.
Results: In both human and 3T3-L1 preadipocytes, iron was required for a timely adipocyte differentiation. Both iron in excess and iron chelation led to either a slight decrease in adipocyte differentiation (decreasing adipogenic gene expression) or to pronounced anti-adipogenic effects (using deferoxamine). In fact, iron replacement reversed the antiadipogenic effects of deferoxamine. As expected, added iron and iron chelation led to reciprocal changes of transferrin receptor (*TFRC*), while iron chelation resulted in decreased ferroportin (*SLC40A1*) and increased ferritin heavy chain (*FTH1*). *TF* gene expression decreased with excess iron. Interestingly *TF* followed a similar gene expression pattern to that of other adipogenic genes with iron chelation, whereas *FTL*significantly increased in these anti-adipogenic conditions. *Tf* knockdown (KD) led to a significant decrease of adipogenic mRNAs (such as *Fabp4, Cebpa, Pparg, Glut4, Adipoq*) while increasing *IL6*gene expression. In contrast, in *Ftl*KD cells, no significant effects were found in adipogenic gene expression. Furthermore, in both *Tf* KD and *Ftl* KD 3T3-L1 cells, the highest dose of iron led to increased expression of adipogenic genes in contrast to control 3T3-L1 cells, suggesting higher iron requirements necessary for adipocyte differentiation in these cells.
Conclusion: A precise and fine-tuned iron handling is essential to ensure a delicate and appropriate cross-talk among adipogenesis, adipose tissue inflammation and insulin action.
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Impaired leptin regulation by epigenetic methylation as a marker of immature preadipocyte function in low birth weight subjects

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Background and aims: Low birth weight (LBW) is associated with increased risk of developing T2D. Immature development of subcutaneous adipose tissue might contribute to risk of lipotoxicity, insulin resistance and T2D in LBW subjects. Leptin is an accepted marker of adipocyte differentiation and plays a key role in the regulation of appetite. We hypothesized that leptin is dysregulated in adipocytes of LBW individuals compared to normal birth weight (NBW) individuals.
Materials and methods: We recruited 14 men born with LBW (mean birth weight 2.7±0.1 kg) and 13 controls born with NBW (mean birth weight 3.7±0.2 kg). Biopsies were obtained from subcutaneous abdominal fat depot and preadipocytes were isolated and cultured. Gene expression of leptin and selected differentiation markers were analyzed using qPCR during preadipocyte differentiation and cell culture media was collected to analyze leptin secretion. Lipid accumulation was analyzed as an end-stage measure of differentiation and lipid storage capacity. DNA methylation of specific CpG sites in the leptin promoter was measured using pyrosequencing.
Results: Differentiating preadipocytes from LBW individuals showed reduced leptin gene expression and a corresponding reduced leptin release compared to NBW individuals. Mean DNA methylation of the proximal LEP promoter was increased in LBW compared to NBW individuals. The notion of a reduced adipocyte function in LBW individuals was supported by a lower expression of fatty acid binding protein 4 (FABP4) mRNA, and a tendency of reduced expression of other differentiation markers.
Conclusion: Differentiating preadipocytes of LBW subjects have an epigenetically dysregulated leptin production which might explain the predisposition of these individuals to T2D.
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Bivalent histone modification of the promoter and its resolution contribute to the epigenetic regulation of PPARγ gene expression and adipocyte differentiation

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Background and aims: Recent genome-wide studies of histone modification in embryonic stem (ES) cells revealed that the promoters of a subset of developmental genes are marked by both active histone H3K4 trimethylation (me3) and repressive H3K27me3, which is termed bivalent domain and proposed to represent the poised status of the developmental genes.We tried to clarify whether bivalent histone modification of the promoter and its resolution could contribute to the epigenetic regulation of PPARγ gene expression and adipocyte differentiation.
Materials, methods and results: Here we show that the PPARγ1 promoter is marked by the bivalent modification in ES cells, murine embryonic fibroblasts (MEFs) and adipocyte progenitor cells in white adipose tissue whereas it is marked by only H3K4me3 in mature adipocytes and adipogenic cell lines including 3T3-L1. Upon differentiation, MEFs lose H3K27me3 at the PPARγ1 promoter and it resolves to H3K4me3 only. Knockdown of the H3K27 demethylases Jmjd3 and Utx by siRNA attenuates the reduction of H3K27me3 and causes suppression of adipocyte differentiation, suggesting that the resolution of the bivalent domain is required for adipocyte differentiation of MEFs. Interestingly, the resolution of the bivalency is not dependent on the addition of the adipogenic inducers and differentiation per se, but rather on the serum context: It resolves to H3K4me3 in fetal bovine serum while it turns to H3K27me3-dominant pattern in calf serum, in which they lose responsiveness to the adipogenic cocktail for differentiation.
Conclusion: Our findings imply that the bivalent modification of the PPARγ1 promoter in *in vivo* adipocyte stem/progenitor cells needs to undergo chromatin remodeling and its resolution is a prerequisite for the subsequent activation of PPARγ expression in response to the adipogenic cue.

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Dual effects of inflammation and insulin resistance on markers of renewal of adipose tissue

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Background and aims: The development of adiposity is dependent on the coordinated interplay between the proliferation and differentiation of adipocytes. The tumor suppressor protein 53 (p53) is a key regulator of various cellular networks, including proliferation, differentiation, and metabolism. The purpose of this study was to investigate the expression of human adipose tissue (AT) *p53* in subjects who varied widely in terms of obesity and insulin resistance. We also analyzed different *in vivo* and *in vitro* models to try to comprehend the associations found in human AT.
Materials and methods: p53 and its modulating partner, the mouse double minute 2 (MDM2), were assessed in subcutaneous (SC) and omental (OM) AT from humans and in isolated fat cells, during *in vitro* adipogenesis, and in high-fat diet induced obese and GLP1R KO mice. The effects of the surgery-induced weight loss and *ex vivo* treatments with metformin in adipose tissue were also studied. The impact of *in vitro* high glucose concentrations, rosiglitazone, and inflammatory conditions such as the macrophage lipopolysaccharide (LPS) - conditioned medium (LPS-MCM) on *p53* expression in adipocytes was evaluated.
Results: OM *p53* gene expression (+27%, p=0.001) and protein (+11%, p=0.04) were increased in obese subjects and high fat diet-induced obese mice (+86%, p=0.018). Although the obesity-associated inflammatory milieu was associated with increased OM p53, AT *p53* was negatively related to insulin resistance and glycated hemoglobin, and positively with biomarkers for insulin sensitivity. Multiple linear regression analyses revealed that glycated hemoglobin (p<0.0001) and BMI (p=0.048) contributed independently to explain 13.7% (p<0.0001) of OM *p53* variance. Accordingly, the improvement of insulin sensitivity with surgery-induced weight loss (+51%, p=0.01) and metformin (+42%, p=0.02) led to increased *p53*, the glucose intolerant GLP-1R KO mice showed decreased AT *p53* (-45.4%, p=0.017), and high glucose led to decreased *p53* in pre-adipocytes (-27%, p<0.0001). Inflammatory treatments led to increased *p53* (+35%, p<0.0001), while rosiglitazone down-regulated this expression (-40%, p=0.005) in mature adipocytes.
Conclusion: Inflammation and insulin resistance exert dual effects on AT *p53*, which seems to be the final result of these opposing forces. Contrary to expected, inverse associations with insulin resistance and type 2 diabetes were reported.

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A novel role of Opa-interacting protein 5 in obesity

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Background and aims: We have shown that visceral fat adiposity plays the central role of metabolic syndrome. We hypothesized that visceral fat accumulation may reflect on gene expression profile in peripheral blood cells and recently reported the association between visceral fat adiposity and gene expressions of peripheral blood cells in human subjects. In a series of study, we searched genes of unknown function in adipocytes or adipose tissues by comparing the cDNA microarray-based gene expression patterns of human peripheral blood cells and mouse adipose tissues. OIP5 (Opa-interacting Protein 5), one of candidate genes, was nominated in this process and herein was investigated in the role of obesity.
Materials and methods: In human study, the estimated visceral fat area (eVFA) was measured by abdominal bioelectrical impedance analysis. Blood total RNA samples were obtained from 28 subjects (BMI 31.9±6.0 kg/m^2, eVFA 199.4±89.4 cm^2) and were subjected to Whole Human Genome 4 × 44 K oligo-DNA microarray (Agilent Technologies, Inc.). For in vivo study, male

C57BL/6J (B6) mice and ob/ob (Ob) mice were analyzed at the same weeks of age. White adipose tissues (WAT) of B6 and Ob mice were subjected to GeneChip Mouse Genome 430 2.0 Array oligo-DNA microarray (Affymetrix, Inc.) at 25 weeks of age. WAT was fractionated into mature adipocytes fraction (MAF) and stromal vascular cell fraction (SVF) by using collagenase. For in vitro Oip5-knockdown experiments, 3T3-L1 adipocytes were transfected with siRNA for Oip5. In the Oip5-overexpression studies, adenovirus expressing Oip5 (Ad-Oip5) was constructed and infected into 3T3-L1 cells stably expressing Coxsackie-Adenovirus Receptor (CAR-3T3-L1). Adenovirus expressing β-galactosidase was used as a control (Ad-βgal). Proliferation of 3T3-L1 preadipocytes was measured by bromodeoxyuridine (BrdU) uptake and cell count.

Results: Fourteen genes, which expressions are similar directions between human and mouse according to adiposity, were extracted by comparing the cDNA microarray-based gene expression patterns of human peripheral blood cells and mouse WAT. We narrowed candidate genes down from 14 to 7 genes in view of its unknown function in adipose tissues. Among 7 genes, OIP5 mRNA levels in peripheral blood cells were positively correlated with eVFA in human subjects. Oip5 mRNA was detected ubiquitously and its adipose mRNA level was significantly higher in Ob mice than B6 mice. Such increase was similarly observed in diet-induced obese (DIO) mice. Oip5 mRNA levels of MAF and SVF in Ob mice were significantly increased 2.1-fold and 5.7-fold, respectively, compared to B6 mice. Next, the functional role of adipose Oip5 was investigated in 3T3-L1 preadipocytes. BrdU uptake and the number of preadipocytes were significantly reduced in Oip5-knockdown cells, while proliferation of CAR-3T3-L1 preadipocytes was significantly increased by the infection of Ad-Oip5 compared to Ad-βgal. Finally, Ad-Oip5 or Ad-βgal was locally administered to subcutaneous WAT of B6 mice. Ad-Oip5-injected WAT was significantly heavier than Ad-βgal-treated WAT. Cell size of adipocytes was generally smaller in Ad-Oip5-administered WAT compared to Ad-βgal-treated WAT.

Conclusion: Oip5 may contribute to adipose tissue hyperplasia and obesity.

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Adipocyte morphology and implications for metabolic derangements in obesity

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Background and aims: Adipocyte size and number have long been suggested to predict the development of metabolic complications in obesity. However, the genetic and environmental determinants behind this phenomenon remain unclear.

Materials and methods: We studied this question in rare weight-discordant (intra-pair difference (Δ) in BMI 3-10 kg/m², n=15) and concordant (ΔBMI 0-2 kg/m², n=5) young adult (22-35 years) monozygotic twin pairs identified from ten birth cohorts of Finnish twins (n=5500 pairs). Subcutaneous abdominal adipocyte size from surgical biopsies was measured under a light microscope and adipocyte number was calculated from cell size and total body fat (DXA).

Results: The concordant pairs were remarkably similar for adipocyte size and number (intra-class correlations 0.91-0.92, p<0.01), suggesting a strong genetic control of these measures. In the discordant pairs the obese co-twins (BMI 30.6±0.9kg/m²) had significantly larger adipocytes (volume 547±59pL), than the lean co-twins (24.9±0.9kg/m²; 356±34pL, p<0.001). Intrapair differences (Δ) in cell number varied widely between pairs but on average the cell numbers did not differ between the obese ($8.29\pm0.6x10^{13}$) and lean ($8.75\pm0.7x10^{13}$, p=0.61) co-twins. In all pairs, Δadipocyte volume correlated positively and Δcell number negatively with ΔHOMA-index and ΔLDL, independent of Δbody fat. In 8/15 pairs, the obese co-twins had less adipocytes than their co-twins. These hypertrophic+hypoplastic obese had significantly higher liver fat (spectroscopy), HOMA-index, CRP and ΔLDL-cholesterol than their lean twin pair members. In the transcriptomics analyses (Affymetrix), transcripts most significantly correlating with Δadipocyte volume were related to reduced mitochondrial function, membrane modifications, DNA-damage and cell death.

Conclusion: Increased adipocyte size and decreased number in obesity are related to metabolic dysfunction, possibly through disturbances in mitochondrial function and increased cell death within the adipose tissue.

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Blockade of lysophosphatidic acid receptors reduces adipose tissue fibrosis and improves insulin resistance

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Background and aims: Fibrosis is one of the obesity-associated complications involved in the etiology of different diseases such as hepatic cirrhosis, nephropathies or cardiomyopathies. Recently, adipose tissue (AT) fibrosis was demonstrated in obese mice and humans and current studies suggest an involvement of this fibrotic process in the onset of associated insulin resistance. Lysophosphatidic acid (LPA) has been described as a potent pro-fibrotic mediator in several organs mainly via its G protein-coupled receptor LPA1R. LPA is a bioactive lipid produced by a lysophospholipase D called autotaxin. In both, human and mice, this enzyme is secreted by the adipose tissue and its expression is up-regulated during obesity in close association with insulin resistance. In the present study, we examined whether LPA might be involved in AT fibrosis and insulin resistance associated with obesity.

Materials and methods: Human subcutaneous AT explants were maintained for 72h in primary culture in the absence or the presence of 1-oleoyl-LPA (10μM) and/or the LPAR antagonist Ki16425 (10μM) and/or the HIF1α inhibitor YC1 (100 μM) before quantifying fibrosis genes and proteins. In parallel, six week-old db/db mice were treated for 7 weeks with the LPAR antagonist Ki16425 (5mg/kg/day, ip) before investigating glucose metabolism and quantify fibrosis genes and proteins in inguinal AT.

Results: Ex vivo, AT explants showed a spontaneous increase in autotaxin mRNAs (4.2 ± 0.8 fold; P<0.001) with accumulation of LPA in the culture medium. This was associated with an up-regulation of fibrosis marker mRNAs (collagen I: 4.8 ± 0.9 fold P<0.001; collagen III: 2.4 ± 0.5 fold P<0.01). Spontaneous fibrosis was not observed in the presence of Ki16425. On the opposite, in the presence of 1-oleoyl-LPA, spontaneous fibrosis was further amplified at both mRNA (collagene I: 7.3 ± 1.1 fold P<0.01; collagen III: 7.0 ± 2.1 fold P<0.001) and protein (collagen staining with sirius red: 29.9 ± 4.7 vs 8.6 ± 3.3 % stained surface; P<0.01 in LPA and control respectively). The pro-fibrotic activity of oleoyl-LPA was blocked by co-treatment with Ki16425 or the HIF1α-inhibitor, YC1. In vivo, db/db mice developed massive obesity, insulin resistance and up-regulation of ATX and fibrosis markers in AT when compared to non obese db/m mice. Chronic treatment of db/db mice with Ki16425 reduced types I, III and IV collagen mRNAs (0.56 ± 0.07, 0.63 ± 0.09 and 0.62±0.07 fold; P<0.05) and reduced Sirius red collagen staining (13.9 ± 1.8 vs 20.4 ± 2.4 % stained surface; P<0.05) in AT when compared to control treated mice. Ki16425-treatment also reduced insulinaemia (7581 ±212 pg/ml vs 8351 ±270 pg/ml; P<0.05), glycaemia (262±28 mg/dl vs 343 ±24 mg/dl; P<0.05) and the AUC of insulin tolerance test (11985 ± 457 vs 13778 ± 312 % x min; P<0.05). AT fibrosis decrease is associated with adipocytes hypertrophy without inflammation increase. HIF1α pathway is currently being studied in this in vivo model.

Conclusion: Our results suggest that increased production of LPA with obesity leads to AT fibrosis through a pathway involving LPARs and HIF-1a. In parallel, LPA impairs insulin-tolerance of db/db mice. Further investigations will aim to determine whether there exists a causal relationship between the pro-fibrotic action of LPA and its impact on insulin-sensitivity.

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Collagen VI mRNA expression is regulated by leptin: a potential physiological feedback mechanism regulating adipose tissue expandability

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Background and aims: Reduced adipose tissue (AT) expandability is now recognised as a driver of ectopic fat deposition, impaired metabolic health and diabetes progression, and is in part modulated by alterations in extracellular matrix (ECM) composition. Collagen VIα3, a pericellular ECM protein, is upregulated in the adipose tissue of diabetic and obese mice whilst knockout of the protein in metabolically compromised animals leads to improved metabolic health. Few studies have assessed the role of *COL6A3* in humans. We therefore characterised the adipose tissue expression of *COL6A3* in obese subjects, subjects with diabetes and in individuals following diet induced weight loss. We also assessed *COL6A3* expression in adipose tissue following treatment with metabolic regulators.
Material and methods: Subcutaneous abdominal and omental biopsies were obtained from nine lean (BMI 22.6±0.5kg/m^2[mean±SEM]) and obese (32.4±4.0 kg/m^2) age and gender matched subjects. Subcutaneous adipose tissue was available from nine subjects with type 2 diabetes (T2DM) and matched non-diabetic controls. To assess expression changes during weight loss, 24 obese subjects (37.6±4.9kg/m^2) were placed on a very low calorie diet (VLCD) for 16 weeks and subcutaneous biopsies obtained pre and post study. To assess regulation of *COL6A3*, AT biopsies were minced into 1mm^3 explants and treated with recombinant human leptin or insulin (0.01nM, 10nM, 100nM) for 24 hours. mRNA expression was determined using Taqman technology or microarray. All data is expressed in Arbitrary Units.
Results: *COL6A3* mRNA was expressed at significantly higher levels in subcutaneous versus omental adipose tissue, independent of BMI (1.15±0.09 vs 0.85±0.12 [mean±SEM], n=12, p<0.05). A trend towards reduced *COL6A3* expression was observed in biopsies from obese subjects versus lean although this did not reach statistical significance. In line with these findings, caloric restriction and weight loss resulted in a significant increase in *COL6A3* mRNA expression in subcutaneous adipose tissue (1725±77.19 vs 2412±100 [mean±SEM], p<0.01). Treatment of subcutaneous explants with leptin resulted in a dose dependent decrease in *COL6A3* expression (1.66±0.23 vs 1.34±0.13 vs 1.11±0.13* vs 1.03±0.23*, Control vs 0.01nM vs 10nM vs 100nM, *=p<0.05). Treatment with insulin did not alter *COL6A3* expression across the same concentration range nor was there any significant alteration in expression in subjects with T2DM.
Conclusion: Unlike rodents, human adipose tissue *COL6A3* expression does not correlate with impaired metabolic health, does not respond to insulin but conversely decreases with obesity. The observation that *COL6A3* mRNA levels increase following weight loss, as leptin levels decrease, coupled with our data highlighting the direct repression of *COL6A3* mRNA following leptin treatment, suggest the presence of a potential paracrine regulatory mechanism in which expanding adipose tissue is able to regulate levels of pericellular collagen to permit matrix remodelling.

PS 057 Autophagy, endoplasmic reticulum and oxidative stresses

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PPARβ/δ attenuates palmitate-induced endoplasmic reticulum stress and provokes autophagy in human cardiac cells

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Background and aims: Apoptosis is an early cellular event in response to diabetes that plays a critical role in the development of diabetic cardiomyopathy. The endoplasmic reticulum (ER) stress is activated in stressed cells with the aim to restore homeostasis through autophagy. However, if it becomes chronic, ER stress will contribute to the apoptotic cell death in the myocardium. ER stress is induced after high fat diet feeding in mice and also after saturated fatty acid treatment in vitro. Therefore, since several studies have shown that PPARβ/δ activation inhibits the ER stress, the main objective of this work was to investigate whether activation of this nuclear receptor prevented lipid-induced ER stress in cardiac cells.
Materials and methods: Wild-type and transgenic mice with reduced expression of PPARβ/δ were fed a standard diet or a high fat diet for two months. For in vitro studies, a cardiomyocyte cell line of human origin, AC16, was treated with palmitate and the PPARβ/δ agonist GW501516, in the presence or absence of several other drugs in order to elucidate the mechanisms involved. After treatment, RNA and total protein extracts were obtained for subsequent gene expression and western blot analyses.
Results: Palmitate induced ER stress in AC16 cells, as confirmed by the i creased levels of spliced XBP1, ATF3 (2-fold vs. control, P<0.001), BiP/GRP78 (4.5-fold, P<0.001), and CHOP (4.5-fold, P<0.001) expression, as well as it enhanced CHOP (4.5-fold, P<0.001) and phosphorylated IRE-1α (2-fold, P<0.05) protein levels. Activation of PPARβ/δ with GW501516 abrogated palmitate-induced effects on ATF3 (P<0.01) and CHOP (P<0.05) expression, phosphorylated IRE-1α (P<0.001), and CHOP (P<0.001) protein levels. In contrast, GW501516 did not prevent the splicing of XBP1 or the enhanced expression of BiP/GRP78. Coincubation with the AMPK inhibitor compound C demonstrated that GW501516 effects on ER stress in AC16 cells were independent of AMPK activation. Interestingly, GW501516 upregulated the protein levels of beclin 1 (1.5-fold vs. control, P<0.001) and LC3II (1.5-fold, P<0.001), two well-known markers of autophagy. In accordance with this, administration of high-fat diet or suppression of PPARβ/δ in transgenic knockout mice, induced ER stress in the heart, as depicted by the augmented BiP/GRP78 and CHOP expression (1.5-fold vs. wild type, P<0.05), and the phosphorylated IRE-1α (2-fold, P<0.05) protein levels. Moreover, these transgenic PPARβ/δ knockout mice also displayed a reduction in autophagic markers (about 50% reduction in beclin 1 protein levels vs. wild type, P<0.05).
Conclusion: PPARβ/δ activation might be useful to prevent the development of diabetic cardiomyopathy and associated cardiovascular disease in obese patients, since it would inhibit the harmful effects of ER stress in heart by inducing autophagy.
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Deletion of hepatic ROCK1 prevents steatosis by reducing lipid synthesis and activating autophagic flux in diet-induced obese mice

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with obesity and insulin resistance and is a risk factor for hepatocellular carcinoma. The physiological mechanisms underlying NAFLD are unclear, although thought to result from impaired lipid homeostasis. Emerging data suggest that Rho-kinase 1 (ROCK1) plays an important role in regulation of glucose metabolism and insulin sensitivity. However, hepatic functions of ROCK1 have not been addressed. Autophagy is a highly regulated process in eukaryotic cells and is determinant for cellular homeostasis. The microtubule-associated protein light chain 3 (LC3) is a key molecule of the autophagy signalling pathway. Evidence shows that autophagy plays a role in lipid metabolism. However, nothing is known about hepatic ROCK1 functions in the autophagy signalling pathway. This study determined the physiological role of hepatic ROCK1 in regulating lipid metabolism in conjunction with autophagy signaling.
Materials and methods: Liver-specific ROCK1-deficient mice (LKO) fed a high-fat diet were studied. Hepatic lipid metabolism was measured by NMR and immunohistochemistry. De novo lipogenesis was determined by using U-^{14}C lactic acid as an indicator of lipogenic rate. Autophagy was assessed by immunoblotting of Beclin1, Atg7 and LC3 I/II in fast versus fed state.
Results: After 6-12 weeks of high-fat diet, hepatic triglyceride was decreased in LKO (54.53±2.58 vs. 40.37±3.49 mg/g, p<0.05), as well as cholesterol (4.96±0.58 vs. 2.86±0.81 mg/g, p<0.05), compared with control mice. Histological analysis also indicated reduced hepatic steatosis by ROCK1 deletion. The physiological mechanism underlying this is, in part, due to decreased lipogenesis; loss of ROCK1 caused a decrease in lipogenic rate (10.46±0.48 vs. 6.75±0.53 nmol/mg/hr, p<0.001), LKO mice have decreased hepatic triglycerides fractional synthetic rate in (100.0±9.91% vs. 66.86±7.61%, p<0.05). To determine whether decreased lipogenesis in LKO could be due to activation of autophagy, protein levels of autophagy signaling components were measured. Hepatic Beclin1 and Atg7 were unchanged by ROCK1 deletion. Levels of LC3-I protein in the fast state were decreased by ~40% (p<0.05) in LKO mice, compared with control mice. This implies elevation of the autophagic-vesicle associated form LC3-II, suggesting an increase of the hepatic autophagic flux in LKO mice.
Conclusion: Our data demonstrate that targeted deletion of ROCK1 in liver protects against diet-induced hepatosteatosis in mice. These effects are most likely due to decreased de novo lipogenesis in hepatocytes. Furthermore, increased autophagic flux in the liver could be involved in this regulation. Together, our data identify hepatic ROCK1 as important regulator of lipid metabolism in the context of the autophagy signalling pathway.
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The PPARβ/δ agonist GW501516 inhibits endoplasmic reticulum stress-induced inflammation in skeletal muscle cells

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Background and aims: Evidence is now emerging that the activation of nuclear receptor PPARβ/δ prevents fatty acid-induced inflammation and insulin resistance (IR) in skeletal muscle cells. Given that endoplasmic reticulum (ER) stress is associated with inflammation and IR, we evaluated whether the PPAR β/δ agonist, GW501516, was able to prevent the ER stress.
Materials and methods: Skeletal muscle cells of human (LHCN-M2) and mouse (C2C12) origin were incubated for 16 hours with thapsigargin (1μM in C2C12 and 100nM in LHCN-M2), tunicamycin (5μg/ml in C2C12 and 2μg/ml in C2C12 and LHCN-M2) and GW501516 (10μM). Levels of mRNA were assessed by reverse transcription-polymerase chain reaction (RT-PCR) and Western Blot analysis was performed to determine protein levels. Data are expressed as means ± S.D. of three independent experiments. Significant differences were established by one-way ANOVA and when significant variations were found, the Tukey-Kramer multiple comparisons post-test was applied. Differences were considered significant at p<0.05.
Results: Exposure of C2C12 and LHCN-M2 cells to the potent ER-stress inducer thapsigargin, increased the mRNA levels of sXBP1 (p<0.001 *vs* control in C2C12 and p<0.01 vs control in LHCN-M2), whereas in the presence of GW501516 these effects were reduced (p<0.001 *vs* thapsigargin in C2C12 and p<0.01 *vs* thapsigargin in LHCN-M2). The ER stress inducer tunicamycin also increased the mRNA levels of CHOP (p<0.001 *vs* control cells) in C2C12 cells, and thapsigargin and tunicamicyn increased CHOP and ATF3 (p<0.001 *vs* control cells) gene expression in LHCN-M2 cells. GW501516 reduced tunicamycin-induced CHOP expression in murine myotubes (p<0.001) as well as tunicamycin-induced CHOP (p<0.001) and ATF3 (p<0.01) mRNA levels in human myotubes. Moreover, GW501516 reduced thapsigargin-induced ER stress in human myotubes (p<0.001 in CHOP and ATF3 mRNA levels). Finally, TNFα mRNA levels (p<0.05 vs control) were increased in C2C12 cells exposed to thapsigargin as well as IL-6 expression (p<0.001 *vs* control) was increased in murine and human myotubes exposed to tunicamycin. Again, GW501516 prevented these effects (p<0.05 vs thapsigargin and p<0.001 *vs* tunicamycin exposed murine myotubes and p<0.01 *vs* tunicamycin exposed human myotubes). GW501516 also prevented the thapsigargin-mediated reduction in IκBα protein levels (p<0.01 *vs* thapsigargin-exposed cells) in C2C12 cells, suggesting that the PPARβ/δ agonist was able to prevent ER stress-induced inflammation.
Conclusion: Our results suggest that the PPARβ/δ agonist, GW501516, might prevent ER stress-induced inflammation in murine and human skeletal muscle cells. At present we are investigating the mechanism responsible for the effects of GW501516 on ER stress and its impact on insulin resistance.
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Inhibition of autophagic flux is associated with increased endoplasmic reticulum stress during the development of non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is an inflammatory chronic disease which is the main hepatic complication of obesity and metabolic syndrome. In fact, epidemiologic and clinical studies have shown a close association between NAFLD and insulin resistance. We have previously reported that hepatic levels of insulin signalling mediators and phosphorylation of Akt and Foxo1 are down-regulated in patients with steatohepatitis (NASH), but not in patients with steatosis (NAS). Furthermore, hepatocyte apoptosis and active caspase 3 were only present in NASH patients. In this study, we have evaluated the relationship between ER stress and the autophagic flux during the onset of NAFLD in human and murine models of hepatic steatosis and NASH.
Materials and methods: This study comprised 59 patients with a clinical diagnosis of NAS or NASH who underwent a liver biopsy and 30 patients with histological normal liver (NL). Real-time PCR, Western blot, immunohistochemistry and electronic microscopy were used to assess markers of ER and autophagy. Mice fed with high fat diet (HFD) for 7 months or methionine-choline-deficient (MCD) diet during 4 weeks were used to study ER stress and autophagy in the liver. Human Huh7 hepatocytes loaded with palmitic acid were used as an in vitro model.
Results: In human liver from patients with NAS and NASH, mRNA levels of ER stress markers were elevated together with increased p62 autophagic substrate and LC3II accumulation. However, LC3 punctuate was less evident in patients with NASH. Mice fed with HFD or MCD diet showed increased phosphorylation of mTOR/S6K1, JNK, PERK, eIF2α together with elevated

expression of ER chaperones GRP78 and CHOP. As observed in patients, p62 and LC3II were up-regulated by both diet interventions. In Huh7 hepatic cells, treatment with palmitate for 8 h activated the autophagic flux by decreasing p62 and cell death was not observed; this effect was blocked at 24 h, time at which ER stress markers and cell death were highly elevated and autophagic flux was blocked. Co-treatment with rapamycin and palmitate restored autophagic flux and protected Huh7 cells against lipoapoptosis.
Conclusion: The impaired autophagic flux associated to NAS and NASH might be the consequence of elevated ER stress in the liver, resulting in apoptosis. Our results strongly suggest that therapies aimed to restore the autophagic flux in patients with severe hepatic steatosis might prevent the progression of the non-alcoholic liver disease.
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Correction of intermittent hypoxia with CPAP improves cardio-metabolic abnormalities, systemic inflammation, and adipose tissue ER stress in obese subjects with OSA
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Background and aims: Morbid obesity is frequently associated with cardio-metabolic abnormalities, low-grade systemic inflammation, inflamed adipose tissue, and obstructive sleep apnea (OSA). It is unclear whether in obesity chronic intermittent hypoxia (CIH) resulting from OSA could be an independent factor for the development of cardio-metabolic abnormalities. We hypothesized that treatment of CIH with continuous positive airway pressure (CPAP) in obesity associated with OSA would modify cardio-metabolic and inflammatory outcomes.
Materials and methods: Sixty-two obese patients with OSA were studied at baseline and after three months of therapeutic CPAP treatment, and compared with twenty-five BMI- and age-matched subjects who had a negative single-night polysomnogram. Both the OSA and control obese groups received general dietary and exercise advice throughout the study. Measurements of anthropometric variables, blood pressure, fasting blood glucose and lipid profile, insulin resistance (HOMA model), HbA1c, white blood cells were obtained at baseline (T0) and after three months (T1). Serum concentrations of inflammatory cytokines were measured at T0 and T1 using Luminex. Subcutaneous (Sc) adipose tissue biopsies were taken at T0 and T1 to assess expression of endoplasmic reticulum (ER) stress-related genes by quantitative RT-PCR.
Results: At T0, neck circumference (41.9 vs. 39.0 cm; p=0.01), systolic (129.8 vs. 121.2 mmHg; p=0.03) and diastolic blood pressure (83.3 vs. 75.1 mmHg; p=0.017), HOMA-IR (8.7 vs. 4.2%; p=0.005), HbA1c (6.01 vs. 5.42%; p=0.01), serum triglyceride (147.8 vs. 121.9 mg/dL; p=0.03) and white blood cells (7,829 vs. 6,135 per μL; p=0.001) were significantly increased in the OSA compared to control subjects. At T1, CPAP treatment resulted in significant mean decreases in BMI (-4 kg/m2; p=0.003), waist (-10.6 cm; p=0.01) and neck (-3 cm; p=0.01) circumferences, systolic (-15.4 mmHg; p=0.03) and diastolic (-12.3 mmHg; p=0.03) blood pressure, serum total cholesterol (-19.6 mg/dL; p=0.04), serum triglyceride (-34.0 mg/dL; p=0.003), and white blood cells (-1,172 per μL; p=0.03) in the OSA obese group, whereas no significant changes in these parameters were observed in the control obese group. Serum concentrations of IL-1ra, IL-2, IL-4, IL-6, MCP-1 and PDGF were also significantly reduced compared to T0 by CPAP therapy in OSA subjects ($p<0.05$), and remained unaltered in the controls. Dietary intakes and levels of physical activity did not differ between groups at T1 vs. T0. In Sc adipose tissue, mRNA expression levels of CHOP were significantly reduced after CPAP treatment as compared to T0 (p=0.02) in OSA subjects, and a tendency toward lower mRNA levels was also observed for ATF4 and ERO-1 (p=0.08). No significant changes in ER stress gene expression were observed in the control obese group.
Conclusion: Three months of CPAP therapy in obese patients with moderate-to-severe OSA improves anthropometric variables, cardio-metabolic abnormalities, systemic inflammation, and fat-specific ER stress, suggesting an independent role of CIH on metabolic, cardiovascular and inflammatory endpoints in obesity.
Clinical Trial Registration Number: 152/C.E.
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Differential effects of oleate and palmitate on beta cell autophagy

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Background and aims: Increasing evidence reveals the importance of autophagy in beta-cell homeostasis and survival. Indeed deterioration of beta-cell induced by chronic exposure to fatty acids is suggested to be associated with alterations in autophagy. However, it is still controversial and unclear how fatty acids regulate autophagy. Here, we compare the effects of free fatty acids palmitate and oleate on beta-cell autophagic flux, and investigate the differential mechanisms underlying their effects.
Materials and methods: Mouse beta-cell MIN6 cells were treated with fatty acids for 0-48h and the levels of autophagic marker, LC3-II, were detected by western blot to show the steady state of autophagy. Autophagic flux was confirmed by incubating the cells with chloroquine to prevent autophagosome degradation. MIN6 cells were transfected with the RFP-GFP-LC3 plasmid prior to fatty acids treatment, the dynamic of GFP disappearance was monitored to show the autophagosome-lysosome fusion. Lysosomes in the cells were localized after fatty acids treatment with the LysoTracker Red and visualized by the microscopy. Autophagy in PBA, an ER stress inhibitor, and fatty acids co-treated cells was also studied. Finally, effect of fatty acids on cathepsin L, a lysosomal enzyme, was investigated by western blot and measuring its activity.
Results: Our results demonstrated that although palmitate treatment only minimally altered steady state of LC3-II, it did increase the autophagic flux. Palmitate enhanced ER stress and PBA reduced ER stress as well as the LC3-II levels. Palmitate also induced autophagosome-lysosome fusion, which might due to either increased lysosome number or size; but it did not alter cathepsin L activity. Oleate, on the other hand, increased both autophagic basal levels and flux. Oleate also augmented autophagosome-lysosome fusion and lysosomal labeling. Cathepsin L activity was also elevated by oleate and its inhibition abolished the oleate-induced autophagy.
Conclusion: Saturated and unsaturated fatty acids exhibit remarkably divergent effects on beta-cell autophagy. Palmitate appears to activate autophagy solely to ER stress and dependently regulates downstream pathways. Oleate, on the other hand, stimulates autophagy via novel mechanism involving activation of catheipsin L.
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DNAJB3/HSP-40 cochaperone role in obesity
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Background and aims: Obesity is a major risk factor for insulin resistance and diabetes. Heat shock response; a crucial host-defence mechanism against stressful conditions, is one of the key pathways that was shown to be deregulated in obesity-induced insulin resistance. Patients with T2D have reduced expression of Heat shock proteins that correlates with increased insulin resistance and therapies known to induce heat shock response such as heat therapy or physical exercise are associated with improved outcomes. The objectives of this study are: 1) Investigate the expression pattern of Hsps and other chaperones and stress related proteins between lean and obese subjects, 2) Evaluate the effect of a defined exercise protocol on their expression pattern and 3) Correlate their expression with the inflammatory, metabolic and oxidative stress biomarkers.
Materials and methods: The current investigation is part of a large cohort study aimed at investigating the effect In the present study, we selected 120 non-diabetic subjects consisting of 66 obese and 54 lean that were enrolled in a defined exercise protocol for 6 months. Blood samples and abdominal subcutaneous adipose biopsies were collected before and after the exercise. Gene expression profile was done by RT-PCR using RT-profiler-heat shock protein array kit consisting of 84 heat shock related genes and validated by Western blotting and immunohistochemistry techniques. Cytokine and metabolic markers were investigated by multiplexing technology (Bioplex) using plasma samples. Markers of oxidative stress, namely ROS and TBARS were measured on plasma or serum using commercially available kits.**Results:**

Our data indicate a downregulation of DNAJB3 mRNA and protein in obese subjects that negatively correlated with triglycerides ($P = 0.013$) and IP-10 inflammatory chemokine ($P < 0.05$). Interestingly, within 6 months of regular exercise, the levels of DNAJB3 in obese subjects were comparable to the lean subjects with a concomitant decrease of phosphorylated JNK, suggesting that exercise can antagonise obesity-mediated DNAJB3 repression. DNAJB3 was also shown to coimmunoprecipiate with JNK and IKKβ stress kinases along with HSP-72 with concomitant inhibition of JNK phosphorylation following treatment with PMA. Using cell lines, DNAJB3 protein was reduced following treatment with palmitate and tunicamycin which is suggestive of the link between the expression of DNAJB3 and the activation of the endoplasmic reticulum stress.
Conclusion: Our result demonstrated a downregulation in the expression of DNAJB3 mRNA and protein in obese subjects that was restored by physical exercise. The existence of a negative correlation between DNAJB3 expression and the inflammatory markers, its restoration by physical exercise and its inhibitory effect on JNK activation is suggestive of a protective role of DNAJB3 against obesity and could therefore represent a potential therapeutic target for the control and management of obesity and insulin resistance.

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Des-acyl ghrelin protects microvascular endothelial cells from oxidative stress-induced apoptosis through sirtuin 1 signalling pathway
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Background and aims: Ghrelin is a stomach-derived hormone. Acylation of ghrelin has been essential for its biological activities such as stimulating growth hormone release and appetite. On the other hand, the function of des-acyl ghrelin (Des-G) has not been fully elucidated. Here, we examined the effects of Des-G on oxidative stress--induced apoptosis in human retinal microvascular endothelial cells.
Materials and methods: After the cells were pretreated with or without 100nM Des-G, apoptosis was induced by incubating the cells with 0.1 mM H2O2. For inhibition of surtuin 1 (SIRT1), the cells were treated with 10 μM Ex-527 which is a SIRT1-selective inhibitor. The quantitative estimation of DNA fragmentation was used as a marker of apoptosis. Furthermore, total SIRT activity in nuclear extracts and mRNA expression levels of SIRT1, manganese superoxide dismutase (MnSOD) and catalase were determined.
Results: Des-G pretreatment protected endothelial cells from oxidative stress-induced apoptosis (3.43 ± 0.92-fold of control vs. 2.12 ± 0.63-fold of control, $p<0.01$). Des-G pretreatment increased total SIRT activity (0.47 ± 0.04 OD/min/mg vs. 1.46 ± 0.10 OD/min/mg, $p<0.01$) and the mRNA expression levels of SIRT1 (3.05 ± 0.86-fold of control, $p<0.01$), while pharmacological inhibition of SIRT1 attenuated the anti-apoptotic effect of Des-G ($p<0.05$) and the increment of activity ($p<0.01$). Moreover, Des-G increased the mRNA expression levels of antioxidant enzymes downstream of SIRT1, MnSOD (2.78 ± 0.84-fold of control, $p < 0.01$) and catalase (2.13 ± 0.38-fold of control, $p < 0.01$).
Conclusion: Our data suggest that SIRT1 signaling pathway contributes to the protective effect of Des-G against oxidative stress-induced apoptosis.

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Plasma URB protein is positively associated with human obesity and URB gene expression is inversely regulated by hydrogen peroxide and IL-1beta in cultured adipocytes
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Background and aims: The URB (upregulated in bombesin receptor subtype-3 (BRS-3) deficient mice)/CCDC80/DRO1/SSG1 gene is mostly expressed in adipose tissue and its expression is downregulated in diet-induced, ob/ob and KKAy obese mice. URB protein is secreted from adipocytes, yet its local or systemic effects remain to be elucidated. The aims of our study were to determine URB plasma levels in relation to obesity and insulin resistance in human subjects and URB gene expression and protein secretion regulation in cultured SGBS adipocytes by obesity-associated factors.
Materials and methods: Subjects: Two independent cohorts, one composed of subjects with different degree of obesity (n= 48, BMI range: 20 to 40 kg/m2) and a second cohort of morbid obese subjects (n=32, BMI range: 35 to 60 kg/m2) were recruited. Study of insulin sensitivity and secretion, artery vasodilation, carotid atherosclerosis (high resolution ecography) and liver steatosis were performed. Cultured cells: human preadipocyte SGBS cell line was differentiated to mature adipocytes and treated with varied factors. Methods: URB gene expression was assessed by quantitative real time PCR relative to the control 18S rRNA. ELISA assay was used to quantify URB protein.
Results: In the first cohort, negative associations of plasma URB with parameters of insulin secretion and circulating IGF1. In contrast, there were positive associations with blood neutrophil count, MCP-1 and lipocalin-type prostaglandin D synthase. In the second cohort, there was a strong and positive association of URB with visceral fat, fatty liver disease and carotid atherosclerosis. In cultured SGBS adipocytes URB gene expression was higher in mature adipocytes than in preadipocytes. URB mRNA levels in mature adipocytes were downregulated by hydrogen peroxide and upregulated by IL-1β, TNFα and insulin, whereas IL-6 had no effect. URB protein cell content decreased in response to hydrogen peroxide and was increased by IL-1β. A small proportion of the IL-1β-mediated increase in URB protein was detected in SGBS conditioned culture media.
Conclusion: The findings observed in plasma URB protein levels suggest a potential role of this protein in metabolic homeostasis mediated by fat accretion and insulin secretion. In human adipocytes, inflammatory and oxidative stress stimuli influence the response of this gene being IL-1β one of the responsible of its secretion by mature adipocytes.
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Palmitate induced repression of GLUT4 protein involves activation of nuclear factor-kappa B (NF-kB)
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Background and aims: High concentrations of saturated fatty acids trigger insulin resistance in skeletal muscle. Reduction in the expression of the glucose transporter GLUT4 is a feature in this process; however the involved mechanisms are not yet fully elucidated. Recent investigations suggest that the endoplasmic reticulum (ER) stress may be involved in this process. GRP78 (glucose-regulated protein 78) is a central regulator for ER stress due to its role as a major ER chaperone with anti-apoptotic properties, as well as its ability to control the activation of transmembrane ER stress sensors as IRE1α (inositol-requiring kinase-1 alpha) through a binding-release mechanism. ER stress results in the activation of a cellular response known as unfolded protein response (UPR), and different studies have demonstrated the activation of the transcription factor NF-kB through these pathways. Therefore, this study aimed to verify the effects of palmitate on GLUT4 expression, ER stress proteins and on the activation of NF-kB in L6 muscle cells.
Materials and methods: L6 myotubes were treated with 0,75 mM of palmitate during different time intervals (2, 6 and 12 hours). Protein (Western blotting), and mRNA (Rq-PCR) were quantified, and NF-kB transcriptional activity as evaluated (EMSA). Data were analyzed using ANOVA followed by Bonferroni post-test.
Results: Palmitate reduced GLUT4 protein content (control: 99.99±3.39, 2H: 66.37±7.20*, 6H: 67.73± 6.52*, 12H: 58.88±5.56* arbitrary units (AU), *P<0.05) and Slc2a4 mRNA (control: 1.00±0.04; 2H: 0.86±0.03*; 6H: 0.78±0.02*; 12H: 0.87±0.05* AU, *P<0.05). GRP78 protein (Control: 100.34±3.46, 2H: 128.23±7.76*, 6H: 117.63±6.26, 12H: 89.16±10.19, AU, n=7) and IRE1α protein content (Control: 100.00±0.00, 2H: 150.72±24.14*, 6H: 105.27±22.44, 12H: 80.18±17.00 AU, *P<0.05) presented a significant increase at the second hour of treatment. Activation of IRE1α results in the recruitment of TRAF2, which also showed an increase within 2 hours of treatment (Control: 100.28±0.35, 2H: 114.49±4.35*, 6H: 89.31±4.87, 12H: 89.52±4.54 AU, *P<0.05). It has been reported that TRAFs may induce the phosphorylation of the IKK complex and participate in the activation of NF-kB. By analysis of NF-kB mRNA there was a significant increase of this transcription factor at 6 and 12 hours (Control: 1.01±0.03, 2H: 1.05±0.03, 6H: 1.18±0.09*, 12H: 1.45±0.05* AU, *P<0.05), and an increase on the binding activity of NF-κB into the Slc2a4 gene promoter at 2 and 12 hours of incubation with the fatty acid (Control: 102.58±2.28, 2H: 131.96±11.54*, 6H: 80.58±6.55, 12H: 119.53±11.23 AU, *P<0.05).
Conclusion: It was observed that palmitate represses the expression of GLUT4 and induces only a transient activation of reticulum stress in L6 mus-

cle cells due to activation of GRP78 and IRE1α. Moreover, without excluding other regulatory mechanisms, palmitate increases NF-kB binding activity, is an important mechanism in the controlling of GLUT4 expression.
Supported by: FAPESP

PS 058 Adipose tissue distribution

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MicroRNA-196a is a potential regulator of body fat distribution in humans

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Background and aims: MicroRNAs are endogenous small non-coding RNAs that have emerged as important post-transcriptional regulators of mRNA expression. There is growing evidence for a role of microRNAs in regulating pathways in adipose tissue that control a range of processes including adipogenesis, insulin resistance and inflammation. It is well established that fat depots differ in terms of the metabolic risk that they confer and that different fat distributions are associated with different metabolic phenotypes. In this study we identify miR-196a as candidate for modulating body fat distribution.
Materials and methods: A microarray screen was performed on human subcutaneous abdominal and gluteal adipose tissue. Significant results were confirmed by qPCR on 40 paired subcutaneous abdominal and gluteal human whole adipose tissue biopsies. Following the identification of miR-196a as being a strongly differentially expressed miRNA between depots the mRNA expression of putative targets of miR-196a was investigated. In addition, 4464 individuals from the Oxford Biobank were genotyped for rs11614913, a SNP in mir-196a-2.
Results: miR-196a was confirmed by qPCR to have 2.1-fold higher expression within subcutaneous gluteal compared with subcutaneous abdominal adipose tissue (P=0.0001). mir-196a is expressed from within *HOX* gene clusters and is predicted to target the developmental genes *HOXA5, HOXB8* and *HOXC8*. The mRNA expression of these *HOX* genes was correspondingly higher in subcutaneous abdominal than subcutaneous gluteal adipose tissue. These findings were replicated in immortalised preadipocyte cell lines and in primary preadipocytes derived from abdominal and gluteal adipose tissue. This suggests that this differential expression is intrinsic to preadipocytes rather than being a function of their environment in different depots. Furthermore, we found that the SNP rs11614913, found in mir-196a-2, was significantly associated with both an increase in waist circumference of ~ 1.6cm (p=0.0004) and an increase in waist hip ratio of 1.5% (p=0.009), independently of BMI.
Conclusion: It is proposed that miR-196a acts as a depot-specific regulator of *HOX* genes, contributing to functional and developmental differences between fat depots, and that a SNP in mir-196a-2 predisposes to increased central body fat distribution.
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Depot-specific changes of fat metabolism with ageing in a type 2 diabetic animal

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Background and aims: Visceral fat accretion is a hallmark of aging and is associated with aging-induced metabolic dysfunction. Many studies have reported that the peroxisome proliferator-activated receptor (PPARγ) agonist rosiglitazone improves insulin sensitivity by redistributing fat from visceral fat to subcutaneous fat. The purpose of this study was to investigate the underlying mechanisms by which aging affects adipose tissue remodeling in a type 2 diabetic animal model and through which PPARγ activation modulates aging-related fat tissue distribution.
Materials and methods: At the ages of 21, 31 and 43 weeks, Otusuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of type 2 diabetes mellitus, were evaluated for aging-related effects on adipose tissue metabolism in subcutaneous and visceral fat depots.
Results: Aging increased adipocyte cell size and the ratio of visceral fat weight to subcutaneous fat weight (V/S ratio). The serum levels of glucose, in-

sulin and free fatty acid (FFA) were also increased. During aging, the mRNA expression of the genes involved in lipogenesis and fatty acid recycling were highly expressed in visceral fat as compared to subcutaneous fat. Aging increased mRNA expression related to lipolysis in both types of fat depots. The changes in the genes involved in lipid oxidation and energy expenditure, such as PDK2, mCPT1 and Acadl, varies during aging. After six weeks of rosiglitazone treatment in the OLETF rats, starting at 15 weeks of age, the PPARγ agonist resulted in fat redistribution with a decreasing V/S ratio and improved glucose intolerance. Compared with the untreated OLETF rats, the genes involved in lipogenesis and fatty acid cycling were decreased in visceral fat, whereas there were no significant changes of mRNA expression associated with lipid oxidation.
Conclusion: Our data suggests that fat distribution was changed by stimulating the lipid uptake and esterification in the visceral fat as compared to the subcutaneous fat, thus altering the lipid oxidation during aging. Conversely, these changes might be modulated by PPAR γ action in a type 2 diabetic animal model.

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Role of PGC-1alpha in exercise induced regulation of inflammatory markers in visceral and subcutaneous adipose tissue
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Background and aims: Obesity and physical inactivity are major risk factors in developing many lifestyle related diseases. Inflammation of adipose tissue is suggested to be involved in the pathogenesis of peripheral insulin resistance and ultimately type 2 diabetes. Regular physical activity reduces the risk of developing type 2 diabetes. Peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α is suggested to mediate many of the beneficial effects of exercise and has been shown to exert anti-inflammatory effects. The aim of the present study was to test the hypothesis that an acute exercise bout reduces the mRNA content of pro-inflammatory markers in both inguinal subcutaneous adipose tissue (SAT) and epididymal visceral adipose tissue (VAT) in a PGC-1α dependent manner in mice.
Materials and methods: Male whole-body PGC-1α knockout (KO) and littermate wildtype (WT) mice were subjected to an acute 1 hour treadmill exercise bout and euthanised either immediately or 2 hours after exercise. Control mice were euthanised after resting for 1 hour on the treadmill. SAT and VAT samples were analysed by reverse transcription real-time PCR.
Results: In the rested state, the mRNA content of interleukin (IL)-6, IL-18 and tumor necrosis factor (TNF)α in SAT was similar in PGC-1α KO and WT mice, whereas in VAT, the mRNA content of IL-6, IL-18 and TNFα was significantly lower in PGC-1α KO compared with WT mice. Immediately after an acute exercise bout, the mRNA content of IL-6 was increased ~50% in SAT in both PGC-1α KO and WT mice, while in VAT, the exercise induced increase in mRNA content of IL-6 was blunted in PGC-1α KO relative to WT mice. No effects of an acute exercise bout were observed on the mRNA content of IL-18 and TNFα in SAT in either genotype. In VAT, the mRNA content of IL-18 was decreased at 2 hours of recovery in WT mice only. An increase in VAT TNFα mRNA content at 2 hours of recovery was observed in PGC-1α KO mice, reaching similar levels as WT mice.
Conclusion: Exercise induces a larger fold acute increase in IL-6 mRNA content in VAT than SAT. Acute exercise only modestly affects the mRNA content of IL-18 and TNFα in adipose tissue. PGC-1α does not seem to regulate the mRNA content of IL-6, IL-18 and TNFα in SAT, whereas in VAT, PGC-1α may be important for both the basal and exercise regulated mRNA content of inflammatory markers.
Clinical Trial Registration Number: 561 1464
Supported by: Lundbeck Foundation

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Identification of CCDC3 as a possible novel biomarker for visceral adiposity
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Background and aims: Obesity is a major health problem associated with various comorbidities, including type 2 diabetes, dyslipidemia, hypertension, and cardiovascular diseases. There is increasing evidence to show that an increase in visceral adiposity is critical for the development of obesity-related morbidities rather than obesity itself. Currently, visceral adiposity is evaluated by measuring waist circumference or waist-hip ratio in clinical practice, but these parameters are still not sufficient for estimating the amount of visceral fat precisely, and thus useful and accurate markers for visceral adiposity remain to be identified. To search for novel markers of visceral adiposity, we conducted comprehensive human study for mRNA expression profiles using visceral (omental) and subcutaneous adipose tissues obtained from subjects with abdominal obesity and lean subjects.
Materials and methods: Omental and subcutaneous adipose tissue biopsies were obtained from 43 men during elective abdominal surgery. We first selected five men with abdominal obesity and five non-obese (control) men, who had normal plasma glucose levels (HbA1c <6.0%), out of the 43 subjects, and conducted a comprehensive analysis for mRNA expression profiles in omental and subcutaneous adipose tissues to search for gene(s) up-regulated specifically in omental adipose tissues in subjects with abdominal obesity. We further compared the expression pattern of candidate genes discovered from the human study in mouse models of adiposity (high-fat diet and db/db mice).
Results: Among 30,500 genes evaluated in the present study, the mRNA expression of *CCDC3* (encoding coiled-coil domain-containing protein 3) was up-regulated in omental adipose tissues from subjects with abdominal obesity (3.1-fold), but not in subcutaneous adipose tissues (0.9-fold) compared to those from control subjects. The omental adipose-specific increase of *Ccdc3* was consistently observed in two distinct mouse models of obesity. In an expanded analysis using all 43 men, *CCDC3* mRNA levels in omental adipose tissues, but not in subcutaneous adipose tissues, were positively correlated with waist circumference ($r = 0.483$, $P < 0.001$) or with body mass index ($r = 0.364$, $P < 0.05$). *CCDC3* was preferentially expressed in human adipose tissues and the expression of murine *Ccdc3* was induced during adipocyte differentiation in cultured 3T3-L1 cells. CCDC3 was predicted to be a secretory protein, and by western blotting analyses using HEK293 cells overexpressing *CCDC3*, we confirmed that overexpressed CCDC3 was secreted into culture media.
Conclusion: We have found that the expression of *CCDC3* is increased specifically in visceral adipose tissues in subjects with abdominal obesity. These results indicate that CCDC3 could be a potential biomarker for estimating visceral adiposity.

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Perihilar fat: another specialised and functionally very active perivascular fat tissue which differs from subcutaneous and visceral fat
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Background and aims: Recently we could show that perivascular adipose tissue (PVAT) represents a novel and important fat compartment for the regulation of insulin sensitivity, vascular function, inflammation and angiogenesis in atherosclerosis which differs substantially from other fat tissues, such as visceral or subcutaneous fat. Furthermore, there is evidence that obesity is also associated with diabetic nephropathy risk. In addition, our group found that perihilar adipose tissue (PHAT) - representing the perivascular fat of renal arteries - could influence microalbuminuria. Thus, the secretion pattern of human primary perihilar fat cells in coculture with human primary human

arterial endothelial cells (EC) and smooth muscle cells (SMC) was examined in the present study.
Materials and methods: Cells from PVAT, PHAT, EC and SMC were isolated from human adipose tissue and artery specimens of the renal hilus or radial arteries of three different patients. These cells were characterized by FACS methods and immunostainings, subcultured in monocultures or cocultured in transwell systems. In one part 600 μg/ml human fetuin-A was added. After 6, 24, 48, and 72 h, supernatants were collected and cells were lysed for mRNA extraction. For the quantification of the release of pro-inflammatory and angiogenic proteins, ELISA assays or the luminex technology was used and for the quantification of the mRNA expression realtime PCR was performed.
Results: Similar to preadipocytes from PVAT, preadipocytes from PHAT expressed and secreted the angiogenic factors IL-6, IL-8, MCP-1, HGF, VEGF, bFGF, PLGF, PAI-1 and TIMP-1. In the presence of cocultured EC or SMC, predominantly the proinflammatory proteins IL-6, IL-8 and MCP-1 were upregulated significantly after 24-72 h, whereas the angiogenic factors HGF, VEGF and PLGF where slightly downregulated. In contrast, the HGF and VEGF mRNA expression in EC and the HGF and bFGF mRNA expression in SMC was upregulated after 48 h by the coculture with preadipocytes. When these preadipocytes were differentiated to mature adipocytes, cocultured EC expressed higher levels of MCP-1. Treatment of preadipocytes with fetuin-A stimulated the IL-6 and IL-8, but inhibited the mRNA expression and protein release of the strong angiogenic factor HGF in both cells from PVAT and PHAT.
Conclusion: In our previous studies we could already show that PVAT secretes not only cytokines but more importantly also angiogenic factors and that the secretion profile differs according to the localisation of PVAT in the vascular tree. We found also that PVAT is a regulator of insulin-induced vasoreactivity. Further, there is evidence that defective podocyte-specific insulin signalling plays a role in the aetiology of proteinuria through podocyte damage. Thus, the described interactions between (pre-)adipocytes from PHAT with vascular cells or also podocytes may explain effects of insulin-signalling on vasoreactivity and the podocyte cytoskeleton leading to proteinuria but maybe also on other vascular diseases. This might lead to a better understanding of processes involved in both human diabetic nephropathy and atherosclerosis.
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Effect of high FFA level on the anti-contractile response of perivascular adipose tissue in rat aorta
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Background and aims: Perivascular adipose tissue (PVAT) plays important roles in the modulation of vascular function and attenuates vasoconstriction response by releasing various relaxation factors. This anti-contractile response of PVAT involves endothelium-dependent and endothelium-independent mechanism. However, PVAT-related anti-contraction was impaired in obesity. As high FFA level is a common feature of obesity and could cause impaired endothelium-dependent vasodilatation through inflammation pathway and oxidative stress, it is logical to hypothesize that PVAT dysfunction in obesity is also related to elevated FFA level. Therefore, the aim of the study was to test whether high FFA level could affect the anti-contractile effects of PVAT.
Materials and methods: Male Wistar rats were randomly divided into normal group, obesity group and fenofibrate group, which were fed with normal diet, high-fat diet, and high-fat diet plus fenofibrate (100mg/kg/d, to reduce FFA level), respectively. The thoracic aorta with or without PVAT (PVAT+ and PVAT−) were prepared with either intact endothelium (E+) or with removed endothelium (E−). To study the acute effect of high FFA on the anti-contraction effect of PVAT, we pretreated the aorta from normal rats with 500 μmol/L palmitic acid (PA) or physiological salt solution (PSS) as control. The concentration-dependent responses of aorta to norepinephrine (NE, 10^{-9}-10^{-5} mol/L) were studied in organ bath.
Results: After 8 weeks, body weight (BW), plasma FFA level, PVAT area and adipocyte area increased significantly in obese rats compared to normal rats (BW, 436 ± 34 g vs 370 ± 25 g; FFA, 620 ± 104 umol/L vs 236 ± 46 umol/L; PVAT, 0.56 ± 0.06 mm^2 vs 0.25 ± 0.05 mm^2; adipocyte,473± 61um^2 vs 201± 45 um^2, $P < 0.05$). In normal rat, the presence of PVAT exerted a significant anti-contractile effect to NE on the aorta both in the presence and absence of endothelium($P < 0.05$). However, the anti-contractile effect of PVAT was attenuated in obese rats in the presence of endothelium (attenuation rates, 5% vs 19%, $P < 0.05$). This attenuation of anti-contractile effect of PVAT was restored by reducing FFA level in fenofibrate group (attenuation rates, 16% vs 5%, $P < 0.05$). Transferring the solution from PVAT+ E+ in normal rats to PVAT- E+ in obese rats caused 12% attenuation of anti-contractile response, compared with 4% when transferred from PVAT+ E+ in the obese rats($P < 0.05$). An in vitro study showed that incubation with PA caused an attenuation of the anti-contractile response to NE in the presence rather than the absence of endothelium and this attenuation of anti-contractile effect was disappeared when PVAT was removed ($P < 0.05$). Transferring the solution from PVAT+ E+ incubated with PA to PVAT- E+ incubated with PSS caused only a 7% attenuation of anti-contractile response, compared with 14% when transferred from the PSS incubation($P < 0.05$). Incubation of the aorta (PVAT+ E+) from obese rats or pretreated with PA in vitro with anti-TNF-α antibody or catalase were able to restore the anti-contractile effect of PVAT($P < 0.05$).
Conclusion: Under both acute and chronic conditions, high FFA level could attenuate the anti-contractile properties of PVAT by endothelium-dependent rather endothelium-independent mechanism, in which inflammation and oxidative stress may play important roles.

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Impact of the peripancreatic adipose tissue on beta cell adaptation to obesity: an integrated, multi-platform analysis
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Background and aims: We have recently demonstrated that changes in the peripancreatic white adipose tissue (pWAT) due to diet induced obesity may play a key role in the regulation of beta cell adaptation to obesity. To elucidate this effect we examined transcriptome variations in pancreatic islets and combined them with changes in pWAT gene expression and protein and metabolite secretion in a rat model of diet induced obesity.
Materials and methods: Male Wistar rats were exposed to a 30 days high caloric cafeteria (CAF) or standard (STD) chow diet (n=6/group). Changes in the transcription profiles of pWAT and pancreatic islets were analyzed using Affymetrix Rat 230.2 arrays. pWAT secretome, obtained by 24 hours culture in serum-free medium, was analysed using 2D Differential Gel Electrophoresis proteomics and untargeted metabolomics (1HNMR, GC/MS). Significant alterations were validated through real time PCR, ELISA and mass spectrometry. The Ingenuity Systems Pathway Analysis software was used for functional enrichment analysis and cross-platform integration in interaction networks.
Results: Transcriptome analysis identified T Cell Signalling and Haematological System Development as major disrupted pathways in both tissues, involving mainly down-regulated genes. Proteomic analysis resulted in the identification of 19 proteins differentially secreted by the pWAT in response to diet induced obesity (T-test, p-value < 0.05). Functional analysis revealed that these proteins were mainly involved in disruption of LXR/RXR receptors activation and acute-phase response signalling pathways. Metabolomic analysis identified significant changes in 18 metabolites secreted from pWAT of CAF in comparison to STD rats (Mann-Witney test, p-value < 0.05). Decreased glucose consumption and lactate secretion, and increased glutamine consumption and α-ketoglutarate and oleic acid secretion suggested decreased pWAT glycolysis and enhanced TCA anaplerosis and lipolysis due to diet induced obesity. Transcriptomic analysis of pWAT revealed the up-regulation of 81 genes and down-regulation of 60 genes, while 59 up-regulated and 52 down-regulated genes were found in islets from CAF with respect to STD rats (Rank Prod test, q-value < 0.05). Increasing the level of data integration significantly enriched the related canonical pathways and increased the network connectivity. The highest level of integration, linking genes differentially expressed in both tissues with secretome perturbations, pointed to LXR/RXR Activation, Triacylglycerol Degradation and Immune Response Signalling as the main molecular pathways involved in the pWAT effect on islet adaptation to obesity. Analysis of functional network clusters allowed correlating key pWAT perturbations with islet transcriptome changes, such as the effect of altered lipid and cholesterol transport on disrupted islet circadian rhythm and increased acute-phase response.
Conclusion: The combination of all data subsets underscored functional clusters and integrative hubs, such as cholesterol, the fatty acid binding pro-

tein 4 and the protease plasminogen, as key mediators of the pWAT effect on beta cell adaptation to obesity.
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In vivo AAV-mediated genetic engineering of white and brown adipose tissue in adult mice
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Background and aims: Obesity and type 2 diabetes (T2D) are very strongly associated and are a major health problem because of their alarmingly growing prevalence worldwide. Deregulation of metabolic and endocrine functions of white adipose tissue (WAT), as well as impaired brown adipose tissue (BAT) activity and/or decreased mass, are considered among the main contributors to obesity, insulin resistance and T2D both in experimental animal models and in humans. To fully understand the metabolic and molecular mechanism(s) involved in adipose dysfunction, in vivo postnatal genetic modification of adipocytes holds great potential. However, this goal has not been achieved to date. In this study, we assessed the ability of adeno-associated viral (AAV) vectors of serotypes 1, 2, 4, 5, 6, 7, 8 and 9 to transduce murine WAT and BAT in vivo.
Materials and methods: AAV vectors encoding for GFP, RFP or the secreted alkaline phosphatase (SeAP) as marker genes, the enzyme hexokinase II (HKII) or the vascular endothelial growth factor (VEGF) were generated by triple transfection in 293 cells and were purified by double cesium chloride gradient. AAV vectors were injected into the epididymal white adipose tissue, into the interscapular brown adipose tissue or systemically in adult mice.
Results: AAV vectors, especially serotypes 8 and 9, mediated efficient transduction of white (WAT) and brown adipose tissue (BAT) in adult mice after intra-depot or systemic administration. The use of short versions of the aP2 or UCP1 promoters enabled highly specific, long-term AAV-mediated transgene expression in white or brown adipocytes. As proof of concept, delivery of AAV vectors encoding for HKII or VEGF to WAT or BAT resulted in increased glucose uptake or increased vessel density in targeted depots. This gene transfer methodology also enabled the secretion of stable high levels of the SeAP marker protein into the bloodstream by transduced WAT.
Conclusion: All together, this work demonstrates the value that AAV vectors hold as new tools for the long-term postnatal genetic engineering of adipose tissue, which could be used in metabolic and pathophysiology studies as well as in the development of new therapies for obesity and type 2 diabetes. Gene therapy strategies based on AAV-mediated genetic engineering of white adipocytes may also be envisioned for diseases in which supply of the therapeutic protein into the bloodstream is required.
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Brown adipose tissue metabolic activity assessed with FDG-PET/CT correlates with BMI and glucose tolerance in insulin resistant subjects
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Background and aims: Unlike white adipose tissue (WAT), brown adipose tissue (BAT) is a heat-generating fat that burns energy, and may have beneficial effects on obesity. This study explored the relationship between FDG-PET assessment of BAT activity, body weight, and glucose tolerance.
Materials and methods: We studied 9 insulin resistant subjects (HOMA-IR=5.2±2.5, mean±SD) and 2 overweight healthy volunteers with fluorodeoxyglucose positron emission tomography and x-ray computed tomography (FDG-PET/CT) of the thorax (C6-T8) to assess the glucose metabolic rate (GMR) of brown and white fat. Subjects were exposed to a 90-min period of either cold (67-68 F) or warm (72-73 F) temperature on separate days and then moved to the PET/CT scanner, which was kept at the same temperature for FDG uptake. CT was used to co-register the cold and warm PET scans. Segmented CT masks of 7 Hounsfield unit (HU) bands: -600 to -160 (mainly lung), -160 to -120, -120 to -80, -80 to -40, -40 to 0, 0 to 100 (muscle) and 100-600 (bone and some muscle) were computed. GMR was quantified as μmol glucose/100g/min using an arterial input function from the aorta image. Cold minus warm GMR values were obtained at 5 thoracic levels. All 11 subjects had BMI measured, but only the 9 IR subjects had glucose metabolism quantification with fasting insulin and oral glucose tolerance tests (OGTT).
Results: A higher cold than warm GMR was observed to the greatest extent in the -120 to -80 and -80 to -40 bands of thoracic levels (Figure 1), consistent with earlier reports of BAT metabolic rate sensitivity to thermal exposure. This was confirmed with MANOVA on the whole thorax and the lower 6 HU bands from -600 to 100 for GMR ($F=4.46$, df=4,5; $p=0.048$). Segmented CT scans (Figure 1) and PET cold-minus-warm overlays demonstrate the distribution of regions of cold condition metabolic increase. In the -120 to -40 HU range for whole thorax, a mean increase of 6.4% in GMR was observed in the cold condition compared to the warm condition. Whole thoracic GMR in the cold condition was correlated with fasting glucose in the two fat HU bands ($r=-0.65$ to -0.68, $p<0.03$), but correlations with fasting insulin levels were not significant. The volume of fat tissue in the -80 to -40 HU band significantly correlated with body weight ($r=0.67$, $p=0.023$), BMI ($r=0.93$, $p=0.0001$), and 2-h OGTT plasma glucose ($r=-0.77$, $p=0.015$) values. The volume in the -40 to 0 HU band in the cold condition also had trend level correlations with fasting insulin levels ($r=0.62$, $p=0.076$) and HOMA-IR values ($r=0.64$, $p=0.062$) for mid-thoracic levels.
Conclusion: These results in humans provide further evidence that BAT is associated with body weight, BMI and glucose homeostasis in IR subjects. CT can quantitatively assess fat volume, locate metabolically thermosensitive fat and provide a correlate of BMI. Additionally, FDG-PET may prove sensitive enough to detect metabolic effects of therapeutic interventions on functional BAT volume and activity.

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The effect of cold exposure on hepatic fatty acid uptake and brown adipose tissue function: relations to glycaemic control
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Background and aims: Functionally active brown adipose tissue (BAT) has been connected to healthy glycemic control. One of the most potent activators of BAT metabolism is cold but virtually non-existent data is available about the effects of cold on liver metabolism - one of the most essential tissues in glucose homeostasis. The aim of this study was to examine the effects of environmental temperature (cold vs warm) on liver fatty acid (FA) uptake and correlations to glycemic control (fasting glucose concentration and HbA1c). BAT perfusion was measured as a marker of BAT functional activity.
Materials and methods: Healthy human volunteers (n = 8) underwent two imaging sessions using 18F-FTHA PET (liver FA uptake) and 15O-H2O PET (BAT perfusion). The studies were performed at room temperature and during nonshivering cold exposure (using a cooling blanket with an adjustable temperature range, started 2 hours prior to the onset of PET study). Fasting blood samples were taken for plasma glucose and HbA1c, and a 2-hour oral glucose tolerance test (OGTT) was performed at room temperature. Correlation analyses were performed with Pearson's correlation and comparisons of mean levels with paired two-way T-test.
Results: BAT perfusion was twofold in cold vs warm conditions. Similarly, liver FA uptake increased almost twofold (0.07±0.02 mmol/l/min vs. 0.04±0.02 mmol/l/min, P=0.14) but nonsignificantly during cold exposure compared to room temperature. Free FA concentration was nonsignificantly higher (0.83±0.30 mmol/l vs. 0.53±0.27 mmol/l, P=0.14) during cold than at room temperature. Liver fractional FA uptake rate (FUR) during cold exposure correlated inversely with plasma HbA1c ($r=-0.75$, $P=0.05$) and directly at room temperature ($r=0.74$, $P=0.04$). Correlations with fasting glucose concentration and 2-hour plasma glucose concentration in OGTT were not found.
Conclusion: Functionally active BAT seems to be in relation to higher hepatic uptake of FAs in cold. Liver FA uptake correlates inversely with serum HbA1c during cold exposure and directly at room temperature. This may partly explain the connection between functionally active BAT and glycemic control.
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Insulin glargine does not increase breast cancer growth in an animal model

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Background and aims: Individuals with type 2 diabetes are at greater risk of developing breast cancer. Of the contributing factors, endogenous hyperinsulinaemia appears to play an important role. Some epidemiological studies suggest that exogenous insulin therapy may increase the risk of developing cancer in those with diabetes, while other studies report no increased risk. We previously found that rhIGF-1 and the insulin analog, AspB10 increase mammary tumor growth in a mouse model of insulin resistance and endogenous hyperinsulinaemia. In this study, we aimed to determine whether insulin glargine and increased orthotopic mammary tumor growth in a mouse with insulin resistance and endogenous hyperinsulinaemia (MKR mouse).
Materials and methods: 50,000 MVT1 (c-myc/vegf overexpressing) and 250,000 Met1 (polyoma virus middle T antigen expressing) cells were injected orthotopically into 8-10 week old MKR mice. Mice were divided into groups with equal tumor volume and treated with vehicle, rhIGF1 (1mg/kg) or glargine (12.5 U/ kg) twice daily for 2 weeks.
Results: rhIGF1 treated mice had significantly larger MVT1 (229.1±19.8mm3) and Met1 tumor volumes (246.9±18.8mm3) than the vehicle treated group (MVT1 121.99±9.6 mm3, $p<0.05$, Met1 162.4±14.1mm3, $p<0.05$). Tumor volumes in mice treated with insulin glargine did not differ from the vehicle treated groups and were significantly lower than rhIGF1 treated groups. Ex-vivo analysis of protein lysates from MVT1 and Met1 tumors revealed that rhIGF1 treatment led to activation of the IGF1 receptor (IGF-1R), hybrid IGF-1R/insulin receptors (IR) and the Akt signaling pathway, with sustained activation of the receptors and Akt an hour after rhIGF1 injection. Treatment with glargine led to Akt phosphorylation that was reduced an hour after injection in both Met1 and MVT1 tumors.
Conclusion: Our studies show that chronic administration of insulin glargine in an animal model of endogenous hyperinsulinaemia did not increase the growth of orthotopic mammary tumors, an important finding for the management of patients with diabetes.
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Insulin glargine (GLA) metabolites do not stimulate cell growth in human cancer initiating cells (CICs)

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Background and aims: [AspB10]insulin (X10) is a long-acting insulin analog that displays greater affinity for IGF1R than either the long-acting insulin analog GLA or human insulin (HI) in vitro. X10 is tumorigenic in animal models whereas GLA is not. GLA is rapidly metabolized to the metabolites, M1 and M2. These metabolites exhibit metabolic and mitogenic profiles similar to that of HI in vitro. CICs are cancer cells with self-renewing, stem cell-like properties with the ability to proliferate and differentiate into specific cells found in tumor types and thus they are able to maintain tumor bulk. Many cancers are thought to be initiated by CICs, and there is evidence that they cause cancer recurrence, metastatic progression and resistance to therapies. Avoiding growth factors, like insulin, estrogen or testosterone, is a current strategy to keep cancer incidence or recurrence under control. Our aim was to compare the mitogenic activity of HI, GLA, X10, M1 and M2 on human CICs in vitro.
Materials and methods: CICs derived from 10 human cell lines including glioma, lung, breast, colon, and prostatic cancers were isolated and grown in defined growth medium (no serum) with either HI, GLA, X10, M1 or M2. Growth was estimated using the MTS colorimetric assay.
Results: HI, GLA and X10 stimulated CIC growth to a similar extent, whereas M1 and M2 had growth promoting activity similar to medium without insulin. Similar results were obtained when measuring diametric sphere growth. Additionally, AKT phosphorylation levels were increased 2-fold after stimulation with HI, GLA or X10, whereas stimulation with M1 or M2 did not produce a significant increase.
Conclusion: This is the first study that has explored the biology of CICs treated with insulin analogs. The data show that GLA displays a mitogenic profile comparable to that of human insulin for all CIC lines tested, whereas M1, the main GLA metabolite in vivo, has even less growth promoting activity. Our results do not support GLA increasing CICs growth and thus the involvement of GLA in cancer promotion or relapse.
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Use of glucose lowering drugs and the risk of adenocarcinoma among patients with type 2 diabetes: a case-control study in the Netherlands

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Background and aims: Epidemiological studies suggest that certain glucose lowering drugs may increase or reduce the risks of cancer among patients with type 2 diabetes. The objective of this study was to compare different glucose lowering drugs with respect to the risk of developing adenocarcinoma.
Materials and methods: The primary care research databases in the Netherlands (IPCI) were used to conduct a case-control study among patients with type 2 diabetes from 1996-2011 (n=29,383). Cases with a first-time diagnosis of colorectal-, mamma-, prostate- or pancreascarcinoma, and 30 controls per case were matched on age, sex, duration of diabetes and duration of follow-up. Odds ratios (OR) and 95% Confidence Interval (CI) were calculated by conditional logistic regression analyses. We repeated the analyses when shifting the index date backwards in time by two years.
Results: In total, 702 case patients were identified. Mean age was 64.9 ± 11.8 years. Diet compared to metformin monotherapy was not associated with a difference in risk of developing adenocarcinoma (matched OR: 0.98, 95% CI: 0.77-1.24). Furthermore, sulfonylurea monotherapy compared to metformin monotherapy did not lead to a significant higher risk of developing adenocarcinoma (matched OR: 0.92, 95% CI: 0.65- 1.31). Also, the adding of sulfonylurea to metformin, insulin to metformin or both sulfonylurea and insulin to metformin was not linked to an increased in risk of developing adenocarcinoma (matched OR: 0.90, 95% CI: 0.72-1.12; matched OR: 0.87, 95% CI: 0.61-1.25; matched OR: 1.27, 95% CI: 0.85-1.90) when compared to metformin monotherapy. Furthermore, shifting the index date backwards in time did not lead to substantial changes in outcomes.
Conclusion: The findings of this study do not provide evidence for an increased or reduced risk associated with the use of commonly used glucose lowering drugs on developing adenocarcinoma.

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Metformin decreases the tumour death ratio accelerated by a high calorie diet in the RasH2 mouse, a model for evaluating and designing tumour prevention or regression therapies

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Background and aims: The objective of the current study was to investigate whether metformin intake changed the rate of tumor death under different diet conditions in the rasH2 transgenic mouse, a model for evaluating and designing tumor prevention or regression therapies.
Materials and methods: Male rasH2 transgenic mice at 8 weeks of age were divided into four groups: one fed regular chow of 352 kcal/100 g and drinking water *ad libitum*; another fed a high calorie diet of 592 kcal/100 g and drinking water *ad libitum*; and the others fed the regular chow or the high calorie diet with metformin at a dose of 25 mg/kg body weight per day added to the drinking water from the initiation of the experimental diets to death.
Results: The rasH2 transgenic mice fed a high calorie diet with or without metformin were significantly heavier compared to those fed regular chow after the age of 10 weeks ($p < 0.0001$). Mice fed the high calorie diet without metformin had a shorter average life span ($n = 15$; 60.9 ± 24.4 weeks) than

those fed regular chow (n = 15; 76.5 ± 17.6 weeks; $p < 0.02$). In contrast, the lifespan of mice fed a high calorie diet with metformin (n = 15; 75.3 ± 17.4 weeks) was longer than those fed a high calorie diet without metformin ($p < 0.05$), regardless of no differences in body weights. However, mice fed regular chow with metformin did not show the gain in longevity (n = 19; 72.7 ± 20.6 weeks) compared to mice fed regular chow without metformin (n = 19; 76.8 ± 16.5 weeks). At autopsy, multiple occurrences of tumors in the lung, liver, and digestive organs were found in all groups and malignancy was considered as the cause of death.

Conclusion: Findings from the current study suggest that a constitutional predisposition to tumor may be enhanced by a high calorie diet, and that high incidence of tumor death may be suppressed by a small dose of orally administered metformin.

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Direct inhibition of hexokinase II underlies a new anticancer property of metformin

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Background and aims: Recent evidence indicates that the widely used anti-hyperglycaemic drug metformin has important anticancer properties. Besides these epidemiological observations, a wide experimental literature documented that this benefit at least partially reflects the drug capability to directly inhibit the proliferation of various types of cancer cells. Most solid cancers avidly use large amounts of glucose as a source for both energy production and cell building blocks. Critical to this phenotype is the production of β-D-glucose-6-phosphate (G6P), catalysed by hexokinases (HK) I and II whose role in glucose retention and metabolism is highly advantageous for cell survival and proliferation. We studied the effect of metformin on the first steps of glucose metabolism in Calu-1 cells, derived from human non-small cell lung cancer.

Materials and methods: Human GLUTs and HKs expression was evaluated by Western blot and Real Time PCR analysis. To assay the HK I and II enzymatic activity, the reduction of NADP+ was followed, through a coupled reaction with glucose-6-phosphate dehydrogenase (G6PD). Mitochondrial membrane potential was determined using the cationic dye JC-1. Apoptosis was measured by annexin V-FITC and propidium iodide double staining. HK I and II mitochondrial localization was determined using MitoTracker probe. Molecular docking on Hexokinase II models simulations were performed with Glide software on a Linux x86_64 platform machine equipped with an Intel Core2 Quad CPU.

Results: Metformin directly inhibits the enzymatic function of HKI and II in the human non-small-cell lung cancer (NSCLC) cell line Calu-1. The inhibition is selective for these isoforms and virtually abolishes cell uptake and phosphorylation of glucose in a dose and time dependent manner, as documented by the reduced entrapment of 18F-fluorodeoxyglycose (FDG). In silico models indicate that this pharmacological action relates to metformin capability to mimic G6P features by steadily binding its pocket in HK II, thus preventing further glucose phosphorylation. The impairment of this energy source results in mitochondrial depolarization and subsequent cell death.

Conclusion: These results demonstrate a novel action of metformin targeting the enzymatic activity of HKI and II. The impairment of these important promoters of cancer cell proliferation contributes to explain the anticancer properties of this biguanide and represents a starting point to open new strategies in cancer prevention and treatment.

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Hyperglycaemia and hyperinsulinaemia promote fibrogenesis: increased risk of pancreatic cancer

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Background and aims: Epidemiological studies clearly indicate that the risk of pancreatic cancer (PC) is increased in type 2 diabetic patients. Insulin resistance and associated hyperglycemia, hyperinsulinemia, and abnormalities in insulin/IGF receptor pathways have been suggested to be the underlying mechanisms contributing to development of diabetes-associated pancreatic cancer. The key effector cells responsible for pancreatic fibrogenesis and pancreatic cancer progression are activated pancreatic stellate cells (PSC). The aim of this study was to investigate the effects of hyperglycemia and hyperinsulinemia on PSC.

Methods: In a novel immortalized mouse pancreatic stellate cell line (imPSC), we examined the effects of different culture conditions. Cells were stimulated with glucose and insulin, either individually or concomitantly. We measured matrix synthesis of Collagen I and Fibronectin, analyzed the expression of insulin/IGF-1 signaling pathway components and their operation as profibrotic and/or proliferative pathways and assessed the potential contributions of imPSC to a procarcinogenic microenvironment.

Results: Both Collagen I and Fibronectin were significantly increased when imPSC were incubated with either glucose or insulin. imPSC express receptors for both insulin (IR) and insulin-like growth factor 1 (IGF-1R), and respond to insulin/IGF-1 stimulation with increased tyrosine phosphorylation at specific autophosphorylation sites. Insulin at 100 nM induced peak stimulation of Akt phosphorylation that remained elevated at levels of 5-8 fold above basal from 10 min to at least 60 min. IGF-1 at 10 nM also produced peak stimulation, maximally at 10 min. Whereas the basal level of ERK1/2 phosphorylation was high even in serum-starved cells, stimulation with either 100 nM insulin or 10 nM IGF-1 produced transient increases which peaked at approximately 3 min. Interestingly, we also found effects of different culture conditions on mRNA and protein expression of IR/IGF-1R β subunits. In particular, in cells shifted to physiologic glucose level (5 mM), marked enhancement of IR and IGF-1R expression levels was exhibited.

Conclusion: In conclusion, this study suggests that hyperglycemia and hyperinsulinemia are key-factors in the activation of PSC and progression of fibrosis. Abnormalities in insulin/IGF receptor signaling network are involved in development and progression of diabetes-associated pancreatic cancer.

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PS 060 Fatty liver: human studies

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Impaired gastrointestinal-mediated glucose disposal in patients with non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with insulin resistance and an increased risk of diabetes. The aim of this study was to evaluate gastrointestinal-mediated glucose disposal (GIGD) in relation to NAFLD and diabetes.
Materials and methods: Healthy controls were compared with patients who had biopsy-verified NAFLD and either normal glucose tolerance (NGT) or type 2 diabetes. All subjects underwent a 50g-oral glucose tolerance test (OGTT) and an isoglycaemic intravenous glucose infusion (IIGI). GIGD was calculated as the percent difference in the amount of glucose given during the OGTT and IGII divided by the amount given during the OGTT [100% × (glucoseOGTT - glucoseIIGI / glucoseOGTT)]. Results are presented as means ±standard errors.
Results: In all three groups of participants: 1) healthy controls (n=10; age: 56±3 years; body mass index (BMI): 29±2 kg/m2; HbA1c: 5.4±0.1%), 2) NAFLD patients with NGT (n=10; age: 51±5 years; BMI: 31±2 kg/m2; HbA1c: 5.4±0.1%), and 3) NAFLD patients with type 2 diabetes (n=9; age: 60±4 years; BMI: 31±1 kg/m2; HbA1c: 6.7±0.3%), isoglycaemia was obtained. Isoglycaemia during IIGIs was obtained using 25±2 g of glucose in the healthy control group and 34±3 g and 42±2 g of glucose in two groups of NAFLD patients, respectively ($p<0.05$ and $p<0.05$ vs. the healthy control group), resulting in a GIGD of 52±6% in the healthy control group and 31±6% and 16±4% in the two groups of NAFLD patients ($p<0.05$ and $p<0.05$ vs. the healthy control group, respectively).
Conclusion: Patients with NAFLD have impaired GIGD independently of their glucose tolerance. This indicates that lipid accumulation in the liver, negatively affects handling of orally delivered glucose - even before prediabetes or overt diabetes has developed.

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Marked reductions in hepatic fat after a cardiovascular prevention programme persist after one year despite weight regain

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Background and aims: We have previously observed marked reductions in total BMI affecting multiple adipose tissue depots to equivalent extents after a 3 month cardiovascular disease (CVD) prevention programme. We have followed-up patients for a further 9 months after cessation of the programme to establish potential reversal of phenotypic changes. We hypothesized that at least some weight regain would occur as is usually observed in weight reduction studies, but with different effects on different fat depots.
Materials and methods: The MyAction CVD prevention programme is a twelve week family based multi-factorial cardiovascular prevention programme including lifestyle and medication changes offered to patients with cardiovascular risk >20% over 10 years. 29 male patients completed MyAction and 25 were followed-up after one year (9 months off the programme). Adipose tissue depots were determined using whole body magnetic resonance (MR) imaging. Ectopic lipid deposition in liver and muscle (tibialis) was determined using MR spectroscopy, and pancreatic lipids by multi-echo MRI.
Results: Participants were aged 68.2±4.0 (mean±SD) years. Weight reduction after the 12 week CVD prevention programme was 2.27± 0.54kg ($p<0.001$ compared with baseline), weight reduction was 1.02±0.68kg ($p=0.13$ compared with baseline) at 1 year. The regain in weight was accompanied by a regain in adipose tissue in subcutaneous and visceral depots (table); such that there was no statistical difference from baseline. In contrast, reductions in hepatic and pancreatic lipids were maintained ($p=0.002$ and $p=0.09$, respectively vs. baseline). The small but non-significant changes in hepatic fat during weight regain were associated with an increase in fasting plasma triglycerides (Spearman's rho 0.51, $p=0.013$).
Conclusion: Half of the weight loss in association with a 12 week CVD prevention programme was regained at 1 year; however the beneficial effects at ectopic sites, specifically in the liver, persisted. The benefits of a CVD prevention programme on accumulation of ectopic lipids may persist for longer periods than reflected by conventional weight measurements.

Immediate and long term effects of the MyAction programme on change in total weight and ectopic fat

	Baseline	Change at 3 months (post programme) vs. baseline	Change at 1 year follow-up (9 months off programme) vs. baseline
Weight, kg (n=25)	81.0±2.42	- 2.27±0.54***	- 1.02±0.68
Waist circumference, cm (n=24)	99.2±1.63	- 2.43±0.81**	- 0.94±1.01
Total adipose tissue, L (n=25)	28.7±1.17	- 2.76±0.5***	- 0.8±0.6
Subcutaneous adipose tissue, L (n=25)	19.8±0.83	- 1.98±0.33***	- 0.67±0.39*
Intra-abdominal adipose tissue, L (n=25)	4.71±0.34	- 0.51±0.14***	- 0.22±0.15
MRS Hepatic lipids, AU (n=25)	2.57 (1.54 to 4.29)	- 1.11 (- 1.38 to - 0.78)***	- 0.892(- 1.28 to - 0.38)**
ME Pancreatic lipids, AU (n=21)	6.04 (4.3 to 8.5)	- 0.82 (- 1.60 to - 0.83)*	- 1.04 (- 2.0 to 0.17)*
MRS IMCL tibialis anterior, AU (n=21)	5.72 (4.66 to 7.04)	0.64 (- 0.36 to 1.83)	0.24 (- 0.69 to 1.35)
HDL cholesterol, mmol/l (n=24)	1.24±0.05	0.009±0.03	0.073±0.039*
Triglycerides, mmol/l (n=23)	1.06 (0.91 to 1.25)	0.04 (- 0.05 to 0.13)	0.16 (0.03 to 0.31)*

Data are mean±standard error of the mean, or mean and 95% confidence interval (for skewed parameters). MRS: Magnetic resonance spectroscopy. ME: Multi-echo magnetic resonance imaging. AU, arbitrary units. IMCL: intramyocellular lipids. *p<0.05, **p<0.01, ***p<0.001 vs. baseline, ° p<0.1, p-values obtained from mixed-linear models.

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The relationship between liver fat content in patients with type 2 diabetes and liver disease outcome

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Background and aims: It has been found that the liver fat content was significantly higher in newly diagnosed type 2 diabetic patients (NT2DM) than who were previously diagnosed (PT2DM) in our past studies. The aim of this study is to investigate the alteration of the liver fat content in type 2 diabetic patients along with diabetic duration, and explore its influencing factors and relationship with the outcome of liver disease.
Materials and methods: A cross-sectional study was performed of 650 type 2 diabetic hospitalized patients, who were recruited from the department of endocrinology, Zhongshan hospital Fudan University, between September 2010 and January 2013. The liver fat content (LFC) were obtained from hepatic ^{1}H magnetic resonance spectroscopy (MRS), and defined fatty liver as LFC≥5.56%. NAFLD fibrosis scores (NFS) were calculated from blood tests taken at time of MRS. The NAFLD fibrosis score was calculated according to the following formula:-1.675+0.037×age (years)+0.094×BMI (kg/m^2)+1.13×impaired fasting glycaemia or diabetes(yes=1,no=0)+0.99×AST/ALT ratio-0.013×platelet(×10^9/litre)-0.66×albumin (g/dl). By applying the high cutoff score (0.676), the presence of advanced fibrosis could be diagnosed with high accuracy, and use of the low cutoff score (-1.455) can reliably exclude advanced fibrosis.
Results: Excluding alcoholic and other systemic diseases that may cause fatty liver, 435 patients (235 male (54%), 82 NT2DM (19%)) were included. The mean age was 57±14 years and mean body mass index was 25.4±3.7 kg/m^2. There were 183 subjects taking insulin secretagogues, 154 subjects taking metformin, 44 subjects taking TZDs, and 50 subjects taking α-glucosidase inhibitors in PT2DM patients. There was no significant difference of LFC between subjects taken insulin-sensitizing drugs or not. Mann-Whitney U test showed that the LFC(%) of NT2DM was significantly higher than that of PT2DM (24.66(14.39-41.38) vs 15.20(7.52-25.94), $P<0.01$). In all subjects that diagnosed of NAFLD (LFC≥5.56%), the chi-square test showed that proportion of patients excluded advanced fibrosis (NFS<-1.455) in NT2DM was significantly higher than that of PT2DM (26.3% vs 15.5%,$\chi^2=4.808$, $P<0.05$). Spearman partial correlation analysis showed that LFC was negatively correlated with duration of diabetes ($\gamma=-0.233$,$P<0.01$), and significantly associated with NFS

(γ=-0.164,P<0.01) after adjustment for gender, age, BMI, and oral anti-diabetic drugs. Meanwhile, duration of diabetes was also correlated with NFS positively (γ=0.236,P<0.01). Multiple linear regression analysis showed that the liver fat content was independently associated with duration of diabetes.
Conclusion: With the extension of the duration of diabetes, the reduction of liver fat content of type 2 diabetic patients with NAFLD is related to the development of advanced fibrosis. Decline in liver fat content of type 2 diabetic patient is associated with poor outcome of non-alcoholic fatty liver disease.

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The change in plasma triglycerides during an OGTT strongly predicts nonalcoholic fatty liver disease and the effectiveness of a lifestyle intervention to reduce liver fat

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Background and aims: It was recently shown that the change of plasma triglyceride levels during a standard oral glucose tolerance test (OGTT) is associated with visceral fat and insulin resistance. We therefore hypothesized that it may also be a predictor of nonalcoholic fatty liver disease (NAFLD) and may be associated with the change of liver fat content during a lifestyle intervention in humans.
Materials and methods: In 330 individuals at risk of type 2 diabetes, liver fat content was quantified by ^{1}H-magnetic resonance spectroscopy. Liver enzymes, lipids and lipoprotein levels were measured during fasting and after a 2hr-75g OGTT. A subgroup of 213 individuals underwent these measurements before and after participating for 9 months in the Tübingen Lifestyle Intervention Program (TULIP).
Results: In all subjects at baseline, the change in plasma triglycerides during the OGTT (120min-0min; ΔTG_{OGTT}) more strongly associated with liver fat content (r=0.51; r=0.28 after adjustment for gender, age, total body- and visceral fat), and more accurately predicted NAFLD (ROC-AUC=0.75 and 0.83) than fasting liver enzymes, lipids and lipoproteins and 2h-triglycerides (all r<0.49 and ROC-AUCs<0.82). During the lifestyle intervention, ΔTG_{OGTT} at baseline strongly predicted (adjusted estimate±SE: 1.04±0.29) change of liver fat content (estimates for all other parameters <0.24±0.08). After correction for confounders, the odds ratio for 1 standard deviation decrease in TG_{OGTT} at baseline for subjects to experience a reduction in liver fat content during the intervention was 1.90 (95% confidence interval, 1.25-2.97).
Conclusion: We provide novel data that, among commonly measured blood parameters, ΔTG_{OGTT} strongly and independently predicts NAFLD. Moreover, ΔTG_{OGTT} may become an interesting parameter being able to predict the change of liver fat content during a lifestyle intervention in subjects who are at risk for type 2 diabetes.

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Alteration in lipid metabolism after an oral fat load in subjects with NAFLD

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Background and aims: Subjects with non alcoholic fatty liver disease (NAFLD) are at high risk to develop type 2 diabetes and cardiovascular diseases. Hepatic fat accumulation (IHTG) is the results of insulin resistance (IR) and the impairment in hepatic lipid metabolism. Hepatic TG accumulate because after synthesis they cannot be exported as VLDL or oxidized. In subjects with NAFLD VLDL secretion is often increased as well as hepatic fat oxidation, so probably these two processes are saturated determining IHTG. Thus, we have studied the effect of a lipid load on peripheral lipolysis and hepatic lipid metabolism.
Materials and methods: We have studied glucose and lipid metabolism after a lipid load (200ml dairy cream and egg yolk) in 21 subjects, 15 with biopsy proven NAFLD and 6 controls, CT (mean age 35, BMI 25 kg/m2). Tracers (6,6-2H2-glucose and 2H5-glycerol) were infused for 120min before meal, and 240min after lipid load to evaluate glucose metabolism (EGP and clearance) and lipolysis. Throughout the test we measured glucose, insulin, FFA, triglyceride, cholesterol profiles. During fasting Peripheral IR was measured as HOMA (peripheral), hepatic IR as Hep_IR=EGPxINS, adipose IR as Adipo_IR=lipolysisxINS)
Results: Subjects with NAFLD had higher LFTs (ALT 68±8 vs 16±2 U/l, AST 34±3 vs 19±2 U/l, GGT 92±20 vs 15±6 U/l, all p<0.03), triglycerides (TG 121±12 vs 45±5 p<0.01), insulin (INS 11±2 vs 6±1 p<0.01) but similar fasting plasma concentrations of glucose (98±3 vs 90±2 mg/dl), FFA (0.57±0.08 vs 0.61±0.06 mmol/l), total cholesterol (198±8 vs 163±13 mg/dl), HDL cholesterol (49±2 vs 54±11 mg/dl), ApoB (90±8 vs 64±9 mg/dl). During fasting EGP was similar in both groups (8.8±0.4 vs 8.2±0.4 umol/kg min) while lipolysis was increased in subjects with NAFLD (2.2±0.2 vs 1.5±0.1 umol/kg min p=0.05) that were also more insulin resistant than CT (HOMA 2.8±0.5 vs 1.3±0.2, Hep_IR 101±18 vs 45±6, Adipo_IR 25±5 vs 9±2 all p<0.01). After the oral lipid load insulin increased slightly and as a consequence EGP and glucose concentrations did not change from baseline in CT and slightly decreased in NAFLD (from 8.8±0.4 at t=0 vs 7.8±0.4 umol/kg min at t=240, p<0.05). On the other hand lipolysis did not change in NAFLD, while it was significantly suppressed at 30 min in CT (to 0.9±0.1 umol/kg min p=0.02) to return to baseline at 240min. Cholesterol profile did not change while FFA concentrations increased similarly in the two groups. On the other hand TG increase after lipid load was more pronounced in NAFLD vs CT, AUC-TG increased by 50% (p<0.02) and iAUC-TG was increased 2 folds (p<0.03), reaching a maximum at 4h.
Conclusion: The metabolic handling of an oral fat load is impaired in subjects with NAFLD: basal increased lipolysis was not suppressed after a lipid load, as occurred in CT, and postprandial triglyceride increase was more pronounced. All these alterations might contribute to the development and progression of fatty liver disease.
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Interaction between obesity status and dietary intakes of sucrose and ω-6/ω-3-polyunsaturated fats and the Ile148Met in the PNPLA3 gene

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Background and aims: The Ile148Met (rs738409) in the patatin-like phospholipase domain-containing protein 3 gene (PNPLA3) associates with non-alcoholic fatty liver disease (NAFLD) and the PNPLA3-148M (G-allele) has been suggested to lead to both loss-of-function (hydrolysis) and gain-of-function [CoA- dependent lysophosphatidic acid acyltransferase (LPAAT)] defects. PNPLA3 is up-regulated by dietary carbohydrates (CHO), it has a higher affinity for unsaturated fatty acids and interactions between rs738409 and dietary intakes of CHO, sucrose and ω-6/ω-3-polyunsaturated fats (ω-6/ω-3-PUFA) on hepatic fat accumulation have been reported. We examined the interaction between rs738409 and obesity status on fasting triglyceride and alanine aminotransferase (ALT) levels, and the interaction between rs738409 and intakes of CHO, sucrose and ω-6/ω-3-PUFA on fasting triglyceride levels.
Materials and methods: From the Malmo Diet and Cancer Cardiovascular Cohort (MDC-CC) 4827 non-diabetic individuals aged 58±6.0y, 3343 with BMI≤27kg/m2 and 1481 with BMI>27kg/m2 were included in analyses. Dietary data was collected by a modified diet history method.
Results: Obesity status modified the association between rs738409 and fasting triglyceride- and ALT-levels (Pinteraction=0.006 and 0.01). The NAFLD-risk G-allele associated with lower triglyceride and higher ALT levels only among overweight individuals (P=0.005, P=0.0002). Significant interactions on triglyceride levels were observed between rs738409 and sucrose among normal weight individuals (Pinteraction=0.02) and ω-6/ω-3-PUFAs among overweight individuals (Pinteraction=0.03). G-allele associated with lower triglycerides only among overweight individuals in the lowest intake tertiles of CHO, sucrose and ω-6/ ω-3-PUFAs (P=0.007, P=0.03, P=0.0004) and with higher triglycerides only among normal weight individuals in the highest intake tertile of sucrose (P=0.02).
Conclusion: Obesity status and dietary sucrose and ω-6/ω-3-PUFA intakes modify the association between rs738409 and fasting triglyceride levels. Our

results suggest that the function of PNPLA3-148Met mutant protein is modulated by the obesity and nutritional status.
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Organ-specific fat accumulation and insulin resistance among the liver, skeletal muscle and adipose tissue in people with non-alcoholic fatty liver disease

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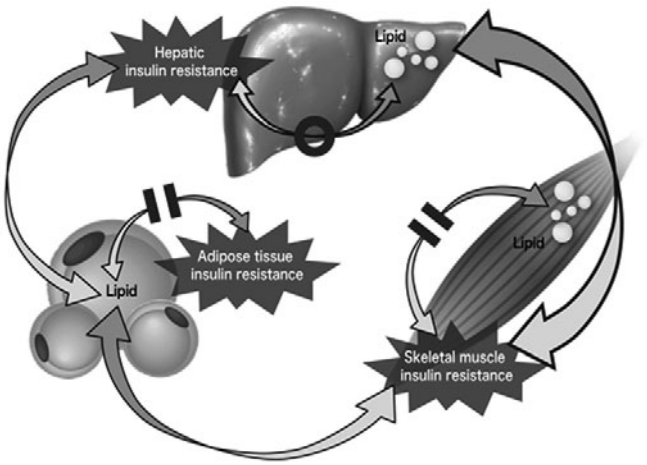
Background and aims: Accumulating evidence suggests that network among insulin-target organs may play a role in the development of distant organ insulin resistance (IR). The humoral and nutritional factors and a neuronal pathway may govern the inter-organ networks that may be disrupted by over-nutrition. However, causal relationship between fat accumulation and organ-specific IR still remains unclear in humans. The aim of this study is to examine the association between ectopic fat and organ-specific IR among the liver, skeletal muscle and adipose tissue in people with nonalcoholic fatty liver disease (NAFLD).
Materials and methods: Organ-specific IR in the liver (hepatic glucose production (HGP) × fasting plasma insulin (FPI) and suppression of HGP by insulin [%HGP]), skeletal muscle (insulin-stimulated glucose disposal [Rd]), and adipose tissue (suppression of free fatty acids by insulin [%FFA]) was measured in 45 patients with NAFLD (age 48±2 years, BMI 30.9±1.2 kg/m^2, mean±SE) using a euglycemic hyperinsulinemic clamp with tracer infusion ([6,6-$^2H_2$]glucose). Liver fat, intramyocellular lipid (IMCL), and body composition were measured by liver biopsy, proton magnetic resonance spectroscopy, and a bioelectrical impedance analysis, respectively.
Results: Both liver steatosis score and IHL were significantly correlated with Rd (r = -0.63, P <0.001; r = -0.50, P <0.01, respectively) as well as HGP × FPI (r = 0.50, P <0.01; r = 0.45, P <0.05, respectively). In the multiple regression analysis, both liver steatosis score and IHL were significantly correlated with HGP × FPI and Rd after adjusting for age, sex, and BMI. When stratified by steatosis score, HGP × FPI was significantly higher and Rd was significantly lower in the score 3 steatosis group compared to the score 1 steatosis group. Unexpectedly, indices of fat accumulation in the skeletal muscle (IMCL) and adipose tissue were not associated with their own organ-specific IR. IMCL and fat-free mass were not correlated with Rd (r = 0.01, P = 0.94; r = -0.24, P = 0.12, respectively). Total fat mass and its percentage were correlated with HGP × FPI (r = 0.60, P <0.001; r = 0.61, P <0.001, respectively) and Rd (r = -0.57, P <0.001; r = -0.65, P <0.001, respectively), but not with %FFA (r = 0.01, P = 0.95; r = -0.16, P = 0.32, respectively).
Conclusion: Unexpectedly, fat accumulation in the skeletal muscle and adipose tissue was not associated with organ-specific IR. Instead, in addition to the previously well-recognized relationship between adipose tissue mass and IR in the liver and skeletal muscle, the present study showed a distinct relationship between liver fat and skeletal muscle IR independently of age, sex, and BMI. Liver fat was also associated with hepatic IR. These findings suggest a central role of fatty liver in systemic IR and that a network exists between liver and skeletal muscle to maintain homeostasis of whole body energy metabolism.



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TWEAK/Fn14 in human hepatic steatosis: sTWEAK reduces triglyceride accumulation in human liver cells

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Background and aims: Liver steatosis is associated with inflammation, cell death, and fibrosis (referred to as Non-alcoholic fatty liver disease; NAFLD); which can progress to cirrhosis. NAFLD is considered as the hepatic manifestation of the metabolic syndrome due to its strong association with obesity, dyslipidemia, and altered glucose regulation. The cytokine TWEAK (TNF-like weak inducer of apoptosis) and its receptor Fn14 have been implicated in the inflammatory/anti-inflammatory imbalance that occurs in obesity and insulin resistance. This cytokine exists in two isoforms: as a full-length membrane bound (mTWEAK) and as a soluble secreted form (sTWEAK). sTWEAK circulating levels have been found decreased in severely obese subjects, Type 2 diabetic patients and Type 1 diabetic patients. Our aim is to study TWEAK/Fn14 axis within the context of human hepatic steatosis from an observational point of view in human liver biopsies of patients with different degree of steatosis and fibrosis and, in vitro by evaluating the effect of sTWEAK treatment on triglyceride (TG) accumulation.
Materials and methods: Subjects: n=30 severely obese subjects with different degree of steatosis and fibrosis. Real time PCR (RT-PCR) from human hepatic biopsies for the genes: *TWEAK*, *Fn14*, *TNFα, CASP3, BAX*, *GRP78* and *IL*-6 were assessed. Human hepatocyte line (HHL) was subjected to different inflammatory stimuli and processed by immunoblot analysis. Steatosis was induced *in vitro* by palmitic acid treatment. Expression levels of TG transport and synthesis/storage genes were assessed. Intracellular TG content was measured by Oil Red staining technique.
Results: In human liver biopsies, *Fn14* gene expression increases progressively with the grade of fibrosis, steatosis and with the presence of T2D. In cultured human hepatocyte cell line, Fn14 protein expression is enhanced by pro-inflammatory stimuli and in a dose dependent manner by palmitic acid stimulus. *In vitro* pretreatment of human hepatocyte cells with sTWEAK reduces significantly TG accumulation induced by palmitic acid by 30%. Gene expression of *PLIN* and *CD36* were significantly reduced after sTWEAK pretreatment followed by palmitic acid stimulus.
Conclusion: While Fn14 expression increased in human steatosis, its soluble ligand, sTWEAK, has protective/modulator effect on TG deposition in the liver.
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PS 061 Fatty liver: rodent studies

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A chronic apelin treatment improves hepatic steatosis in obese and insulin resistant mice by modulating de novo lipogenic pathway

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Background and aims: Apelin, a widely expressed peptide, acting through the GCPR receptor APJ, has recently emerged as a new player in energy metabolism. Notably, a chronic apelin treatment improves insulin sensitivity in high fat diet (HFD)-fed obese and insulin resistant mice, by enhancing glucose uptake and lipid utilization through fatty acid oxidation in skeletal muscle. However, the role of apelin on the liver in this model has not been addressed so far, although the liver is a major player in energy metabolism. In fact, APJ expression in the liver is very weak, but it is upregulated in severe liver pathologies. Liver steatosis, the lipid accumulation in hepatocytes, is one of the major features of obesity and insulin resistance. We aimed to evaluate the effects of apelin treatment on hepatic lipid metabolism, as well as the regulation of APJ expression in insulin resistance, in order to determine whether apelin can directly act on the liver.
Materials and methods: Male adult C57BL/6J mice were fed with a normal diet (ND) or a HFD containing 45% fat, during 12 weeks until the onset of insulin resistance, and then daily treated with an intraperitoneal apelin dose of 0.1µmol/kg or with PBS as the control group, during 28 days. Body weight, fasted glycemia and insulinemia were monitored during the treatment. At sacrifice, liver was taken to assess *ex vivo* ^{14}C-palmitate beta-oxidation on fresh homogenate, triglyceride content, histology and gene expression, to explore the hepatic lipid metabolism.
Results: As previously shown, this chronic apelin treatment reduced fasted glycemia and insulinemia and fat mass gain of HFD-fed mice, with no significant effect on the body weight. In the liver, triglyceride content was decreased after apelin treatment (ND: 0.241 ± 0.023 gTG/g tissue, HFD-PBS: 0.728 ± 0.110 gTG/g tissue, p<0.001 vs ND, HFD-Apelin: 0.440 ± 0.071 gTG/g tissue, p<0.05 vs HFD-PBS). The Fatty Acid Transporter (FAT) expression wasn't modulated by the treatment, neither was the *ex vivo* liver ^{14}C-palmitate beta-oxidation. However, Sterol Regulatory Element Binding Protein 1c (SREBP1c) expression was decreased (ND: 100 ± 10.6 AU, HFD-PBS: 267.4 ± 19.3 AU, p<0.001 vs ND, HFD-Apelin: 191.1 ± 15.8 AU, p<0.01 vs HFD-PBS) as well as its target gene Fatty Acid Synthase (FAS) expression (ND: 100 ± 12.9 AU, HFD-PBS: 182.4 ± 22.2 AU, p<0.01 vs ND, HFD-Apelin: 101.6 ± 15.4 AU, p<0.01 vs HFD-PBS) after 28 days of apelin administration. Compared to ND-fed mice, the insulin resistant HFD-fed mice exhibited an increase in APJ expression in the liver (ND: 100.0 ± 5.56 AU, HFD: 143.0 ± 16.1 AU, p<0.05), that was not modified by the apelin treatment.
Conclusion: Liver steatosis in HFD-induced insulin resistant mice was attenuated after a 28-days apelin treatment. The *de novo* lipogenic pathway seems to be the privileged target of apelin in our model, since the expression of FAT wasn't modified by the apelin treatment, neither was the ^{14}C-palmitate beta-oxidation performed on fresh liver homogenates.The small increase in the expression of APJ in HFD mice suggests that it could be localized in a particular cell type, such as stellate cells, as previously reported in cirrhosis. APJ needs to be precisely localized in the liver, in order to determine whether the Apelin/APJ system has a direct effect in the liver.
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P2X$_7$ receptor deficiency attenuates non-alcoholic steatohepatitis (NASH) induced by high-fat diet: possible role of the NLRP3 inflammasome

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Background and aims: Visceral obesity is associated with inflammatory, metabolic and vascular abnormalities including insulin-resistance, atherosclerosis and non-alcoholic steatohepatitis (NASH). The purinergic receptor P2X$_7$ (P2X$_7$R) might contribute to progression from steatosis to NASH, since it is modulated by NEFA and, in turn, mediates ATP-induced activation of the NLRP3 inflammasome. This study was aimed at investigating the role of P2X$_7$R through activation of the NLRP3 inflammasome in NASH induced by a high fat diet (HFD), an established experimental model of the metabolic syndrome.
Materials and methods: To this end, P2X$_7$R knockout (KO) mice and coeval wild type (WT) controls were fed a HFD (60% saturated fat) or a normal fat diet (NFD, 10% saturated fat) for 4 months. Metabolic parameters were assessed using standard methods. Liver sections stained with hematoxylin-eosin and Masson's trichrome were analysed for grading and staging of non-alcoholic fatty liver disease (NAFLD) and NASH using the American Association for the Study of Liver Disease (AASLD) criteria. Immunohistochemistry and real time PCR were used to assess the expression of markers of inflammation, fibrosis and lipid metabolism.
Results: Body weights were significantly higher in KO vs. WT mice and increased in both genotypes upon HFD. Likewise, blood glucose and insulin concentrations, the HOMA-IR index, and triglyceride and cholesterol levels were higher HFD- vs. NFD-fed mice, with no difference between the two genotypes. HFD-induced hepatic lesions were attenuated in KO vs. WT mice. This was confirmed by morphometric analysis, showing mixed, macro- and micro-vesicular, steatosis in both genotypes on a HFD, though it was less severe and predominantly of the micro-vesicular type in the KO as compared with the WT mice. The majority of WT animals (4 out of 7) met the AASLD criteria for diagnosis of moderate-to-severe NASH, i.e. lobular inflammation, ballooning, Mallory's bodies and fibrosis. In contrast, only 1 out of 7 KO mice showed signs of NASH of mild degree, with the remaining 6 animals showing stage 1 or 2 NAFLD, i.e. simple steatosis, predominantly micro-vesicular, with little or no inflammation. The expression of pro-fibrotic (fibronectin, collagen I and TGF-β), pro-inflammatory (MCP1, CXCR3 and TNF-alfa), and lipid metabolism (FAS, SREBP1c, CPT1 and LXR-alfa) genes increased to a significantly lesser extent in KO vs. WT mice on a HFD. Immunohistochemical analysis showed a strong positivity for P2X$_7$R and NLRP3 (in sinusoids, bile ducts and infiltrating inflammatory cells) in the WT mice, whereas staining was absent for P2X$_7$R and very low for NLRP3. These data were confirmed by RT-PCR analysis of gene expression. Preliminary experiments in liver sinusoidal endothelial cells showed that P2X$_7$R and the NLRP3 inflammasome components (NLRP3, caspase-1 and IL-1ß) are expressed under basal conditions and are upregulated in response to the pro-inflammatory cytokine TNF-alfa.
Conclusion: These data show that P2X$_7$R ablation protects mice from HFD-induced NASH, possibly through a blunted activation of NLRP3 inflammasome. This suggests that P2X$_7$R and NLRP3 may represent novel targets for therapeutic intervention.

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Beta cryptoxanthin improves hepatic insulin resistance and inflammation through alternative activation of macrophages in diet-induced nonalcoholic fatty liver disease

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) can be defined as a lipotoxic liver injury, and could worsen whole-body insulin resistance as well as progress to steatohepatitis (NASH). In our previous study, we developed a cholesterol- and saturated FA-induced model of lipotoxic NASH and revealed that excessive hepatic lipid accumulation promotes activation of macrophage/kupffer cells, resulting in exacerbating insulin resistance and hepatic inflammation. β-cryptoxanthin is a carotenoid compound that is known to have a potent anti-inflammatory effect by primarily modulating innate immune response. In the present study, we examined the effect of β-cryptoxanthin on diet-induced NAFLD to clarify the significance of lipotoxicity-mediated hepatic activation/polarization of macrophages in insulin resistance and inflammation in the pathogenesis of NAFLD.
Materials and methods: 7-week-old C57BL/6 mice were fed on a high-cholesterol/high-fat diet (CL) or a CL diet containing β-cryptoxanthin 3 mg/kg (CL+CX), for a total of 12 weeks. The liver histology, insulin sensitivity, and hepatic gene expression profile were examined. Next, we quantified intrahepatic immune cells by fluorescence-activated cell sorter (FACS).
Results: After 12 weeks of feeding, histological examination revealed hepatic steatosis and inflammation in mice fed CL diet. They showed hyperinsulinemia (CL 2.5±0.3 vs normal chow (NC) 0.5±0.1 ng/ml, p<0.01) even

though weight and adiposity were similar. β-cryptoxanthin administration improved glucose intolerance, hyperinsulinemia, and enhanced insulin signal assessed by IRβ and Akt phosphorylation in the liver. β-cryptoxanthin reduced hepatic TG, TC, and FA levels by 36%, 22%, and 48% respectively (all p<0.01), and decreased F4/80$^+$ macrophage infiltration in liver of CL group. Integrated pathway analysis using cDNA microarray showed that β-cryptoxanthin significantly downregulated both macrophage activation signal and T cell differentiation-related genes without affecting most of either FA, cholesterol or bile acid metabolism-related genes. To further assess the impact of β-cryptoxanthin on hepatic inflammation, intrahepatic leukocytes were quantified by FACS. Hepatic macrophages identified as CD45$^+$CD11b$^+$F4/80$^+$ cells were increased in CL group by 7.1-fold compared with NC-fed mice. In addition to slight reduction of total macrophage content in liver, mice fed CL+CX had 51% fewer CD11c$^+$CD206$^-$ (M1)-type macrophages whereas 170% more CD11c$^-$CD206$^+$ (M2)-type macrophages than CL diet fed mice, resulting in a predominance of M2 over M1 macrophage population. Moreover, the numbers of either CD3$^+$, CD4$^+$, or CD8$^+$ T cells were decreased by 39%, 42%, or 34% respectively (all p<0.05) in liver of CL+CX group. In parallel experiments, β-cryptoxanthin (50-200 nM) decreased LPS-induced M1 markers mRNA expression (TNF-α, IL-1β, RANTES) of peritoneal macrophage whereas it augmented IL-4-induced M2 markers mRNA expression (IL-10, CD209a, Mrc2) in a dose-dependent manner.

Conclusion: β-cryptoxanthin improves hepatic insulin resistance and inflammation through an M2-dominant shift in macrophge/Kupffer cells in diet-induced NAFLD. Hepatic M2 polarization or alternative activation of macrophages could contribute to the attenuation of lipid-induced insulin resistance and inflammation in liver.

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Dysfunctional adipose tissue-liver crosstalk contributes to short-term HFD-induced insulin resistance

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Background and aims: Short-term high-fat diet (HFD) leads to an impairment of glucose tolerance and hepatic insulin sensitivity. Whether this is due to an acute lipid overload or the result of induced adipose tissue inflammation, or both, remains unclear. Besides its well documented apoptosis-promoting effect, Fas (CD95) was demonstrated to activate inflammatory pathways in white adipose tissue (WAT) among others. The aim of the present study was to investigate whether a dys-functional adipose tissue-liver crosstalk contributes early to the development of insulin resistance.

Materials and methods: Adipocyte-specific Fas knockout (Fas$^{\Delta adipo}$) mice, generated on a C57BL/6J background, and control mice (Fas$^{f/f}$) were put on a 4-day HFD or chow control diet. Intraperitoneal glucose tolerance test and hyperinsulinemic-euglycemic clamp studies were performed. Total liver lipids were extracted and measured by a sulfo-phospho-vanillin reaction. Protein levels and mRNA expression were determined by Western blot technique and quantitative real-time PCR, respectively.

Results: Four days of HFD led to marked glucose intolerance in control mice (AUC, 1761 ± 78 mmol/l*min in HFD vs. 1433 ± 50 mmol/l*min in chow, p<0.01). Clamp studies revealed a reduction in glucose infusion rate (GIR) in HFD vs. chow animals (74.5 ± 2.3 vs. 122.8 ± 6.2 mg/kg*min, respectively, p<0.001) as well as a blunted response to insulin-mediated inhibition of endogenous (mainly reflecting hepatic) glucose production (18.7 ± 4.6 mg/kg*min and -5.8 ± 11.0 mg/kg*min for HFD and chow-fed animals, respectively, p <0.05). In a second set of HFD experiments, Fas$^{\Delta adipo}$, compared to Fas$^{f/f}$, had markedly improved glucose tolerance (AUC 1556 ± 66 mmol/l*min vs. 1717 ± 42 mmol/l*min, p<0.05). In clamp studies, GIR was slightly, albeit not significantly improved in Fas$^{\Delta adipo}$ mice. However, insulin-mediated inhibition of endogenous glucose production was almost completely preserved in Fas$^{\Delta adipo}$ whereas it was clearly blunted in Fas$^{f/f}$ mice. Similarly, insulin-stimulated Akt phosphorylation was sustained in livers of Fas$^{\Delta adipo}$ when compared to Fas$^{f/f}$ mice. Total liver lipid content increased with 4-day HFD, however, was not different between HFD-fed Fas$^{\Delta adipo}$ and Fas$^{f/f}$ mice (5.1 ± 0.1 vs. 5.1 ± 0.3 % of total liver weight) suggesting a similar degree of hepatic steatosis. Four days of HFD, compared to chow, did not affect gene expression of inflammatory or macrophage markers in liver, but, specifically, increased tumor necrosis factor alpha (TNF-α) expression in epididymidal WAT and, to a higher degree, in mesenteric WAT. Strikingly, HFD-fed Fas$^{\Delta adipo}$ mice revealed an overall reduced inflammatory profile in both epididymidal and mesenteric WAT compared to Fas$^{f/f}$ mice.

Conclusion: Our results identify adipose tissue inflammation and consecutive dys-functional adipose tissue-liver crosstalk as an early event in the development of HFD-induced deterioration of hepatic insulin sensitivity.

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Dietary lipids do not contribute to fructose induced hepatic triglyceride accumulation in mice

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Background and aims: Consumption of western diets rich in fat and fructose - present in soft-drinks - contribute to excessive energy intake and development of non-alcoholic fatty liver disease (NAFLD). In these patients, fructose intake is associated with alterations on hepatic high energy phosphate (HEP) content. Hepatic fructose metabolism bypasses key regulatory steps, and "fructolysis" represents an unrestrained ATP use and a constant supply of acetyl-CoA carbons for de novo lipogenesis (DNL). Fructose also stimulates the enterocyte secretion of apoB48 and chylomicron formation, which altogether may expose the liver to greater dietary lipid levels. The aims of this study were to test in vivo, these hypotheses: 1- lipogenic effects of fructose are independent of caloric intake; 2- dietary fructose supply disturbs hepatic HEP content; 3- fructose promotes greater dietary lipid absorption to hepatic triglyceride (HTG) pool.

Materials and methods: C57Bl6J mice (n=29) were fed with 60% fructose or glucose diets for 8 weeks. Caloric intake was determined for 24h in metabolic cages. Intraperitoneal glucose and insulin tests were performed (1.5g/Kg and 0.75U/Kg respectively). Abdominal adipose tissue (WAT) volume was determined by magnetic resonance imaging (MRI) at 0, 4 and 8 weeks of diets. Intracellular lipid pools in muscle and liver were determined at the same dietary time points by 1H magnetic resonance spectroscopy (MRS). Intramyocellular lipids (IMCL) were normalized to total creatine (tCr) and HTG was normalized to water (%). Hepatic HEP content was determined by 31P MRSI after 6 weeks of diets. Dietary lipid incorporation into HTG was determined 5h after a bolus of [U-13C]algal lipids (5g/Kg) by 1H{13C}MRS. Contribution of DNL to HTG pool was determined by 2H nuclear magnetic resonance (NMR) using 2H2O as tracer (21g/Kg).

Results: Caloric intake was similar between the fructose and glucose fed mice (0.31±0.08 vs 0.47±0.13Kcal/gbw/24h). After 8 weeks of diet glucose tolerance was decreased in both mice groups (p<0.05 vs baseline). Glucose clearance was similar between the mice groups. Throughout the course of the diets, glucose and fructose fed mice had a constant WAT volume of ~4%. IMCL/tCr ratios were unaltered after 8 weeks of diet, range [1.4-1.9] for fructose and [1.5-2.0] for glucose fed mice. HTG levels were more elevated after 8 weeks of fructose (3.2±2.0% vs 7.8±2.4%) than after glucose diet (2.4±1.2% vs 4.8±2.5%), p<0.05. The contribution of dietary lipids to the HTG pool was 1.6±0.8% vs 2.2±1.1%, respectively for fructose and glucose fed mice. Simultaneously, DNL contributed to 2.5±1.5% vs 1.1±0.7% of HTG pool in fructose and glucose fed mice, p=0.01. Hepatic ATP levels were 1.9±1.1mM vs 1.6±1.2mM and inorganic phosphate levels were 2.9±0.7mM vs 3.0±1.4mM for fructose and glucose fed mice respectively. These data show that fructose diet did not alter HEP content differently from glucose diet.

Conclusion: Independently of the caloric intake, fructose diet specifically induced HTG accumulation but abdominal adipose tissue and IMCL levels remained unaltered. HTG accumulation is better explained by fructose stimulation of DNL rather than by an increase of dietary lipid absorption. Fructose diet did not alter hepatic HEP content.

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Depletion of S-adenosylmethionine promotes non-alcoholic steatohepatitis by upregulating sphingomyelin synthase 1

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Background and aims: Methionine and choline-deficient diet (MCDD)-induced fatty liver disease is a widely used animal model of nonalcoholic steatohepatitis (NASH). Depletion of S-adenosylmethionine (SAMe) is considered a major contributor to the development of NASH.
Materials and methods: We examined the expression of enzymes involved in sphingolipid metabolism in C57BL/6J mice and *db/db* mice given MCDD for 6 weeks (n = 6 each). Hepatic levels of ceramide, sphingomyelin and diacylglycerol were measured by LC/MS/MS. In a separate set of experiments, C57BL/6J mice were given MCDD supplemented with SAMe.
Results: Feeding MCDD caused NASH, and also increased the expression of ceramide biosynthetic enzymes. Contrary to our expectation, it did not change hepatic total ceramide level, but increased diacylglycerol and sphingomyelin levels. Interestingly, feeding MCDD increased hepatic expression of sphingomyelin synthase 1 (SMS1), which converts ceramide and phosphatidylcholine into sphingomyelin and diacylglycerol, and acid sphingomyelinase (ASMase), two enzymes having apparently opposite action in sphingolipid metabolism. Adeno-associated virus-mediated knockdown of SMS1 decreased hepatic ASMase expression and prevented NASH in MCDD-fed mice. Feeding MCDD in mice and incubation of hepatocytes with MCD medium profoundly decreased expression of stearyl CoA desaturase 1 (SCD1). Knockdown of SCD1 increased SMS1 expression in cultured hepatocytes. Administration of SAMe reversed changes in the expression of SCD1, SMS1 and ASMase, and prevented NASH in MCDD-fed mice.
Conclusion: MCDD-induced depletion of SAMe causes downregulation of SCD1 and upregulation of SMS1 and ASMase, contributing to the progression of simple steatosis to NASH. Our findings identify SMS1 as a key mediator of NASH development.

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Protection of high-fat diet induced non-alcoholic steatohepatitis with the combination of n-3 polyunsaturated fatty acids and IgY targeted for NPC1L1

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), the most severe form of NAFLD, has an increased risk for progression to liver cirrhosis and accompanied with comorbidities such as cardiovascular disease. Treatment of NAFLD includes diet and lifestyle modification, some pharmacological interventions without clear benefits and there is no definitive treatment for NASH. Nieman Pick C1 like 1 (NPC1L1) transporter located in hepatocytes and enterocytes is essential for dietary cholesterol absorption and molecular target for ezetimibe (EZM). Since EZM blocked the internalization of NPC1L1 and caused their retention in the plasma membrane, this drug is a clinically used cholesterol absorption inhibitor. Recent evidence suggests that EZM has a beneficial effect in the treatment of NAFLD. Based on our previous publication that n-3 polyunsaturated fatty acid (n-3 PUFA) significantly ameliorated high fat diet-induced NAFLD, in this study, we have explored the therapeutic efficacy of combination of n-3 PUFAs and immunoglobulin Y targeted to NPC1L1 (N-IgY) on high fat diet-induced NASH.
Materials and methods: We generated N-IgY and confirmed its blocking efficacy in hepatoma cell line HepG2 and intestinal cell line Caco2. Wild-type and *fat-1* transgenic mice were fed normal chow diet (AIN-76A) or high-fat diet (HFD, 40% beef tallow modified AIN-76A purified rodent diet) with or without N-IgY (daily 9 mg per kg body weight) or EZM (positive control, daily 1.8 mg per kg body weight) administration in drinking water for 5 months beginning at 6 weeks of age.
Results: On functional assay to block NPC1L1, N-IgY showed similar blocking efficacy of EZM in HepG2 and Caco2 cells. Animal study revealed that N-IgY and endogenously synthesized n-3 PUFAs led to significant reductions in either body weight or liver wet weight, but EZM showed significant reduction in body weight only. N-3 PUFAs combined with either N-IgY or EZM led to statistically significant decreases in either hepatic fibrosis or steatosis. The changes in biochemical parameters were more significant; Either serum alanine transaminase (ALT) or aspartate transaminase (AST) was significantly decreased and the total cholesterol and LDL cholesterol was also significantly decreased with n-3 PUFAs and N-IgY/EZM. Combination of n-3 PUFAs and N-IgY led to significant reductions in either liver triglyceride (TG) or serum TG levels. Gene expression analysis showed that N-IgY and n-3 PUFAs synergistically up-regulate genes involved in cholesterol uptake (LDLR), bile cid biosynthesis (CYP7A1) or excretion (ABCG5, ABCG8 and *Ostb*) and down-regulate cholesterol synthetic gene (*Hmgcs2*) in liver. N-IgY and n-3 PUFAs also upregulate genes involved in bile acid transporter (*Asbt, Osta and Ostb*) and downregulate cholesterol synthase (*Hmgcs2*) in small intestine.
Conclusion: This study provide first evidence that combination of n-3 PUFAs and N-IgY can be promising treatment strategy to prevent HFD-induced NAFLD. However, extensive clinical trial will be required to stand as potential therapeutics under the well-acknowledged safety.
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Liver overexpression of GLUT2 and Slc2a2 are associated with nonalcoholic steatohepatitis in obese diabetic mice

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Background and aims: Glucose transporter GLUT2, encoded by Slc2a2 gene, participates in the final step of hepatic glucose efflux, playing a key role in glycaemic homeostasis. The nonalcoholic steatohepatitis (NASH) is associated with insulin resistance, diabetes and obesity, situations that could induce alterations of GLUT2/Slc2a2 expression. Thus, we hypothesize that alterations in GLUT2/Slc2a2 expression can occur during development of NASH. The present study aims to investigate GLUT2/Slc2a2 expression during evolution of NASH in a model of obese mice fed with hyperlipidic diet.
Materials and methods: Obesity was induced by neonatal treatment with monosodium glutamate (MSG): 2mg/g (subcutaneously) during 5 days, 4mg/g at the 7th day of life. Control animals (C) were treated with saline during the same period. After the weaning, the animals were fed with normal (ND) and high fat diet (HD - 60% of fat) by 4, 8 and 16 weeks. Four groups of animals were investigated: C-ND, C-HD, MSG-ND and MSG-HD. Obesity was evaluated by measurement of Lee index (body weight related to naso-anal length) and weigh of periepididymal adipose tissue. Plasma glucose and cholesterol were analyzed by commercial kits. NASH was evaluated by regular hematoxylin-eosin (HE) staining of liver samples, analyzing hepatocellular steatosis, ballooning and inflammation).The GLUT2 and Slc2a2 content were analyzed by Western blotting and Rq-PCR, respectively). Data were statistically analyzed by ANOVA, Student Newman-Keuls as post test.
Results: Based on Lee index and fat mas, it was observed that obesity progressively increased from 4 to 16 weeks of treatment in MSG mice, and high fat diet exacerbated the development of obesity. Glycaemia and cholesterolemia also progressively increased, reaching the highest values in 16-week MSG mice, despite high fat diet ($P<0.05$ vs ND and HD, at same age) group. Although signals of steatosis could be observed in early periods, nonalcoholic steatohepatitis was detected only in MSG-HD mice fed by 16 weeks. Finally, increased GLUT2 protein and Slc2a2 mRNA was also observed only in 16-week-treated MSG-HD mice ($P<0,05$).
Conclusion: The present study shows that treatment of MSG obese mice with high fat diet by 16 weeks leads to development of nonalcoholic steatohepatitis, which is accompanied by increased expression of Slc2a2 mRNA and GLUT2 protein, This suggests that increased Slc2a2/GLUT2 expression can contribute to impair hepatic glucose fluxes, and points out Slc2a2/GLUT2 as a potential molecular marker of NASH.
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Implication of mitochondria associated membranes regulation in insulin resistant and fatty liver

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Background and aims: Hepatic Insulin Resistance (HIR) and fatty liver disease (FLD) are associated with both mitochondrial and endoplasmic reticulum (ER) dysfunction. These two organelles are tightly link through contact points, known as Mitochondria Associated Membranes (MAMs). Even if an increasing number of studies point to several roles of MAMs in cellular processes such as lipid and Ca2+ exchange or mitochondrial dynamics, there is currently no data describing any potential physiological and/or pathological regulation. Our laboratory recently demonstrated a new role of altered MAMs in HIR. The aim of the study was to determine whether nutritional status was able to regulate MAM amount in liver in both physiological and pathological context, and identifying the different actors and cellular pathways involved.
Materials and methods: Using cell fractionation, in situ proximity ligation assay (PLA) and electronic microscopy stereology we quantified the interaction between ER and mitochondria in liver of fasted and fed wild type (wt) and ob/ob mice, as a model of HIR and FLD. In addition, we measured the repercussions of several metabolites and hormones related to nutritional state like glucose, lipids, glucagon and insulin on organelle interactions in rodent primary hepatocytes and HuH7 cells by quantifying the VDAC1/IP3R1 interaction, two interactors mediating Ca2+ transfer between both organelles, by in situ PLA.
Results: We demonstrated a reduction of MAM amount in the liver of fed wt mice compared to fasted ones (-60%, p<0,001). In addition we observed a reduction of MAM amount in liver of ob/ob mice in both fasted and fed states (-50% and -60% respectively, p<0.001), which is confirmed by in situ PLA on primary hepatocytes (-50%, p<0.01). Interestingly there was no significant difference between fed and fasted obese mice, suggesting the absence of a nutritional regulation of MAMs in the liver of obese and diabetic mice. In vitro, the addition of glucose (25mM, 4H) in the culture medium of either HuH7 cells or primary hepatocytes reduced VDAC1/IP3R1 interactions (-50% and -60% respectively, p<0,001). Insulin (10-7M, 4H) also reduced these interactions in primary hepatocytes (-70%, p<0.001). Interestingly, addition of 2-deoxyglucose (25mM, 4H) or fructose (25mM, 4H) reproduced the reduction of interorganelle interactions in HuH7 cells (-50% p<0.01 and -40 % p<0,001, respectively), whereas glucagon (100nM, 4H) and palmitate (200µM, 4H) showed no significant effect.
Conclusion: Our data point to an alteration of the ER-mitochondria interactions, as well as their regulation by nutritional state, in the insulin resistant and fatty liver. The fact that either glucose, fructose, 2-deoxyglucose and insulin similarly reduced VDAC1/IP3R1 interactions suggest that the pentose phosphate pathway is potentially involved, requiring further investigations to identify the key players.

PS 062 Signalling in adipocyte metabolism

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Involvement of Tpl2 kinase in COX-2 expression and prostaglandin production in adipocytes

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Background and aims: Obesity is associated with a low-grade inflammation of adipose tissue that leads to insulin resistance and to the onset of type 2 diabetes. A crosstalk between adipocytes and macrophages within adipose tissue contributes to the production of inflammatory mediators and promotes adipocytes dysfunction. Prostaglandins (PG) play a key role in inflammatory response and are produced by cyclooxygenases (COX) from arachidonic acid. COX-2 expression and PGE2 production are increased in adipose tissue from obese patients. However, inflammatory signaling pathways controlling its expression in adipose tissue remain ill-defined. Our previous work has shown that the MAP3Kinase Tpl2 is overexpressed in adipose tissue of obese patients and rodents and is involved in lipolytic effect of cytokines. The aim of this work is to study the involvement of Tpl2 in COX-2 induction and PGE2 production in adipocytes.
Materials and methods: Different in vitro experiments were performed : 1- treatment of 3T3-L1 adipocytes with IL-1β or IL-1β+TNF-α ; 2- direct coculture between adipocytes and RAW264.7 macrophages ; 3- incubation of adipocytes with conditionned medium from macrophages treated with LPS 0.5 ng/ml. In each approach, Tpl2 was inhibited by using a pharmacological inhibitor or a siRNA. Moreover, explants of adipose tissue from obese wild-type and Tpl2 KO mice were incubated with LPS 100 ng/ml. COX-2 gene expression was analysed by RT-qPCR. COX-2 protein expression was studied by Western-blot. PGE2 production was measured by enzyme immunoassay.
Results: In 3T3-L1 adipocytes, IL-1β and IL-1β+TNF-α induce COX-2 gene and protein expression and PGE2 production. These effects are decreased by 50% with Tpl2 inhibitor or siTpl2. COX-2 expression and PGE2 production are also increased in a direct coculture between adipocytes and macrophages. A pharmacological inhibition of Tpl2 in the coculture or Tpl2 silencing in adipocytes markedly reduces these inductions. Further, a pharmacological inhibition of Tpl2 in adipocytes reduces by 60% COX-2 expression induced by a conditionned medium from LPS-treated macrophages. Collectivelly, these data demonstrate that Tpl2 activation in adipocytes is necessary for COX-2 epxression and PGE2 production induced by cytokines-derived from macrophages. Importantly, Tpl2 expression in obese adipose tissue is necessary for LPS-induced COX-2 expression since we found that LPS is less efficient to induce COX-2 mRNA in explants of adipose tissue from obese Tpl2 KO mice as compared to obese wild-type mice (2.4 vs 6.5 fold respectively).
Conclusion: We have identified Tpl2 as an important inflammatory kinase necessary for COX-2 expression and PGE2 production in adipocytes in response to inflammatory cytokines derrived from macrophages. Thus, a deregulation of Tpl2 in adipocytes could participate to the overexpression of PG and to inflammatory state in adipose tissue.
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Bombesin receptor subtype-3: expression/function/role in cell signalling pathway and glucose transport, in adipose tissue from human and animal model

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Background and aims: previously, we described that Bombesin Receptor Subtype-3 (BRS-3) expression is lower than normal (N), in skeletal muscle from obese (OB) or type 2 diabetic (T2D) patients. Moreover, agonist [D-Phe^{6},β-Ala^{11},Phe^{13},Nle^{14}]bombesin$_{6-14}$ (BRS-3-AP) induced a concentration-related lipogenesis stimulation, in isolated adipocytes from normal rats (totally abolished by wortmanin -PI3K/PKB inhibitor-, and partially by rapamicin -p70s6K inhibitor-). Here we studied: A) BRS-3 expression in human and rat pieces from adipose tissue with/without metabolic alterations, B) characteristics of the receptor signalling pathways by using BRS-3-AP, and

its effect on glucose transport (GT) in normal human mature and rat isolated adipocytes.
Material and methods: RNA and protein were extracted from human (5 N, 5 OB and 6 T2D) and rat models [5 normal (n), 5 Hyperlipidemic (HL) and 4 T2D]. Human mature adipocytes were differentiated from a normal preadipocyte cell line, and normal rat adipocytes isolated by enzymatic digestion from epididymal fat pads (18 normal male Wistar rats 250 g, kept in a standard chow and water *ad libitum*). We measured: BRS-3 gene expression -qPCR-, BRS-3 protein, PKB and p70s6K -Western Blot-, glucose transport as ^{3}H-2-deoxy-D-glucose incorporation, in cells incubated in the absence (control) or presence of BRS-3-AP (10^{-13}-10^{-7}M) and with/without 10^{-6}M wortmanin, 10^{-7}M rapamycin.
Results: Human BRS-3-mRNA level was lower than normal in fat tissue from OB and T2D patients (11.2±0.9; 13.5±0.9 times down-regulated, $p<0.001$; respectively), as detected in rat adipose tissue from HL and T2D models (1.96±0.04; 10.3±0.36 times down-regulated, $p<0.001$; respectively). Human (T2D: 66±5% of N control, $p<0.05$) and rat (T2D: 57±11% of n control, $p<0.05$) protein values corresponded with mRNA expression reduced levels. In human adipocytes, 10^{-8}M BRS-3-AP increased ($p<0.05$) PKB and p70s6K phosphorylation (130±2% of control; 183±28%, respectively) like insulin (10^{-9}M: 147±5% and 152±10%, $p<0.05$, respectively); at 10^{-9}M, BRS-3-AP clearly induced p70s6K (152±5%, $p<0.05$), but not PKB phosphorylation (117±6%). However, in rat adipocytes, 10^{-8} and 10^{-9}M agonist produced a significant increase, in both, phosphorylation enzymes (PKB: 134±5% of control and 133±5%, $p<0.05$; p70s6K: 156±10% of control and 195±20%, $p<0.05$) like insulin (144±11% of control; 163±22%, $p<0.05$, respectively). In human adipocytes, BRS-3-AP caused a concentration-related stimulation of GT, maximal at 10^{-8}M (165±17% of control, $p<0.05$) which was blocked by wortmanin (98±5% control), and partially reduced by rapamacin (118±6% of control, $p<0.05$ *vs* control; $p<0.05$ *vs* 10^{-9}M BRS-3-AP); however, in rat adipocytes, BRS-3 maximal effect on GT (10^{-9}M: 155±8% of control, $p<0.02$) was abolished, by the additional presence of both, wortmanin (102±6%) and rapamycin (106±7%, $p<0.05$).
Conclusion: The results confirm a specific role of BRS-3 in human and rat adipose tissue, and not only describe the insulin-mimetic effect of its synthetic agonist on glucose transport, but also reveal the receptor signaling pathway. These facts support the notion that BRS-3 receptor, and/or its agonist peptide, might be considered as a molecular target with therapeutic purposes in diabetes and obesity.
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CDK4 is an essential insulin effector in adipocytes
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Background and aims: Insulin signaling is one of the most versatile systems that coordinate growth, proliferation and development with metabolic processes that accommodates energy needs to cellular function. Insulin response depends on tissue and cellular function. In white adipose tissue insulin signaling regulates lipid synthesis, lipolysis and glucose transport. However, the exact mechanism of how the insulin signaling produces its own specific pattern of regulated cellular functions is not yet fully understood.
Materials and methods: CDK4-/- mice were used in this study. Primary cultures of adipocytes and 3T3L1 cell line were also used.
Results: We show in this study that the cell cycle regulatory kinase CDK4 is a key insulin effector in adipocytes. Adipose tissue from CDK4-/- mice, or adipose tissue from mice treated with a CDK4 inhibitor have decreased lipogenesis and glucose transport and increased lipolysis. Consequently CDK4-/- mice are lean. In sharp contrast, mice that express a mutant hyperactive CDK4 (R24C) are obese as a result of decreased lipolysis and increased lipogenesis. We show here that CDK4 genetic or chemical inhibition blocks, as measured by whole kinome analysis AKT insulin signaling. Most interestingly we show that CDK4 phosphorylates and activates the insulin receptor substrate IRS2 creating a positive feed-back loop that maintains insulin action in adipocytes.
Conclusion: These findings place CDK4 at the initiation of the signaling pathway triggered by insulin in adipocytes.
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Down-regulation of calcineurin-like phosphoesterase domain containing 1 (CPPED1) expression improves glucose metabolism in vitro in adipocytes
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Background and aims: We have shown that the expression of CPPED1 is down-regulated in adipose tissue (AT) in response to weight reduction in persons with features of metabolic syndrome. However, the function of CPPED1 in AT is unknown. In this study we aimed to investigate the localization of CPPED1 protein expression in cultured SGBS preadipocytes and adipocytes, and to elucidate the role of CPPED1 in adipocyte glucose metabolism.
Materials and methods: Immunofluorescence was used for localization of CPPED1 protein expression in SGBS preadipocytes and adipocytes. The effect of reduced CPPED1 expression by RNAi technique on insulin action was measured using insulin-stimulated glucose uptake. Furthermore, the effect of CPPED1 knock-down on the expression of genes related to adipocyte function was studied in SGBS adipocytes by RT-qPCR and western blot.
Results: Immunofluorescence showed cytoplasmic expression of CPPED1 in preadipocytes and adipocytes. CPPED1 knock-down increased insulin-stimulated glucose uptake by 74% ($p<0.05$) which was abolished by wortmannin treatment ($p<0.01$). Moreover, CPPED1 knock-down increased the mRNA expressions of adiponectin ($p<0.01$), adiponectin receptor 1 ($p<0.01$) and glucose transporter 4 ($p<0.01$), and decreased glucose transporter 1 ($p<0.001$) and leptin ($p<0.05$). CPPED1 knock-down increased adiponectin protein expression time-dependently leading to a significant increase at 96h after CPPED1 knock-down (+32%, $p<0.05$). Furthermore, CPPED1 knock-down tended to increase adiponectin secretion ($p=0.057$) into the conditioned medium.
Conclusion: We demonstrate that CPPED1 is a novel molecule involved in adipocyte biology, potentially related to insulin action and improved glucose metabolism in adipocytes.
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Role of ChREBP and PPARa in the regulation of glucose and lipid metabolism in brown adipocytes
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Background and aims: Carbohydrate response element binding protein (ChREBP) plays an important role in the regulation of lipogenesis in the liver. Chrebp mRNA levels in brown adipose tissue are as abundant as those in the liver; however the function of ChREBP in brown adipose tissue is still unclear. In this study, we clarified the role of ChREBP in brown adipocytes and identified a functional involvement of peroxisome proliferator-activated receptor alpha (PPARα).
Materials and methods: Mouse immortal brown adipose HB2 cells were obtained from the interscapular brown adipose tissue of mice deficient for the tumor-suppressor gene p53. Six-week-old male wild-type (WT) and ChREBP-/- C57BL/6J mice were starved for 24 h or fed ad libitum (n=3, each group). To measure mRNA levels of ChREBP and ChREBP target genes in brown adipocytes, we performed quantitative RT-PCR and DNA microarray analysis. Relative mRNA levels were determined by real-time RT-PCR and normalized to mouse RNA polymerase II mRNA as the invariant control. To measure PPARα and ChREBP transactivities in differentiated HB2 cells, we performed a reporter assay using a pGL3 promoter vector containing the PPAR response element of acyl-CoA oxidase or carbohydrate response element of Fasn and Chrebpb.
Results: Chrebpb mRNA levels in brown adipose tissue from fasting mice were lower than those in mice fed ad libitum (0.042±0.021 and 3.98±0.042, respectively), while Ppara mRNA levels in brown adipose tissue of fasting mice were much higher than those in fed mice (1.23±0.033 and 0.69±0.006, respectively). In differentiated HB2 cells, glucose increased mRNA levels of ChREBP target genes such as Fasn and Glut4 in a dose-dependent manner, but reciprocally decreased Chrebp and Ppara mRNA levels. DNA microar-

ray data revealed that most ChREBP target genes (Fasn, Acaca, Fgf21, Scd1, Elovl6, Tkt, Acly, and Me1) were reduced more than two-fold (22.1 fold, 18.4 fold, 49.9 fold, 8.63 fold, 5.66 fold, 3.13 fold, 23.06 fold, and 5.27 fold, respectively), while Ppara mRNA levels were elevated 3.06 fold. Quantitative RT-PCR confirmed that (1) Fasn mRNA levels in brown adipose tissue from ChREBP-/- mice were much lower than those in WT mice (0.079±0.0026 and 0.72±0.0028, respectively), and (2) Ppara mRNA levels in ChREBP-/- mice were 3.38±0.17 fold higher than those in WT mice (2.64±0.13 and 0.78±0.075, respectively). Adenoviral overexpression of ChREBP and reporter assays showed that ChREBP suppressed PPARα transactivity in HB2 cells. In contrast, 100 μM Wy14643, a selective PPARα agonist, partially suppressed glucose-mediated induction of Chrebpb and Fasn mRNA and ChREBP transactivity in HB2 cells.
Conclusion: ChREBP and PPARα coordinately regulate glucose and lipid metabolism in brown adipocytes.
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Unsuppressed lipolysis in adipose tissues by insulin is critical for glucose homeostasis under high fat diet
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Background and aims: Excessive consumption of animal fat has been considered to contribute to the recent upsurge in the incidence of diabetes mellitus. However, molecular mechanism of impaired glucose metabolism under high fat diet (HFD) still remains uncertain, since few animal models develop overt hyperglycemia only under HFD. We found that marked hyperglycemia was induced by HFD feeding in a mutant IR (mIR) mouse, which harbors a loss of function mutation in the insulin receptor. In this study, we examined the pathophysiology of HFD-induced diabetes mellitus using mIR.
Materials and methods: mIR and wild-type mice were fed with high fat diet (60% fat in calorie) and the metabolic changes in insulin target tissues, including white adipose tissue (WAT) and liver, were examined.
Results: Marked hyperglycemia developed in mIR under HFD (mIR/HFD) but not in wild-type (WT) mice under HFD (WT/HFD) (mIR/HFD, 376 ± 30 mg/dl; WT/HFD, 189 ± 9 mg/dl, n=9-10, $p < 0.001$, after 8 week HFD at 16 weeks of age). By contrast, the increase in body weight was significantly less in mIR/HFD than in WT/HFD. Oral glucose tolerance test and insulin tolerance test revealed that mIR/HFD have apparent glucose intolerance and severe insulin resistance. Considering that gluconeogenesis might be increased in mIR/HFD, we measured the mRNA expressions of PEPCK and glucose-6-phosphatase (G6P) in the liver, both of which are critical in the regulation of gluconeogenesis. The PEPCK expression in mIR/HFD was similar to that in WT/HFD, while the G6P expression in mIR/HFD was significantly increased (1.9 folds in mIR/HFD vs WT/HFD, n=10-12, $p < 0.05$). PEPCK mediates gluconeogenesis from amino acids and pyruvate, while G6P is also involved in the gluconeogenesis from glycerol as well as from amino acids and pyruvate. Thus, we measured the increase in blood glucose levels after pyruvate or glycerol administration in vivo to assess the activity of gluconeogenesis from these substrates. Interestingly, gluconeogenesis from glycerol but not from pyruvate was found to be increased in mIR/HFD. Glycerol is generated from triglyceride through lipolysis. Lipolysis in WAT is inhibited by insulin through inhibition of phosphorylation of hormone sensitive lipase (HSL). Thus, we performed Western blot analysis of phosphorylated HSL in white adipose tissues (WAT), and found that phosphorylation of HSL was inhibited markedly by HFD in WT, but much less in mIR (3 folds in mIR/HFD vs WT/HFD, n=6-7, $p < 0.05$). These results indicated that suppression of lipolysis by HFD is impaired in mIR and this leads to the increase in glycerol influx to the liver and resultant rise in gluconeogenesis, contributing to the development of overt hyperglycemia in mIR/HFD.
Conclusion: Unsuppressed lipolysis in WAT and increased gluconeogenesis in the liver from glycerol both contribute to the development of hyperglycemia under HFD in insulin resistance.
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Cyclosporin A and tacrolimus impair dynamics of GLUT4 traffic in insulin-responsive cells
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Background and aims: The calcineurin inhibitors cyclosporin A (CsA) and tacrolimus (FK), are immunosuppressive agents (IAs) frequently used after transplantation and they are associated with several side effects including hyperglycaemia and new-onset diabetes. However, the mechanism for glucose intolerance is not known and the direct effects of the IAs on insulin-responsive cells including human adipocytes have not been well characterized previously.
Materials and methods: Glucose uptake and protein expression of insulin signalling proteins were measured in human isolated adipocytes, obtained from 42 non-diabetic volunteers, incubated in the absence and presence of either CsA or FK and insulin (1000 μU/mL). Effects of either CsA or FK on cellular distribution of GLUT4 in human preadipocytes differentiated into adipocytes and in 3T3-L1 adipocytes, was evaluated by immunohistochemistry and fluorescence microscopy. In addition, effects of CsA or FK on endocytotic and exocytotic rates of the GLUT4 transporter were studied in L6 myoblasts stably expressing GLUT4 with an exofacially directed Myc-tag, by an enzyme-linked immunosorbent-like assay.
Results: CsA and FK had a concentration dependent-inhibitory effect on basal and insulin-stimulated 14C-glucose uptake in human adipocytes (up to 40% reduction, $p<0.05$). Although the phosphorylation of the insulin receptor at Tyr1146 was inhibited by CsA and FK, phosphorylation and/or protein levels of insulin signalling proteins (IRS1/2, p85-PI3K, PKB, AS160, mTORC1) and GLUT4 and 1 content were not changed. Furthermore, CsA or FK reduced the insulin-induced redistribution of GLUT4 to the cell surface of differentiated human adipocytes (~60%, $p<0.05$) and 3T3-L1 adipocytes. In addition, CsA and FK similarly reduced the cell surface levels of GLUT4 in L6 muscle cells and increased the GLUT4 endocytosis rate, by up to 30%, with no change in exocytosis rate.
Conclusion: In conclusion, these results suggest that therapeutic concentrations of cyclosporin A and tacrolimus, inhibit glucose uptake by removing GLUT4 from the cell surface via increased endocytosis and this is independent of the insulin signalling cascade. The described effects of immunosuppressive agents in adipocytes and other insulin-sensitive cells may contribute to the development of insulin resistance and new-onset diabetes associated with immunosuppressive therapy.
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Amyloid beta42 administration impairs energy metabolism in vivo and in vitro
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Background and aims: Amyloid β_{42} ($A\beta_{42}$) is a protein implicated in the onset of Alzheimer's Disease (AD), in part through impaired neuronal metabolism. Obese and diabetic patients have increased circulating $A\beta_{42}$, yet it is unknown whether circulating $A\beta_{42}$ contributes to altered metabolism in these conditions. The aim of our study was to determine whether $A\beta_{42}$ alters metabolism of insulin sensitive cells *in vitro*, and whole body metabolism *in vivo*.
Materials and methods: Both 3T3-L1 adipocytes and FAO hepatocytes were treated with either $mA\beta_{42}$ or aggregated $A\beta_{42}$ ($aA\beta_{42}$) for 48h, with respective controls being monomeric or aggregated scrambled $A\beta_{42}$ ($scrA\beta_{42}$). After 48h of treatment, basal and insulin stimulated glucose uptake and glucose production was measured in 3T3-L1 adipocytes and FAO cells respectively. For *in vivo* studies, 8 week old, male C57Bl/6J mice (n=10 per group) were treated with $mA\beta_{42}$ or $scrA\beta_{42}$ (control) for two weeks (1μg/day; I.P injections). Bodyweight and food intake were monitored daily. Indirect calorimetry was performed after 14 days of treatment and oxygen consumption, respiratory quotient and substrate oxidation determined.

Results: Monomeric $A\beta_{42}$ ($mA\beta_{42}$) increased glucose production in FAO hepatocytes, while $aA\beta_{42}$ had no effect. Similarly, $mA\beta_{42}$ impaired glucose uptake in 3T3-L1 adipocytes, while $aA\beta_{42}$ had no effect. Administration of $mA\beta_{42}$ had no effect on bodyweight or food intake compared with control treated mice. However, administration of $mA\beta_{42}$ reduced oxygen consumption and total carbohydrate oxidation compared with control animals ($p \leq 0.05$).
Conclusion: This data shows that monomeric $A\beta_{42}$ impairs glucose metabolism while aggregated $A\beta_{42}$ had no effect. This data suggests that not only is $A\beta_{42}$ involved in the pathology of AD, but it may also be involved in the dysregulation of metabolism in obesity and type 2 diabetes, where circulating $A\beta_{42}$ levels are elevated.
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Metabolic profiling of cultured human adipocytes from metabolically malign versus benign obese individuals
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Background and aims: Among obese subjects, benign (metabolically healthy) and malign obesity exists; the latter revealing whole-body insulin resistance, hepatic steatosis, and subclinical inflammation. The underlying pathomechanisms aren't well understood yet, the prevention strategies insufficient. Thus, aim of this study was to characterize the metabolic profile of subcutaneous human adipocytes from insulin-resistant (IR) versus comparatively insulin-sensitive (IS) morbidly obese subjects.
Materials and methods: 10 IR versus 10 IS non-diabetic Caucasians (BMI>40kg/m2) were matched for gender, age, BMI, and percentage of body fat; primary preadipocytes were isolated and in vitro differentiated to adipocytes. Metabolites were detected by a targeted metabolomic approach in cell lysates and supernatants. Multi- and univariate methods were used for statistical analysis, correction for multiple testing was done.
Results: Among others, aspartate was found to be lower in IR adipocyte lysates (3-fold, p=0.0005), maybe pointing to a relative depletion of citric acid cycle metabolites. Regarding the supernatants, four phosphatidylcholines emerged (PC aa C32:3, PC aa C40:4, PC aa C40:5, PC ae C34:3, p<0.0004; all lower in IR subjects) as well as an altered arachidonic acid (AA) metabolism: 15(S)-HETE (15-hydroxy-eicosatetraenoic acid; 0 vs. 120pM; p=0.0089), AA (1.5-fold; p=0.0046) and docosahexaenoic acid (DHA, C22:6; 2-fold; p=0.0041) were higher in IR group, emphasizing a direct contribution of adipocytes to the inflammatory state of insulin resistance. As DHA serves as an inhibitor of prostaglandin synthesis, higher DHA levels might be a hint for counterregulatory mechanisms in IR subjects.
Conclusion: With our study we were able to identify adipocyte-specific metabolic alterations that are associated with malign (insulin-resistant) obesity.
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PS 063 Lipoproteins

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Protective effect of physical activity on atherogenic lipoproteins in type 1 diabetes
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Background and aims: Cardiovascular disease is the main cause of mortality in type 1 diabetes (T1D), with physical activity (PA) creating controversial effects in this population. The aim of this study was to analyze the lipoprotein subclass profiles in the physically active T1D population.
Materials and methods: 94 T1D patients and 125 control (CT) subjects were recruited. PA was assessed by IPAQ questionnaire, and subjects were classified as active (A) or non-active (NA) following IPAQ criteria. Serum samples were analyzed using LipoProfile (LipoScience Inc.). A subgroup of 32 T1D and 66 CT was also analysed by a new test based on diffusion-ordered nuclear magnetic resonance spectroscopy (DOSY).
Results: Both groups were similar in terms of age, gender, hypertension and smoking and physical activity habits. T1D patients presented HbA1c 7.4 ± 1.3%. Any macro or microvascular complications were present, except for 4 subjects that presented incipient retinopathy. There were no differences in the usual diets of T1D and CT groups concerning cholesterol, saturated and unsaturated fat intake, but there was a higher intake of total carbohydrates, simple carbohydrates and alcoholic beverages in the CT group. No participants were using lipid lowering drugs. DOSY test revealed that LDL-Ps were similar in A and NA CT subjects (751.0 nmol/L ± 206.7 vs. 781.1 ± 150.3), but different between A and NA T1D ones (649.8 nmol/L ± 165.4 vs. 917.8 ± 222.6, p=0,015). Total LDL-cholesterol (LDL-c) was similar in A and NA CT subjects (73.8 mg/dL ± 16.5 vs. 74.3 ± 9.1), but again different between A and NA T1D ones (66 mg/dL ± 13.2 vs. 89.6 ± 15.0, p=0.010), with this difference mainly due to Medium LDL-c (29.6 mg/dL ± 5.8 vs. 40.2 ± 6.1, p=0.006, in A and NA T1D subjects, respectively). VLDL-triglyceride (VLDL-TG) was lower in A CT subjects than in NA ones (33.8 mg/dL ± 19.5 vs. 50.3 ± 29.4, p=0,049) and was similar in A and NA T1D subjects (28.1 mg/dL ± 13.1 vs. 28.1 ± 13.7). This difference in CT groups was confined to the Large VLDL-TG (27.1 mg/dL ± 16.6 vs. 41.6 ± 25.3, p=0,031, in A and NA, respectively). Additional trends identified by LipoProfile showed that the T1D group presented lower Medium VLDL particles (medium VLDL-P) than the CT group (10.84 nmol/L ± 8.59 vs. 17.01 ± 14.92, p=0.014). Total LDL particles (LDL-P) were similar in A and NA CT subjects (1083.1 ± 290 vs. 1104.9 ± 337.3), but there was a significant difference between A and NA T1D subjects (948.7 ± 233.5 vs. 1264.3 ± 209, p=0.027), confirming observations made with DOSY. For clinical data, the Wilcoxon rank sum test was used, and for lipoprotein profiles, lineal models, adjusted for BMI, hypertension, active smoking, tryglicerides and HDL cholesterol.
Conclusion: T1D subjects that regularly practice physical activity present a lower proaterogenic lipid profile than their non-active T1D counterparts, suggesting that physical activity should be encouraged in T1D.
Supported by: METADIAB/CIBERDEM

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Anti-oxidant and anti-inflammatory properties of HDL are inhibited by apolipoprotein A-I antibodies isolated from type 2 diabetes patients
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Background and aims: Several evidences points the a role for islet inflammation in pathogenesis of type 2 diabetes. HDL suppression of the lipid induced macrophage inflammation enhances insulin sensitivity. Moreover infusions of recombinant HDL in patients with type 2 diabetes increase the anti-inflammatory properties of the resulting plasma HDL fraction. Recently, our group identified the presence of antibodies towards HDL and its main

apolipoprotein (ApoA-I) in patients with type 2 diabetes. Herein we intend to investigate the effect of anti-ApoA-I (aApoA-I) antibodies isolated from patients with type 2 diabetes in the anti-inflammatory and anti-oxidant activities of HDL *in vitro*.
Materials and methods: aApoA-I antibodies were isolated from patients serum by immunoaffinity chromatography using an HiTrap NHS- activated HP (1 mL) column. A possible inhibitory effect of aApo A-I antibodies on paraoxonase 1 (PON1) activity was addressed by performing dose dependent inhibition assays by incubating HDL (100 μg/mL) plus aApo A-I antibody (0.001-10μg/mL) isolated from patients. PON1 activity was assessed by quantification of nitrophenol formation by spectrophotometry. To investigate the effect aApo A-I antibodies on the expression of vascular adhesion molecules (VCAM-1), HUVECs were incubated with human HDL (1.6 mg/mL) without or with aApoA-I antibodies (50 μg/mL) isolated from patients serum and/or TNF-α (10 ng/mL). Expression of VCAM-1 was assessed by flow cytometry using a fluorescein-conjugated mouse monoclonal anti-human VCAM-1.
Results: PON1 activity was inhibited in a dose-dependent fashion from 5% to 37% by the aApoA-I antibodies isolated from patients, after correction for a non-specific human IgG used as control. Pre-incubation of HDL with aApo A-I antibodies abrogated the inhibitory effect of HDL on VCAM-1 expression, in more than 65%, when compared with the non-specif human IgG.
Conclusion: This study shows that aApo A-I antibodies isolated from patients with type 2 diabetes inhibit HDL-associated anti-oxidant and anti-inflammatory properties in vitro, and may contribute to the pathogenesis of type 2 diabetes.
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VLDL-Apo B 100 fractional synthesis rate (FSR) is decreased in type 2 diabetes mellitus patients with diabetic nephropathy and hypertriglyceridaemia: possible defect in VLDL-Apo B 100 removal
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Background and aims: Patients with diabetic nephropathy (DN) and albuminuria often exhibit increased triglyceride concentrations, suggesting an altered lipoprotein metabolism. We have previously shown that in T2DM patients with albuminuria, the hepatic fractional synthesis rate (FSR) of both albumin and fibrinogen, two liver-synthesized secreted proteins, are increased, that of albumin possibly representing a compensatory mechanism for the increased urinary albumin loss. Since VLDL-Apo B 100 is synthesized by the liver too, we argued whether also the FSR of this protein is increased in T2DM subjects with DN and albuminuria, thus accounting for their hypertriglyceridaemia.
Materials and methods: We employed isotope dilution, precursor-product methods, using leucine stable isotope infusion to measure VLDL-Apo B 100 FSR (in addition to that of albumin and fibrinogen FSR, data previously published), in T2DM male subjects with DN (n=9, age: 53±2 yrs; BMI: 31±2 kg/m^2) and without DN (n=4; age: 44±6; BMI: 28±1 kg/m^2), as well as in healthy male controls (n=6, age: 42±7 yrs; BMI: 28±2 kg/m^2). Of the T2DM patients with DN, four had microalbuminuria and five macroalbuminuria. A primed continuous infusion of ^{13}C-leucine was administered to reach a tracer steady state of the precursor pool plasma α-ketoisocaproate (KIC). Plasma protein synthesis were assessed by modelling analysis of tracer-to-trace ratio dynamics in the protein product pool, monitored in the 6 hr period following tracer infusion. Data are expressed as Mean±SE.
Results: In T2DM patients with DN, both plasma triglyceride (TG) (2.2±0.3 mmol/L) and VLDL-Apo B 100 concentrations (16.4±3.3 mmol/L) were ≈1-fold greater than in nondiabetic controls (TG: 1.2±0.2 mmol/L, p<0.01; VLDL-Apo B 100: 8.1±0.6 mmol/L, p<0.05). In contrast, VLDL-Apo B 100 FSR in the T2DM patients with DN was ≈60% lower (5.2±0.7 pools/day) than that in controls (12.5±3.7 pools/day, p<0.04). In the T2DM patients without DN, plasma triglyceride (1.2±0.1 mmol/L) and VLDL-Apo B 100 concentrations (8.6±0.7 mg/dl), as well as VLDL-Apo B 100 FSR (10.7±2.3 pools/day) were similar to those of controls.
Conclusions: The hypertriglyceridaemia accompanying T2DM patients with DN is not due to hepatic VLDL-Apo B 100 overproduction, which is instead decreased, but it is should rather be due to decreased removal rates. Protein synthesis of liver-synthesized, secreted proteins appears to be selectively regulated.
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LDL receptor knock-out mice impaired spatial cognition with hippocampal vulnerability to apoptosis and synapse
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Background and aims: Evidences from clinical studies support that abnormal cholesterol metabolism in the brain lead to the progress of cognitive dysfunction. LDL receptor (LDLR) is well known for its role in regulating brain cholesterol homeostasis. We investigated whether LDLR played roles in spatial cognition and the potential mechanisms.
Materials and methods: Twelve-month-old Ldlr$^{-/-}$ mice (n =10) and wild-type littermates C57BL/6J (n =14) maintained on a standard lab chow diet were subjected to the morris water maze test. All animals were killed for synapse and apoptosis study uses one week after the completion of the behavioral test.
Results: The plasma cholesterol concentrations of Ldlr$^{-/-}$ mice increased limitedly than C57BL/6J ($t(4) = 4.076$, $P = 0.015$). The results of behavioral test revealed that Ldlr$^{-/-}$ mice displayed impaired spatial memory with decreased expression levels of synaptophysin and number of synaptophysin-immunoreactive presynaptic bouton in the hippocampal CA_1 and dentate gyrus areas (all $P<0.05$). Ultrastructural changes in DG area of the hippocampus were observed by transmission electron microscopy. Furthermore, the apoptosis occurred in the hippocampus of Ldlr$^{-/-}$ mice was discovered with the elevated Bax/Bcl-2 ratio for gene and protein expression (t=4.369, P=0.012; t= 6.163, P=0.004, respectively), and activated-caspase3.
Conclusion: LDLR deficiency induced deteriorations of brain cholesterol homeostasis contributing to impaired spatial cognition, probably via its negative effects on hippocampal vulnerability to apoptosis and synapse deficits.

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Early improvement of postprandial lipaemia after bariatric surgery in obese type 2 diabetic patients
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Background and aims: Bariatric surgery (BS) for the treatment of morbid obesity is able to positively influence glucose homeostasis in obese type 2 diabetic patients (T2DM). This improvement occurs early after surgery and is related, at least in part, to restoration of entero-insular axis. Fasting lipid profile also improves after BS, but no data is available on the impact of BS on postprandial lipid metabolism. To evaluate the short-term effects (2 weeks) of BS on fasting and postprandial lipid metabolism in patients with T2DM and obesity and the relationship between changes in lipid levels and incretin hormones (GLP-1 and GIP).
Materials and methods: We studied 25 patients with T2DM and obesity (age = 46 ± 8 years, BMI = 44 ± 7 kg / m²), of which fifteen patients underwent sleeve gastrectomy and ten underwent roux-en-y gastric bypass. Since the changes in body weight and in the main metabolic parameters were similar with the two interventions, data were pooled all together. Lipid and incretin hormone levels were evaluated fasting and for 3 hours after ingestion of a standard liquid mixed meal both at baseline and 2 weeks after BS.
Results: After surgery, there was a significant reduction in body weight (p<0.001), fasting plasma glucose (p<0.001), fasting plasma insulin (p<0.05), HOMA-IR (p<0.005) and fasting plasma lipids (-26% in plasma triglycerides, p<0.005; -13% in total cholesterol, p<0.005; -16% in LDL cholesterol, p<0.05; -19% in HDL cholesterol, p<0.001). During the meal, the response of plasma triglycerides, total cholesterol and HDL cholesterol was significantly lower compared to baseline (p<0.05 - p<0.001). In particular, the incremental area under the curve (IAUC) of plasma triglycerides decreased by 70% (p <0.001). The meal-response of GLP-1, rather flat preoperatively, increased significantly after 2 weeks (IAUC 36 ± 30 vs. 1794 ± 374 pmol / l • 180 min, p <0.001), while the response of GIP did not change significantly. No relation was found between changes in incretin hormones and postprandial triglyceride concentration. The reduction of fasting triglycerides correlated positively with the reduction of insulin resistance (p <0.05).
Conclusion: BS leads to an early improvement of postprandial lipemia, in particular triglyceridemia, which decreases by 70%. This effect does not seem not to be influenced by the decrease in body weight and/or changes in incretin hormones, but is likely to be ascribed to reduced intestinal lipid absorption.

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Short-term changes of HDL-C related parameters during transition from insulin to GLP-1 in type 2 diabetics

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Background and aims: We previously observed that HDL-C decreased during the therapeutic transition from insulin to GLP-1 analogue (GLP1a). We made a closer examination of HDL metabolism in patients with GLP1a monotherapy (Gm) and with GLP-1 analog (GLP1a) and basal insulin (INS) combination therapy (GbIc).
Materials and methods: Fifty-three Japanese type 2 diabetics treated with INS monotherapy were enrolled in this study. Liraglutide (LIR) was introduced during the three-week admission period. GbIc was introduced to those with elevated fasting glucose over 126 mg/dl. Fasting blood samples were obtained before and 15 days after LIR introduction for the following measurements; TC, TG, HDL, Apolipoproteins (AI, AII, B, B48, CII, CIII, E), lathosterol, sitosterol, pre beta1-HDL, CETP activity, LCAT activity, soluble phospholipase A2 (sPLA2), lipoprotein lipase mass (LPL), bile acid (BA), free fatty acid (FFA), glucagon, adiponectin, resistin and C-peptide (CPR).
Results: Clinical features of all subjects were as follows; M/F 37/16, age 65±13 years old, HbA1c 7.5±1.4 %, TC 166±33 mg/dl, TG 124±79 mg/dl, HDL 50±15 mg/dl. To achieve similar pre-meal plasma glucose levels, 40 patients recieved Gm and 13 patients received combination therapy with GIc. The following parameters decreased in both Gm and GbIc; TC (166→145 mg/dl), LDL-C (92→76 mg/dl), HDL-C (50→43 mg/dl), ApoAI, ApoAII, ApoB, ApoE, preβ1HDL, sitosterol, FFA, BMI, BA, sPLA2. The following additional parameters showed no change in both Gm and GbIc; TG, lathosterol, LPL, glucagon. Gm specific changes were shown as follows; an increase in ApoB48, sPLA2, BA, resistin, and CPR, and a decrease in adiponectin. GbIc specific changes were as follows; a decrease in ApoCII, LCAT, and CETP.
Conclusion: From the results, three possible mechanisms for HDL-C reduction should be considered. The first is reduced insulin signaling for lipoprotein metabolism. Generally, insulin resistance-like profiles were observed in Gm, and some of these profiles looked corrected in GIc. We speculated that previous treatment with insulin suppressed TG generation and activated LPL. The second is weight reduction. GLP1a induces weight reduction for short periods which would have contributed to the observed changes. The third is increased BA, which suggested the activation of farnesoid X receptor (FXR) resulting in increased ApoAI efflux into the gastrointestinal tract. Even with similar glycemic control, the elimination of extra portal insulin administration affects lipoprotein metabolism relating to HDL.
Clinical Trial Registration Number: UMIN000005565

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Comparison of the HDL-C-raising effects of statins in high-risk hypercholesterolaemia with diabetes: TOHO-LIP diabetes sub-analysis for 96 weeks

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Background and aims: HDL-Cholesterol (HDL-C) has attracted notice for its anti-atherosclerotic properties. A lower HDL-C level is definitively linked to a higher risk of cardiovascular disease. Few clinical studies, however, have examined how potent statins administered over a long term affect HDL-C in diabetic subjects with hyperlipidemia.
Materials and methods: The TOHO-LIP (TOHO-Lipid Intervention trial using Pitavastatin) is a prospective study to compare how two agents, pitavastatin 2mg/day and atorvastatin 10mg/day, affect cardiovascular prognosis, lipid profiles, laboratory parameters, and safety in patients with high-risk hypercholesterolemia over a treatment period of 5 years. The trial is conducted with 652 enrolled subjects at three TOHO University hospitals in Japan. In this report we present laboratory data at 96 weeks of treatment in 414 diabetic patients as a sub-study for TOHO-Lip.
Results: TC, LDL-C, and non HDL-C were significantly improved at 96 weeks with both statin treatments. These statins slightly increased HDL-C, but not to a statistically significant level. We conducted a quartile analysis with respect to baseline HDL-C, since baseline HDL-C values affected the percent change of the HDL-C at 96 weeks (as showen in the table). In quartile 1, the lowest HDL-C group (baseline HDL-C≦45mg/dl), the subjects receiving pitavastatin exhibited a significant increase of HDL-C (13.1%) at 96 weeks, whereas those receiving atorvastatin exhibited an increase of only 3.3%. This analysis in quartile 1 also brought about statistically significant difference between the two treatment depending on the prescribed statins (p = 0.0090). In another analysis in which hypo-HDL-C was defined as baseline HDL-C below 40 mg/dl, the differential effect of pitavastatin was confirmed by a significant percent increase in HDL-C (14.5%) at 96 weeks (versus a 6.2% increase in the subjects receiving atorvastatin). This effect of pitavastatin did not differ between genders. Glucose metabolism parameters (FPG or HbA1c) remained unchanged in both groups. In univariate analysis, the change in HDL-C was significantly correlated with the changes in HbA1c and the baseline HDL-C below 40 mg/dl. In a stepwise multi-variate analysis, the changes in HbA1c and baseline HDL-C below 40 mg/dl had significant independent effects in the total group, whereas the treatment drug did not.
Conclusion: Both strong statins showed comparable effects on lipid profiles in diabetic subjects. In patients with hypo-HDL cholesterolemia, HDL-C was improved only in the pitavastatin group. The evidence suggests that pitavastatin was effective in preventing cardiovascular events.

Time-course of changes in the HDL-C of quartile1

	week	0	12	24	48	72	96	p
HDL-C	pitava	39	43	45	44	45	45	0.012
	atrova	39	42	42	42	42	40	
n	pitava	56	46	46	45	42	39	
	atrova	45	42	41	38	37	38	

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JAZF1 improves hepatic insulin resistance in C57bl/6j mice and attenuates atherosclerosis in apoE-knockout mice

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Background and aims: Genomewide association studies have suggested an association of Juxtaposed with another zinc finger gene 1(JAZF1) with type 2 diabetes mellitus (T2DM). As an inhibitor of the TAK1/TR4 signaling pathway, JAZF1 has been shown to be involved in gluconeogenesis, lipid metabolism and insulin sensitivity. In previous studies, we reported that the overexpression of JAZF1 in 3T3-L1 adipose cells and hepatic carcinoma Hepa1-6 cells suppressed lipid synthesis and accumulation and increased lipolysis. It remains unclear, however, whether chronic, ubiquitous JAZF1 activation in vivo ultimately benefits or impairs metabolic control, insulin sensitivity and atherosclerotic vascular disease. To address this question, we sought to determine whether an increase in JAZF1 expression in vivo was sufficient to affect both metabolic control and atherosclerotic plaque development.
Materials and methods: Adenovirus-mediated JAZF1 overexpression was used to characterize the role of JAZF1 in the regulation of lipid metabolism and the development of atherosclerosis in normal chow- or high fat diet(HFD)-fed ApoE-/- mice. Insulin sensitivity was examined by euglycaemic-hyperinsulinaemic clamping. Glucose rates of appearance (GRa) were determined with 3-[3H] glucose. Whole body GRa and glucose uptake (GRd) were calculated using the non-steady-state equation. Liver tissue was procured for histological examination, real-time RT-PCR and western blot analysis. Cholesterol de novo synthesis was measured by intraperitoneal [1-14C] acetate injection and atherosclerotic plaques were quantified by histological analysis.
Results: JAZF1 overexpression improved HFD-induced hepatic insulin resistance in C57BL/6J mice. In HFD-fed ApoE-/- mice, JAZF1 overexpression decreased serum cholesterol levels and hepatic cholesterol synthesis by inhibiting expression of 3-hydroxy-3methylglutaryl coenzyme A reductase (HMGCR). Furthermore, the en face and cross-sectional plaque areas of aorta and aortic sinus were reduced, and CD68+ macrophages in the central region of the plaques were significantly decreased.
Conclusion: We report the first evidence that JAZF1 overexpression was protective against development of atherosclerosis and insulin resistance in-

duced by a HFD in apoE-/- mice and C57BL/6J mice. Our findings illustrate a heretofore unrecognized link between JAZF1 and atherosclerosis and insulin resistance in the HFD-fed a mouse model. Whether or not JAZF1 has also antiatherogenic and anti-insulin activity in human subjects remains to be explored.
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Cholesterol as a novel activator of HIF-1 pathway in hepatocytes under hypoxia
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Background and aims: Non alcoholic steatohepatitis (NASH) is associated with obesity, insulin-resistance and diabetes and promotes progression to liver damage. Recently, free cholesterol has been linked to the pathogenesis of NASH. A growing body of evidence demonstrates a role for hypoxia inducible factor-1 (HIF-1) in the development of liver fibrosis, inflammation, hepatic cellular carcinoma as well as other liver diseases. The aim of the present study was to investigate possible activation of HIF1 by cholesterol in hepatocytes.
Materials and methods: AML12-hepatocytes were incubated with water-soluble cholesterol under normoxic conditions. HIF-1 and iNOS induction and mitochondrial dysfunction were evaluated by the use of reporter gene plasmids, RT-PCR, western blot, flow cytometry and biochemical analysis.
Results: Increased HIF-1α protein accumulation was evident in hepatocytes after 2h of incubation with cholesterol while enhanced HIF-1 transcriptional activity was observed after 6h of incubation ($p<0.05$). Investigations into the molecular mechanism showed that cholesterol inhibited HIF-1α subunit hydroxylation and thus proteasomal degradation ($p<0.001$) whereas HIF-1α gene expression was not significantly altered by cholesterol. Hepatocytes incubated with cholesterol exhibited augmented Nitric oxide (NO) levels at 2h and 6h, which corresponded with increased iNOS induction ($p<0.05$). Treating the cells with L-NAME, a potent inhibitor of iNOS, had no effect on HIF-1α stabilization at 2h but effectively attenuated cholesterol-induced HIF-1 accumulation and transcriptional activity at 6h ($p<0.05$). The role of reactive oxygen species (ROS) in initial HIF-1α stabilization was further determined. Mitochondrial dysfunction and enhanced mitochondrial ROS generation were observed in 2h cholesterol-treated cells ($p<0.05$). In order to establish a role for mitochondria-derived ROS in HIF-1α protein stabilization, mitochondrial DNA-depleted cells (p0AML12) were utilized. In these cells, the ability of cholesterol to induce HIF-1 stabilization and transactivation was significantly abolished.
Conclusion: Together, these results demonstrate that cholesterol promotes HIF-1 activation in hepatocytes in a ROS-dependant manner. Therefore, it is speculated that alterations of HIF-1 may contribute to the deleterious effects of cholesterol in the liver.

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Deficiency of clusterin aggravates insulin resistance in high fat fed mice
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Background and aims: Clusterin is a multifunctional glycoprotein and is known to be involved in lipid transportation. Clusterin levels in high-densitiy lipoprotein are lower in man with reduced insulin sensitivity and clusterin single nucleotide polymorphism in Japanese diabetic patients is associated with the prevalence of type 2 diabetes. Although the physiological role of clustserin and previous epidemiological studies implicates the close relationship between clusterin and insulin resistance or diabetes, there is no direct evidence suggesting the involvement of clusterin in insulin sensitivity. The aim of present study was to investigate whether clusterin is involved in insulin resistance.
Materials and methods: Male 8-week-old C57B/6 wild-type (WT) and clusterin knockout (KO) mice were randomly assigned into chow fed and high fat fed mice for 8 weeks and insulin sensitivity was measured with intraperitoneal glucose tolerance test (IPGTT) and hyperinsulinemic euglycemic clamp test.
Results: After high fat feeding, body weight, fat mass, and plasma levels of triglyceride and free fatty acids were not different between the two groups. Fasting glucose and insulin levels were higher in clusterin KO mice. In IPGTT, glucose levels were not different except for basal glucose levels (0 min) between these two groups in high fat fed group. However, insulin levels tended to be higher in clusterin KO mice at all time points. Area under curve for insulin was higher in clusterin KO mice. In hyperinsulinemic euglycemic clamp study, clamp insulin levels were higher in clusterin KO mice than WT mice after high fat feeding. High fat feeding reduced glucose infusion rate (GIR) and whole body glucose uptake (WB) both in WT and clusterin KO mice and there was no difference between these two groups. However, after adjusted for increased plasma insulin level during clamp, GIR (GIR/insulin) and WB (WB/insulin) were lower in clusterin KO mice. Skeletal muscle glucose uptake was reduced both in WT and clusterin KO mice after high fat feeding and there was no difference between these two groups. After adjusting increased clamp insulin levels, it was lower in clusterin KO mice. C-peptide levels were higher in clusterin KO mice than WT mice after fasting or feeding in high fat fed mice. Clamp c-peptide levels were also higher in clusterin KO mice. Gene expression of insulinase was higher in liver and lower in kidney. Clusterin mRNA level was higher in the skeletal muscle of high fat fed WT mice. Gene expression of antioxidant enzyme was lower and gene expression of cytokines levels was higher in clusterin KO mice than WT mice in high fat fed group.
Conclusion: These results suggest that deficiency of clusterin induces insulin resistance in high fat fed mice through increased oxidative stress and inflammation.
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PS 064 Adipokines and secreted proteins in obesity and diabetes

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In vivo proteomics reveals increased galectin-1 levels in subcutaneous interstitial fluid in newly diagnosed type 2 diabetes patients

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Background and aims: Obesity spreads over the world like an epidemic and a third of all obese individuals will develop type 2 diabetes (T2DM). Adipose tissue becomes insulin resistant when adipocytes increase in size and inflammatory cells invade the tissue. This transformation is followed by a changed expression and release of proteins manifesting adipose tissue as a significant endocrine organ. Galectin-1 was recently suggested to be a novel circulating biomarker in T2DM. The source of galectin-1 is less clear, but it is known that preadipocytes secrete galectin-1 during differentiation. Moreover, galectin-1 is regulated by hypoxia-inducible factor-1 and shows an association with neovascularization. Interestingly, subcutaneous adipose tissue is considered poorly circulated and hypoxic in T2DM patients. Our overall aim was, using avante-guard methodology, to unconditionally identify novel adipose tissue secretory factors relevant in the development of T2DM.
Materials and methods: Subcutaneous microdialysis enables sampling from the interstitial fluid and can be perfomed in vivo in human subjects with minimal invasiveness. Combining protein sampling using an ultrafiltrating membrane (Asahi, Plasmaflo OP-02, cut off 3 MDa) with LC-MS/MS is an innovational approach to characterize the adipose tissue secretome. We employed these procedures and investigated 7 male newly diagnosed T2DM patients in good metabolic control and 8 gender- and age-matched (55±3 vs 54±3 years, mean±SEM) healthy controls after fasting over night. Average duration of T2DM was 1.6±0.8 years, while BMI was higher in T2DM patients than in controls (25.9±0.7 vs 23.4±0.6 kg/m2, p<0.05).
Results: In total, 856 proteins were identified in the subcutaneous interstitial fluid. Of the proteins found in all samples > 80% were known secreted proteins. Based on the relative detection rate of peptides in the LC-MS/MS, we demonstrated that 36 proteins had a significantly different expression (students t-test, p<0.05) in T2DM patients compared with healthy controls. One of these proteins was galectin-1, the previously proposed biomarker in T2DM patients. We showed that galectin-1 is increased in subcutaneous interstitial fluid in T2DM (p< 0.05) and we also confirmed previous observations that serum galectin-1 was increased in T2DM patients compared with controls (p<0.05).
Conclusion: Our in vivo proteomics method for characterization of the subcutaneous interstitial fluid has identified 36 proteins as potential biomarkers in newly diagnosed T2DM patients. Furthermore, we show that insulin-resistant adipose tissue may be a source of galectin-1.

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Circulating chemerin level is associated with arterial stiffness in patients with type 2 diabetes

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Background and aims: The prevalence of diabetes is rapidly increasing worldwide, and cardiovascular disease (CVD) is the main cause of mortality in patients with type 2 diabetes (T2DM). A number of studies have demonstrated that several adipokines are implicated in the development of atherosclerosis. Recently, chemerin and omentin-1 have been shown as potential adipokines linking inflammation, obesity, and CVD. However, there have been scant clinical data relating these adipokines to atherosclerosis in T2DM patients. Therefore, we investigated the relationship of circulating chemerin and omentin-1 levels with atherosclerosis in patients with T2DM.
Materials and methods: A total of 113 T2DM subjects (61 M/52 F, age 58 ± 1 yr) were enrolled. Subjects with known CVD, cancer, chronic kidney disease, or active infection were excluded. We assessed subclinical atherosclerosis by measuring doppler-derived aortic pulse-wave velocity (PWV) and carotid intima-media thickness (IMT). Circulating chemerin, omentin-1, leptin, adiponectin, resistin, and high-sensitivity C-reactive protein (hsCRP) levels were also measured.
Results: Chemerin was significantly related to leptin (r=0.26, $P < 0.01$), estimated glomerular filtration rate (eGFR; r= -0.29, $P < 0.01$), and albumin-to-creatinine ratio (ACR; r=0.20, $P < 0.05$). Also, chemerin was associated with aortic PWV (r=0.31, $P < 0.01$), but not carotid IMT. Aortic PWV was significantly correlated with age, body mass index (BMI), eGFR, ACR, resistin, and chemerin. Multiple regression analysis showed that circulating chemerin level was independently associated with aortic PWV. Omentin-1 was significantly related to age (r=0.26, $P < 0.01$), BMI (r= -0.27, $P < 0.01$), hsCRP (r= -0.19, $P < 0.05$), adiponectin (r=0.31, $P < 0.01$), triglyceride (r= -0.30, $P < 0.01$), high-density lipoprotein cholesterol (r=0.26, $P < 0.01$). However, there was no association of omentin-1 with aortic PWV, or carotid IMT.
Conclusion: Circulating chemerin level was independently associated with arterial stiffness in T2DM patients without known CVD. These results suggest that chemerin is related to subclinical atherosclerosis in T2DM.

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Serum omentin significantly predicts cardiovascular events both in patients with the metabolic syndrome and in subjects who do not have the metabolic syndrome

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Background and aims: Some recent small cross-sectional studies have described associations of the novel adipocytokine omentin with atherosclerosis. However, no prospective data on the power of omentin to predict cardiovascular events are available.
Materials and methods: We therefore measured serum omentin in a series of 297 patients undergoing coronary angiography for the evaluation of established or suspected stable CAD; the metabolic syndrome (MetS) was defined according to national cholesterol education programme adult treatment panel III criteria; cardiovascular events were recorded over a mean follow-up period of 3.2 years.
Results: During the follow-up period, 18.4% of our patients suffered cardiovascular events, corresponding to an annual event rate of 5.8%. In the total study population, serum omentin significantly predicted cardiovascular events both univariately (standardized adjusted HR = 1.47 [1.21-1.78]; p <0.001) and after adjustment for age, gender, BMI, diabetes, hypertension, LDL cholesterol, HDL cholesterol and smoking (HR = 1.49 [1.21-1.82]; p <0.001). From our patients, 98 had the MetS and 199 did not have the MetS. In both of these patient subgroups serum omentin strongly predicted cardiovascular events both univariately (HRs = 1.51 [1.15-2.00]; p = 0.003 and 1.41 [1.08-1.84]; p = 0.011, respectively) and after adjustment for age, gender, BMI, diabetes, hypertension, LDL cholesterol, HDL cholesterol and smoking (1.56 [1.09-2.25]; p = 0.016 and 1.48 [1.12-1.97]; p = 0.006, respectively).
Conclusion: From this first prospective evaluation of the cardiovascular risk associated with serum omentin we conclude that elevated serum omentin is a strong predictor of cardiovascular events both among patients with the MetS and among subjects who do not have the MetS.

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Ghrelin and its relationship with insulin resistance markers in myocardial infarction complicated with acute heart failure

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Background and aims: The main reason for myocardial infarction (MI), which remains one of the most common causes of mortality and morbidity, is atherosclerotic lesions of large or medium-sized coronary arteries with their further thrombotic occlusion. The most severe MI complication having an impact on both in-hospital and post-hospital periods as well as patient's wellfare is acute heart failure (AHF). The mechanisms of AHF are being studied, adipokines and insulin resistance (IR) markers being considered to contribute greatly. In particular, the role of ghrelin, which was first a marker of cardiac cachexia syndrome, is being discussed: its positive impact on cardiovascular system, including left ventricular pump function, was described. Considering the fact that ghrelin is able to inhibit insulin secretion,

it can have a role in IR development, being a part of MI pathogenesis and its complications. The study was aimed at assessing the levels of ghrelin and its relationship with IR markers in MI patients complicated with AHF.
Materials and methods: 133 patients (90 males and 43 females, aged 60,8±1,69 years) with ST elevation MI were enrolled in the study. According to the presence of acute heart failure at day 1 from MI onset all the patients were divided into 2 groups: group 1 included 102 Killip I MI patients and group II enrolled 31 Killip II-IV MI patients. The control group included 33 patients with no cardiovascular disease; their age and gender were similar to those of the treatment group patients. At days 1 and 12 from MI onset serum ghrelin and insulin concentrations were measured by ELISA.
Results: Both groups had lower ghrelin concentrations at days 1 and 12 of the hospital stay as compared to the healthy subjects; group II having a more pronounced decrease. In MI patients with no clinical manifestations of AHF serum insulin levels at day 1 had a tendency towards increasing but were not significantly different from those in the controls and decreased by day 12. In patients with clinical symptoms of AHF these levels were 1.4-fold higher than those in the controls and 1.2-higher than those in Killip I patients; they significantly decreased by day 12. Glucose concentrations in both groups at day 1 were higher than those in the controls, at day 12 glucose levels slightly decreased in Killip I patients but were still higher than the control ones; and, on the contrary, glucose levels increased in Killip II-IV patients. The correlation analysis showed a negative correlation between ghrelin and glucose concentrations in group I patients (R=-0,3 p=0,01) and a negative correlation between ghrelin and insulin concentrations in group II patients (R=-0,72 p=0,0007). A stepwise selection method was used to determine the most significant parameters in AHF development and ghrelin and insulin were selected to be included in the logistic regression models. In order to assess the efficacy of the parameters under study ROC-analysis was applied and it demonstrated that the areas under the curve (AUC) of the studied parameters were good: AUC=0,78 p=0,047 for insulin and AUC=0,79 p=0,002 for ghrelin. Consequently, these parameters can be used for AHF prediction in MI patients.
Conclusion: Thus, ghrelin can be regarded not only as IR marker but also as a predictor of AHF in these patients.

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Alterations in the levels of the endocrine factors FGF21 and FGF19, and in the expression of their receptors, are associated with impaired glucose homeostasis in obese patients

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Background and aims: FGF19 and FGF21are endocrine factors involved in glucose homeostasis. Studies in rodents indicate that FGF21 and FGF19 protect against type II diabetes and obesity. However, the levels of FGF21in obese patients are high. Based on rodent models, it has been proposed that obesity may be a situation of FGF21 resistance which contributes to impaired glucose homeostasis. We investigated the circulating levels of FGF21 and FGF19 in obese patients with varying degrees of abnormal glucose homeostasis and type II diabetes. We also determined gene expression for the components of tissue responsiveness to FGF21 and FGF19, namely FGF receptor-1 and -4 (FGFR1, FGFR4), and the co-receptor beta-Klotho (KLB), in liver and adipose tissue from patients.
Materials and methods: We analysed 61obese patients (BMI, median: 40.5, IQR: 34.7-46.2), sub-classified as normoglycaemic (NG, 36), showing impaired glucose tolerance (IGT,15) or with type II diabetes (T2D,10); and lean healthy controls (35). Biopsies from liver, visceral fat and subcutaneous fat from obese patients were obtained on occasion of bariatric surgery. Tissue samples from healthy controls were also analysed (5, liver; 7, subcutaneous fat; 6, visceral fat). Anthropometric data, and DEXA analysis of adiposity were recorded. Plasma metabolic and hormone parameters were determined using standard procedures. FGF19 and FGF21 levels were measured using ELISA (Biovendor). Gene expression was analysed by quantifying specific transcripts using TaqMan probes and qRT-PCR(Applied Biosystems).
Results: FGF21 serum levels were significantly increased in obese patients compared to controls, while FGF19 levels were decreased. FGF21 levels were positively correlated with markers of insulin resistance (HOMA-R; r=0,37, P=0,0002; insulin, r=0,32, P=0,0012. Obese patients with compromised glycaemia (IGT and T2D) showed a significant increase in serum FGF21 compared to obese normoglycaemic patients (P=0.03 versus IGT, P=0.02 versus T2D). Conversely, FGF19 levels were negatively correlated with insulin levels (r=-0.28, P=0.0034) and HOMA-R (r=-0.27, P=0.0073). In obese patients, the hepatic expression of FGF21 and KLB were increased relative to healthy controls (P = 0.049, P = 0.040, respectively), but no clear trend was found in relation to the glycaemic homeostasis status. FGF21 expression was undetectable in adipose tissues. FGFR1 expression was unaltered in adipose tissues from patients while KLB expression was strongly decreased in visceral fat from obese patients (P = 0.0027). The reduction in KLB was progressively more marked as glycaemic status was worsened, being the lowest in T2D obese patients.
Conclusion: Our results are consistent with the hypothesis of FGF21 resistance in obesity, being much more intense in patients with type II diabetes. Resistance to FGF21 may be mediated by the reduction in KLB expression, an essential component of FGF21 action, in visceral fat. The decrease in FGF19 levels, in combination with reduced KLB expression, may also contribute to metabolic dysregulation in obese patients.
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Circulating osteopontin levels are associated with whole body insulin resistance in type 2 diabetes mellitus in humans

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Background and aims: Obesity and type 2 diabetes (T2DM) are associated with insulin resistance and low-grade inflammation. Osteopontin (OPN) is a multifunctional protein expressed in activated macrophages and T-cells, osteoclasts, hepatocytes, smooth muscle and endothelial cells. OPN plays a role in the release of inflammatory cytokines such as TNF-α and IL-6 which are known to be increased in atherosclerosis, diabetic vascular diseases and hepatic inflammation. Plasma OPN levels are increased in obese as compared to lean subjects. The aim of the study was to compare the circulating concentrations of OPN in normal glucose tolerance (control subjects) and T2DM subjects and its relation with insulin sensitivity and insulin secretion. Low-dose pioglitazone (15 mg/day) effects on OPN levels were evaluated in T2DM patients.
Materials and methods: In a cross-sectional study, 20 T2DM patients treated with metformin and/or sulfonylurea and 18 control subjects were studied. T2DM patients were randomized to receive low-dose pioglitazone or placebo for 6 months. All subjects received: (I) OGTT; (II) euglycemic hyperinsulinemic clamp to directly evaluate whole body insulin sensitivity in vivo; and (III) measurement of plasma OPN levels. In T2DM patients all procedures were repeated after 6 months treatment.
Results: Type 2 diabetics exhibited significantly increased circulating OPN concentrations as compared with control subjects (6.1±1.2 vs. 3.1±0.7 ng/ml, p=0.04). A correlations were found among OPN levels, fasting plasma glucose (r=0.45, P=0.005), 1 hour OGTT plasma glucose (r=0.37, p=0.02), 2 hours OGTT plasma glucose (r=0.36, P=0.02) and fasting insulin (r=0.42, p=0.008). A negative correlations were found between OPN levels and M/I value (r=-0.41, p=0.01) and disposition index (r=-0.32, P=0.04). The area under the ROC curve for OPN levels as a predictor of M/I value less than 4 (insulin resistant state) was 0.79 (95% CI, 0.63-0.94; p=0.004). Adipose tissue insulin resistance index (fasting FFA x fasting insulin) was higher in T2DM subjects as compared to control subjects (4.1±0.9 vs. 3.0±0.5, p=0.03) and it was correlated with OPN levels (r= 0.33, p=0.04). OPN levels were inversely correlated with total cholesterol (r=-0.36, p=0.02) and LDL cholesterol (r=-0.48, p=0.002) while were directly positively correlated with triglycerides levels (r=0.33, p=0.04).OPN levels correlated with MCP-1 (r=0.52, p=0.001), TNF-α (r=0.45, p=0.006), IL-6 (r=0.38, p=0.02) and Fractalkine (r=0.38, p=0.03) levels. After pioglitazone treatment, baseline OPN levels were reduced although not significantly (5.5±1.5 vs. 5.1±1.4 ng/ml, P=0.63). OPN levels were reduced during clamp after pioglitazone treatment although not significantly (5.4±1.5 to 4.0±1.5 ng/ml at 120 min, p=0.2 and 5.6±1.6 to 2.9±1.3 ng/ml at 180 min, p=0.1) as compared to placebo (7.8±2.5 to 7.7±2.2 ng/ml at 120 min, p=0.3 and 7.7±2.5 to 6.9±1.7 ng/ml at 180 min, p=0.2).
Conclusion: Higher OPN levels are associated with reduced whole body insulin sensitivity and reduced disposition index. These findings reveal a potential therapeutic impact of targeting OPN and inflammation, which could lead to improvement in insulin sensitivity while reducing cardiovascular risk in T2DM patients.

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DPP4 shedding is mediated by ADAM17 and is upregulated by severe hypoxia

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Background and aims: DPP4 has recently been identified as a novel adipokine by comprehensive proteomic profiling of the adipocyte-secretome by our group. Expression of this protease is upregulated in visceral adipose tissue of obese patients and circulating DPP4 correlates with the metabolic syndrome. How DPP4 is shedded from the membrane is currently unknown. Our aim was to understand how DPP4 release is regulated and which enzymes are responsible for the shedding of DPP4 from the cell membrane.
Materials and methods: We used human smooth muscle cells (hSMC) and primary human adipocytes, which showed a high DPP4 release (300 pg/mL and 1000 pg/mL respectively) to elucidate the shedding mechanism *in vitro*. Inhibitors for different classes of proteases were used to identify potential shedding enzymes. To narrow down the prospective members of the involved protease family we used specific inhibitors or silencing of the respective enzyme. To study the regulation of DPP4 release, we applied acute hypoxic conditions of 1% oxygen in a Biospherix hypoxia work chamber. DPP4 release to the culture medium was measured by ELISA. Expression of target genes was assessed by qRT-PCR measurements. Selected experiments were also conducted in primary human adipocytes.
Results: Of the tested protease inhibitors, the general MMP and ADAM inhibitor Batimastat, the serine protease-inhibitor AEBSF and the cysteine protease-inhibitor E64 showed significant reduction of DPP4 release in hSMC (remaining DPP4 release: Batimastat 50%, E64 70%, AEBSF 60%). We could observe no additive effects of AEBSF, E64 and Batimastat in combination, which suggests that the effects are not independent. A specific inhibitor against MMP2 showed a significant decrease of DPP4 release, whereas a specific MMP9 inhibitor and silencing of MMP1 and 14 showed only slight effects on DPP4 shedding in hSMCIn contrast, silencing of ADAM17 (aka TACE) resulted in a decrease of DPP4 release by about 40% in hSMC. In this cell type, we observed a significant increase of DPP4 release by hypoxia of 1% oxygen for 48 hours (1,5-fold), which is associated with increased expression of ADAM17 (1,4-fold) and MMP1 (2,5-fold). Hypoxia had no effect on MMP14 and DPP4 expression itself. To confirm that the involvement of the ADAM or Metalloproteinase family in DPP4 shedding is not only restricted to hSMC, we treated primary human adipocytes with the broad spectrum inhibitor Batimastat. Also in adipocytes, Batimastat significantly reduced DPP4 release by about 40%. Further experiments to prove the involvement of ADAM17 in the shedding process are ongoing at the moment.
Conclusion: Our results suggest that MMPs contribute only to a minor extent to DPP4 shedding whereas ADAM17 is a key player in the shedding process. DPP4 shedding is sensitive to severe hypoxia. A disbalanced interaction between MMPs and ADAMs in obesity and artherosclerosis in relation to hypoxia could be responsible for the elevated serum levels of DPP4.

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Serum follistatin is reduced in gestational diabetes mellitus: association with anthropometric parameters in offspring

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Background and aims: The Activin A-Follistatin system has emerged as an important regulator of lipid and glucose metabolism with possible repercussions on fetal growth. In the current study we analyze circulating activin A, follistatin and follistatin-like-3 (FSTL3) levels and their relationship with glucose metabolism in pregnant women and influence on fetal growth and neonatal adiposity.
Materials and methods: A prospective cohort was studied comprising 207 pregnant women, 129 with normal glucose tolerance (NGT) and 77 with gestational diabetes mellitus (GDM) and their offspring. Activin A, follistatin and FSTL3 levels were measured in maternal serum collected in the early third trimester of pregnancy. Serial fetal ultrasounds were performed during the third trimester to evaluate fetal growth. Neonatal anthropometry was measured to assess neonatal adiposity.
Results: Serum follistatin levels were significantly lower in GDM than in NGT pregnant women (9.22 $\pm$ 3.41 vs. 8.21 $\pm$ 2.32 ng/ml, $P = 0.012$) whereas serum FSTL3 and activin A levels were comparable between the two groups. Serum follistatin concentrations were negatively correlated with HOMA-IR ($r = -0.153$; $P = 0.030$) and positively with ultrasound growth parameters such as fractional thigh volume estimation in the middle of third trimester ($r = 0.212$; $P = 0.005$) and percent fat mass at birth ($r = 0.239$; $P = 0.002$). Also, in the stepwise multiple linear regression analysis serum follistatin levels were negatively predicted by HOMA-IR (B= -0.199; $P = 0.008$) and the diagnosis of gestational diabetes (B= -0.138; $P = 0.049$). Likewise, serum follistatin levels positively predicted fractional thigh volume estimation in the middle of third trimester (B= 0.214; $P = 0.005$) and percent fat mass at birth (B= 0.231; $P = 0.002$).
Conclusion: Circulating follistatin levels are reduced in GDM compared with NGT pregnant women and they are also independent positive determinants of fetal growth and neonatal adiposity. The Activin-Follistatin system should be considered as a new player influencing maternal and fetal metabolism during pregnancy.
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The novel role of oncostatin m, a GP130 cytokine secreted in adipose tissue, in the development of obesity and type 2 diabetes

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Background and aims: Adipose tissue is a highly active endocrine organ secreting several factors that comprise a new hormonal network linking adipose tissue with other tissues. The adipocytes, the primary constituent of adipose tissue, are responsive to several gp130 cytokines, which have been targeted as potential therapeutic agents in obesity. Oncostatin m (OSM) is one of these cytokines, but its effects on adipocytes have not been previously examined. The aim was to assess the effects of OSM *in vitro* (adipocytes) and to examine its expression *in vivo* (adipose tissue from mice and humans with obesity-induced insulin resistance).
Materials and methods: *In vitro:* Murine 3T3-L1 adipocytes were treated with OSM in different conditions and cell monolayers were harvested to test activation of STATs by western blot, and PAI-1 and IL-6 gene expression by PCR. *In vivo: Animals-* Fifty male C57BL/6J mice 6 weeks of age were fed either low- or high-fat diets for 2, 4, 6 and 12 wk. Visceral adipose tissue was isolated to examine OSM levels by western blot. *Humans-* Subcutaneous and visceral adipose tissue from obese patients prior to bariatric surgery were used to examine the OSM gene expression by PCR.
Results: OSM was up-regulated in adipose tissue of obese/type 2 diabetic mice and humans. The specific receptor of OSM (OSMRβ) was identified in adipocytes demonstrating that OSM activates STAT5 in a dose-dependent manner. In addition, OSM induced the expression of target genes implicated in metabolic diseases such as PAI-1 and IL-6.
Conclusion: All these novel findings suggest a key role of OSM in the development of metabolic disorders including type-2 diabetes.

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LBP is not merely a liver acute phase reactant: adipose tissue is an important source of this proinflammatory adipokine

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Background and aims: Circulating lipopolysaccharide binding protein (LBP) is an acute phase reactant known to be increased with obesity. We hypothesised LBP production by the adipose tissue in association with obesity.
Materials and methods: *LBP* mRNA and protein levels expression were analyzed in adipose tissue from 3 cross-sectional (n=210, 144, 28) and 3 longitudinal human cohorts, in adipose tissue fractions, during adipocyte differentiation, in genetic mice models and testing the effects of high fat diet, LPS and PPARγagonists. Functional *in vitro* and *ex-vivo* experiments were also performed.
Results: LBP synthesis and release is demonstrated in whole human and mice adipose tissue (AT), in isolated adipocytes and in different human and mice cell lines (hMADs, and 3T3-L1 cells), increasing with adipocyte differentiation. AT LBPexpression was robustly associated with inflammatory markers and increased with obesity, metabolic deterioration and insulin resistance in 2 independent cross-sectional human cohorts. AT LBP also increased longitudinally with weight gain and excessive fat accretion (in both humans and mice); and decreased with weight loss (in 2 other independent cohorts), in humans with acquired lipodystrophy and after PPARγ agonists ex-vivo. Inflammatory agents such as LPS and TNF-α led to increased adipose tissue LBP expression in vivo in mice and in vitro, while this effect was prevented in CD14 knockout mice. Functionally, LBP gene knockdown using shRNA or antibodies anti-LBP led to increased markers of adipogenesis and decreased adipocyte inflammation in human adipocytes.
Conclusion: LBP is a novel adipokine that seem to display an essential role in inflammation- and obesity-associated adipose tissue dysfunction.
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PS 065 Myeloid cells and type 2 diabetes

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Deletion of myeloid-cell protein tyrosine phosphatase-1B improves glucose tolerance and suppresses inflammatory responses in high-fat fed and endotoxaemic mouse models

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Background and aims: *Protein tyrosine phosphatase-1B* (*PTP1B*) negatively regulates insulin and leptin signalling; properties which render it an attractive drug target for the treatment of conditions associated with metabolic syndrome. However, some studies suggest caution when targeting macrophage-*PTP1B*, due to its potential anti-inflammatory role. The aim of this study was to investigate *in vivo*, the role of macrophage-*PTP1B* in inflammation and whole body metabolism, using myeloid-cell specific (*LysM*) *PTP1B*-knockout mice.
Materials and methods: Male *LysM-PTP1B* and control littermates were fed high-fat (HF) diet and whole body glucose/lipid metabolism, inflammatory status, and response to inflammatory challenges were analysed.
Results: *LysM-PTP1B* mice demonstrated improved glucose tolerance compared to age-matched controls after prolonged HF-feeding despite no alteration in body weight/adiposity. In HF obese mice injected intraperitoneally with low-dose lipopolysaccharide (LPS), *LysM PTP1B* displayed no evidence of hyperinsulinemia after three hours, in contrast to controls. Moreover, endotoxin-induced liver damage was decreased in *LysM-PTP1B* mice, as demonstrated by lower levels of serum alanine aminotransferase ($P<0.01$). qRT-PCR of white adipose tissue revealed lower F4/80 expression ($P<0.01$), indicating reduced macrophage migration, and diminished levels of TNFα ($P<0.01$) in the absence of *PTP1B*. Remarkably, *LysM-PTP1B* mice had increased basal and post-LPS ($P<0.05$ and $P<0.01$, respectively) serum IL-10 levels, suggesting a possible mechanism for the improvements in whole body glucose and inflammatory status.
Conclusion: This is the first study to demonstrate that myeloid-specific *PTP1B* deletion improves glucose tolerance and protects against endotoxic shock in mouse models of HF-feeding and LPS challenge. This is contrary to the popular hypothesis that myeloid *PTP1B* negatively regulates TLR4-mediated inflammatory signalling.
Supported by: BHF, EFSD/LILLY

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Siglec-7 is down-regulated in inflamed islets and activated peripheral mononuclear cells and restores beta cell function and survival

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Background and aims: In, both, type 1 and type 2 diabetes mellitus; cytokine and chemokine production and infiltrating immune cells are the hallmarks of islet inflammation. Siglecs (Sialic-acid binding immunoglobulin like lectins) are cell surface receptors expressed on haematopoietic cells which participate in such immune responses. Upon investigation, a β-cell specific expression of Siglec-7 was observed, which was altered in diabetic conditions in islets and in activated PBMCs. Here, we aimed to investigate whether such changes in Siglec-7 expression directly modulate β-cell function and survival.
Materials and methods: To investigate the role of Siglec-7 in β-cell function and survival, over-expression was carried out in isolated human islets with or without diabetic stimuli, followed by glucose stimulated insulin secretion (GSIS) and TUNEL assay. Cytokine secretion was analyzed by the Meso Scale Discovery® multi array technology. To ascertain its role in immune cells, Siglec-7 expression was analyzed by RT-PCR and FACS in freshly isolated PBMCs from non- diabetic individuals cultured with the same diabetogenic conditions or 100 ng/ml Lipopolysaccharide (LPS) for 12 hours.
Results: Siglec-7 was markedly down-regulated in islets isolated from patients with T2DM and in pancreases from autopsy from patients with T2DM (70% decrease vs non-diabetic islets; 85% vs non-diabetic pancreases). Overexpression of Siglec-7 improved β-cell function and survival in human islets isolated from patients with T2DM. The overexpression also prevented

glucolipotoxicity- and cytokine-induced β-cell apoptosis. To investigate the mechanisms of this protective effect cytokine secretion was analyzed in these cultured islets. Cytokines IL1β, IL-6 and TNFα were induced under glucolipotoxicity (1.75-, 4- and 2-fold respectively) and cytokine treatment (19-; 4.6-, 1.7-fold respectively), which were significantly inhibited by Siglec-7 overexpression. In isolated human islets from patients with T2DM, Siglec-7 overexpression caused a reduction in basal IL1β (40%) and TNFα (50%) expression. In PBMCs, LPS, glucolipotoxicity, as well as cytokine mixture induced activation as seen by the induction of IL-6 mRNA and CD25 expression; a simultaneous decrease was observed in Siglec-7 mRNA levels. The flow cytometric analysis of cell surface expression of Siglec-7 showed 35% decrease in percentage of Siglec-7 positive cells as well as a 25% decrease in the mean fluorescence intensity.
Conclusion: Our data suggest that Siglec-7 is regulated in T2DM and influences β-cell function and survival. Also, decreased Siglec-7 in activated PBMCs, hints towards the inhibitory role of Siglec-7 in the activation of these immune cells during the development and progression of diabetes mellitus.
Supported by: ERC

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The relationships between macrophage infiltration and amyloid deposition in the islets of humans with type 2 diabetes
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Background and aims: In humans, β-cell dysfunction and the loss of β-cell mass are often considered as two defining features of type 2 diabetes and it is suggested that these responses represent the culmination of prolonged exposure of islet cells to various stressors. Among such factors are amyloid deposition and chronic islet macrophage infiltration. This is important since it has been suggested that islet amyloid deposits may lead to inflammasome activation within islet cells and that this could contribute to the development of a chronic inflammatory milieu. However, it is not known whether islet amyloid deposits serve as a direct stimulus for macrophage infiltration. In the present study, we have assessed this relationship.
Materials and methods: Formalin-fixed, paraffin embedded pancreatic sections from 24 type 2 diabetes (T2D; 68.2±7.7y; range 46-79y) cases were examined using immunohistochemical techniques. To confirm earlier findings that macrophage infiltration is enhanced in the islets of patients with T2D, sections from 5 non-diabetic adults (NDA; age 51.4±9.3y; range 35-58y) were also included for comparative purposes. Sections were analysed for macrophage infiltration by staining with anti-CD68 serum and for amyloid deposition by thioflavin T staining.
Results: Amyloid deposition was found in the islets of 17 of 24 (71%) patients with type 2 diabetes. Macrophages were detected at a low frequency in both the islets of control individuals and in those with type 2 diabetes, although the frequency was increased 4.5-fold in type 2 diabetes (0.7±0.1 macrophages per islet in NDA to 3.2±0.1 in T2D; $p<0.001$). Among the patients with T2D, islets containing amyloid had increased macrophage numbers compared to those with no amyloid deposits. Analysis of 41 amyloid negative islets across 6 T2D patients revealed that a mean of 1.3 ± 0.21 macrophages were present per islet section. By contrast, analysis of 116 islets with amyloid deposits revealed the presence of 3.7 ± 0.3 macrophages per islet section ($p<0.001$). However, in patients with type 2 diabetes macrophage numbers also increased with islet size, irrespective of amyloid deposition. Islets with a cross-sectional area less than 350μm2 contained an average of 1.8 ± 0.24 macrophages per islet, whereas islets with an area more than 650μm2 contained a mean of 4.5 ± 0.3 macrophages per islet; $p<0.001$ (analysed across 343 islets from 12 patients). Importantly, amyloid negative islets were usually smaller in cross-sectional area than amyloid positive islets suggesting that macrophage infiltration might be related mainly to islet size rather than to the presence of amyloid per se. Therefore, islets of equivalent surface area were compared. In islets matched for cross-sectional area, a mean of 2.9±0.3 macrophages per islet were seen in amyloid negative islets (164 islets analysed in 4 T2D patients) whereas a mean of 2.1 ± 0.2 macrophages per islet section were found in amyloid positive islets (105 islets analysed).
Conclusion: Islets from patients with type 2 diabetes contain significantly more infiltrating macrophages than those without diabetes, consistent with the presence of chronic low grade inflammation under these conditions. Both macrophage number and amyloid content are correlated with islet size but that these relationships are independent. Therefore, it is concluded that the enhanced infiltration of islets by macrophages seen in type 2 diabetes is not a direct response to amyloid deposition.
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TIMP3 modulates CD11c$^+$ recruitment through IL6/Il6Ralpha complex in a model of metabolic disease
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Background and aims: Immune cells recruitment into adipose, hepatic, and vascular tissues disturbs tissue homeostasis and promotes the development of insulin resistance and diabetes. The net effect of immune cells infiltration into tissues depends on a delicate balance between salutary, reparative functions and pathogenic, proinflammatory influences. The complex formed by transmembrane protein A Metalloprotease and Disintegrin Domain 17 (ADAM17) and its tissue inhibitor of metalloproteinase 3 (TIMP3) is a unique central switch to the recruitment of circulating leukocytes from the bone marrow to inflamed sites. Among TIMP3 substrates, ADAM17 regulates TNF and its receptors, EGFR, and IL6R signaling pathways. Here we plan to investigate whether this axis affect immune cells phenotype and activity elucidating their function during the progression of metabolic diseases. We hypothesize that the modulation of ADAM17/TIMP3 axis on IL6 signalling will deviate immune cells from acute inflammation toward protection.
Materials and methods: In this proposal we applied emerging technologies in immune cell biology, animal models of metabolic disease, large-scale cytokine profile, transcriptomics and metabolomics to address a new role to ADAM17/TIMP3 pathway for the treatment of metabolic inflammation.
Results: (1) We characterized systemic and tissue resident F4/80$^+$, F4/80$^+$ CX-3CR1$^+$, CX3CR1$^+$, CX3CR1$^+$CD11c$^+$ that were found increased in TIMP3KO mice compared to the WT animals (1.9, 3.8, 2.3, 2.9 fold increase respectively, $p<0.001$) under high fat diet conditions; (2) level of circulating IL6 and IL6Rα were found significantly increased in TIMP3 ko animals compared to WT animals; IL6 was increased in adipose and hepatic tissue of TIMP3KO animals at 4 months of HFD; we determined the IL6 transignaling pathway as the mechanism responsible for the recruitment of circulating and tissue resident dendiritic and macrophagic cells like. (4) When the IL6/IL6R complex pathway was blocked the number of circulating and tissue resident inflammatory cells was reduced. As a consequence, the metabolic dysfunction was significantly improved.
Conclusion: Because myeloid cells accumulation and resident macrophage polarization is a critical event regulated by TIMP3 for the coordination of inflammatory events within fat tissue, increasing its release from cells directly at tissue injury site may be a valid approach for decreasing inflammatory signals in adipose tissue. If successful these studies will lead to a novel therapeutic paradigm for metabolic disorders.
Supported by: EFSD/Lilly

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Fructose supplementation exacerbates glucose intolerance and causes adipose tissue inflammation in PPARb/d-deficient mice
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Background and aims: Strong evidence exists that chronic moderate fructose intake (e.g. 30% fructose solution as only fluid source) results in increased de novo lipogenesis and insulin resistance. On the other hand, PPARb/d-has been proposed as a therapeutic target for the treatment of inflammation and insulin resistance. In this study we aimed to examine whether fructose intake exacerbates metabolic dysfunction in PPARb/d-deficient mice.
Materials and methods: Wild-type and transgenic mice with reduced expression of PPARb/d were fed with plain water or water containing 30% fructose ad libitum for a period of 8 weeks. After treatment, RNA and total protein extracts were obtained from epididymal white adipose tissue for subsequent mRNA expression and western blot analyses.
Results: Fructose supplementation exacerbated the glucose intolerance (glucose tolerance test) already present in PPARb/d-null mice. Body weight,

liver weight and hepatic triglycerides increased only in PPARb/d-null mice fed with fructose ($p<0.05$). Likewise, chronic intake of fructose increased the size of adipocytes in both wild type and PPARb/d-null mice, but only in white adipose tissue of knockout mice an increase was observed in TNF-α (approximately 2-fold, $p<0.05$) and Mcp-1 (4-fold, $p<0.05$) expression. Interestingly, a significant increase was observed in the mRNA levels of F4/80 (approximately 2.5-fold, $p<0.05$) and Cd68 (5-fold, $p<0.05$) only in PPARb/d-deficient mice fed with fructose, suggesting macrophage infiltration in white adipose tissue. Interestingly, when we explored whether the activation of the CD36-JNK pathway was involved in inflammation and glucose intolerance, we noticed an increase in CD36 protein levels and JNK phosphorylation in PPARb/d knockout mice fed with water, but these changes were of higher intensity in fructose-fed PPARb/d-deficient mice.
Conclusion: Fructose exacerbates the CD36-JNK pathway in the white adipose tissue of PPARb/d-deficient mice. These changes might contribute to the increase in inflammation and glucose intolerance observed in these mice when fed with fructose.
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Obesity induces CD11c$^+$ macrophages in murine adipose tissue which are distinctive from, but resemble, dendritic cells
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Background and aims: Obesity is hallmarked by immunological events in adipose tissue (AT) that contribute to the development of a chronic inflammatory state, leading to insulin resistance and atherosclerosis. During obesity, AT resident anti-inflammatory (so-called M2) macrophages are replaced by inflammatory (so-called M1) macrophages. These "M1" AT macrophages (ATMs) express the dendritic cell marker CD11c. Dendritic cells are important immune regulators shown to promote insulin resistance and atherogenesis. Currently, it is unclear whether and how CD11c$^+$ ATMs differ from dendritic cells and if they are activated by obesity. Our aim was to define a strategy for discriminating between resident CD11c$^-$ ATMs, inflammatory CD11c$^+$ ATMs and dendritic cells and to investigate the effect of obesity on their presence and phenotype.
Materials and methods: Male C57Bl/6 mice were fed chow or high fat diet (45 kcal% from lard) for 10 weeks and the stromal vascular fraction, containing immune cells, from epididymal AT was analyzed by flow cytometry. Furthermore, ATMs were isolated for mRNA analysis.
Results: Monocytes, but not CD8 and CD4 T-cells, B-cells or granulocytes, were increased in obese vs lean epididymal AT (6.1 ± 0.77 vs 3.6 ± 0.21% of stromal vascular cells, $p< 0.05$). Additionally, CD11c$^-$ macrophages were markedly increased in obese vs lean AT (15.08 ± 0.79 vs 7.95 ± 1.04 % of stromal vascular cells, $p<0.01$). Within CD11c$^+$ cells, we identified 2 cell populations based on CD11b and F4/80 surface expression, *i.e.* CD11b^{high} F4/80$^+$ CD11c$^+$ cells, corresponding to "M1" macrophages and CD11b^{low} F4/80$^-$ CD11c$^+$ cells, which are most probably DCs. Indeed, expression of the dendritic cell marker FLT3 was high and expression of the key macrophage genes F4/80 and CD64 was absent, confirming their identity as dendritic cells. Obesity resulted in the accumulation of CD11c$^+$ ATMs (5.98 ± 0.72 vs 3.43 ± 0.22 % of stromal vascular cells, $p<0.05$), but not of dendritic cells. Intriguingly, CD11c$^+$ ATMs expressed the typical macrophage markers F4/80 and CD64 but also intermediate levels of FLT3 and even higher CD11c levels compared to dendritic cells, indicating that these macrophages are distinctive from, but resemble, dendritic cells. These so-called "M1" CD11c$^+$ ATMs were characterised by high expression of TNF and Arginase-1 while expression of Mannose Receptor and MCP1 was lower compared to the putative "M2" CD11c$^-$ ATMs. Additionally, gene expression levels of both M1 and M2 markers in all macrophage subsets were only marginally affected by 10 weeks of high fat diet.
Conclusion: We present a novel and highly discriminative flow cytometry strategy to discriminate CD11c$^+$ ATMs from dendritic cells. Furthermore, we identify a "pro-inflammatory" CD11c$^+$ ATM subset, enriched in obese AT, expressing DC-markers and displaying a mixed M1/M2 profile. Importantly, obesity affects cell numbers rather than inflammatory phenotype of ATMs.
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Ubc13 haploinsufficiency protects against high-fat diet-induced insulin resistance via TRAF-mediated inflammatory responses
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Background and aims: Obesity is associated with a low-grade inflammation, leading to insulin resistance and type 2 diabetes via Toll-like Receptor (TLR) and TNF-family cytokine receptor (TNFR) signaling pathways. Ubc13 is an ubiquitin-conjugating enzyme responsible for non-canonical K63-linked polyubiquitination of TNF receptor-associated factor (TRAF)-family adapter proteins involved in TLR and TNFR pathways. However, the relationship between Ubc13 and metabolic disease remains unclear. In this study, we investigated the role of Ubc13 in high-fat diet (HFD)-induced obesity and insulin resistance.
Materials and methods: We compared Wild-type (WT) and Ubc13 haploinsufficient (Ubc13+/-) mice under normal diet (ND) and HFD, because homozygous knockout mice (Ubc13-/-) were embryonic lethal. Animals were subjected to metabolic characterization: body weight (BW), food intake, energy expenditure, glucose tolerance test (GTT), and insulin tolerance test (ITT). HFD-fed WT and Ubc13+/- mice were sacrificed and metabolically active organs were collected for molecular and histological studies.
Results: No significant differences were observed in BW, GTT, and ITT between WT and Ubc13+/- mice under ND. However, HFD-fed Ubc13+/- mice showed lower levels of blood glucose and insulin secretion in GTT than HFD-fed WT mice, whereas BW was not significantly different. HFD-fed Ubc13+/- mice also showed increased insulin sensitivity in ITT compared to HFD-fed WT mice. Consistent with the improved insulin sensitivity measured by GTT and ITT, Akt Ser473 phosphorylation was enhanced in livers of HFD-fed Ubc13+/- mice after insulin stimulation. In addition, Ubc13+/- macrophages showed reduced TLR and TNFR signaling after lipopolysaccharide (LPS) and TNFα stimulation compared to WT macrophages. These results indicate that Ubc13 haploinsufficiency improves insulin resistance caused by HFD.
Conclusion: Ubc13 may play an important role in HFD-induced insulin resistance by regulating obesity-related inflammatory responses. Reducing Ubc13 activity could have therapeutic potential in the prevention of insulin resistance associated with obesity.

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Obesity-associated macrophage infiltration has a paracrine effect to induce skeletal muscle insulin resistance
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Background and aims: Insulin resistance (IR) in skeletal muscle is commonly associated with overweight or obesity, accompanied by elevated levels of triglycerides and fatty acids (FAs) in plasma. Obesity is now recognised to be a chronic sub-clinical inflammatory condition, characterised by increased macrophage (mφ) infiltration into adipose tissue. Although ectopic deposition of lipids is known to induce IR directly, it is not known whether local infiltration of mφ into muscle also plays a role in mediating the local effects of elevated FA levels, nor whether there are distinct effects of different FA classes.
Materials and methods: Cohorts of rats (n=8) were fed a chow or high fat diet (HFD; 60% calories as fat) for 5 weeks and the number of mφ present were counted in adipose tissue and muscle after anti-CD68 immunostaining. To explore the mechanisms of this effect, J774 mφ were incubated with or without saturated FA (palmitic acid), unsaturated FA (palmitoleate) or lipopolysaccharide (LPS; positive control) for 8 hours to generate conditioned medium. Medium content of cytokines/chemokines was assayed by ELISA. Mφ phenotype was established by measurement of nitric oxide (Griess assay) and arginase activity. Differentiated C2C12 myotubes were incubated with each type of mφ-conditioned medium for 16 hours and the relative effects on intracellular signalling and glycogen synthesis assessed. All experiments consisted of n=4-5 independent repeats.
Results: HFD-feeding resulted in increased macrophage infiltration into adipose tissue and a 138% increase in tibialis cranialis muscle compared to

chow (P<0.01). In the in vitro model, both LPS and palmitic acid resulted in increased nitric oxide production (p<0.05) and reduced arginase activity (p<0.05), indicative of an M1-type pro-inflammatory mφ phenotype. Conditioned medium contained TNF-α, IL-1β, CXCL2 and MCP-1. Whereas LPS treatment of mφ increased the concentration of all of these, only TNF-α was significantly increased by ~3-fold in palmitic acid-treated mφ compared to control medium (P<0.001). Addition of palmitoleic acid negated the effects of palmitic acid, implying an anti-inflammatory effect of unsaturated FAs on mφ. Incubation of myotubes with palmitic acid-treated mφ-conditioned medium lead to reductions in phosphorylation of PI3-kinase pathway intermediates of 45% for AS160 (Thr 642; p<0.05) and 65% for GSK3β (Ser9; p<0.01), accompanied by a 53% reduction in insulin-stimulated glycogen synthesis (p<0.01). These changes were mirrored by the effects of LPS-conditioned medium. In contrast, palmitoleic acid-treated mφ-conditioned medium enhanced glycogen synthesis by 36% and increased phosphorylation of IRS-1, AS160 and GSK3β. When added in combination, palmitoleic acid-conditioned medium negated the effect of palmitic acid to impair signalling.
Conclusion: Our data demonstrate that obesity in rats is associated with infiltration of mφ into skeletal muscle as well as adipose tissue. We have shown that saturated FA induce a pro-inflammatory phenotype in mφ, which can then directly induce IR in myotubes. This effect is strikingly abrogated in the presence of unsaturated FA. Thus, local mφ infiltration associated with obesity and elevated saturated FA can induce IR in skeletal muscle by a paracrine mechanism.
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Characterisation of macrophage infiltration and polarisation in adipose tissue of children
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Background and aims: Obesity is a disease that is accompanied by a low-grade chronic inflammatory state. It has recently been shown that adipose tissue (AT) from adults contained increased amounts of macrophages which may contribute to the inflammatory state. Human macrophages can be classified into proinflammatory M1 and anti-inflammatory M2 macrophages. However, there is only little information about the occurrence and polarisation of macrophages in AT of children.
Materials and methods: Therefore, we aimed to characterize macrophage infiltration and polarisation in AT of children. In order to assess macrophage infiltration and the polarisation status we performed immunohistochemical analysis and *qRT*-PCR of the stromal vascular fraction (SVF) of AT obtained from 169 biopsies lean (n=112; age= 0-18) and obese (n= 57; age= 0-18) children.
Results: *CD68* expression indicated a positive correlation of macrophage infiltration with BMI-SDS (r=0.56; *P*<0.001), adipocyte size (r=0.59; *P*<0.001) and the number of crown-like structures (CLS) (r=0.4; *P*<0.001) in subcutaneous AT of children. CLS were detected in 37% of obese, but only in 9% of lean children. Furthermore, obese children showed an increased expression of *CD68* (*P*<0.001) in the SVF which was accompanied by an increased expression of the specific M1 marker *CD86* (*P*<0.001). In contrast, no expression changes of the M2 marker *MRC1* were detectable in obese children compared to lean controls.
Conclusion: Adipocyte hypertrophy is a hallmark of obesity, which is thought to trigger an inflammatory response. Here we show a positive correlation of macrophage infiltration, adipocyte size and obesity in children. Furthermore, obese children showed an increased expression of M1 macrophages which could indicate a shift in the M1/M2 ratio.
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Circadian regulation of MCP1 serum levels and expression in monocytes and adipose tissue upon switching from high to low carb diets with replacement by fat in humans
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Background and aims: The monocyte chemotactic protein-1 (MCP-1)/chemokine (C-C motif) ligand 2 (CCL2) is a proinflammatory chemokine primarily secreted by monocytes, macrophages and adipocytes and contributing to the pathogenesis of obesity, diabetes and cardiovascular disease. Exercise and weight loss induced by caloric restriction reduce circulating levels of MCP1. However, there is little information about the effect of nutrient composition on MCP1 levels and mRNA expression in humans. To address this, we analysed MCP1 levels in human serum, and its mRNA expression in monocytes and adipose tissue after 6 weeks high carbohydrate - low fat (HC/LFD, 55% carbohydrate, 30% fat) followed by 6 weeks of low carbohydrate - high saturated fat (LC/HFD, 40% carbohydrate, 45 % fat) isocaloric diet interventions.
Materials and methods: The NUtriGenomics Analysis in Twins (NUGAT) study included 92 non obese healthy twins (35 monozygotic and 11 dizygotic twin pairs). Three investigation days were dated after 6 weeks HC/LFD and after 6 and 42 days of the LC/HFD. MCP1 serum level and mRNA gene expression in blood monocytes was measured at three time points (in the morning, at noon and in the afternoon). Gene expression in subcutaneous adipose tissue biopsies was studied at one time point at noon.
Results: Serum levels of MCP1 demonstrated significant circadian variation with the highest level in the morning, a trough level at noon and increase again in the afternoon. The same trend was found for mRNA expression in monocytes. Moreover, serum MCP1 and its mRNA expression in adipose tissue correlated with the expression of core clock genes in adipose tissue. MCP1 serum level correlated with age, waist circumference, BMI and waist-hip ratio, as well as with total cholesterol and LDL cholesterol level in the afternoon. In the stepwise multivariate regression analysis, only age, waist circumference, waist-hip ratio and total cholesterol remained in the final predictive model as significant variables associated with serum MCP1 (adjusted R2= 0.435, p<0.01). Serum MCP1 was significantly decreased after six weeks of LC/HFD with the largest effect in the afternoon. In contrast, the MCP1 mRNA expression was increased in adipose tissue, and not changed in monocytes under the LC/HFD diet intervention. Furthermore, the correlation of MCP1 serum level with mRNA expression was found only in adipose tissue for CID1, but not in monocytes. Estimating of heritability in twins using ACE model revealed that the serum MCP1 and its circadian rhythms were about 70% genetically determined, whereas the diet-induced changes had a lower genetic impact.
Conclusion: Serum MCP1 level in humans demonstrates circadian regulation and correlates with total and LDL cholesterol levels. Isocaloric LC/HFD induces the decrease of MCP1 serum level obviously due to posttranscriptional regulation. The mRNA expression in adipose tissue or monocytes is not a good predictor of serum MCP1 levels in humans.
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A community-based intervention for diabetes risk reduction in inner-city obese adolescents

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Background and aims: Childhood obesity has been accompanied by an increasing prevalence of type 2 diabetes (T2D), particularly in minority children. 20-30% of obese youth have "pre-diabetes" a precursor to diabetes marked by insulin resistance, β-cell dysfunction and impaired glucose tolerance (IGT). The Diabetes Prevention Program demonstrated T2D could be prevented/delayed by lifestyle modification in adults with pre-diabetes, but efficacy of similar interventions in youth has not been established. Therefore, we evaluated the effects of the Bright Bodies Healthy Lifestyle Program on 2-hr OGTT glucose in comparison to children receiving standard of care.
Materials and methods: Parallel-group randomized controlled trial comparing Bright Bodies (BB) with standard clinical care (CC) in obese adolescents (10-16 yo) with elevated OGTT 2-hr blood glucose (130-199 mg/dl) from a racial/ethnically-diverse population. OGTTs, including anthropometric and metabolic syndrome criteria assessments, were conducted at baseline and 6 months. Children attended BB twice weekly for exercise and nutrition/behavior modification at one of two community sites and CC group received clinical care from their pediatrician. Primary outcome was change in 2-hr OGTT glucose and % conversion from elevated 2-hr blood glucose to non-elevated (<130 mg/dl) 2-hr blood glucose. Changes in outcomes were compared between groups using a mixed model with covariate adjustment for baseline outcome and multiple imputation for missing data. Least squares means and 95% CIs were estimated for changes in outcomes.
Results: Reductions in 2-hr glucose were more favorable in BB compared to CC (-27.2 vs. -10.1 mg/dl; diff=-17.1, 95% CI ;p= 0.005). Moreover, greater conversion to <130 mg/dl 2-hr glucose occurred in BB than CC (p=0.03). Other insulin sensitivity indices were significantly improved, as well as the prevalence of metabolic syndrome in the BB group (p=0.004).
Conclusion: Compared to standard of care, the Bright Bodies Program is a more effective means of reducing the risk of T2D in obese adolescents with elevated 2-hr blood glucoses.
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Big breakfast rich in protein improved glycaemic control and satiety feeling in adults with type 2 diabetes mellitus

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Background and aims: Consuming breakfast has been inversely associated with BMI, fasting lipids and postprandial insulin sensitivity. The present study was designed to evaluate the effect of breakfast size and composition on glycemic control, and its association with hormone profile in adults with type 2 diabetes.
Materials and methods: The present study is a randomized, controlled, open clinical trial, including overweight/obese, non-insulin-dependent adults with type 2 diabetes. Participants were randomized to balanced hypocaloric diabetic diets with either big breakfast (BB) or small breakfast (SB), (33% vs. 12.5% of total daily energy intake). The BB diet included higher percentage of protein and fat. Anthropometric measures were assessed every 2 weeks. Fasting adipokines, hormones, proinflammatory cytokines and lipid profile were performed at baseline and after a follow-up period (Week 13).
Results: Of the 59 enrolled participants, 47 completed the study. At end of follow-up, greater HbA1c and systolic blood pressure reductions were observed in the BB than SB group (HbA1c: -4.62% vs. -1.46 %, p=0.047; SBP -9.58 vs. -2.43 mmHg; p=0.04). Additionally, DM medication doses were reduced in a greater proportion of the BB participants (31% vs. 0%; p=0.002) while in the SB, a greater proportion of participants had a dose increases (16.7% vs. 3.4%; p=0.002). Hunger scores were lower in the BB group and greater improvements in fasting glucose were observed in the BB group comparison to the SB group.
Conclusion: A simple dietary manipulation of BB diet rich in protein and fat appears to have additional benefits compared to a conventional low-calorie diet in individuals with type 2 diabetes.
Clinical Trial Registration Number: NCT01178723

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The impact of high fat meal frequency on metabolic profile and energy expenditure in obese subjects

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Background and aims: Whilst obesity remains a critical risk factor for developing type 2 diabetes mellitus (T2DM), it is clear that dietary habits also have a key role in the earlier onset of metabolic dysfunction, as well as late complications of T2DM. Clinically, consumption of small frequent meals versus fewer large meals is considered beneficial for weight loss and to reduce future cardiovascular (CVD) risk, although there is limited evidence to support this popular belief. Therefore, in this study, our aim was to (1) compare the effect of 2 versus 5 isocaloric high fat meals on the metabolic profile in lean and obese women; and (2) examine the effect of meal frequency on 24 hour energy expenditure.
Materials and methods: In a crossover study, 24 white Caucasian non-diabetic lean (age: 34 (mean±SD) ± 10 years, BMI: 22.9 ± 2 kg/m^2) and obese (age: 42 ± 9 years, BMI: 36 ±8 kg/m^2) women were given 2 or 5 isocaloric high fat meals (50% fat) per day. Each subject attended on two separate visits, less than two weeks apart. On each occasion, following an overnight fast, blood samples were taken every 2 hours at 7 time points, from 9am to 9pm. Biochemical assessment was undertaken for glucose, insulin, HOMA-IR, lipid profile and non-esterified fatty acids (NEFA). Concurrently, subjects were assessed for 24 hour energy expenditure in a purpose built whole body room calorimeter, on both instances. Subjects also completed visual analogue scales for hunger, satiety, fullness and appetite every 2 hours, for the duration of each visit.
Results: The obese subjects had significantly increased insulin and decreased HDL ($p<0.001$), as well as increased glucose, HOMA-IR and triglyceride (TG) levels ($p<0.01$), throughout the day, compared with the lean subjects for both the 2 and 5 meal visits. Despite the change in meal size and frequency, there were no significant differences in either weight group for glucose, insulin, HOMA-IR, cholesterol, LDL, HDL, TG or NEFA. However, there was a noted difference in metabolic response to an individual standardised high fat meal at lunchtime between lean and obese subjects (insulin, HOMA-IR, TG, $p<0.01$), independent of meal frequency. Furthermore, there was no significant difference in 24 hour expenditure, whether 2 or 5 meals were consumed in either the obese group (2124 ± 312 vs 2142 ± 365 Kcal/day) or lean group (1724 ± 160 vs 1683 ± 166 Kcal/day). The obese group had higher hunger scores and lower fullness scores on the visual analogue scale in the 2 meal visit compared with the 5 meal visit ($p<0.05$); whilst this was not observed in the lean group.
Conclusion: In summary, consumption of 2 large or 5 small high fat meals in either obese or lean women had no significant impact on their respective metabolic profiles or 24 hour energy expenditure. Obese women had a much more dysfunctional metabolic profile compared with lean subjects, which was further exacerbated by individual high fat meal challenges. Taken together, these data suggest that the amount of calories and content per day may have a more significant impact on metabolic profile and energy expenditure than the frequency of meals over a 24 hour period.
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Combining dietary polyphenols increase energy expenditure and improve parameters of adipose tissue and skeletal muscle in healthy overweight humans

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Background and aims: Impaired regulation of lipid oxidation (metabolic inflexibility) is associated with obesity, insulin resistance and type 2 diabetes mellitus. Dietary polyphenols have recently gained attention attributed to their modulating effects on lipolysis, fat oxidation and mitochondrial function. The objective of the present study was to test short-term (3 days) additional or synergistic effects of combinations of polyphenols, with distinct mechanisms of action, on energy and substrate metabolism in overweight subjects.
Materials and methods: 18 healthy overweight volunteers (9 females, 9 males, age 35±2.5 yrs, BMI 28.9±0.4 kg/m2) participated in a randomized, double-blind cross-over study. Combinations of Epigallocatechin-gallate (EGCG, 282mg/d) + Resveratrol (RSV, 200mg/d), and EGCG+RSV + 80mg/d soy isoflavones (SI), or placebo capsules (PLA) were supplemented twice daily, for a period of 3 days. On day 3, circulating metabolites, energy and substrate metabolism (indirect calorimetry) were measured during fasting and postprandial conditions (high-fat-mixed meal (2.6MJ, 61.2 E% fat)) and a subcutaneous adipose tissue biopsy was taken. Differences between supplementation periods are analyzed by ANOVA using SPSS 19.0 for MAC and P,0.05 was considered statistically significant. Post-hoc analyses with Bonferroni correction were applied when ANOVA indicated significant effects or interactions.
Results: EGCG+RSV increased resting energy expenditure (EE) compared to placebo (5.45±0.17 vs 5.22±0.18 kJ/min, p<0.05). Postprandially, EE was increased by both treatments vs PLA 2-4h after the meal (p=0.01). Metabolic flexibility, calculated as difference between fasting and maximal postprandial RQ, tended to be improved by EGCG+RSV as compared to PLA in men (0.11±0.02 vs 0.06±0.02, p=0.079), whilst in women there was no effect. EGCG+RSV tended to increase the glucose-insulin-ratio, a marker for insulin sensitivity during early postprandial phase (0-120 min, p=0.06). The latter effects were not seen during additional SI supplementation. EGCG+RSV+SI increased circulating free fatty acid and free glycerol as compared to PLA.
Conclusion: EGCG+RSV increased fasting and postprandial EE, whilst there was a tendency towards improved postprandial insulin sensitivity. This was accompanied by an improved metabolic flexibility in males. These metabolic changes were not seen with EGCG+RSV+SI, suggesting that addition of SI reversed these effects. If these effects of EGCG+RSV remain after long-term treatment (under investigation), this may have positive consequences for body weight control and insulin sensitivity.
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Combination naltrexone/bupropion therapy resulted in clinically meaningful improvements in weight and quality of life (QOL): integrated analysis of four phase 3 trials

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Background and aims: Comprehensive treatment for obesity ideally will improve physical, psychological, and social aspects of health in addition to facilitating weight loss. This integrated analysis of the four Phase 3, 56-week trials of combination naltrexone sustained-release (SR) / bupropion SR (NB) vs placebo (PBO) investigated changes in body weight and weight-related QOL using the Impact of Weight on Quality of Life-Lite (IWQOL-Lite) scale at baseline and at 8, 16, 28, and 56 weeks of treatment.
Materials and methods: All patients (NB [n=2043], PBO [n=1319]) were overweight/obese (BMI: ≥27 and ≤45 kg/m^2). Treatment group differences (LS mean±SE) in the modified ITT-LOCF population (≥1 post-baseline weight while on study drug) were evaluated by ANCOVA with treatment, study, and baseline values as covariates.
Results: Baseline characteristics (mean±SD) were similar between treatment arms: 81% female, 79% Caucasian, 46±11 y, 36±4 kg/m^2, type 2 diabetes: 12.6%, IWQOL-Lite total score: 71±18. Completion rate was 66% (NB) and 59% (PBO). Weight loss with NB was significantly greater than PBO at all time points (Week 56: -7.0±0.2% NB vs -2.3±0.2% PBO; p<0.001). At Week 56, NB resulted in significantly greater improvement in IWQOL-Lite total score (+11.9±0.3 vs +8.2±0.3; p<0.001) and all subscores (Physical function, Self-esteem, Sexual life, Public distress, Work; all p<0.05 vs PBO). At Week 56, significantly more patients reduced body weight by ≥5% with NB (53% vs 21%; p<0.001), and greater mean improvement in IWQOL-Lite total score was observed in these patients. The safety/tolerability profile of NB was consistent with its individual components; the most common adverse events were nausea (32%), constipation (19%), headache (18%), and vomiting (11%).
Conclusion: NB improved both weight and psychosocial outcomes, with 36% of NB patients (vs 12% PBO; p<0.0001) achieving clinically meaningful improvement in both IWQOL-Lite and body weight (≥5% reduction) after 1 year.
Clinical Trial Registration Number: NCT00474630; NCT00456521; NCT00532779; NCT00567255
Supported by: Orexigen Therapeutics, Inc.

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An integrated analysis of weight loss with combination naltrexone/bupropion therapy by BMI (obesity) classification

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Background and aims: Naltrexone sustained-release (SR) / bupropion SR (NB) significantly reduced body weight vs placebo (PBO) in the four, 56-week, Phase 3 trials of overweight/obese patients (BMI ≥27 and ≤45 kg/m^2). This integrated analysis investigated weight loss in these 4 trials when stratified by baseline BMI (obesity class): Class-I (30.0-34.9 kg/m^2), Class-II (35.0-39.9 kg/m^2), Class-III (≥40 kg/m^2).
Materials and methods: Treatment group differences (LS mean±SE) in the modified ITT-LOCF population (≥1 post-baseline weight on study drug) were evaluated by ANCOVA with treatment, study, and baseline values as covariates.
Results: Baseline characteristics (mean±SD) were similar between treatment arms (NB [n=2043] or PBO [n=1319]): 81% female; 79% Caucasian; 46±11 y; 36±4 kg/m^2; obesity Class-I: 37%, Class-II: 36%, Class-III: 25%. Completion rate was 66% for NB and 59% for PBO. At Week 56, NB resulted in significantly greater weight loss vs PBO (-7.0 ±0.2% NB vs -2.3±0.2% PBO; p<0.001) and proportion of patients who attained ≥5% weight loss (53% NB vs 21% PBO; p<0.001). NB patients experienced favorable shifts in BMI class from baseline relative to PBO (NB vs PBO: improved = 45% vs 20%, no change = 53% vs 74%, worsened = 2% vs 6%; p<0.001), and a larger proportion of NB vs PBO subjects shifted to a non-obese BMI from Class-I obesity (17% NB vs 6% PBO) and from Class-II obesity (3% vs 1%). Of note, weight loss with NB was similar across the 3 obesity classes (NB range: -6.1% to -7.3%; PBO-corrected range: -4.0% to -5.0%, all p<0.001), as was achievement of ≥5% weight loss (NB range: 49% to 54%, odds ratio relative to PBO: 3.5 to 4.3; all p<0.001). The safety/tolerability profile of NB32 was consistent with its individual components; the most common adverse events were nausea (32%), constipation (19%), headache (18%), and vomiting (11%).
Conclusion: The clinically significant weight loss associated with NB was consistent across a wide BMI range, and resulted in an approximately 3-fold greater transition from Class-I/II obese to non-obese BMI.
Clinical Trial Registration Number: NCT00474630; NCT00456521; NCT00532779; NCT00567255
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Early improvement in control of eating is associated with long-term weight loss: integrated analysis of four phase 3 trials of combination naltrexone/bupropion treatment

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Background and aims: Combination treatment with naltrexone sustained-release (SR) / bupropion SR (NB) results in substantial and sustained weight loss that is postulated to be partially mediated by effects on the mesolimbic dopaminergic reward system that modulate control of eating behavior. In the Phase 3 trials of NB in overweight/obese individuals, eating behavior was evaluated with the Control of Eating Questionnaire (CoEQ, twenty 100mm

visual analog scales) at baseline and Weeks 8, 16, 28, and 56. This integrated analysis of all four Phase 3, 56-week clinical trials of NB in overweight/obese patients (BMI ≥27 and ≤45 kg/m^2) evaluated the change in CoEQ question 19 (CoEQ 19: "Generally how difficult has it been to control your eating?") and the relationship between changes in CoEQ 19 and body weight.
Materials and methods: Treatment group differences (LS mean±SE) in the modified ITT-LOCF population (≥1 post-baseline weight on study drug) were evaluated by ANCOVA with treatment, study, and baseline values as covariates.
Results: Baseline characteristics (mean±SD) were similar between treatment arms (NB [n=2043] or placebo [PBO; n=1319]): 81% female, 79% Caucasian, 46±11 y, 36.3±4.3 kg/m^2, CoEQ 19 score: 60±24. Completion rate was 66% for NB and 59% for PBO. At Week 56, weight loss was significantly greater with NB (-7.0±0.2%) vs PBO (-2.3±0.2%; p<0.001). NB-treated subjects reported significantly greater improvement in CoEQ 19 at all time points (p<0.001 vs PBO), with the greatest effect observed at Week 8 (-23.3 vs -13.5 mm; p<0.001). In both groups, reduction in CoEQ 19 at Week 8 was positively correlated with body weight reduction at Week 56 (r=0.20 (NB), r=0.22 (PBO); both p<0.001), such that the quartile of NB subjects with the greatest CoEQ 19 improvement at Week 8 (> 43 mm) exhibited -9.4% mean weight change at Week 56.
Conclusion: These results suggest that NB is associated with rapid improvement in control of eating behavior that may, in part, contribute to the weight loss associated with treatment.
Clinical Trial Registration Number: NCT00474630; NCT00456521; NCT00532779; NCT00567255
Supported by: Orexigen Therapeutics, Inc.

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Oral formulation of UGP302, a novel amylin and calcitonin dual receptor agonist, exerts anti-obesity effects in diet-induced obese (DIO) rats
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Background and aims: Salmon calcitonin (sCT) is associated with anorectic effects mediating weight loss, and oral delivery of sCT have previously been published to have glucoregulatory and weight-reducing effects in obese diabetic rats; however, increasing peptide potency could have further beneficial effects. Here we describe a novel sCT mimetic (UGP302), and we performed a thorough head-to-head comparison of UGP302 and sCT in vitro, as well as orally formulated UGP302 and sCT in vivo.
Materials and methods: The in vitro studies were conducted in mammalian cell lines from DiscoverX expressing human calcitonin receptor (CTR) or human amylin receptor (AMYR). GPCR downstream signalling was investigated with dose response profiles for cAMP accumulation and β-arrestin recruitment by sCT and UGP302 during short term (< 2 hours) and prolonged (up to 72 hours) stimulation. Binding affinity for both ligands was assessed by competitive binding using I-125 radio-labeled sCT for both receptors. The in vivo study was conducted in the diet-induced obese rat model (DIO). Male DIO rats were treated with oral sCT (1mg/kg) or UGP302 (1 mg/kg) or oral vehicle twice daily for 4 weeks. A pair-fed group to UGP302 was included to evaluate the impact of food intake on body weight regulation. Furthermore, an oral glucose tolerance test (OGTT) was performed to investigate postprandial glycaemic control. Levels of plasma insulin and leptin were measured at study end to evaluate the resistance to the action of these hormones.
Results: In vitro, UGP302 was demonstrated to be more potent compared to sCT with respect to cAMP accumulation and β-arrestin recruitment for both receptors (CTR p < 0.01, AMYR p < 0.01), and UGP302 scored a lower IC50 value in a competitive binding assay when compared to sCT for both receptors, indicating improved receptor affinity. Intriguingly, UGP302 and sCT did not activate the CGRP-receptor. In vivo, over the 4 week period oral UGP302 caused a significant reduction in accumulated food intake, when compared to sCT and oral vehicle (p < 0.01 vs. sCT and p<0.001 vs. oral vehicle). At study end, oral UGP302 administration induced a 10% reduction in bodyweight compared to vehicle (p < 0.001). In contrast, only a 6% vehicle-corrected weight-loss was observed for pair-fed and oral sCT groups (p < 0.01), thus, resulting in a more pronounced anti-obesity effect for oral UGP302 (p < 0.05). Furthermore, oral sCT and UGP302 improved glucose intolerance evaluated as reduced incremental area under the curve (iAUC) during OGTT when compared to vehicle (p < 0.001) and UGP302 pair-feeding (p < 0.01). At study end, oral UGP302, but not oral sCT, significantly reduced hyperinsulinemia (p<0.05, vehicle and pair-fed) indicating improved insulin sensitivity. In addition, UGP302 and sCT both significantly reduced hyperleptinemia when compared to vehicle (UGP302 p< 0.001, sCT p<0.05) indicating reduced leptin resistance. In contrast, the pair-fed group showed no reduction in any of the measured hormones.
Conclusion: In conclusion, we have demonstrated oral UGP302 to be superior to oral sCT in both an in vitro and in vivo setting. The results support that this novel dual receptor agonist could be a new treatment for obesity and potentially T2DM due to the improved glycaemic control, improved hormonal homeostasis and weight reducing effects.
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Beneficial effect of lingonberry, blackcurrant and bilberry consumption on weight gain and adiposity in C57BL/6J mice
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Background and aims: The prevalence of obesity and diabetes is increasing dramatically worldwide. Obesity is associated with several metabolic disorders such as cardiovascular disease, non-alcoholic fatty liver disease and type 2 diabetes. Many of these multifactorial conditions are preventable by adopting dietary patterns that promote maintenance of a healthy body weight. Diets rich in fruits and berries have been associated with reduced risk of type 2 diabetes. This study was designed to screen eight species of berries for their ability to counteract negative effects induced by a high-fat diet.
Materials and methods: Male C57BL/6J mice were fed a high-fat diet (45 E%) for 13 weeks supplemented with different berries (20%); lingonberry, blackcurrant, bilberry, raspberry, blackberry, açai, crowberry and prunes. The control group received high-fat diet without berries. Body weight and food intake was monitored continuously, and in the end of the study organs were collected. Plasma parameters were measured and epididymal fat pads and liver tissue were subjected to gene and protein expression analysis.
Results:

Table 1. Metabolic parameters after 13 weeks on high-fat diet with berry supplement

	Lingonberry	Blackcurrant	Bilberry	Raspberry	Açai	Crowberry	Prune	Blackberry	Control
Body weight (g)	28.9 ±0.9***	30.3 ±1.2***	31.2 ±1.3*	31.5 ±1.4**	37.9 ±2.3***	31.7 ±1.5	33.9 ±1.8	33.0 ±1.7	33.3 ±1.7
Body fat (%)	24.3 ±1.7***	30.1 ±1.6**	32.4 ±1.6*	36.6 ±2.8	37.0 ±0.9	36.0 ±1.4	39.0 ±1.3	39.7 ±0.8	38.5 ±0.9
Liver triacylglycerol (mg/g)	13.24 ±0.6***	25.04 ±2.3***	33.21 ±3.2*	50.03 ±9.0	100.7 ±6.2***	51.01 ±7.3	55.79 ±6.6	54.17 ±7.7	58.37 ±6.9
ALT (U/l)	24.0 ±1.2*	23.9 ±1.7*	23.6 ±1.2*	28.8 ±3.6	40.9 ±2.2	28.5 ±3.7	27.6 ±1.7	32.2 ±3.8	35.4 ±35.5
Plasma glucose (mmol/l)	7.6 ±0.31**	7.6 ±0.38**	9.1 ±0.48	10.5 ±0.73	12.6 ±0.53	10.2 ±0.60	12.6 ±0.95	12.0 ±0.66	11.0 ±0.53
Plasma insulin (pmol/l)	419 ±34***	546 ±68***	619 ±81**	704 ±81*	1168 ±28	833 ±117.3	878 ±79	906 ±82	1008 ±61
HOMA-IR	21.0 ±1.8***	26.6 ±3.8***	35.6 ±4.4**	50.6 ±8.8	94.7 ±5.5	56.0 ±9.6	71.6 ±8.4	68.7 ±6.8	71.4 ±6.3
Total cholesterol (mmol/l)	2.6 ±0.11***	2.9 ±0.14**	3.4 ±0.15	3.6 ±0.24	4.3 ±0.13*	4.1 ±0.11	4.1 ±0.15	4.0 ±0.17	3.7 ±0.16
LDL/HDL-ratio	0.84 ±0.04	0.76 ±0.06	0.87 ±0.03	0.87 ±0.05	0.90 ±0.04	0.91 ±0.04	0.94 ±0.08	0.92 ±0.06	0.88 ±0.05
PAI-1 (ng/ml)	1.4 ±0.2	1.8 ±0.3	1.8 ±0.2	3.0 ±0.6	4.4 ±0.4	2.3 ±0.3	2.9 ±0.4	2.7 ±0.6	3.5 ±0.4

*p<0.05, **p<0.01, ***p<0.001 statistical comparisons are to the control group. Mean ±SEM (n=12).

Supplementation with lingonberries, blackcurrants or bilberries reduced weight gain, decreased body fat content and had positive effects on glucose homeostasis (Table 1). In addition to improving weight regulation, the lingonberries lowered inflammatory marker plasminogen activator inhibitor-1 (PAI-1), reduced liver weights and decreased levels of the liver enzyme alanine aminotransferase (ALT). The opposite was true for the group receiving açai berry. In addition, phosphorylation of protein kinase B as well as expression of hormone-sensitive lipase and other proteins involved in adipose lipid handling were significantly altered by lingonberries.

Conclusion: Lingonberries, as well as blackcurrants and bilberries, prevented the high-fat diet-induced metabolic abnormalities. In the case of lingonberries, this could to be mediated by mechanisms involving the adipose tissue.
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Proteomic analysis in type 2 diabetes patients before and after a very low calorie diet reveals disease state and intervention specific biomarkers

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Background and aims: Very low calorie diets (VLCD) with and without exercise programs lead to major metabolic improvements in obese type 2 diabetes (T2DM) patients. Using proteomic analysis, we aimed to further investigate the underlying mechanisms of a VLCD with or without exercise and to uncover possible biomarkers associated with these interventions.
Materials and methods: Blood samples were collected from 27 obese T2DM patients before and after a 16-week VLCD (Modifast®, ± 450 kcal/day). Thirteen of these patients followed an exercise program in addition to the VCLD. Plasma was obtained from 27 lean and 27 obese controls as well. Proteomic analysis on all available subjects was performed using mass spectrometry (MS) and targeted multiple reaction monitoring (MRM) for 13 proteins. A large scale Isobaric tags for relative and absolute quantitation (iTRAQ) approach was used in the T2DM patients only to identify a diet and/or exercise effect.

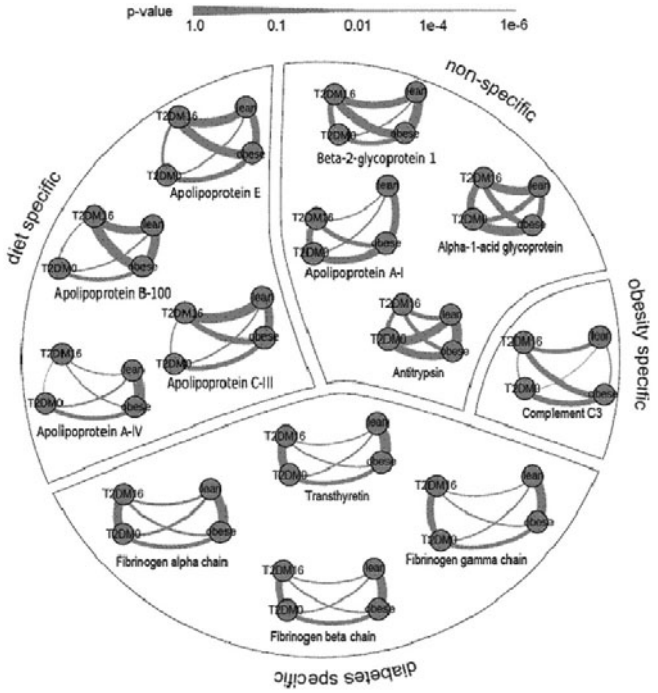

Figure 1. Graph representation of group wise comparisons for the proteins in the MRM data set. Comparisons between the four groups (diabetes patients at baseline (T2DM0) and after the 16-week VLCD (T2DM16) and obese and lean controls) are represented by edges. The thickness of the edge represents the p-value. Proteins have been clustered into groups (obesity, diabetes, diet and non-specific) based on similarity in patterns of differences between the groups.

Results: After the 16-week VLCD, there was a significant decrease in body weight (−27.2±1.9 kg VLCD+exercise; −23.7±1.6 kg VLCD-only, p=NS) and HbA1c (VLCD+exercise 7.8±0.4 vs. 6.3±0.4%; VLCD-only 7.8±0.3 vs. 6.7±0.3%, p=NS). Targeted MRM analysis in the T2DM patients and controls revealed differences in several proteins, which could be divided in obesity specific (complement C3), diet specific (apolipoproteins, especially apolipoprotein A-IV) and diabetes specific markers (Figure 1). The diabetes specific markers were related to coagulation (fibrinogens) and transport of thyroid hormones (transthyrethin). With the large scale iTRAQ analysis, for 87 of the 232 proteins that could be measured in all 27 patients at both time points, a significant VLCD effect after multiple-test correction was found. No proteins were found showing an exercise effect.
Conclusion: This study shows that proteomic analysis reveals possible biomarkers that are differentially expressed in type 2 diabetes patients versus controls and before and after a VLCD.
Clinical Trial Registration Number: ISRCTN76920690
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PS 067 Preventing and treating diabetes: the nutrition impact

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Consuming snacks at mid-afternoon reduced the incremental area under the glucose curve after dinner in patients with type 2 diabetes

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Background and aims: Postprandial plasma glucose and glycaemic spikes are associated with cardiovascular diseases in patients with diabetes. The aim of this study was to evaluate whether consuming snacks at different time of the day could change the postprandial glucose excursions in Japanese patients with type 2 diabetes.
Materials and methods: This was a randomized, controlled, two-treatment, crossover study. Thirteen patients with type 2 diabetes (Male 46%, Age 68.5 ± 6.1 yrs, HbA1c 7.4 ±1.2%: mean ± SD) were assigned to perform continuous glucose monitoring system (CGMS, Medtronic Minimed Gold, Northridge, CA) for 72-h by eating snacks at different time of the day. At 12:00 of the first day, each participant wore a CGMS at the clinic, and the 2nd and the 3rd day all subjects consumed the test meals at 7:00, 12:00, and 19:00, and snacks at 12:30 or 15:30 at home, and at noon of the 4th day a CGMS was removed at the clinic. The test meals consist of rice/bread, meat/fish, 500 g of vegetables, and contain 21 g of dietary fiber: the energy ratio was 58, 17, and 25% from carbohydrates, proteins, and fat, respectively. Snacks (biscuits 18 g, 75 kcal, carbohydrate 13.5 g, protein 1.2g, fat 1.8 g) were consumed after lunch (12:30) or between lunch and dinner (15:30) in a random order on the 2nd and the 3rd day. The daily glucose fluctuations were assessed by the following parameters obtained from CGMS and compared between two different days: the mean blood glucose (MBG), standard deviation (SD), mean amplitude of glycaemic excursions (MAGE), large amplitude of glycaemic excursions (LAGE), incremental glucose peak (IGPs), postprandial blood glucose, and the incremental area under the curve for blood glucose (IAUCs).
Results: The IAUCs 0-5 after lunch, and dinner were significantly reduced 20 to 30% by consumption of the snacks at 15:30 compared to consuming immediately after lunch. The incremental glucose peak after lunch was significantly higher by consumption of the snacks at 12:30 compared to consuming at 15:30. However, the levels of MBG, SD, MAGE, and LAGE did not differ between two treatments.
Conclusion: Consuming snacks at mid-afternoon leads to a reduction by 30% the IACU after dinner in patients with type 2 diabetes, possibly because of second-meal like effect.

Characteristics of glycaemic excursion when patients consumed snacks at 12:30 and 15:30

	Patients with type 2 diabetes (n=13)						
	Snacks at 12:30			Snacks at 15:30			P
MBG (mmol/L)	9.5	±	2.6	9.5	±	2.5	n.s.
SD (mmol/L)	2.1	±	0.6	2.0	±	0.7	n.s.
MAGE (mmol/L)	6.8	±	1.9	6.0	±	2.3	n.s.
LAGE (mmol/L)	9.1	±	2.2	8.3	±	3.0	n.s.
IGP after lunch (mmol/L)	6.6	±	2.7	5.4	±	2.1	< 0.01
IGP after dinner (mmol/L)	5.0	±	2.0	4.1	±	3.2	n.s.
IAUC 0-5 after lunch (mmol•min/L)	1144	±	588	936	±	493	< 0.05
IAUC 0-5 after dinner (mmol•min/L)	761	±	439	547	±	476	< 0.05

Data are expressed as mean ± SD. Snacks consumed at 12:30 vs.15:30.

Abbreviations: MBG, mean blood glucose; SD, standard deviation; MAGE, mean amplitude of glycaemic excursions ; LAGE, large amplitude of glycaemic excursions; IGP, incremental glucose peak; IAUC: incremental area under the curve for blood glucose.

Clinical Trial Registration Number: UMIN-CTR 6537
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Effects of vitamin D supplementation on insulin sensitivity and secretion in subjects with type 2 diabetes: a randomised controlled trial

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Background and aims: Low serum levels of 25 hydroxy-vitamin D (25(OH) D) are common and have been linked to impaired glucose metabolism and development of type 2 diabetes. We tested whether cholecalciferol supplementation could improve insulin sensitivity and insulin secretion.
Materials and methods: In a 6-month parallel group, placebo-controlled, double-blind, randomized trial patients with type 2 diabetes and hypovitaminosis D (25(OH)D)<50 nmol/L) received oral vitamin D_3 or placebo. A dose of 400,000 IU vitamin D_3 was given at baseline, followed by 200,000 IU after 6 weeks if 25(OH)D <100 nmol/L. Insulin sensitivity was measured by euglycaemic, hyperinsulinaemic clamp, with endogenous glucose production measured by tracer dilution method, giving the total glucose disposal rate (GDR). Insulin secretion was measured as insulin incremental area under curve from 0-10 minutes ($iAUC_{0-10}$) after an intravenous glucose tolerance test (IVGTT). Vitamin 25(OH)D was measured using radioimmunoassay (DiaSorin).
Results: Subjects (n=61, 48% females) were of Nordic (n=43) and South-Asian (n=18) ethnicity: Mean (SD) age was 57.7 (9.6) years, BMI 31.9 (5.2) kg/m2, HbA1c 7.8 (1.4)% and diabetes duration 10.3 (6.2) years. Serum 25(OH)D was 37.4 (12.1) nmol/L at baseline, and increased in the intervention group (D_3 group) to 73.2 (13.7) nmol/L after 3 months, and remained unchanged in the control group, p<0.001. Supplementation with vitamin D_3 had no effect on glycaemic control. We observed no changes in insulin sensitivity or insulin secretion between baseline and six months, neither within nor between groups (**table 1**).
Conclusion: In a 6-month placebo-controlled, double-blind randomized trial of oral vitamin D_3 or placebo, patients with type 2 diabetes and hypovitaminosis D did not improve glycaemic control, insulin sensitivity or insulin secretion.

Table 1. Effects of vitamin D supplementation on insulin secretion and insulin sensitivity

	Vitamin D_3 group		Placebo group		p-value (D_3 vs pl)
	Baseline	Change	Baseline	Change	
Glucose disposal rate (μmol/m²/min)	1163[661]	105 (-173, 383)	969[730]	106 (-70, 235)	0.99
Insulin $iAUC_{0-10}$ (pmol/l/min)	118 [164]	- 1,5 (-49, 46)	205[367]	16 (-144, 175)	0.83
HbA1c (%)	7.5 [2.1]	0.3 (-0.1, 0.6)	7.4 [1.6]	0.3 (0.0, 0.4)	0.82

Baseline data are median [inter quartile range], changes are mean with 95% confidence interval. iAUC: incremental area under the curve

Clinical Trial Registration Number: NCT00992797
Supported by: EXTRA-foundation, Norwegian Diabetes Association, South East Health Authority

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Vitamin D is positively related to the increase of pulse wave velocity during 4 years in men with type 2 diabetes while PTH levels are related to less such arteriosclerosis in women

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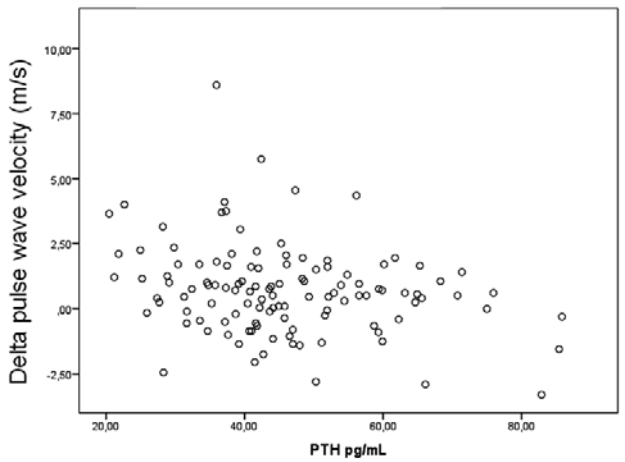
Background and aims: Levels of Vitamin D have been shown to be negatively associated with obesity in many cross-sectional studies and some studies have suggested, although not entirely conclusive, that Vitamin-D levels relate favourably to pulse wave velocity (PWV). The aim of this analysis was to cross-sectionally, and also prospectively, explore associations between levels of Vitamin-D and PTH to PWV in patients with type 2 diabetes.
Materials and methods: PWV was measured with applanation tonometry over the carotid and femoral arteries. The baseline measurements of PWV were started in 2006 and it was repeated at a follow-up investigation four years later. Vitamin-D, PTH and other potential cardiovascular risk markers were measured in serum at baseline
Results: The cohort comprised of 454 male and 239 female patients with type 2 diabetes aged 55--65 years at the baseline investigation. Sagittal abdominal

diameter (SAD) correlated negatively with Vitamin-D in both genders (men: r = -0.129, p = 0.006, women: r= -0.189, p= 0.003). In men there was no correlation between PWV at baseline and Vitamin-D or PTH levels (p >0.4 for both) while there was a borderline significant correlation between Vitamin-D and PWV in women (Vitamin-D r= -0.124, p= 0.063, PTH p= 0.98). At follow up 278 men and 117 women were alive and participated in the re-investigation. Baseline Vitamin-D, but not PTH, correlated positively with the change (increase) in PWV at follow-up in men (Vitamin-D r= 0.134, p= 0.02, PTH p=0.7) while PTH levels at baseline were negatively correlated with increased PWV in women (Vitamin-D r= 0.105, p= 0.24, PTH r= -0.26, p=0.004). In multivariate analyses the correlations between Vitamin-D in men and PTH in women with delta PWV were independent of ApoB/ApoA1, corrected s-Ca, SAD, statin-use and ambulatory systolic blood pressure levels.

Conclusion: While we verified a cross-sectional negative relationship between Vitamin-D levels and obesity in this cohort, Vitamin-D levels were related to increased arterial stiffness in men while serum PTH in women was associated with a lower increase in this measure of arteriosclerosis. These prospective findings do not support the idea that an increased use of Vitamin-D would be beneficial with regard to cardiovascular disease in men with type 2 diabetes.



Relationship between baseline serum PTH and 4-year change (increase) of PWV in women with type 2 diabetes. r= -0.265, p= 0.004

Clinical Trial Registration Number: NCT 01049737
Supported by: ALF, FORSS, MRC, HU Linköping

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Randomisation to a low-carbohydrate diet improves health related quality of life compared with a low-fat diet at similar weight loss in type 2 diabetes

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Background and aims: While some studies have investigated the effect of weight reduction on health related quality of life (HRQoL) in patients with obesity or type 2 diabetes there are very few data comparing the effect of different diet regimens. The aim was to compare the effects on HRQoL of a 2-year intervention in patients with type 2 diabetes with a low-fat diet (LFD) or a low-carbohydrate diet (LCD) based on four group-meetings to achieve compliance.

Materials and Methods: Prospective, randomized trial of 61 adults with type 2 diabetes. The SF-36 questionnaire was used at baseline, 6, 12 and 24 months. Patients on LFD aimed for 55-60 energy percent (E%) and those on LCD for 20E% from carbohydrates.

Results: Mean body-mass-index was 32.7±5.4 kg/m^2 at baseline. Weight-loss did not differ between groups and was maximal at 6 months, LFD: -3.99±4.1 kg, LCD: -4.31±3.6 kg, (p<0.001 within groups). At 12 months the Physical Function, Bodily Pain, General Health and Vitality improved in the LCD group compared to baseline (Table 1) while there was no change in the LFD group. There was an increase in the Physical Component Score of SF-36 from 44.1 (10.0) to 46.7 (10.5) at 12 months in the LCD group (p<0.009) while no change occurred in the LFD group (p<0.03 between groups). Mental Component Score was unchanged in both groups.

Conclusion: Weight-changes did not differ between the diet groups while improvements in HRQoL only occurred after one year during treatment with LCD. No changes of HRQoL occurred in the LFD group in spite of a similar reduction in body weight. Thus based on the results from SF-36 our data suggest that a LCD is preferable compared with a LFD for HRQoL after one year follow-up.

SF-36 domain	baseline	12 months	P value
Physical Function	77.7 (19.3)	83.6 (18.2)	0.009
Bodily Pain	61.0 (23.0)	71.4 (22.1)	0.021
General Health	63.2 (22.3)	70.7 (22.7)	0.031
Vitality	62.2 (19.9)	69.8 (19.3)	0.042

Table 1. SF-36 domains at baseline and after 12 months in the LCD group

Clinical Trial Registration Number: NCT01005498
Supported by: ALF, FORSS

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Nutritional screening in elderly type 2 diabetic patients admitted in a ward

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Background: Elderly patients are more prone to malnutrition and, among many factors, Diabetes can contribute to this increased risk. There are few data correlating Diabetes and malnutrition in older people (≥ 65 years).

Aim: To evaluate nutritional state in type 2 Diabetic patients over 65 years admitted for various reasons to the internal medicine ward in our hospital.

Materials and methods: The authors performed an observational study evaluating consecutively 244 elderly diabetic patients from 1st June 2009 to 31st January 2013 and compared them to a group of 358 non-diabetic elderly patients. The clinical tool was the Mini Nutritional Assessment (MNA).The MiniNutritional Assessment (MNA) stratifies nutritional state from 0 to 30: malnutrition (<17), malnutrition risk (17,5-23,5) and normal (>24).This tool was validated in Portugal in 2008.Statistical analysis was performed by SPSS 17.0 for Windows, when appropriate.

Results: The 244 elderly diabetic patients (59% female and 41% male) had a mean age of 81,89 ± 7,77 years. There was no statistical significant difference from the 358 non-diabetic control group (56,1% female, 43,9% male with a mean age of 81,06 ± 7,87 years, p > 0,05). Mean MNA score in diabetic elderly patients was significantly lower than in the control group (15,39 ±5,62 vs 18,89±5,28, p < 0,001). In diabetic patients, 14,3% were well nourished, 64,8 % were classified with malnutrition e 20,9 % with malnutrition risk (values of 26,5%, 35,8 % e 37,7% respectively for control group, p< 0,001). Overall, we have found 64,2% Diabetic patients with nutritional problems vs 35,2% in the control group. The item that was observed more frequently in the diabetic population and therefore responsible for this nutritional state was limited mobility (83% vs 58% (p<0,001)). We have also observed significantly more elderly diabetic patients with malnutrition coming from institutions than from home setting. (MNA score diabetic 14,42 +/-4,79 vs MNA score controls 19,52 +/- 5,25,p < 0,001).

Conclusion: Our results show that malnutrition is more prevalent in elderly people with type 2 diabetes admitted to a medical ward for various reasons, which implies that they must be identified for specific nutritional intervention. There will be necessarily more studies to evaluate the causes and the complex relationship between type 2 diabetes and malnutrition in elderly patients.

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Higher urinary sodium excretion is weakly associated with albuminuria, but not with retinopathy in type 1 diabetes: the EURODIAB study

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Background and aims: Nutritional advice that favourably affects cardiovascular risk factors, such as blood pressure (BP), may also have a favourable effect on microvascular complications. Restriction of salt intake is known to reduce BP. Population-based studies have shown positive associations of 24-hour urinary sodium excretion, which is currently regarded the reference standard for salt intake estimation, with microalbuminuria. In individuals with type 1 diabetes (T1DM), urinary sodium excretion has been associated with all-cause mortality and end-stage renal disease. The association of salt intake with (early) microvascular complications in individuals with T1DM, however, has not yet been established. We studied the association of urinary sodium excretion with the prevalence of microalbuminuria, macroalbuminuria, non-proliferative and proliferative retinopathy in individuals with T1DM.
Materials and methods: We measured sodium and potassium concentrations in two 24-hour urine samples in 1,212 individuals with T1DM who participated in the EURODIAB Prospective Complications study. We used multiple logistic regression analyses to investigate cross-sectional associations of urinary sodium excretion with the prevalence of microvascular complications. Adjustments were made for age and sex and additionally for BMI, smoking, urinary potassium excretion, use of antihypertensive medication, physical activity, and total energy, alcohol, saturated fat and fiber intake.
Results: Individuals (51% men) were (mean ± SD) 40 ± 10 years old, with a diabetes duration 22 ± 9 years, HbA1C 8.4 ± 1.5%, SBP 121 ± 19 and DBP 74 ± 12 mm Hg. Of the 1,212 individuals currently investigated, 205 and 143 individuals had micro- and macroalbuminuria; 507 and 224 individuals had non-proliferative and proliferative retinopathy, respectively. The 24-hour urinary sodium excretion was 3.96 ± 1.60 g/day, which corresponds to a dietary salt intake of 9.90 g/day. After adjustment for age and sex, 1 g/day higher salt intake was positively associated with microalbuminuria [OR: 1.06 (95%CI: 1.02; 1.10)], but not with macroalbuminuria [1.00 (0.95; 1.04)], non-proliferative retinopathy [1.02 (0.98; 1.05)] or proliferative retinopathy [0.99 (0.94; 1.03)]. After excluding individuals with CVD and/or antihypertensive medication (n=433), the association with microalbuminuria became borderline significant [1.05 (1.00; 1.10)], with no significant associations for macroalbuminuria [1.04 (0.96; 1.12)], non-proliferative retinopathy [1.01 (0.97; 1.05)] or proliferative retinopathy [1.02 (0.95; 1.09)]. Essentially the same results were found in the fully adjusted models.
Conclusion: In individuals with T1DM, high salt intake, as determined by urinary sodium excretion, may be positively associated with albuminuria, but not with retinopathy. Further investigation in prospective studies is warranted to address the role of dietary salt intake in the development of microvascular complications in T1DM.

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Breastfeeding and the risk of developing type 1 diabetes or islet autoimmunity

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Background and aims: The increased incidence of type 1 diabetes (T1D) suggests a role for environmental factors in the development of the disease. Early nutrition, as an important environmental source of exposure, may contribute significantly to the development of T1D, but there is a lack of evidence for this from prospective studies in humans. The effect of breastfeeding on T1D and islet autoimmunity clearly varies between studies, showing inconsistent results. The aim of this study was to investigate the association between breastfeeding and the risk of developing T1D or islet autoimmunity.
Materials and methods: Data were derived from a prospective cohort study (MIDIA) with inclusion of children identified with the high-risk HLA genotype, with follow-up from three months up to 15 years of age. Close to 48000 newborns were genotyped, and 1047 were identified with the high-risk genotype. A questionnaire was filled out by the parents when the child was 3, 6, 9 and 12 months of age. Blood samples were obtained from children at the same intervals. The WHO definitions were used to categorise breastfeeding into "full breastfeeding" and "any breastfeeding". Full breastfeeding was categorised into two groups: <3 months and ≥3 months. Any breastfeeding was categorised into two groups: ≤6 months and >6 months. Logistic regression analyses were applied to study T1D and islet autoimmunity in relation to full breastfeeding, any breastfeeding and other parental and infant characteristics. Data were then adjusted for variables associated with T1D and islet autoimmunity.
Results: The multivariate analysis included full breastfeeding and any breastfeeding separately, and having a first degree relative with T1D as the only variable associated with T1D or islet autoimmunity ($p < 0.001$). There was no significant association between T1D and full breastfeeding (OR 1.28; $p = 0.66$), or any breastfeeding (OR 1.01; $p = 0.99$), after adjusting for first degree relatives with T1D. There was no significant association between islet autoimmunity and full breastfeeding (OR 1.30; $p = 0.41$), or any breastfeeding (OR 1.25; $p = 0.51$), after adjusting for first degree relatives with T1D.
Conclusion: Based on the data from the MIDIA study, we explored the association between breastfeeding and the risk of developing T1D or islet autoimmunity. Our key finding was that full breastfeeding was not significantly associated with either T1D or islet autoimmunity, and neither was any breastfeeding.
Supported by: Norwegian Extra Foundation for Health and Rehabilitation

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Effect of omega 3/ALA supplementation on inflammatory markers and ER stress in diabetic rats

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Background and aims: A state of chronic and subclinical inflammation is often observed in diabetic patients and more recently it has been associated to activation of the endothelium reticulum stress (ERS). Previous studies have shown that polyunsaturated fatty acids ω3 eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) can reduce inflammation, but little is known about the effect of ω3 α-linolenic acid (ALA; C18:3n-3). The ω3/ALA is the primary fatty acid of the n-3 pathway found in seeds oils, notably those of flaxseeds and chia. The aim of this study was to evaluate the effects of ω3/ALA supplementation on inflammation and ERS in animal models of diabetes mellitus.
Materials and methods: 40 wistar rats (150g) were divided into four groups: control, control+ω3, diabetes and diabetes+ω3. Induction of diabetes was performed using 40mg/kg of streptozotocin iv. The control+ω3 and diabetes+ω3 received supplementation of 3g of ω3/ALA from flaxseed, daily for a period of 8 weeks. Measurements of serum glucose, lipid profile serum cytokines (TNF-α, IL-6 and INF-γ) and body weight were performed before the beginning of the supplementation (T1) and by the end of this period (T2). Data of liver and epididimal fat weight were collected no T2. Protein expression of AKT, TNF-α, IL-6, BIP, IRE1a, PERK, XBP-1, DAPK1 and GADD153 were evaluated by western blot in liver tissue of the diabetic groups.
Results: The diabetes+ω3 group had lower liver ($p<0.001$) and greater epididimal adipose tissue ($p=0.04$) weight in relation to the diabetes group, besides no difference in total body weight. On the other hand, the control+ω3 group showed increased liver ($p=0.001$), epididimal ($p<0.001$) and body weight ($p<0.001$). Diabetes+ω3 group showed lower glucose ($p=0.01$) and triglyceride ($p<0.001$) levels on T2, but no difference in total cholesterol. Serum levels of TNF-α and IL6 were lower in the diabetic groups compared to the control groups but the ω3/ALA supplementation did not determine significant changes. However, the diabetic+ω3 group showed a decrease in the INF-γ serum levels after the supplementation period. We also observed that diabetes+ω3 group had increased expression of BIP (50.4%;$p=0.01$) and AKT (37.5%;$p=0.03$) and decreased expression of XBP-1 (7.0%;$p=0.004$), IRE1a (23.3%;$p=0.03$) and TNF-α (12.2%;$p=0.04$). There were no difference in the expression of PERK, GADD153, DAPK1 and IL-6.
Conclusion: Supplementation of ω3/ALA was able to reduce blood glucose and triglyceride in diabetic rats associated to a reduction in systemic inflammation as observed by the lower levels of INF-γ. Our data also shows that ω3/ALA supplementation was able to influence important pathways involved in the ERS, suggesting that this polyunsaturated fatty acid may modulate the ERS.

	CONTROL		CONTROL+ω3		DIABETES		DIABETES+ω3	
	T1	T2	T1	T2	T1	T2	T1	T2
Body Weight(gr)	169,1±17,7	283,5±42,5	168,7±10,5	336±71,7	146,9±11,2	245,3±1,2	158,5±11,7	225,4±0,3
Glucose(mg/dL)	153,6±17,2	100,3±9,9	141,1±8,3	92,8±6,3	471,7±54,4	517,4±67,7	482,6±35,7	431,4±58,4
Total Cholesterol(mg/dL)	78,7±12,5	57,7±6,9	70,1±10,4	55,4±10,0	74,7±7,6	91±17,7	79,2±13,6	91±14,4
Triglycerides(mg/dL)	70,7±15,7	86±22,7	68,5±21,7	114,5±30,1	75,8±8,8	216,8±49,7	71,9±29,5	89,4±39,5
Serum TNF-a(pg/mL)	40,1±21,6	19,1±12,7	32,4±12,8	18,6±18,0	13,0±7,9	30,6±5,5	29,3±28,1	30,3±13,4
Serum IL-6(pg/mL)	130,3±38,4	81,4±25,3	116,1±36,9	95,7±23,1	44,1±28,3	37,5±17,6	74,9±31,7	50±35,3
Serum INF-g(pg/mL)	798,2±190,8	542,0±237,7	694,4±159,7	634,9±170,0	617,8±186,6	402,4±236,7	636,1±121,9	378,3±174,7

Supported by: FAPESP

PS 068 Mechanistic insights: GLP-1-based medications and GPR40 agonists

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Glucose tolerance and gastrointestinal-mediated glucose disposal in women with previous gestational diabetes mellitus

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Background and aims: Women with previous gestational diabetes mellitus (GDM) are at high risk of developing type 2 diabetes mellitus later in life, and constitute an excellent opportunity for studying the early stages of diabetes. Gastrointestinal-mediated glucose disposal (GIGD) is the result of all factors affecting glucose disposal differently during oral glucose ingestion versus an i.v. glucose infusion. GIGD is decreased in patients with type 2 diabetes, and the aim of this study is to explore whether reduced GIGD precedes the type 2 diabetic state and already can be detected in high-risk individuals, i.e. women with previous GDM.
Materials and methods: Twenty-six non-diabetic women with previous GDM (age: 37±1 years (mean±SEM); BMI: 30.9±1.0 kg/m^2; waist-hip ratio: 0.9±0.0; fasting plasma glucose (FPG): 5.5±0.1 mM; HbA_{1c}: 5.4±0.1 % (36±1 mmol/mol) were examined on two separate occasions: 1) 4h 75g-OGTT and 2) isoglycaemic i.v. glucose infusion (IIGI). Based on the FPG and the 2h value of the OGTT, the women were classified as having normal glucose tolerance (NGT) or prediabetes (defined as impaired fasting glucose (IFG), and/or impaired glucose tolerance (IGT)). Using the isoglycaemic clamp technique, GIGD was calculated as the percent difference in the amount of glucose given during the OGTT and IGII divided by the amount given during the OGTT [100% × (glucose$_{OGTT}$ - glucose$_{IIGI}$ / glucose$_{OGTT}$)].
Results: Nine out of 26 women were NGT (35%) and 17 (65%) were diagnosed with prediabetes. Two-hour plasma glucose concentrations during OGTT differed significantly between the groups (6.9±0.2 vs. 9.3±0.3, p<0.001). The groups were similar with respect to age, BMI, waist-hip ratio, FPG, and HbA_{1c}. No difference in GIGD was seen (57±7 vs. 49±3%, p=0.26).
Conclusion: Our results show that prediabetes is prevalent in women with previous GDM. No significant difference in GIGD was seen between women with NGT and prediabetes.

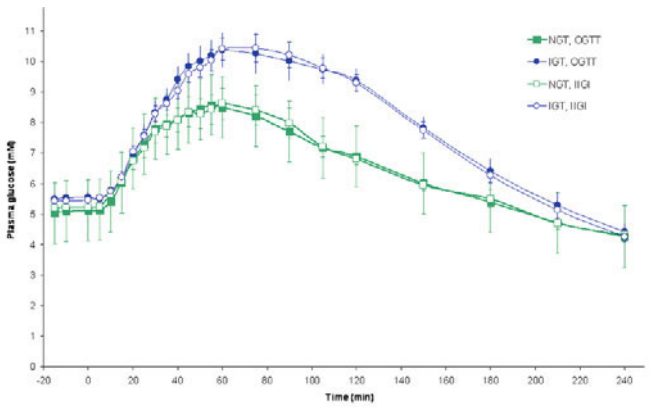

Figure 1. Plasma glucose profile during OGTT (filled symbols) and isoglycaemic i.v. glucose infusion (IIGI) (open symbols) for normal glucose tolerant women (green) and prediabetic women (blue). NGT: normal glucose tolerant, IGT: impaired glucose tolerance.

Clinical Trial Registration Number: NCT01795248
Supported by: IIS grant from Novo Nordisk

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The impact of dipeptidyl peptidase 4 inhibition on incretin effect, glucose tolerance, and gastrointestinal-mediated glucose disposal in healthy subjects

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Background and aims: Inhibition of the enzyme dipeptidyl peptidase 4 (DPP-4), which under normal circumstances degrades and inactivates the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), is thought to intensify the physiological effects of incretins. We aimed to investigate the effects of DPP-4 inhibitor-induced increments of active GIP and GLP-1 levels on glucose tolerance, incretin effect, gastrointestinal-mediated glucose disposal (GIGD) and gastric emptying in healthy subjects.
Materials and methods: Ten healthy subjects (age: 40±5 years (mean±SEM); body mass index: 24±3 kg/m2, fasting plasma glucose: 5.1±0.2 mmol/l; HbA1c: 5.3±0.1% [34 mmol/mol]) were subjected to two paired study days each constituting a 4h 50 g-OGTT (A) and an isoglycaemic i.v. glucose infusion (IIGI) (B) with (A1+B1) and without (A2+B2) preceding administration of the DPP-4 inhibitor sitagliptin (100 mg 12 hours and 2 hours before each experimental day).
Results: Isoglycaemia was obtained with and without DPP-4 inhibition in all subjects. Significant increases in active GLP-1 and active GIP levels were seen during fasting conditions and especially during OGTT after DPP-4 inhibitor administration. No significant impact of DPP-4 inhibition on fasting plasma glucose (5.1±0.1 vs 4.9±0.1 mmol/l, p=0.3), glucose tolerance (evaluated as AUC for plasma glucose: 151±35 vs 137±26 mmol/l×min, p=0.7) or peak plasma glucose during OGTT (8.5±0.4 vs 8.1±0.3 mmol/l, p=0.3) was observed. Neither incretin effect (40±9 without DPP-4i vs. 40±7% with DPP-4i, p=1.0), glucagon responses (1,395±165 vs. 1,223±195 pmol/l, p=0.41), GIGD (52±4 vs. 56±5%, p=0.40) or gastric emptying (measured as peak concentration time (86±9 without vs. 80±12 min with DPP-4i, p=0.60) changed following DPP-4 inhibition.
Conclusion: These results suggest that acute increases in active incretin hormone levels (achieved via inhibition of DPP-4) do not affect GIGD, incretin effect, glucagon responses, glucose tolerance or gastric emptying in healthy subjects.
Clinical Trial Registration Number: NCT01342939

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Substantial posprandial appearance of active GLP-1 in the gut lymphatics in mice with marked increase after DPP-4 inhibition

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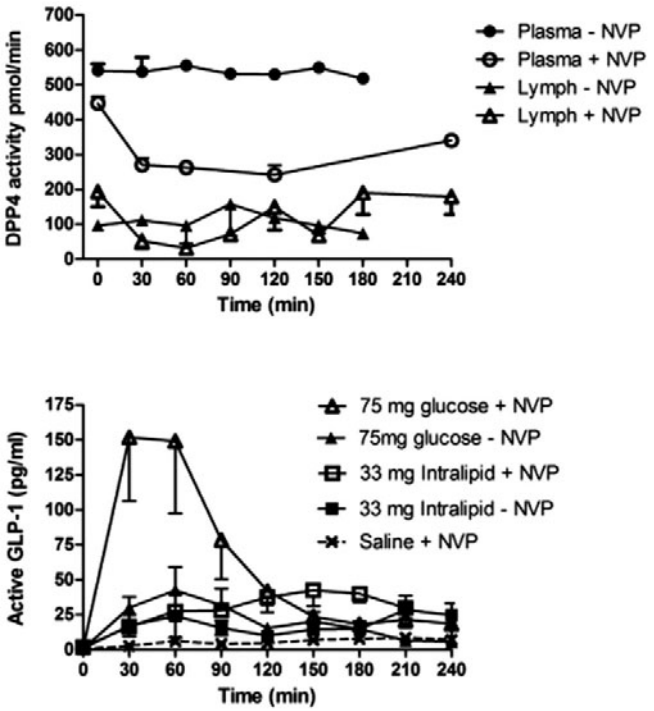
Background and aims: After secretion from intestinal L-cells, the incretin hormone glucagon-like peptide-1 (GLP-1) is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) before reaching the portal system leaving only a small fraction of active GLP-1 in peripheral blood. This inactivation hampers measurements of GLP-1 secretion under in vivo conditions, since peripheral plasma GLP-1 determinations only weakly reflect secretion rates. To quantify GLP-1 secretory rates, we have harnessed on the fact that GLP-1 appears in the gut lymphatics after its release from L-cells, and have developed a technique analyzing lymphatic GLP-1 levels after macronutrient ingestion. The aim of this study was to validate this novel technique and examine gut lymphatic GLP-1 after macronutrient ingestion with or without concomitant DPP-4 inhibition.
Materials and methods: Anesthetized C57BL6/J mice were fitted with both a mesenteric lymph duct and stomach cannula before being infused with either glucose or fat emulsion (IntralipidR) (both 0.3 kcal) into the stomach, with or without concomitant administration of an orally active DPP-4-inhibitor (10 µmol/kg NVP DPP728). Lymph was collected sequentially in 30 min intervals up to four hours. DPP-4 activity (enzymatic method) and active GLP-1 concentrations (ELISA) were measured in lymph and plasma (orbital plexus) at corresponding time points.
Results: DPP-4 activity in lymph was only 18±2% of that in peripheral plasma (P<0.001) with no significant variation after macronutrient ingestion. The DPP-4-inhibitor NVP DPP728 decreased DPP-4 activity by 57% and 43 % in lymph and plasma for 2 hours (both P<0.001). Basal concentrations of active GLP-1 in lymph were 4.7 times higher than in plasma (1.17 ± 0.2 vs. 0.25 ± 0.05 pg/ml, p=0.04), whereas peak levels after glucose infusion were 140 times higher in lymph than in plasma (42±17 vs. 0.3±0.02 pg/ml, p= 0.09). Peak GLP-1 was raised three-fold by DPP-4 inhibition (to 152±46 and 1.2 ± 0.21 pg/ml p=0.08). The concentrations of active GLP-1 in lymph after intralipid were similar with and without NVP DPP728 during the initial 60 minutes (27± 5.8 and 24 ± 16 pg/ml). Thereafter, GLP-1 levels decreased without NVP DPP728, but remained high for a prolonged period of time with NVP DPP728 (40±15 pg/ml after 150 min).
Conclusion: There is a substantial release of active GLP-1 into mesenteric lymph after macronutrient ingestion, resulting in a many fold higher GLP-1 concentration in lymph than in peripheral plasma. DPP-4 inhibition further increased the concentration of active GLP-1 by a factor of three in both lymph and plasma. Thus, we conclude that measurement of active GLP-1 in mesenteric lymph is a valuable tool for quantifying GLP-1 secretion in vivo, and oral DPP-4 inhibition has substantial effect on the pool of active GLP-1 emerging from the lymph into the subclavian vein after intake. Figure: DPP4 activity in plasma and lymph (a) and GLP-1 concentration in lymph (b) after intake of glucose or Intralipid.



Supported by: Påhlssons Stiftelse

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Effects of miglitol, sitagliptin and combination of both on plasma incretins' responses to a mixed meal and visceral fat in over-weight Japanese type 2 diabetic patients

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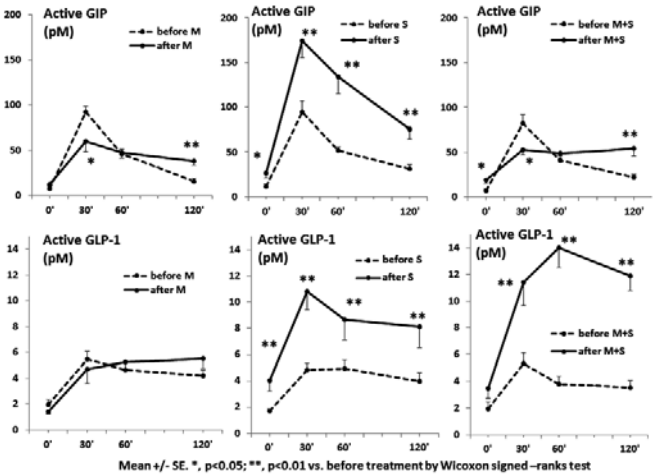
Background and aims: Alpha-glucosidase inhibitors (AGIs) induce reduced gastric inhibitory polypeptide (GIP) and enhanced glucagon-like peptide-1 (GLP-1) responses after a meal. Dipeptidyl peptidase-4 (DPP-4) inhibitors induce enhanced active GIP and GLP-1 levels. In combination of both, although additive enhancement of plasma active GLP-1 levels has been reported, changes in active GIP levels have not been examined. Further, effects of combination of both on body fat compositions have not been examined although AGIs induce body weight (BW) reduction and DPP-4 inhibitors have neutral effect on BW.

Materials and methods: In this multicenter, open 24-week trial, Japanese over-weight (BMI of 25 or more) type 2 diabetic patients (T2Ds) without medication or with metformin and/or sulfonylurea were randomized assigned to receive 50 mg of miglitol (M, n=14) three times per day, sitagliptin (S, n=14) 50 mg once a day or combination of both (M+S, n=13). We measured plasma active GIP and GLP-1 at 0, 30, 60, 120 min during a meal tolerance test (MTT) at baseline and 24 weeks after the treatment. Active GLP-1 was measured using a commercially available kit (Linco Research). Active GIP was measured by LC-MS/MS/MS method. Visceral fat (VF) mass was measured with body composition analyzer X-Scan plus (JAWON MEDICAL). Data are expressed as mean (SE). *, ** indicate p<0.05 and p<0.01 by Wilcoxon signed-ranks test, respectively.
Results: During MTTs before and after M, S and M+S, plasma glucose-AUCs were significantly reduced from 364.9 (13.1), 394.4 (17.8), 414.4 (19.4) to 320.2 (17.1)*, 346.9 (15.4)*, 289.7 (13.6)** mg•hr/dl, respectively. M and M+S significantly reduced serum insulin-AUCs whereas S increased serum insulin-AUC significantly. As seen in figure, M decreased active GIP at 30 min and increased 120 min during MTT. S increased active GIP significantly throughout MTT. The combination of both significantly increased active GIP at 0 and 120 min during MTT despite of significant decrease at 30 min during MTT without an AUC change. S and M+S increased active GLP-1 with significantly increased AUCs (approximately two and three folds, respectively). Changes in VF mass by M, S and M+S were -0.27 (0.11), -0.15 (0.17) and -0.45 (0.11)** kg.
Conclusion: The effects of the combination of M+S on glycemic control and active GLP-1 were additive whereas enhancement of active GIP by S is abolished by M+S with reduced VF mass. In over-weight T2Ds, the combination of both drugs may have a beneficial effect on adiposity with relation to different effects on two incretins.



Clinical Trial Registration Number: UMIN000006098

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Pharmacological characterisation of a novel long-acting CarboCarrier® D-Ala[8]GLP-1 for treatment of type 2 diabetes
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Background and aims: Novel long-acting GLP-1 analogues were synthesized using CarboCarrier® technology. Pharmacological properties of Org 249305-1 in which an ATIII binding pentasaccharide, derived from Idraparinux, was conjugated to Lys37 of D-Ala[8]-GLP-1 amide are reported.
Methods and results: In vivo half-life of Org 249305-1 in rat and mouse was ~ 11h, with resistance to DPP-IV degradation. Insulin secretion from clonal BRIN-BD11 cells and mouse islets was increased 2.1 - 4.1- fold (P<0.01-0.001) by Org 249305-1 at 10^{-8}M - 10^{-6}M. Org 249305-1 had EC-50 values of $10^{-6.7}$M and $10^{-7.0}$M for GLP-1 receptor binding and cAMP generation, respectively. Glucose tolerance in normal mice both immediately and 4-h post Org 249305-1 administration (25 nmol/kg) resulted in significant (P<0.001) reductions in blood glucose (AUC 41% and 65% lower, respectively). These effects persisted 48h post administration. Long-term treatment (21 days) of glucose intolerant high fat-fed Swiss NIH mice and C57/6J ob/ob mice with 25 nmol/kg Org 249305-1 resulted in significant improvement in glucose tolerance and reductions in HbA_{1c} of 0.5 - 1.0% (P<0.05-0.01). Treatment of diabetic C57/KsJ db/db mice for 15 days with 100 nmol/kg Org 249305-1 reduced (P<0.05-0.001) non-fasting glucose with raised plasma insulin. Oral glucose tolerance was significantly improved (P<0.001) and HbA1c reduced 1.8% compared to saline controls. Islet size and pancreatic insulin content were increased whereas glucagon content was decreased.
Conclusion: Org 249305-1 exerts significant antidiabetic actions and has a projected PK/PD profile compatible with once-weekly subcutaneous dosing in humans.
Supported by: MSD

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Long-term effects of liraglutide on pancreatic beta cell function and glycaemic control in type 1 diabetes with residual insulin secretion
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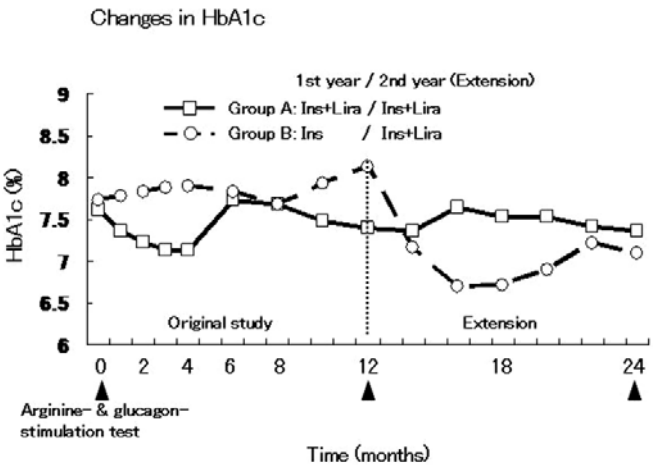
Background and aims: Previously we reported that one-year liraglutide (Lira) added on ongoing insulin (Ins) therapy improved glycaemic control without increase in hypoglycaemia in patients with type 1 diabetes (T1D) with residual insulin secretion. In the study, however, no obvious beneficial effect on beta-cell function could be observed with Lira treatment. To investigate the longer-term effects of Lira and to compare the effects between Ins only and Lira plus Ins in the same arm, extension study was performed for another one year over the original study.
Materials and methods: This is an open-labelled, randomised, parallel-group study. A total of 15 subjects with type T1D (age 51.5±13.2yrs, BMI 22.5±3.1; mean±SD) who were positive for GADAb or IA-2Ab and had been treated with insulin more than one year with residual insulin secretion [C-peptide (CPR) level >0.3ng/ml] were enrolled in the original study, and 8 subjects have completed second-year evaluation. The subjects were randomised into two groups; Group A was administered Lira 0.9mg in addition to Ins therapy for 2 years, and group B continued the ongoing Ins for the first year and the second year Lira 0.9mg was added-on. As a primary outcome, changes in C-peptide response to glucagon- and arginine-stimulation test that were performed annually were evaluated. Changes in HbA1c, blood glucose (BG) by self-monitoring (SMBG), body weight, daily insulin dose, and adverse events (AE) were also investigated. For the first year, comparison was performed between the two arms, and as for the second year, the comparison was also performed between the first year and second year in the same arm.
Results: In group A, HbA_{1c} tended to decrease from 7.6±1.4 to 7.4±1.5% with decreased Ins dose from 0.66±0.29 to 0.58±0.24U/Kg/day in the first year but did not change in the second year. In group B, HbA_{1c} remained unchanged in the first year (from 7.7±0.6 to 8.1±0.5%) but decreased from 8.1±0.9 to 7.1±0.4% by addition of Lira with a significant decrease in Ins dose (from 0.66±0.29 to 0.58±0.29U/Kg/day, P<0.05). CPR response to arginine-stimulation varied among the subjects (mean 9.3±54.8%, range -55.6% to +66.4% compared to baseline in group A, and mean 7.1±33.2%, range -19.5% to +44.4% in group B in the first year; and mean -5.2±43.1%, range -53.5% to +47.4% in the second year in group A&B) suggesting that the response to Lira was different in the individual cases. Addition of Lira decreased glucagon response to arginine-stimulation by -11.9±13.9% (p<0.05 at the peak level).
Conclusion: The present findings suggest that adding Lira on Ins therapy may have a therapeutic potential to improve glycaemic control in T1D with a decrease in insulin dose. Although the effects of Lira on pancreatic beta-cell showed variation among the subjects, the major effect was considered to be exhibited through extra-beta-cell effects such as suppression of glucagon secretion rather than an increase of insulin secretion.



Clinical Trial Registration Number: UMIN000006990

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Dipeptidyl peptidase-4 inhibitors improve glycaemic control in latent autoimmune diabetes in adults (LADA) patients insufficiently controlled despite insulin therapy

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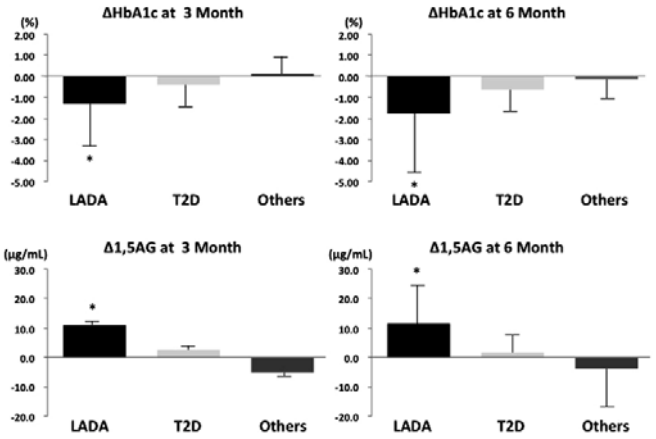
Background and aims: Latent autoimmune diabetes in adults (LADA) patients share many genetic and immunological similarities with type 1 diabetes (T1D), suggesting that LADA is an autoimmune disease. Dipeptidyl peptidase-4 inhibitors (DPP4i) have emerged as a new class of agents for the treatment of type 2 diabetes (T2D), and currently have been extending for their use to early T1D, because of their potential for shutting off autoimmunity and stimulating beta cell regeneration. It is possible that the therapeutic benefit of the DPP-IV inhibition on the immune system, in addition to resulting in increased circulating levels of GLP-1, may improve pathophysiological process of autoimmune mediated diabetes, we compared the impact of treatment with the DPP4i (sitagliptin) on HbA1c reductions and improvements of 1,5-anhydroglucitol (1,5AG) value in patients retrospectively identified with LADA who had insufficient glycemic control despite insulin therapy.
Materials and methods: Patients were classified as LADA if the GAD65 autoantibody was present at baseline or any on-treatment visit. GAD antibody was assessed with RIA.
Results: Of 118 patients with assumed T2D, 10.2% (n=12) had LADA, and 9.3%(n=11) had others (pancreatitis, liver cirrhosis, steroids diabetes, etc.). At baseline, patients age (64.7±12.6 yrs, mean ± SD), BMI 24.2±3.9 kg/m2, HbA1c (8.09±1.40 %), and dose of daily insulin (19.5±13.8 units) were not significantly different between LADA, T2D, and others. HbA1c reductions in LADA at 3 month and 6 month after the initiation of administration of sitagliptin (25mg; n=47, 50mg; n=71, once daily) were significantly (*; $p<0.05$) better than those of T2D and others (Figure). 1,5 AG at the same time points in LADA were significantly (*; $p<0.05$) increased than those of T2D and others.
Conclusion: DPP4i could have more beneficial impacts on glycemic control in LADA than T2D and others who were treated with insulin. Further research exploring the molecular mechanism of potential modulating effects of DPP4i on LADA is required.

Figure: Changes in HbA1c and 1,5AG over time by treatment with DPP4i and insulin



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Exenatide blunts hyperactivation in CNS reward and satiety circuits elicited by viewing food cues in obese individuals

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Background and aims: It has been hypothesised that changes in central nervous system (CNS) reward and satiety responses to food stimuli play an important role in the development of obesity and type 2 diabetes (T2DM). Food ingestion activates the secretion of several gut-derived mediators, including the incretin glucagon-like peptide 1 (GLP-1). Based on animal studies, these hormones have been proposed to relay information to the CNS about nutritional status and regulate appetite. The GLP-1 receptor agonist (GLP-1RA) exenatide improves glycaemic control and promotes satiety, leading to reductions in food intake and body weight. GLP-1RA actions on the brain may partly mediate satiety and weight effects. We hypothesised that exenatide reduces food intake by affecting CNS reward and satiety circuits.
Materials and methods: Normoglycaemic obese (n=11; mean± SD age 58±8 yrs, BMI 32±3 kg/m^2; 6 males) and gender and age-matched normoglycaemic healthy lean controls (n=11; age 57±9 yrs, BMI 24±2 kg/m^2) were studied in a randomised, cross-over, placebo-controlled trial. We determined the acute effect of iv infusion of exenatide (infusion duration approximately 120 min, spanning the entire test) on CNS reward and satiety responses to visual food-related stimuli, using functional magnetic resonance imaging (fMRI), after an overnight fast. Pictures of high calorie food, low calorie food and non-food objects were presented. To avoid the effects of metabolic and hormonal changes induced by exenatide, a somatostatin pituitary-pancreatic clamp (glucose 5 mmol/L) was performed, with replacement of basal insulin, glucagon and growth hormone. After the fMRI, subjects were presented a choice buffet to assess energy intake. Imaging data were analysed using SPM8. For each subject, activation contrasts were computed (food versus non-food).
Results: Obese versus lean subjects showed increased brain activation, measured as changes in blood oxygenation level dependent (BOLD) signal, in bilateral insula and right caudate nucleus, when watching food versus non-food pictures ($p<0.005$). Exenatide versus placebo blunted these activations in bilateral caudate nucleus, left insula and left thalamus in obese individuals ($p<0.005$), but in lean controls the effects of exenatide did not reach statistical significance. Exenatide effects were more pronounced in obese women than in men. Exenatide versus placebo reduced caloric intake by 25% in lean and by 21% in obese subjects.
Conclusion: Exenatide blunted hyperactivation in reward- and satiety-related brain regions, elicited by viewing food cues in obese subjects, thus restoring an activation pattern that resembled that of lean controls. These findings lend support to the hypothesis that GLP-1RA affects food-related CNS reward and satiety, which may contribute to treatment-related weight loss.
Clinical Trial Registration Number: NCT01281228
Supported by: BMS / Amylin / Eli Lilly

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Liraglutide is applicable in elective perioperative patients with type 2 diabetes

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Background and aims: Diabetic patients in sedentary states during perioperative periods tend to gain weight, and their glycemic control frequently deteriorates. There is substantial evidence linking hyperglycemia in surgical patients with worse outcomes. On the other hand, temporary insulin therapy needs frequent monitoring of blood glucose levels and carries a risk of hypoglycemia. The recent largest study NICE-SUGAR suggested intensive insulin therapy significantly increased the risk of hypoglycemia and resulted in no overall mortality benefit among critically ill patients. Analogues of incretins have been widely used to treat type 2 diabetes (T2DM), but data about the therapeutic applications of these drugs instead of insulin for perioperative glycemic control are scarce. To investigate the efficacy and safety of perioperative liraglutide (LGT) therapy as a non-insulin agent for perioperative glycemic control, we performed a retrospective case analysis on a total of 73 elective surgeries on 68 patients with T2DM in two separate hospitals.
Materials and methods: Informed consents were obtained, and LGT was introduced after cessations of OADs. Sixty-eight subjects with T2DM (male/female; 41/28, Age; 69.8±11.2 years old, HbA1c; 7.9±1.3 %, BMI; 26.9±4.0, Disease duration 12.9±11.7 years) were initiated with LGT therapy before elective operations, e.g. orthopedic operations (39 cases), cardiac catheterizations for ischemic heart disease (17), cataract (10), prostate cancer (2), uterus cancer (1), renal cancer (1), bladder cancer (1), breast cancer (1), plastic surgery (1). In case of hyperglycemia during perioperative period, regular insulin was added if needed. As LGT decreases gastrointestinal motility, surgical cases with gastrointestinal tracts were excluded. BMI change, glycemic level fluctuation, hypoglycemia, doses of insulin, if required, and perioperative complications were analyzed.
Results: LGT was continued through the entire period of hospital stay including the operation day (day 0). Meals were resumed on day1. The mean glucose levels on LGT initiation day, day 0, and day 1 were 194.5±52.5, 146.2±30.6 (*p<0.01*) and 162.2±35.1 (*p<0.01*) mg/dl, respectively. The BMI of almost all patients decreased (-1.1±1.4) with LGT therapy before operation. LGT achieved good glycemic control throughout the perioperative period. Additional regular insulin was not needed except in the case of 9 patients. HbA1c stayed within a good range (6.8±0.8 %) at 6th months after operation. There was an additional benefit of body weight loss enabling efficient rehabilitation. Adverse incidents such as hypoglycemic episodes, wound healing retardations, or other complications were not observed. Discussion: Insulin is a gold standard for treating hyperglycemia in surgical patients. It needs frequent glucose measurement and adjustment of dose by well-trained medical providers especially during perioperative periods when the caloric intake is variable. The present results showed the possible application of LGT in selected stable diabetic patients who are expected to consume meals at regular intervals without adjustment of dose.
Conclusion: LGT provides an effective and optional way as a perioperative non-insulin agent to safely achieve good glycemic control in perioperative subjects with T2DM, especially those with limited exercise ability and those at risks of hypoglycemia.

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Pharmacokinetics (PK) and pharmacodynamics (PD) of the GPR40 agonist fasiglifam (TAK-875) and glimepiride following co-administration in type 2 diabetes subjects

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Background and aims: This phase 1, single-blind, placebo-controlled, sequential, single-site study evaluated the effects of multiple oral doses of fasiglifam (TAK-875) on the PK of a single oral dose of glimepiride and effects of a single dose of glimepiride on steady-state PK and PD of fasiglifam in T2DM subjects.
Materials and methods: T2DM subjects (n=30) received a single dose of glimepiride 2 mg on Day 1 followed by fasiglifam 50 mg QD on Days 3-19. On Day 18, glimepiride was also given with fasiglifam. Serial plasma PK samples were collected on Days 1-3 & Days 18-20 for glimepiride and on Days 13-19 for fasiglifam & M-I. Serial PD samples for markers of fasting glucose, insulin, C-peptide, and glucagon were collected on Days 17-18. All PK & PD parameters were estimated using non-compartmental methods. PK interactions were assessed by point estimates and 90% confidence intervals (CIs) of the ratios of the central values for C_{max} and AUC values when treatments were coadministered relative to each treatment alone using paired t-test. The effects of glimepiride on fasiglifam PD response (AUEC) were similarly assessed for each marker.
Results: Glimepiride was absorbed (T_{max} ~ 3 hr) and eliminated ($T_{1/2}$ ~ 7.5 hr) following dosing of either glimepiride alone or with fasiglifam. Fasiglifam was absorbed (T_{max} ~ 4 hr) following dosing of either fasiglifam alone or with glimepiride. The 90% CIs for C_{max} and AUC of glimepiride, fasiglifam, and M-I fell within the no-effect range of 0.80 to 1.25. The mean AUEC values were lower for fasting glucose and higher for other PD markers after dosing of fasiglifam with glimepiride relative to fasiglifam alone; differences were statistically significant ($p<0.005$) for all markers.
Conclusion: Co-administration of fasiglifam with glimepiride was well-tolerated with no hypoglycemia and no effects on the PK of glimepiride, fasiglifam, or M-I. Changes in PD markers with coadministration of glimepiride suggest a potential synergistic effect between fasiglifam and glimepiride on insulin secretion mediated via different pathways.
Supported by: Takeda Pharmaceuticals International

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Efficacy and safety of fasiglifam (TAK-875), a GPR40 selective agonist, in Japanese type 2 diabetes mellitus patients: a phase 3, double-blind, placebo controlled, comparative study

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Background and aims: G-protein-coupled receptor 40 (GPR40), which is highly expressed in pancreatic β-cells, functions as a receptor for free fatty acids and potentiates glucose-stimulated insulin secretion. Fasiglifam (TAK-875) is a potent and highly selective agonist of GPR40, and previous clinical data suggest that fasiglifam has the potential to be a safe and effective treatment for type 2 diabetes mellitus with glucose-lowering effect comparable with sulfonylureas and less risk of hypoglycemia. We aimed to evaluate the efficacy and safety of fasiglifam monotherapy in Japanese patients with type 2 diabetes mellitus.
Materials and methods: This randomized, double-blind, placebo-controlled, phase 3 study was designed to evaluate the efficacy and safety of fasiglifam 25 mg and 50 mg administered once daily before breakfast for 24 weeks in Japanese patients with type 2 diabetes inadequately controlled by diet and exercise. The primary endpoint was the change from baseline in HbA1c at Week 24. Secondary endpoints included proportions of patients with HbA1c < 6.9%, and changes in HbA1c and fasting blood glucose (FPG) at each assessment point.
Results: A total of 192 subjects were randomized to receive fasiglifam 25 mg (n=63), fasiglifam 50 mg (n=62), or placebo (n=67). Baseline characteristics were as follows: mean age of 60.4 years, mean BMI of 24.97 kg/m^2, mean HbA1c of 7.79%, and mean FPG of 155.6 mg/dL. There was no major difference among the treatment groups. At Week 24, all fasiglifam groups had statistically significant reductions in HbA1c compared with placebo ($p<0.0001$). Least-square mean HbA1c difference versus placebo was -0.75% (95% CI: -0.985 to -0.521) in the fasiglifam 25 mg group and -1.01% (95% CI: -1.244 to -0.777) in the fasiglifam 50 mg group. The mean change from baseline in HbA1c showed a time-dependent decrease from Weeks 2 to 24 in all fasiglifam groups and significant decreases versus placebo occurred as early as Week 4. The percentage of subject who achieved the HbA1c level of <6.9% at Week 24 was also significantly higher in the fasiglifam 25 mg (30.2%) and 50 mg (54.8%) groups than in the placebo group (13.8%). The mean change in FPG at Week 24 was -25.3 mg/dL, -25.5 mg/dL and -3.1 mg/dL in the fasiglifam 25 mg, 50 mg and the placebo groups, respectively, resulting in a significant decrease in all fasiglifam groups compared with the placebo group. There were no significant differences in incidence or types of adverse events between the fasiglifam groups and the placebo group. Hypoglycemia was reported only in 1 subject in the fasiglifam 50 mg. There were no changes in body weight in any groups.
Conclusion: Fasiglifam significantly improved glycemic control with minimum risk of hypoglycemia and was well tolerated in Japanese patients with type 2 diabetes inadequately controlled by diet and exercise.
Clinical Trial Registration Number: NCT01585792

PS 069 Incretin-based drugs: predictors of clinical outcomes

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Markers of insulin resistance and beta cell function do not predict response to incretin based treatments in type 2 diabetes

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Background and aims: Glycaemic response to the incretin based agents (GLP-1 agonists (GLP-1A) and DPPIV inhibitors (DPPIVI)) is highly variable. Determining predictors of treatment response could potentially aid the selection of the most appropriate treatment for an individual. We aimed to determine whether markers of insulin resistance and beta cell function are associated with response to incretin based therapies in Type 2 diabetes.
Methods: We prospectively studied 530 predominantly white European participants with HbA1c >58mmol/mol (7.5%) commencing GLP-1A or DPPIVI therapy as part of their usual diabetes care. We assessed markers of insulin secretion (fasting C-peptide, post largest home meal urine c-peptide:creatinine ratio (UCPCR), HOMA2B, duration of diabetes) and insulin resistance (HOMA2IR, BMI, triglycerides) immediately prior to commencing therapy. We measured HbA1c at baseline and at 3 and 6 months on therapy: response was defined by 6 month HbA1c unless treatment was changed or stopped after 3 months. Participants who completed <3 months treatment, increased other treatment or stopped >1 OHA were excluded from analysis (number analysed 280 GLP-1A, 156 DPPIVI). We assessed the relationship between baseline markers and both absolute HbA1c change and achieving an HbA1c <7.5% (58mmol/mol) using linear and logistic regression respectively with adjustment for the following covariates: baseline HbA1c, discontinuation of OHA, number of baseline OHAs, insulin co-treatment and change in insulin dose (%). We assessed potential utility of predictors identified using Receiver Operating Characteristic (ROC) curve analysis.
Results: Participant characteristics were: GLP-1A - 51% male, 41% insulin treated, median (IQR) age 55 (48-62), diabetes duration 9 (5-13) years, BMI 38 (35-43)kg/m^2 baseline HbA1c 84 (72-96)mmol/mol, HbA1c change -14(-3 to -26)mmol/mol, DPPIVI - 60% male, 7% insulin treated, age 63 (56-70), diabetes duration 8 (5-13) years, BMI 32 (29-37)kg/m^2, HbA1c 75 (67-84)mmol/mol, HbA1c change -8.9 (-1 to -16)mmol/mol. In univariate analysis (Spearman's correlation) UCPCR and fasting C-peptide were weakly associate with GLP-1A response (absolute HbA1c fall over 6 months) with better response seen in those with higher insulin secretion: UCPCR r=0.13, p=0.04, fasting C-peptide r=0.18, p=0.04. No markers of insulin secretion or resistance were associated with DPPIV response. After adjusting for covariates no marker of insulin secretion or resistance was associated with absolute HbA1c fall for either therapy (p>0.12 for all). Both low fasting C-peptide and low HOMA2IR were associated with achieving an HbA1c <58mmol/mol (7.5%) for DPPIV inhibitors (C-peptide p=0.03, HOMA2IR p=0.02) but not for GLP-1 agonists (p=0.35 and 0.49 respectively), other markers were not associated with this outcome (p for all >0.11). In ROC analysis fasting C-peptide and HOMA2IR had low utility in predicting DPPIVI response (achieved HbA1c<58mmol/mol): C-peptide AUC ROC 0.63 (CI 0.52-0.78), HOMA2IR 0.68 (CI 0.55-0.81), no better than fasting glucose alone (AUC ROC 0.67 (0.57-0.77)).
Conclusion: Markers of insulin resistance and beta cell failure are not clinically useful predictors of response to incretin based treatments in Type 2 diabetes.
Supported by: NHS NIHR Fellowship Programme

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"Real-life" experience in use of dipeptidyl peptidase 4 inhibitors at a large Scottish teaching hospital

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Background and aims: Dipeptidyl Peptidase 4 Inhibitors (DPP4I) are a relatively new class of hypoglycaemic agent shown in clinical trials to improve HbA_{1c} whilst being weight neutral and well tolerated. There is little information however on real-life use and efficacy of these drugs. This study aimed to assess effect of DPP4I on glycaemic control and weight in a real-life clinical setting and review adherence to renal dosing guidance.
Materials and methods: Retrospective observational review of patients with type 2 diabetes attending the diabetes centre at Gartnavel General Hospital, Glasgow. Patients prescribed a DPP4I were identified using the Scottish Care Information - Diabetes Collaborative (SCI-DC) database. Electronic medical records were reviewed for initiation of DPP4I, concomitant hypoglycaemic drugs and the specific DPP4I and dose selected. HbA_{1c} (mmolmol^{-}1), weight (kg) and renal function as eGFR (ml/min/1.73m^2) were recorded at initiation and at the first follow-up appointment at least 3 months post initiation.
Results: 263 patients were identified as being initiated on DPP4I therapy between March 2008 and June 2012. Mean time between initiation and follow-up HbA_{1c} measurement was 207days (SD 81.5). Sitagliptin 100mg daily was the most commonly prescribed DPP4I (90.5%). In patients where DPP4I initiation was the only pharmacological intervention at initiation (n=195), mean change in HbA_{1c} at follow-up was -8.8mmolmol^{-1} (95%CI -10.8, -6.7; p-value 0.000). Largest mean change in HbA_{1c} was observed in patients with HbA_{1c} at initiation of 90-99molmol^{-1}, 100-109mmolmol^{-1} and 80-89mmolmol^{-1}: -17.2mmolmol^{1}(95%CI -26.2, -8.1; p-value 0.002). -13.6mmolmol^{-1}(95%CI -24.2, -2.98; p-value 0.016) and -9.6mmolmol^{-1}(95%CI -17.3, -1.99; p-value 0.0015) respectively. This is compared with initial HbA_{1c} 60-69mmolmol^{-1} and 70-79mmolmol^{-1}; -7.9mmolmol^{1}(95%CI -10-2, -5.5; p-value 0.000), -6.8mmolmol^{1}(95%CI -10.5, -3.1; p-value 0.000). Comparison of concomitant therapy showed significant changes in mean HbA_{1c} in DPP4I regimes including; metformin (-11.6mmolmol^{-1}, 95%CI -15.3, -7.8, p-value 0.000); metformin and sulphonylurea (SU) (-6.56mmolmol^{-1}, 95%CI -10.0, -3.0, p-value 0.000); metformin and thiazolidinedione (TZD) (-7.0mmolmol^{-1}, 95%CI -13.7, -0.26, p-value 0.043); metformin and insulin (9.2mmolmol^{-1}, 95%CI -15.1, -3.4, p-value 0.003); and metformin, SU and TZD(-14.5mmolmol^{-1}, 95%CI -20.6, -8.4, p-value 0.000). A reduction in weight was observed at follow-up; mean change: -0.79kg (95% CI -1.3, -0.3, p-value 0.001). Comparison of concomitant therapy showed significant weight changes in patients taking DPP4I with metformin and SU (mean change -1.6kg; 95%CI -2.3, -0.9; p-value 0.000), and metformin and insulin (mean change +1.3kg, 95% CI 0.02, 2.5, p-value 0.047). 87.7% of patients were initiated on a dose of DPP4I appropriate for degree of renal function. Examination of subdivisions of renal impairment found that 75% patients with moderate and 50% of patients with severe renal impairment were not prescribed doses in accordance with guidance.
Conclusion: DPP4I are effective in reducing HbA_{1c} in a "real-life" setting. Patients with higher initial HbA_{1c} values or on regimes including metformin exhibited larger mean reductions in HbA_{1c}. A small reduction in weight was observed at follow-up after at least three months. Dose adjustment of DPP4I in renal impairment was infrequent suggesting a lack of awareness.

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INdividualised Treatment targets for ElderRly patients using Vildagliptin Add-on or Lone therapy (INTERVAL) study

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Background and aims: Management of type 2 diabetes mellitus (T2DM) in elderly patients is challenging and complicated by multiple comorbidities, polypharmacy, high prevalence of renal impairment and increased risk of hypoglycaemia. Further, the benefit of aggressive glycaemic control has been questioned recently in the advent of outcome trials such as ACCORD, in which weight gain and hypoglycaemia were thought to contribute to the 25% increase in mortality. Guidelines are increasingly suggesting individualising treatment targets for vulnerable elderly adults, despite no evidence-base for such an approach. We evaluated the feasibility of such an approach. In order to achieve individualised targets, we used the DPP-4 inhibitor vildagliptin vs. placebo, in view of its weight neutrality and low risk of hypoglycaemia.
Materials and methods: We enrolled 278 drug-naïve or inadequately controlled (HbA1c ≥7.0% to ≤10.0%) T2DM patients aged ≥70 years in a multinational, double-blind, placebo-controlled 24-week study. Investigators were asked to set unique, individualised treatment targets for participants based on age, baseline HbA1c, comorbidities, polypharmacy, frailty status and local recommendations for glycaemic targets before 1:1 randomisation to vildagliptin (50 mg qd or bid as per label) or placebo.
Results: Mean age was 74.4 ± 4.0 and 75.1 ± 4.3 years with a maximum age of 89 and 97 years for the placebo and vildagliptin arms respectively; 76.6% of patients had mild or moderate renal failure and 9.4% were frail according to

the modified Fried definition. Baseline HbA1c was 7.9% for both groups. On average the target HbA1c reduction was set at 0·9% (range -4.4% to -0.1%). Simple advice and nursing intervention enabled 27% of elderly patients to achieve their target. After adjustment for baseline variables, patients treated with vildagliptin had a higher likelihood of achieving their individualised treatment targets (OR=3.16), accompanied by clinically relevant reductions in overall, conventional HbA1c assessment (Table). Change in FPG from baseline to endpoint was -1.34 and -0.47 mmol/L in the vildagliptin and placebo groups, respectively, and the between-group difference was statistically significant (p<0.001). Vildagliptin was well tolerated in this elderly cohort with low incidence of hypoglycaemia and no new safety signals emerging.
Conclusion: INTERVAL is the first study to introduce investigator-defined, individualised HbA1c targets as an endpoint in any T2DM population. It demonstrates the feasibility of evaluating individualised targets when exploring diabetes management in the growing elderly population. It also confirmed that individualised glycaemic target levels are achievable using vildagliptin without any tolerability issues.

Investigator-defined individualised HbA1c target (Full analysis set)

Treatment	N	Number of responders	Proportion of responders (%)	Treatment difference (Vildagliptin - Placebo)		
				Adjusted Odds ratio	96.2% CI	p-value
Vildagliptin	137	72	52.6	3.16	1.81, 5.52	<0.001*
Placebo	137	37	27.0			

HbA1c reduction (Full analysis set)

Treatment	N	Baseline mean (SE)	Adjusted Mean change (SE)	Mean (SE)	98.8% CI	p-value
Vildagliptin	137	7.9 (0.06)	-0.9 (0.12)	-0.6 (0.10)	-0.81, -0.33	<0.001**
Placebo	137	7.9 (0.06)	-0.3 (0.12)			

*statistical significance at one-sided 1.9% level; **statistical significance at one-sided 0.6% level.

Clinical Trial Registration Number: NCT01257451
Supported by: Novartis Pharma AG

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Efficacy and safety of linagliptin as add-on therapy to basal insulin and metformin in patients with type 2 diabetes
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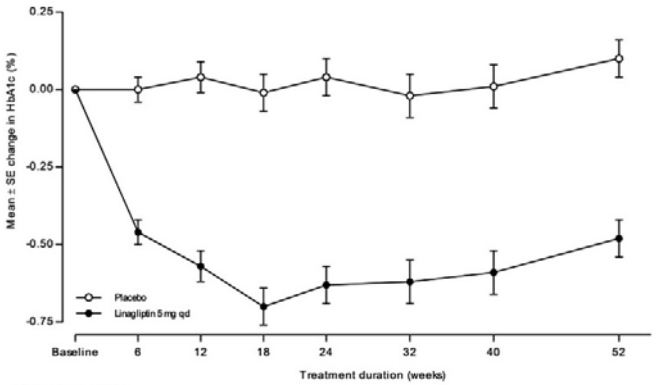
Background and aims: The combination of basal insulin and metformin (MET) is a common second-line treatment option for patients with type 2 diabetes mellitus (T2DM), potentially providing superior glycaemic control to either agent alone. Adding a dipeptidyl peptidase (DPP)-4 inhibitor to this combination may further improve glycaemic control without increasing risk of hypoglycaemia and weight gain. A Phase III study evaluated the efficacy and long-term safety of the DPP-4 inhibitor linagliptin (LINA), added to basal insulin alone or in combination with MET and/or pioglitazone. We performed a sub-analysis to evaluate the efficacy and safety of LINA in combination with insulin and MET which was the study's most common treatment.
Materials and methods: This sub-analysis of a global, placebo (PBO)-controlled, Phase III trial evaluated LINA 5 mg once daily in patients on background insulin and MET. Patients were randomised (1:1) to double-blind treatment with LINA or PBO for ≥52 weeks. The basal insulin dose was kept stable for 24 weeks but could be adjusted after this period. The primary endpoint was the change in HbA1c from baseline at Week 24.
Results: There were a total of 950 patients in this sub-analysis (LINA and PBO, n=475). Mean ± SD baseline characteristics were similar in the LINA vs PBO groups: age, 59.3 ± 9.9 vs 59.9 ± 9.8 yrs; BMI, 30.9 ± 5.4 vs 31.1 ± 4.9 kg/m^2; HbA1c, 8.3 ± 0.9% vs 8.3 ± 0.8%. At Week 24, adjusted mean ± SE changes from baseline in HbA1c were -0.63 ± 0.06% with LINA and +0.04 ± 0.06% with PBO (treatment difference -0.67 ± 0.06% [95% CI -0.78, -0.56; *P*<0.0001]). After 52 weeks, the adjusted mean ± SE change in HbA1c from baseline was -0.49 ± 0.06% with LINA and +0.10 ±0.06% with PBO (Figure). Fasting plasma glucose decreased only with LINA at Week 24 (adjusted mean ± SE: -0.5 ± 0.2 mmol/L vs +0.2 ± 0.2 mmol/L) and Week 52 (mean ± SE: -0.3 mmol/L vs +0.1 mmol/L). Although up-titration of basal insulin was allowed after 24 weeks, background insulin dose was only adjusted by +1.5 ± 0.7 IU/day with LINA vs +3.3 ± 0.7 IU/day with PBO (treatment difference -1.75 ± 0.6 [95% CI -2.95, -0.55; *P*<0.005]) at Week 52. The overall frequency of patients with ≥1 adverse event (LINA, 79.8%; PBO, 81.3%) and investigator-defined hypoglycaemia (LINA, 30.7%; PBO, 31.6%) appeared to be similar in both groups. The percentage of patients with severe hypoglycaemia was low (LINA, 1.7%; PBO, 0.8%). There were no relevant changes in mean ± SD body weight from baseline to Week 52 (LINA, -0.5 ± 3.2 kg; PBO, 0.0 ± 3.1 kg).
Conclusion: The addition of LINA to patients with T2DM inadequately controlled on basal insulin and MET significantly improved glycaemic control, with no additional risk of hypoglycaemia or weight gain.

Figure
Adjusted mean change in HbA1c over time



Data are mean ± SE
P<0.0001 for the placebo-corrected change in HbA_{1c} at all time points
FAS; LOCF analysis

Clinical Trial Registration Number: 00954447
Supported by: Boehringer Ingelheim

893

Predictors of attaining a composite endpoint with exenatide once weekly (EQW): recursive partitioning analysis
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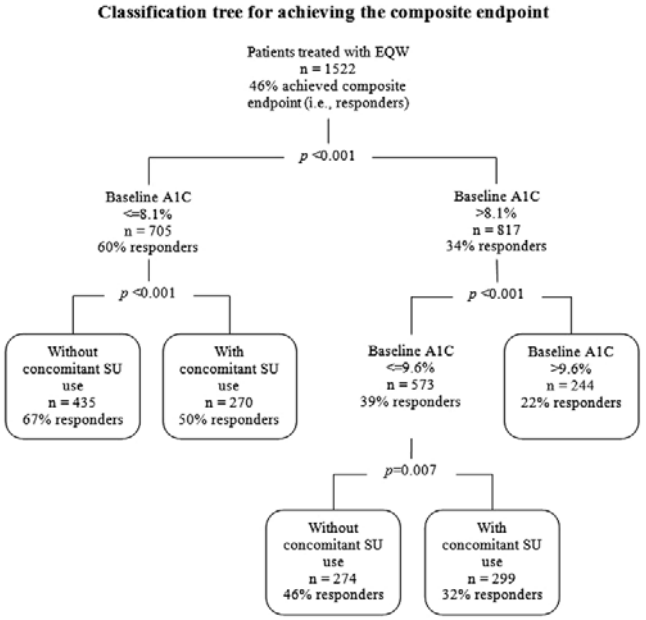
Background and aims: Current treatment guidelines (ADA/EASD) advocate a patient-centered approach in the medical management of hyperglycemia. In addition to setting glycemic targets, individualized factors and goals should be considered in the choice of therapy, including minimizing risk of hypoglycemia and weight gain. Prediction of responders to specific therapies based on patient characteristics may further advance such a patient-centered approach. A recursive partitioning analysis was performed to identify patient characteristics that are potential predictors of response to EQW treatment, defined as attainment of the composite endpoint of HbA_{1C} <7%, without weight gain or hypoglycemia, following 24-30 weeks of treatment.
Materials and methods: Data from T2DM patients who received EQW for 24-30 weeks in 8 randomized, comparator-controlled clinical trials were pooled to identify predictors of attaining the composite endpoint using the recursive partitioning (classification tree) method. Potential predictors evaluated were age, gender, BMI, weight, ethnicity, HbA_{1C}, fasting glucose, concomitant antihyperglycemic use, diabetes duration, and renal function (creatinine clearance). Logistic regression with stepwise selection to find the best model to describe the current data, as opposed to predict future data, was explored.
Results: Among the 1522 patients (mean baseline age 55 yrs, HbA_{1C} 8.5%, weight 88 kg, BMI 31.4 kg/m^2, 45% with concomitant SU), 46% achieved the composite endpoint and the majority of patients attained at least one endpoint (HbA_{1C} <7%, 59%; no weight gain, 78%; no hypoglycemia, 93%). Baseline HbA_{1C} was the most important predictor of attaining the composite endpoint, with a 60% probability if baseline HbA_{1C} ≤8.1%, vs 34% if baseline HbA_{1C} >8.1% (*P*<0.001). The probability increased to 67% among patients who were not on SU if baseline HbA_{1C} ≤8.1% (*P*<0.001), vs 46% if baseline HbA_{1C} was >8.1% and ≤9.6% (*P*=0.007). For patients on SU, probabilities of attaining the composite endpoint were 50% and 32%, respectively. Among all patients, baseline HbA_{1C} >9.6% (*P*<0.001) conferred the least likelihood (22%) of achieving the composite endpoint, regardless of background concomitant antihyperglycemic use. No other significant predictors were identified for achieving the composite endpoint. Similar results were found in the logistic regression analysis.
Conclusion: A majority of patients reached glycemic target of HbA_{1C} <7% with the addition of EQW treatment, and 46% achieved a composite goal

that also included no weight gain or hypoglycemia. Among the 10 predictors examined, baseline HbA_{1C} and concomitant antihyperglycemic use were the most important predictors for attainment of the composite endpoint with EQW following 24-30 weeks of treatment. Results from such analysis may assist clinicians in patient-centered treatment approaches to hyperglycemia management in T2DM.



Clinical Trial Registration Number: NCT00308139 NCT00877890 NCT00637273 NCT01003184 NCT00641056 NCT00676338 NCT00917267 NCT01029886

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Diabetes duration and background diabetes therapies in predicting liraglutide treatment response: data from the post-marketing EVIDENCE study

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Background and aims: We used data from a post-marketing study in France to determine whether liraglutide treatment is more effective in lowering HbA_{1c} when used early in the course of type 2 diabetes.
Materials and methods: EVIDENCE is a multicentre, observational, post-marketing outpatient study requested by the French National Health Authority to evaluate the efficacy and safety of the human GLP-1 analogue liraglutide. Its primary objective is to determine the percentage of patients still taking liraglutide and at HbA_{1c} target (<7%) after 2 years. This analysis examined HbA_{1c} reductions 1 year after initiating liraglutide treatment, stratified according to diabetes duration (0-5 years; 6-10 years; >10 years) or extent of background diabetes therapy (1, 2, ≥3 oral anti-diabetes drugs or insulin [±OAD]). Statistical analyses: for quantitative variables a normality Kolmogorov-Smirnov test was used. For comparison of 2 independent groups the Mann-Whitney Wilcoxon test was used, and for >2 groups the Kruskal-Wallis test was used for quantitative variables. Comparisons of independent groups were performed by the Chi-square test with qualitative variables. The Wilcoxon signed rank test was used for paired quantitative variables, and the McNemar test for paired qualitative variables.
Results: The study included 3137 patients, 2433 (77.6%) of whom remained in the study at 1 year. Of these, diabetes duration was available for 2297 (94.4%) (duration 0-5 years, n=689; 6-10 years, n=800; >10 years, n=808) and background therapy was available for 2161 (88.8%) (1 OAD, n=413; 2 OADs, n=791; ≥3 OADs, n=640; insulin (±OAD), n=317). Mean±SD HbA_{1c} reductions for the 3 diabetes duration groups were: -1.22±1.73%, -1.01±1.39% and -0.76±1.33% (all p<0.0001) from baseline HbA_{1c} values of 8.53, 8.58 and 8.53%, respectively. Subjects with diabetes duration 0-5 years and 6-10 years achieved greater HbA_{1c} reductions than those with duration >10 years [between-treatment differences: -0.46% (p<0.001) and -0.25% (p=0.002), respectively]. HbA_{1c} reductions in the background treatment groups (1, 2, ≥3 OADs and insulin [±OAD]) were: -1.40±1.52%, -1.14±1.50%, -0.74±1.38% and -0.73±1.53% (all p<0.0001) from baseline HbA_{1c} values of 8.50, 8.55, 8.60 and 8.62%, respectively. Subjects taking 1 or 2 OADs achieved greater HbA_{1c} reductions than those treated with ≥3 OADs (-0.66% and -0.40%, respectively; both p<0.001) or insulin (±OAD) (-0.67% and -0.41%, respectively; both p<0.001). Increasing diabetes duration correlated with increasing number of background therapies; 6.3, 9.2, 10.9 and 12.5 years for 1, 2, ≥3 OADs and insulin (±OAD), respectively. Withdrawals were similar across diabetes duration groups (22-24%), but greater among patients receiving ≥3 OADs (25%) and insulin (±OADs) (30%) compared with patients on 1 or 2 OADs (19-21%). The most common reasons for withdrawal were gastrointestinal disorders experienced early in the study.
Conclusion: In this observational EVIDENCE study, treatment response to liraglutide was greater in patients with shorter duration of diabetes and fewer background treatments.
Clinical Trial Registration Number: NCT01226966
Supported by: Novo Nordisk

895

The effect of liraglutide on HbA_{1c} and body weight is largely independent of baseline diabetes duration

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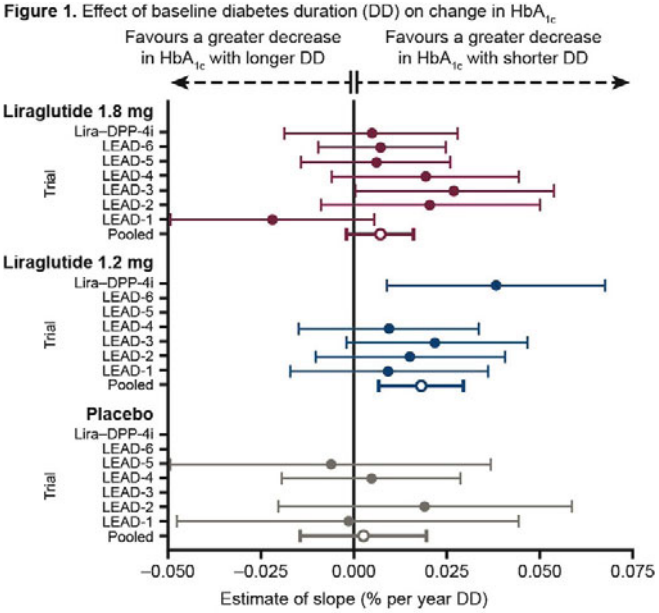
Background and aims: The LEAD phase 3 clinical programme demonstrated liraglutide's effectiveness across the continuum of type 2 diabetes (T2D); however, the effect of baseline diabetes duration is unclear. Using 26/28-week data from seven phase 3 trials, this post-hoc, pooled analysis evaluated the effect of diabetes duration on changes in HbA_{1c} and body weight from baseline with liraglutide 1.8 mg (n=1581), liraglutide 1.2 mg (n=1117) and placebo (n=524).
Materials and methods: The slope for change in HbA_{1c} or body weight vs. diabetes duration was determined by linear regression. The statistical model included the baseline value of the endpoint, diabetes duration, and age as continuous covariates. Previous treatment and country were categorical covariates.

Figure 1. Effect of baseline diabetes duration (DD) on change in HbA_{1c}



Data are modelled estimates with 95% confidence intervals, from ITT population, LOCF. The model included baseline endpoint value (HbA_{1c}/body weight; BW), diabetes duration and age as continuous covariates, previous treatment, and country as categorical covariates

Results: Across treatment arms, diabetes duration ranged from <1 to >40 years, with a mean of ~8 years for pooled groups. There was a trend towards

greater HbA_{1c} reduction with shorter diabetes duration with liraglutide but this was not clinically relevant (Figure 1); statistical significance was achieved for pooled liraglutide 1.2 mg group ($p<0.05$) but the difference only equated to -0.2% HbA_{1c}/10-year shorter diabetes duration. The effect of liraglutide on body weight was independent of diabetes duration.
Conclusion: The proven effectiveness of liraglutide to reduce HbA_{1c} and body weight is largely independent of diabetes duration. Therefore, liraglutide can be successfully used across the continuum of T2D.
Supported by: Novo Nordisk

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Efficacy of lixisenatide in patients with different levels of beta cell function as assessed by C-peptide/glucose ratio

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Background and aims: Over time, beta-cell mass declines progressively in type 2 diabetes and this decline is closely related to loss of glucose control. Previous studies have suggested that a fasting C-peptide/glucose ratio can predict pancreatic beta-cell mass in humans. Lixisenatide is a once-daily prandial GLP-1 receptor agonist that has been recently approved in Europe for the treatment of type 2 diabetes. Because lixisenatide is believed to act predominantly via delay of gastric emptying, we speculated that its glucose-lowering effect should be maintained even in patients with low beta-cell function. Therefore, the glucose-lowering efficacy was analysed in relation to beta-cell function at baseline.
Materials and methods: Data were extracted from two 24-week, randomized, double-blind, placebo-controlled trials from the lixisenatide Phase III clinical development programme: GetGoal-S (lixisenatide in patients insufficiently controlled on sulphonylurea ± metformin [MET]) and GetGoal-M (lixisenatide in patients insufficiently controlled on MET). Patients were grouped into four quartiles according to their baseline pre-prandial C-peptide/glucose ratios as a measure of beta-cell function (Table).
Results: C-peptide/glucose ratios were closely correlated with diabetes duration and degree of glucose control. HbA_{1c} and fasting blood glucose were reduced in all quartiles, with no correlation with C-peptide/glucose ratios. In contrast, reduction in postprandial glucose (PPG) was negatively correlated with C-peptide/glucose ratio, with a significant difference between quartile groups (p=0.0001). The greater PPG reduction with lixisenatide in patients with the lowest baseline beta-cell function suggests that most of the effect is probably driven by delaying gastric emptying. The relationship between the C-peptide/glucose ratios and PPG was similarly observed whether patients were receiving sulphonylureas or not. The incidence of symptomatic hypoglycaemia was low in all quartiles with no significant difference between groups.
Conclusion: Lixisenatide improves glycaemic control regardless of the baseline beta-cell function, presumably because of its effect on delaying gastric emptying. These data suggest that lixisenatide treatment is effective in reducing postprandial hyperglycaemia even in the more advanced stages of type 2 diabetes.

Baseline characteristics and endpoints

	Quartile 1 n=105	Quartile 2 n=115	Quartile 3 n=107	Quartile 4 n=110	p-value
Baseline clinical characteristics					
C-peptide/glucose ratio (nmol/mg), mean (SD)	0.0026 (0.0007)	0.0042 (0.0004)	0.0056 (0.0004)	0.0088 (0.0022)	p<0.0001
Diabetes duration, years (SD)	10.3 (6.3)	8.1 (5.7)	7.2 (5.8)	6.9 (5.7)	p=0.0001
Baseline HbA_{1c} (%), mean (SD)	8.4 (0.8)	8.3 (0.9)	8.1 (0.9)	7.9 (0.9)	p<0.0001
Change from baseline to endpoint					
C-peptide/glucose ratio (nmol/mg), mean (SD)	0.0014 (0.0016)	0.0016 (0.0021)	0.0017 (0.0032)	0.0006 (0.0044)	p=0.0205
HbA_{1c} (%), mean (SD)	-0.9 (0.9)	-1.0 (0.9)	-0.7 (1.0)	-0.9 (0.9)	p=0.0766
FBG (mg/dL), mean (SD)	-15.3 (40.0)	-15.8 (40.0)	-14.6 (41.7)	-20.0 (35.1)	p=0.7392
PPG (mg/dL), mean (SD)*	-135.3 (104.9)	-111.4 (92.4)	-85.0 (95.6)	-86.7 (77.2)	p=0.0001
Symptomatic hypoglycaemia (<60 mg/mL), %	13.3	10.4	12.2	3.6	p=0.0750

SD=standard deviation; HbA_{1c}=glycated haemoglobin; FBG=fasting blood glucose; PPG=postprandial glucose after standardized meal test; *n=104, 114, 105, 110 for Quartiles 1, 2, 3, 4, respectively

Clinical Trial Registration Number: NCT00712673; NCT00713830
Supported by: Sanofi

897

Effectiveness of prandial insulin and glucagon-like peptide-1 treatment regimens in patients with type 2 diabetes on basal insulin in a real-world setting in the United States

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Background and aims: Data from clinical trials suggest that glucagon-like peptide-1 (GLP-1) receptor agonists are attractive treatment options for certain populations of patients with type 2 diabetes mellitus (T2DM), either alone or in combination with other agents. However, availability of real-world data is limited. This study aims to document real-world demographic characteristics and outcomes of patients with T2DM, treated with basal inulin, who initiate add-on therapy with a GLP-1 analog or prandial insulin.
Materials and methods: Using electronic medical records (EMR), this retrospective study was conducted in T2DM patients aged ≥18 years old who initiated prandial insulin or a GLP-1 analog between January 2006 and January 2011, while on basal insulin therapy. Patients were required to have had EMR activity of ≥6 months prior to and ≥12 months after their prandial insulin or GLP-1 analog initiation date. Standardized differences were used to compare baseline characteristics and control for observed differences in baseline characteristics (including age, HbA_{1c}, and body mass index). Primary endpoints included change in HbA_{1c} and body weight at 6 and 12 months follow-up. Rates of hypoglycemia and adverse events, and utilization of health care resources were measured over 12 months' follow-up.
Results: After variable matching, the study group comprised 1,143 GLP-1 analog users and 5,557 prandial insulin users with the following mean baseline characteristics: age 56.0 and 56.6 years; HbA_{1c} of 8.5% and 8.6%; and body weight of 111.1 kg and 108.9 kg, respectively. Outcomes of the matched analysis are presented in the Table. At 6 and 12 months post-treatment initiation, reduction in HbA_{1c} levels was similar for the prandial insulin and GLP-1 analog users. GLP-1 analog users had a significantly greater reduction in body weight at both 6 and 12 months post-initiation. Throughout follow-up, significantly lower rates of hypoglycemia and utilization of health care resources were observed for patients adding GLP-1 analog therapy. The tolerability of both add-on therapies was similar (Table). Proportions achieving different composite endpoints favored the GLP-1 arm over the prandial insulin arm: HbA_{1c} goal <7.0% at 6 months was achieved by 28.8% vs 27.1% (34.9 vs 30.9% at 12 months), respectively. Also, 26.2% vs 22.6% (30.5% vs 25.2%) achieved this glycemic target without hypoglycemia, and 20.7% vs 13.7% (28.3% vs 20.3%) of patients did so without weight gain.
Conclusion: This study showed that, among real-world T2DM patients, the addition of a GLP-1 analog to basal insulin therapy was associated with similar glycemic control, but significantly greater reduction in body weight, lower incidence of hypoglycemia, and lower health care resource utilization compared with prandial insulin.

Primary efficacy endpoints	Prandial insulin (n=5,577)	GLP-1 analog (n=1,143)
HbA_{1c}, %		
Change from baseline at 6 months, mean (SD)	−0.5 (1.8)	−0.4 (1.5)
Change from baseline at 1 year, mean (SD)	−0.6 (1.9)	−0.6 (1.8)
Body weight, kg		
Change from baseline at 6 months, mean (SD)*	1.7 (7.7)	−0.9 (6.4)
Change from baseline at 1 year, mean (SD)*	−1.7 (13.2)	−3.7 (9.9)
Adverse events of interest for patients with ≥1 event during follow-up		
Hypoglycemia, n (%)*	68 (1.2)	6 (0.5)
Any gastrointestinal-related event, n (%)	1,278 (22.9)	274 (24)
Health care resource utilization for patients with ≥1 event during follow-up		
ED visits, mean (SD)*	0.9 (3.3)	0.6 (1.6)
Hospitalizations, mean (SD)*	1.7 (4.2)	1.1 (2.7)
Specialist referrals, mean (SD)*	4.2 (8.8)	3.6 (8.7)

ED: emergency department; SD: standard deviation.
*P <0.05 between groups.

Supported by: Sanofi US, Inc.

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Adding rapid-acting insulin or GLP-1 receptor agonist to basal insulin in patients with type 2 diabetes: real-world health care costs from a US managed care setting

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Background and aims: In patients with type 2 diabetes mellitus (T2DM) where basal insulin alone does not provide glycemic control, additional injections of rapid-acting insulin (RAI) or glucagon-like peptide-1 receptor agonists (GLP-1) may be an effective therapeutic approach. Few studies have evaluated the addition of GLP-1 or RAI to basal insulin. This study evaluated the real-world economic outcomes of basal insulin plus RAI (Basal+RAI) vs basal insulin plus GLP-1 (Basal+GLP1) among US patients with T2DM.
Materials and methods: This was a retrospective cohort study using data from a US managed care claims database (IMPACT) conducted in adult patients with T2DM on basal insulin (insulin glargine, insulin detemir, NPH) who added GLP-1 (exenatide, liraglutide) or RAI (insulin glulisine/aspart/lispro) between 1/07/07 and 31/12/11. Study index date was the first RAI/GLP-1 prescription. Included patients were prescribed basal insulin in the quarter before and the quarter after index date, and had continuous health plan coverage ≥6 months before (baseline) and 1 year after index date (follow-up). All baseline characteristics, clinical and economic outcomes were summarized and compared, with P values provided by *t* tests for continuous variables or X^2 tests for categorical variables. Propensity score matching (1 up to 3 ratio) was used to control for baseline demographics, clinical characteristics (including comorbidity score) and health resource utilization differences. Endpoints included 1 year follow-up, health care utilization and costs.
Results: Among the 11,338 patients identified, at baseline Basal+RAI patients were more often >65 years, had more comorbidities (except for hypertension, hyperlipidemia and obesity), higher hypoglycemia rates, and higher rates of hospitalizations and emergency room visits. For the outcomes analysis 6,718 matched patients were included: 5,013 Basal+RAI (7% insulin glulisine, 49% insulin aspart, 44% insulin lispro) and 1,705 Basal+GLP1 (82% exenatide, 18% liraglutide), with similar baseline characteristics (47% female; 54 years; 79% insulin glargine, 16% insulin detemir, 5% NPH). During follow-up, patients in both groups experienced a similar proportion of any type of hypoglycemic event (*P*=0.4079), but events leading to hospitalization were significantly higher for Basal+RAI (2.7% vs 1.8%, *P*=0.0444). The table shows health care utilization and costs in the follow-up period. Basal+RAI patients had a higher proportion of patients with a hospitalization and higher total health care costs, but lower pharmacy costs, than Basal+GLP1 patients.
Conclusion: This study demonstrates that in a real-world setting, the addition of RAI to basal insulin in T2DM patients is associated with a higher proportion of hospitalizations and higher total costs when compared to those patients who added GLP-1.

	Basal+RAI (n=5,013)	Basal+GLP1 (n=1,705)	*P* value
All cause annual utilizations, n (%)			
Any hospitalization	933 (18.6)	231 (13.6)	<0.0001
Any ER visit	1,594 (31.8)	512 (30.0)	0.1740
Any office visit	4,988 (99.5)	1,701 (99.8)	0.1508
Any endocrinologist visit	1,949 (38.9)	788 (46.2)	<0.0001
Mean all cause annual costs, $ (SD)			
Total costs	20,821 (31,794)	18,413 (19,649)	0.0002
Inpatient costs	5,474 (20,899)	3,423 (13,426)	<0.0001
Outpatient costs	7,404 (16,173)	5,722 (9,532)	<0.0001
Diabetes supply costs	667 (637)	502 (467)	<0.0001
Pharmacy costs	7,336 (5,957)	8,747 (5,628)	<0.0001

Table: Follow-up health care utilizations and costs.
ER, emergency room; SD, standard deviation

Supported by: Sanofi US, Inc.

PS 070 Incretin-based drugs: treatment strategies

899

Efficacy of the human GLP-1 analogue liraglutide in patients with type 2 diabetes who switched from a DPP-4 inhibitor: 1-year data from the EVIDENCE study

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Background and aims: Baseline and 1-year data from a post-marketing study in France were compared to assess the change in glycaemic control and body weight after 1 year's treatment with the human GLP-1 analogue liraglutide in patients who previously received a DPP-4 inhibitor (DPP-4i).
Materials and methods: EVIDENCE is a multicentre, observational, post-marketing outpatient study requested by the French National Health Authority to evaluate the efficacy and safety of liraglutide. Its primary objective is to determine the percentage of patients still taking liraglutide and at HbA_{1c} target (<7%) after 2 years. Statistical analyses: for quantitative variables a normality Kolmogorov-Smirnov test was used; comparisons of two independent groups were performed using the Mann-Whitney Wilcoxon test for quantitative variables and chi-square test for qualitative variables; for paired quantitative variables the Wilcoxon signed rank test was used; for paired qualitative variables the McNemar test was used.
Results: Data were collected from 3137 subjects of whom 1255 (40%) were receiving a DPP-4i prior to liraglutide initiation. A total of 1004 (32%) subjects switched from a DPP-4i to liraglutide at study start, while 251 (8%) added liraglutide to a DPP-4i. The table shows baseline characteristics of subjects who switched. Subjects who switched and continued liraglutide treatment for 1 year (n=734) achieved significant reductions from baseline in mean HbA_{1c} (-0.84%, p<0.0001), fasting plasma glucose (-0.27 g/L, p<0.0001) and body weight (-3.53 kg, p<0.0001). A greater percentage of subjects had HbA_{1c} <7% at 1 year (31.6%) vs. baseline (8.0%; p<0.0001). Withdrawals (30% in overall study cohort) were mostly due to gastrointestinal disorders experienced early in the study.

Baseline characteristics	
N	1004
Age, years	58 ± 10
Diabetes duration, years	9.8 ± 6.3
HbA_{1c}, %	8.5 ± 1.4
Proportion of patients with HbA_{1c} <7%	8%
Fasting plasma glucose, g/L	1.8 ± 0.6
Body weight, kg	95 ± 18
BMI, kg/m²	34 ± 6
Data are mean±SD	

Conclusion: In this observational study, significant improvements in glycaemic control and body weight from baseline were seen after 1 year of liraglutide treatment in a subgroup of patients who switched from a DPP-4i at study start. A limitation of this study is the lack of control arm, making it difficult to evaluate whether observed improvements are attributable to liraglutide alone. It was previously shown by Pratley *et al.* in 2012 that switching from the DPP-4i sitagliptin to liraglutide in a randomised controlled trial setting provided significant HbA_{1c} (-0.5%; p<0.0001) and body weight reductions

(-2.5 kg; $p<0.0001$). The cohort enrolled in EVIDENCE is more representative of patients routinely treated in the clinic; therefore results reported here may provide a better indication of the outcomes of liraglutide treatment in patients who switch from a DPP-4i in daily clinical practice.
Clinical Trial Registration Number: NCT01226966
Supported by: Novo Nordisk

900

A randomised trial comparing the addition of liraglutide to high dose intensive insulin therapy vs insulin up-titration in type 2 diabetes
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Background and aims: Patients with type 2 diabetes may benefit from GLP-1 RA therapy in combination with basal insulin, which results in improved glycemic control, weight loss and lower insulin doses than addition of basal insulin alone . However, GLP-1 RA's have not been studied in combination with intensive insulin therapy (prandial plus basal insulin). This study compares the effects of the addition of the GLP-1 RA liraglutide to high dose basal/bolus insulin therapy vs. insulin up-titration alone in patients with type 2 diabetes requiring > 100 units of insulin per day.
Materials and methods: 30 subjects with type 2 diabetes and HbA1c > 6.5% using intensive insulin therapy of >100 units of insulin per day, either by MDI or CSII, with or without metformin, were randomized in a 1:1 fashion to receive either liraglutide with insulin (LIRA) or standard insulin up-titration (control). Liraglutide was initiated at 0.6 mg daily and increased to 1.2 or 1.8 mg. Subjects in each group were evaluated with HbA1c, weight, and 72 hour CGM (Medtronic iPro) at 0 and 6 months. The differences between groups in HbA1c, weight, total daily insulin dose (TDID), time spent in hypo-, hyper- and euglycemia by CGM, and glycemic variability as determined by average daily standard deviation (SD) of glucose by CGM were compared by ANOVA using XLSTAT2012 for Windows.
Results: Both treatment groups experienced a significant decrease in HbA1c at 6 months, from a mean baseline of 7.76% to 7.14% in the LIRA group ($p < 0.0001$) and from 7.88% to 7.38% in the control group ($p < 0.0001$). There was no significant difference in HbA1c between groups at 6 months. Subjects in the LIRA group experienced a weight loss of 12.25 lbs (5.57 kg) at 6 months ($p <0.001$) compared to a weight gain of 1.8 lbs (0.86 kg) (p=NS) in the control group ($p < 0.0001$ between groups). The TDID in the LIRA group decreased by 31%, from a mean baseline dose of 203 U to 140 U at 6 months ($p < 0.0001$), whereas the control group had an increase of 9% in TDID from 181 U to 198 U (p=NS). The LIRA group had an increase in time spent in the euglycemic range (BG 70 to 180 mg/dl) from 57% at baseline to 74% at 6 months (p=0.001), whereas the control group had no increase in time spent in the euglycemic range from baseline (68%) to 6 months (69%). The LIRA group reduced time in the hyperglycemic range (BG > 180 mg/dl), from 39% at baseline to 23% at 6 months (p=0.002); there was no change in time spent in the hyperglycemic range in the control group (29% at baseline and at 6 months). Neither group had any change in the amount of time spent in hypoglycemia (BG < 70 mg/dl) by CGM (LIRA group: 3.7% at baseline to 2.9% at 6 months; control group: 2.7% at baseline to 1.7% at 6 months; p=NS for both groups and between groups). Glycemic variability, expressed as the average daily SD of blood glucose, declined significantly by 25% in the LIRA group, from 40 mg/dl at baseline to 30 mg/dl at 6 months ($p < 0.0001$), whereas the control group had no reduction in glycemic variability (from a baseline of 38 mg/dl to 37 mg/dl at 6 months).
Conclusion: In obese subjects with type 2 diabetes on intensive insulin therapy with >100 units of insulin per day, the addition of liraglutide to insulin resulted in similar improvement in HbA1c to the current standard therapy of insulin up-titration alone, but with additional benefits of weight loss, reduction in TDID, increased percent of time in the euglycemic range and reduced glycemic variability by CGM.
Clinical Trial Registration Number: NCT0165412
Supported by: Novo Nordisk

901

A novel fixed-ratio combination of insulin degludec and liraglutide improves postprandial glycaemic control in patients with type 2 diabetes: results from a standardised meal test
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Background and aims: Improvements in postprandial glycaemic control with the GLP-1 analogue, liraglutide (Lira), are in part attributed to the stimulation of postprandial insulin secretion. IDegLira is a novel fixed-ratio combination of the ultra-long-acting basal insulin, insulin degludec (IDeg), and Lira. Here we compare IDegLira vs IDeg or Lira given alone with respect to postprandial glycaemic control as assessed by a standardised 4-h mixed-meal test.
Materials and methods: In this 26-week randomised open-label trial in patients with type 2 diabetes inadequately controlled on metformin ± pioglitazone (n=1663; mean age: 55 yrs; diabetes duration: 6.6 yrs; HbA_{1c}: 8.3%; BMI: 31.2 kg/m^2), IDegLira was added once daily and compared to IDeg or Lira (1.8 mg) given alone. IDegLira and IDeg were titrated to achieve a similar pre-breakfast plasma glucose (PG) target of 4-5 mmol/l. A subgroup of patients (n=260) were randomised to a standardised 4-h liquid mixed-meal test (fasting ≥8 h) at baseline and after 26 weeks. PG, plasma glucagon and serum insulin were measured at timed intervals. For each parameter, mean incremental increase was calculated as the area under the curve (AUC) above the pre-meal value from t=0 to 4 h ($iAUC_{0-4h}$). Insulin secretion adjusted for postprandial glucose levels was expressed as $AUC_{insulin0-4h}/AUC_{glucose0-4h}$.
Results: After 26 weeks, IDegLira produced a significantly greater decrease from baseline in mean postprandial PG increment over the meal test ($iAUC_{0-4h}$) than IDeg (-0.87 vs. -0.17 mmol/l; estimated treatment difference (ETD): -0.71 mmol/l [-1.17; -0.26], p=0.0023). Similar reductions were observed for IDegLira and Lira (-0.87 vs -0.78 mmol/l; ETD: -0.09 mmol/l [-0.56; 0.37], p=0.70). Changes in postprandial insulin secretion ($iAUC_{0-4h}$) from baseline to week 26 were 20.6 vs -26.7 pmol/l for IDegLira vs IDeg (p=0.077), and 20.6 vs 64.8 pmol/l for IDegLira vs. Lira (p=0.083). Adjusting insulin secretion for postprandial glucose levels indicated that insulin secretion during the meal test was significantly higher for IDegLira than IDeg (33.8 vs 25.7 pM/mM; p=0.048), whereas insulin secretion was similar for IDegLira and Lira (33.8 vs 36.8 pM/mM; p=0.45). Postprandial glucagon ($iAUC_{0-4h}$) was suppressed similarly for IDegLira and IDeg (ETD: 0.00 pg/ml [-3.51; 3.50], p=0.998) but significantly more suppressed by IDegLira vs Lira (ETD: -5.38 pg/ml [-8.91; -1.86], p=0.0029).
Conclusion: IDegLira provides significantly better postprandial glycaemic control following a standardised mixed meal than IDeg. Because IDegLira and IDeg suppress glucagon secretion to a similar extent, the reduced postprandial PG increment associated with IDegLira may to some extent be explained by the higher endogenous insulin secretion associated with IDegLira compared to IDeg. The effect on postprandial glycaemic control and endogenous insulin secretion of the Lira component is maintained when used in a fixed-ratio combination with exogenous insulin (IDeg).
Clinical Trial Registration Number: NCT01336023
Supported by: Novo Nordisk A/S

902

HARMONY 1 results at week 52 primary endpoint: once-weekly albiglutide vs placebo in patients with type 2 diabetes mellitus not controlled on pioglitazone ± metformin
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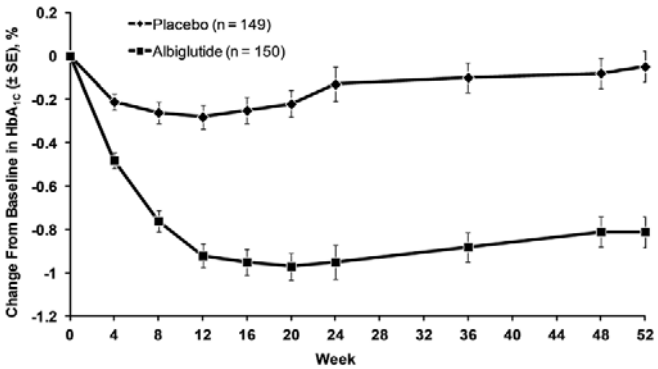
Background and aims: The aim was to evaluate the efficacy and safety of the GLP-1 receptor agonist albiglutide administered subcutaneously once weekly vs placebo in patients with type 2 diabetes mellitus who had inadequately controlled haemoglobin A_{1c} (HbA_{1c}: 7–10%) on pioglitazone ± metformin.

Materials and methods: The trial was designed as a 3-year, randomised, double-blind, placebo-controlled, Phase III study. Patients on background pioglita zone ± metformin were randomised to receive either albiglutide 30 mg or placebo subcutaneously once weekly and were allowed to continue in the study if hyperglycaemic rescue was required.The primary objective was to evaluate HbA_{1c} change from baseline to Week 52 using an analysis of covari ance model adjusting for baseline HbA_{1c},region, prior myocardial infarc tion history, age, and background therapy.
Results: Baseline demographics were similar between the groups; mean HbA_{1c} 8.1%, mean age 55 years, BMI 34 kg/m^2, and duration of diabetes 8 years, Week 52 change from baseline HbA_{1c} was −0.05% for placebo and −0.81% for albiglutide, treatment difference [TD]: −0.75% (95% CI −0.95%, −0.56%), p <.0001. Fasting plasma glucose mirrored HbA_{1c} and showed a rapid improvement which was maintained out to 52 weeks (+0.35 mmol/l for placebo, −1.28 mmol/l for albiglutide [TD]: −1.64 mmol/l, p <.0001). There was a non-significant difference in weight change from baseline between the groups (+0.45 kg for placebo, +0.28 kg for albiglutide, TD: −0.1 8 kg). Adverse events (% participants including rescue) reported included nausea and vomiting, which was comparable between albiglutide and placebo (10.7%/11.3% and 4.0%/4.0%, respectively), diarrhoea, which was higher with albiglutide (11.3% vs 8.6%, respectively) and injection site reactions, which were also higher for albiglutide (11.3% vs 7.9%, respectively). The incidence of pre-rescue documented symptomatic (≤3.9 mmol/l) and severe hypoglycaemia was low: 1% and 0%, respectively, for placebo vs 3% and 1%, respectively, for albiglutide.
Conclusion: Albiglutide added on in combination therapy resulted in a robust and sustained glycaemic improvement with good tolerability in patients with type 2 diabetes mellitus.

Figure. Model-Adjusted[1] Change From Baseline in HbA_{1C} Through Week 52 (Intent-to-Treat; Last Observation Carried Forward[2]) in Patients Not Controlled on Pio ± Met



[1] Analysis of covariance model adjusted for baseline HbA_{1C}, region, history of prior myocardial infarction, age category, and background antidiabetic therapy
[2] Last observation prior to study discontinuation or hyperglycaemia rescue

Clinical Trial Registration Number: NCT00849056
Supported by: GlaxoSmith Kline

903

HARMONY 2 results at week 52 primary endpoint: once-weekly albiglutide monotherapy for patients with type 2 diabetes mellitus inadequately controlled with diet and exercise

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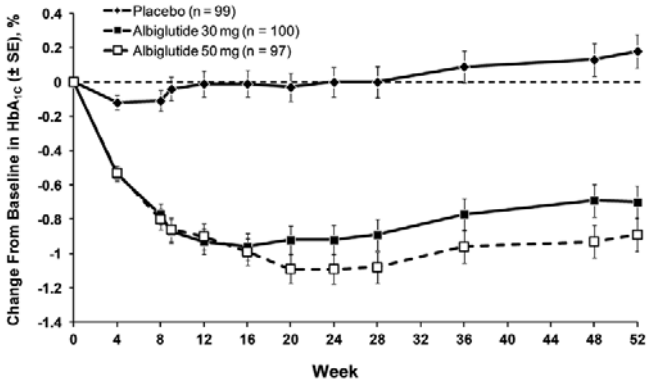
Background and aims: The aim was to evaluate the efficacy and safety of 2 doses of the GLP-1 receptor agonist, albiglutide, compared to placebo in patients with type 2 diabetes mellitus who had inadequately controlled haemoglobin A_{1c} (HbA_{1c}: 7–10%) with diet and exercise alone.
Materials and methods: The trial was designed as a 3-year, randomised, double-blind, placebo-controlled, Phase III study. Patients on diet and exercise were randomised to receive either albiglutide 30 or 50 mg (all began at 30 mg, while those in the high-dose group uptitrated to 50 mg at Week 12) or placebo subcutaneously once weekly. Patients were allowed to continue in the study if hyperglycaemic rescue was required. The primary objective was to evaluate HbA_{1c} change from baseline at Week 52, using an analysis of covariance model adjusting for baseline HbA_{1c}, region, prior myocardial infarction history, age, and background therapy. Step-wise statistical analysis was first performed for high-dose albiglutide vs placebo followed by low-dose albiglutide vs placebo.
Results: Baseline demographics were similar across treatment groups; mean HbA_{1c} 8.1%; mean age 53 years; BMI 34 kg/m^2; and duration of diabetes 4 years. Week 52 change from baseline HbA_{1c} difference (albiglutide − placebo) was −0.84% (95% CI −1.11, −0.58) for albiglutide 30 mg and −1.04% (95% CI −1.31, −0.77) for albiglutide 50 mg; both p <.0001. Fasting plasma glucose decreased rapidly and the improvement mirrored HbA_{1c} out to 52 weeks (−1.89 mmol/l (95% CI −2.55, −1.22) for albiglutide 30 mg and −2.38 mmol/l (95% CI −3.05, −1.71) for albiglutide 50 mg, both p <.0001). Weight decreased in all groups: placebo, −0.7 kg; albiglutide 30 mg, −0.4 kg; and albiglutide 50 mg, −0.9 kg. Gastrointestinal adverse events (% participants including rescue) were low and comparable for placebo/albiglutide 30 mg/albiglutide 50 mg: nausea 8%/10%/9%; diarrhoea 12%/10%/13%; and vomiting 1%/3%/3%. Injection site reactions (% participants including rescue) were higher in the low-dose (18%) and high-dose (22%) albiglutide groups as compared to placebo (10%). The incidence of pre-rescue documented (≤3.9 mmol/l) symptomatic hypoglycaemia (%) for placebo/albiglutide 30 mg/albiglutide 50 mg were 2%/1%/0%; no severe events were reported in the study.
Conclusion: Once-weekly monotherapy with albiglutide in drug-naïve patients with type 2 diabetes mellitus resulted in a dose-dependent robust and durable glycaemic improvement out to 52 weeks of therapy.

Figure. Model-Adjusted[1] Change From Baseline in HbA_{1C} Through Week 52 (Intent-to-Treat; Last Observation Carried Forward[2])



[1] Analysis of covariance model adjusted for baseline HbA_{1C}, region, history of prior myocardial infarction, and age category
[2] Last observation prior to study discontinuation or hyperglycaemia rescue

Clinical Trial Registration Number: NCT00849017
Supported by: GlaxoSmith Kline

904

HARMONY 4: 52-week efficacy of albiglutide vs insulin glargine in patients with type 2 diabetes mellitus

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Background and aims: To assess the efficacy and safety of the GLP-1 receptor agonist albiglutide administered once weekly vs daily glargine in patients with type 2 diabetes mellitus who had inadequately controlled haemoglobin A_{1c} (HbA_{1c}: 7–10%) on a regimen of metformin ± sulphonylurea therapy.
Materials and methods: The trial was a 3-year, randomised, open-label, parallel-group, multicentre, Phase III study. Albiglutide was administered at 30 mg once weekly vs glargine. Patients were treated to a target HbA_{1c} ≤7.0% and fasting plasma glucose (FPG) ≤5.6 mmol/l. If needed, patients could uptitrate to albiglutide 50 mg once weekly and glargine (per pre-specified criteria). Patients were allowed to continue hyperglycaemic rescue was required. The primary objective was to evaluate the HbA_{1c} change from baseline to Week 52 in albiglutide vs glargine.
Results: Baseline demographics were similar between groups; mean age 56 years, BMI 33 kg/m^2, HbA_{1c} 8.3%, duration of diabetes 8.8 years, 82% receiving metformin + sulphonylurea. HbA_{1c} decreased in both groups. The Week 52 treatment difference was 0.11% (95% CI: −0.04%, 0.27%). The upper bound of the CI was below the non-inferior margin of 0.3%, indicating non-inferiority of albiglutide to glargine. Change in FPG significantly favoured glargine, whereas change in weight significantly favoured albiglutide. Adverse

events through Week 52 for albgilutide/glargine were: nausea 9.9%/3.7%; diarrhoea 7.5%/4.1%;vomiting 3.8%/3.7%, respectively. Documented (≤3.9 mmol/l) symptomatic and severe hypoglycaemic events (prior to the addition of hyperglycaemia rescue medications) were fewer with albiglutide vs glargine (18%/0.4% and 27%/0.4%, respectively). Injection site reactions occurred in 13.9% of albiglutide and 8.7% of glargine patients.

Conclusion: Albiglutide treatment resulted in HbA_{1c} improvement at Week 52 that was non-inferior to glargine with modest weight loss. Both drugs were well tolerated.

Table. Week 52 Model-Adjusted Change From Baseline Least Square Mean for Key Efficacy Parameters

	Albiglutide (n = 496)	Glargine (n = 239)
HbA_{1c}, least square mean[a] (SE), %	−0.67 (0.04)	−0.79 (0.06)
p value (noninferiority)	.0086	
p value (superiority)	.1463	
FPG, least square mean (SE), mg/dl	−15.7 (2.30)	−37.1 (3.31)
p value	< .0001	
Weight, least square mean (SE), kg	−1.05 (0.17)	+1.56 (0.25)
p value	< .0001	

[a]Last observation prior to study discontinuation or hyperglycaemia rescue carried forward. Treatment comparison based on analysis of covariance model was adjusted for baseline HbA_{1c}, region, history of prior myocardial infarction, age category, and background antidiabetic therapy.

Clinical Trial Registration Number
Supported by: GlaxoSmith Kline

905

52-week efficacy of albiglutide vs placebo and vs pioglitazone in triple therapy (background metformin and glimepiride) in patients with type 2 diabetes: HARMONY 5 study

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Background and aims: To evaluate the efficacy and safety of the GLP-1 receptor agonist albiglutide (Albi) administered once weekly vs placebo (Pbo) and vs pioglitazone (Pio) in patients on dual therapy (all on background metformin and glimepiride) with baseline haemoglobin A_{1c}(HbA_{1c}) 7.0–10.0% (53–85 mmol/mol).

Materials and methods: The trial was designed as a randomised (N=685), double-blind, multicentre (234 centres) study. Dual-therapy dose stability (metformin >1500 mg, glimepiride 4 mg) was required after screening before randomisation. Uptitration of Albi 30 mg to 50 mg once weekly and Pio 30 mg to 45 mg once daily (both masked) was allowed if needed. Patients were allowed to continue if hyperglycaemic rescue was required. The primary objective was adjusted HbA_{1c} change from baseline at Week 52. Step-wise statistical analysis was first vs Pbo, then non-inferiority (margin: 0.30%) testingvs Pio.

Results: Baseline age w as 55.2 years (mean, SD 9.5), BMI 32.2 kg/m^2 (5.5), HbA_{1c} 8.2% (0.9%), duration of diabetes 8.9 years (6.2). Week 52 HbA_{1c} difference (Albi - Pbo) was −0.87% (95%CI: −1.07%, −0.68%) (−9.5 mmol/mol [−11.7, −7.4]; *p* <.0001)and vs Pio 0.25% (0.10%, 0.40%) (2.7 mmol/m ol [1.1, 4.4]) (non-inferiority *p* value .27, not shown non-inferior). Changes in fasting plasma glucose (FPG) mirrored those of HbA_{1c} (Table). Albi and Pio differed in weight change direction, difference −4.9 kg (−5.5, −4.2) (*p* <.0001) (Table). Adverse events included more gastrointestinal and injection site reaction reports with Albi vs Pbo and vs Pio, and more documented symptomatic hypoglycaemic events with Pio and Albi, with severe events being too infrequent to analyse (Table).

Conclusion: Albiglutide in triple therapy effectively lowers glucose with no unexpected side effects.

Table. Efficacy (Adjusted Mean Difference From Baseline [SE]) and Adverse Event (%) Findings at 52 Weeks

	Albiglutide	Pioglitazone	Placebo
Participants (n)	269	273	115
HbA_{1c} (%)	−0.55 (0.06)	−0.80 (0.06)[a]	+0.33 (0.08)[b]
FPG (mg/dL)	−12.4 (2.9)	−31.4 (2.9)[b]	+11.5 (4.4)[b]
Weight (kg)	−0.4 (0.2)	+4.4 (0.2)[b]	−0.4 (0.4)[c]
Adverse events (% participants)			
Diarrhoea	8.9	5.4	2.6
Nausea/vomiting	9.6/2.6	4.3/1.8	3.5/0.9
Injection site reactions	12.9	3.2	3.5
Documented symptomatic hypoglycaemia[d]	13.7	25.3	7.0
Severe hypoglycaemia[d]	0.4	1.1	0.0

[a]Last observation prior to study discontinuation or hyperglycaemia rescue carried forward. Treatment comparison based on analysis of covariance model was adjusted for baseline HbA_{1c}, region, history of prior myocardial infarction, and age category; *p* (non-inferiority) not significant (albiglutide not noninferior to pioglitazone)
[b]*p* <.001 vs albiglutide; [c]Not significant; [d]Events prior to addition of hyperglycaemia rescue meds

Clinical Trial Registration Number: NCT00838916
Supported by: Glaxo Smith Kline

906

HARMONY 8: once-weekly glucagon-like peptide 1 receptor agonist albiglutide vs sitagliptin for patients with type 2 diabetes with renal impairment: week 26 results

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Background and aims: Therapies for patients with type 2 diabetes mellitus with renal impairment are limited and may require dose adjustment. This study evaluated the efficacy and safety of the GLP-1 receptor agonist albiglutide administered once weekly vs sitagliptin in patients with type 2 diabetes mellitus and renal impairment who had inadequately controlled haemoglobin A_{1c} (HbA_{1c}: 7–10%).

Materials and methods: Thistrial was designed as a 52-week, randomised, double-blind, active-controlled, Phase III parallel-group study. Once-weekly albiglutide 30 mg (uptitrated to 50 mg, if needed) vs daily sitagliptin was administered to patients with type 2diabetes mellitus and renal impairment (estimated glomerular filtration rate [eGFR] ≥15 and <90 ml/min per 1.73 m^2) on lifestyle only (11% of patients) or metformin, thiazolidinedione, sulphonylurea, or any combination (89% of patients). Sitagliptin was dosed by the degree of renal impariment per prescribing information; albiglutide dose did not require modification. Patients were allowed to continue if hyperglycaemic rescue was required. Primary endpoint wasHbA_{1c} change at Week 26 for albiglutide vs sitagliptin (non-in feriority with subsequent superiority analysis).

Results: Demographics matched between groups, including the degree of renal impairment; mean age 63 years, weight 83 kg, and HbA_{1c} 8.2%. HbA_{1c} decreased in both groups: −0.83% for albiglutide (n=246) and−0.52% for sitagliptin (n=240) at Week 26; treatment difference = −0.3 2% (95%CI: −0.49%, −0.15%). Albiglutide was superior to sitagliptin (*P* =.00 03). Mean HbA_{1c} change was greater with albiglutide vs sitagliptin regardless of degree of renal impairment. 35% of albiglutide patients uptitrated to 50 mg. HbA_{1c} <7% was achieved by 42.6% of albiglutide and 30.5% of sitagliptin patients. Fasting plasma glucose (−1.42 mmol/l vs −0.22mmol/l , *p* <.0001) and weight change (−0.8 kg vs −0.2 kg, *p* =.0281) favoured albiglutide. Adverse event rates for albiglutide/sitagliptin were low in both groups: nausea: 4.8%/2.8%,diarrhoea: 8.8%/6.1%, and vomiting:1.6%/0.8%. Documented (≤3.9 mmol/l) symptomatic hypoglycaemia rates (prior to the addition of hyperglycaemia rescue medications) were 10.4% (0 sev ere) for albiglutide v 5.7% (0.8% severe) for sitagliptin. Injection site reaction rate was 6.8% for albiglutide vs 2.8% for sitagliptin. Anti-albiglutide antibody rate was 2.8% (0% neutralising).

Conclusion: In patients with type 2 diabetes mellitus and renal impairment, once-weekly albiglutide had superior reductions of HbA_{1c} at Week 26 vs sitagliptin without requiring dose adjustment. Both agents were well tolerated.

Table. Change From Baseline in HbA_{1c} at Week 26 by Severity of Renal Impairment

	Albiglutide				Sitagliptin			
Renal impairment severity (eGFR range, ml/min/1.73 m²)	Mild (60–89) n=125	Moderate (30–59) n=98	Severe (15–29) n=19	Any (15–89) n=246	Mild (60–89) n=122	Moderate (30–59) n=99	Severe (15–29) n=15	Any (15–89) n=240
Baseline HbA_{1c}, mean, %	7.96	8.26	8.05	8.08	8.16	8.28	8.32	8.22
Week 26 HbA_{1c}, mean, %	7.23	7.37	6.97	7.27	7.50	7.91	7.67	7.68
Change from baseline HbA_{1c}, mean (SD), %	−0.72 (0.81)	−0.88 (1.00)	−1.08 (0.91)	−0.82 (0.90)	−0.66 (0.88)	−0.37 (1.33)	−0.65 (1.24)	−0.54 (1.12)
Model-adjusted change from baseline HbA_{1c},* least square mean (SE), %	−0.80 (0.09)	−0.83 (0.10)	−1.08 (0.22)	−0.83 (0.06)	−0.67 (0.09)	−0.31 (0.10)	−0.61 (0.25)	−0.52 (0.06)
Difference of least square means from sitagliptin (95% CI),* %	−0.13 (−0.37, 0.11)	−0.53 (−0.80, −0.26)	−0.47 (−1.12, 0.18)	0.32 (−0.49, −0.15)	–	–	–	–
Superiority *p* value	–	–	–	.0003	–	–	–	–

*Last observation prior to study discontinuation or hyperglycaemia rescue carried forward. Treatment comparison based on analysis of covariance model was adjusted for baseline HbA_{1c}, treatment, renal impairment, prior myocardial infarction history, age, and region.

Clinical Trial Registration Number: NCT01098539
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Meta-analysis of GLP-1 agonist lixisenatide use in patients insufficiently controlled with OADs

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Background and aims: Lixisenatide is a new once-daily prandial glucagon-like peptide-1 (GLP-1) receptor agonist, which was granted marketing authorization in Europe in February 2013 for the treatment of type 2 diabetes mellitus (T2DM).
Materials and methods: The safety and efficacy of lixisenatide was evaluated in combination with oral antidiabetic drugs (OADs) in patients with T2DM in five GetGoal Phase III studies (GetGoal-M, GetGoal-P, GetGoal-S, GetGoal-M-Asia and GetGoal-F1). The current meta-analysis reports composite efficacy and safety outcomes for lixisenatide compared with placebo.
Results: There were no significant differences in baseline characteristics between the lixisenatide and placebo groups (mean age: 55.5 years, mean BMI: 31.5 kg/m^2, duration of diabetes: 7.4 years, duration of OAD use: 4.5 years, metformin use: 92.3%, sulfonylurea use: 35.6%, thiazolidinedione use: 16.5%). The total patient population included n=1828 in the lixisenatide group and n=932 in the placebo group. A significantly greater proportion of patients treated with lixisenatide achieved HbA_{1c} <7% compared with placebo (odds ratio [95% confidence interval (CI)] 2.7 [2.1, 3.4], p<0.0001). Lixisenatide was also significantly better than placebo for the composite endpoints of HbA_{1c} <7% and no weight gain (odds ratio [95% CI]: 2.6 [2.0, 3.5], p<0.0001), HbA_{1c} <7% and no documented symptomatic hypoglycaemia (odds ratio [95% CI]: 2.6 [2.0, 3.2], p<0.0001) and HbA_{1c} <7%, no weight gain and no documented symptomatic hypoglycaemia (odds ratio [95% CI]: 2.5 [1.9, 3.3], p<0.0001) (Table). Only one patient reported documented severe hypoglycaemia (0.05%) (lixisenatide in combination with sulfonylurea).
Conclusion: Treatment with lixisenatide in patients with T2DM inadequately controlled on OADs significantly reduces HbA_{1c} (-0.9% vs -0.3%; p<0.0001) and is 2.5 times more likely than placebo to result in HbA_{1c} <7% with no documented symptomatic hypoglycaemia and/or no weight gain.

	LIXI	PBO	Odds Ratio LIXI vs PBO	95% CI	p-value
HbA_{1c} <7%, no weight gain†, %	35.8	18.9	2.6	2.0, 3.5	<0.0001
HbA_{1c} <7%, no documented symptomatic hypoglycaemia‡, %	40.9	23.0	2.6	2.0, 3.2	<0.0001
HbA_{1c} <7%, no weight gain, no documented symptomatic hypoglycaemia†,‡, %	33.5	18.2	2.5	1.9, 3.3	<0.0001

*Meta-analysis includes GetGoal-M, GetGoal-P, GetGoal-S, GetGoal-M-Asia and GetGoal-F1; †Δ Weight: Endpoint -Baseline ≤0; ‡Symptomatic hypoglycaemia with blood glucose <60 mg/dL; LIXI=lixisenatide, PBO=placebo, CI=confidence interval, HbA_{1c}=glycated haemoglobin. Lixisenatide was recently granted marketing authorization in Europe for the treatment of type 2 diabetes mellitus

Clinical Trial Registration Number: GetGoal-M: NCT00712673; GetGoal-P: NCT00763815; GetGoal-S: NCT00713830;GetGoal-M-Asia: NCT01169779; GetGoal-F1: NCT00763451
Supported by: Sanofi

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Meta-analysis of randomised controlled trials of lixisenatide as add-on to basal insulin and/or oral antidiabetic drugs in patients with type 2 diabetes mellitus

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Background and aims: Lixisenatide is a new once-daily prandial glucagon-like peptide-1 (GLP-1) receptor agonist, which was granted marketing authorization in Europe in February 2013 for the treatment of Type 2 diabetes mellitus (T2DM).
Materials and methods: The safety and efficacy of lixisenatide has been evaluated in combination with basal insulin with or without oral antidiabetic drugs in three Phase III, randomized, placebo-controlled trials (GetGoal-L, GetGoal-Duo-1 and GetGoal-L-Asia) in T2DM. Endpoints included HbA_{1c}, weight, insulin dose, fasting blood glucose, postprandial glucose and hypoglycaemia. A meta-analysis was performed on the safety and efficacy outcomes in 1198 patients (mean age: 57.2 years; diabetes duration: 11.7 years; BMI: 30.3 kg/m^2) using a random effects model (RevMan).
Results: Mean HbA_{1c} at baseline for lixisenatide compared with placebo-treated patients was 8.2% vs 8.1%, respectively. At endpoint, mean HbA_{1c} for lixisenatide, compared with placebo-treated patients, was 7.5% vs 7.9%, respectively. A significantly higher proportion of lixisenatide-treated patients achieved HbA_{1c} <7% compared with placebo (odds ratio [95% CI]: 3.7 [1.6, 8.2], p=0.0016). Furthermore, lixisenatide was more than three times more likely than placebo to result in HbA_{1c} <7% and no weight gain (odds ratio [95% CI]: 3.4 [1.7, 6.8], p=0.0008), and more than 2.5 times more likely than placebo to result in HbA_{1c} <7% together with no documented symptomatic hypoglycaemia (odds ratio [95% CI]: 2.7 [1.3, 5.4], p=0.0073). Lixisenatide was also more than 2.5 times as likely to result in HbA_{1c} <7% and no weight gain and no documented symptomatic hypoglycaemia (odds ratio [95% CI]: 2.6 [1.5, 4.7], p=0.0009) (Table).
Conclusion: In patients with T2DM, lixisenatide in combination with basal insulin with or without oral antidiabetic drugs is significantly more effective than placebo in combination with basal insulin with or without oral antidiabetic drugs in achieving HbA_{1c} <7%, and is more than 2.5 times as likely to result in HbA_{1c} <7% with no documented hypoglycaemia and no weight gain. Lixisenatide is a treatment option as add-on to treatment with basal insulin.

Meta-analysis results: composite endpoints*

	LIXI + basal insulin (n=665)	PBO + basal insulin (n=533)	Odds ratio LIXI + basal insulin vs PBO + basal insulin	95% CI	p value
HbA_{1c} <7%, no documented symptomatic hypoglycaemia†, %	26.3	17.3	2.7	1.3, 5.4	0.0073
HbA_{1c} <7%, no weight gain, %	27.4	12.4	3.4	1.7, 6.8	0.0008
HbA_{1c} <7%, no weight gain, no documented symptomatic hypoglycaemia, %	18.5	9.8	2.6	1.5, 4.7	0.0009

*Includes GetGoal-Duo-1, GetGoal-L and GetGoal-L-Asia; †Symptomatic hypoglycaemia with blood glucose <60 mg/dL; LIXI=lixisenatide; PBO=placebo; CI=confidence interval; HbA_{1c}=glycated haemoglobin

Clinical Trial Registration Number: NCT00975286; NCT00715624; NCT00866658
Supported by: Sanofi

PS 071 Incretin-based drugs: potential risk and use in special populations

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Effect of metformin on glycotoxic intermediate-methylglyoxal metabolism in patients with type 2 diabetes

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Background and aims: Hyperglycaemia increases the formation of intracellular reactive oxigen species and glycotoxic intermediates. Methylglyoxal (MG) is a highly potent glycatic agent that is throught to contribute to late diabetic complications either as a toxic agent or as a precursor for advanced glycation end products. Metformin, a biguanide is the most widely prescribed oral glucose-lowering agent for the treatment of type 2 diabetes. Its primary mechanism of action is to increase hepatic and peripherial tissue sensitivity to insulin. Metformin has also been proposed as a scavenger of MG. The effect of metformin on MG metabolism was studied with respect of its effects on MG levels, triosphosphate intermediates (TPINT) and intracellular activity of glyoxalase-1 (Glo1), the GSH dependent enzyme for MG detoxification in a prospective non-randomized 24 weeks trial of 12 patients with type 2 diabetes.
Materials and methods: Whole blood was sampled from newly diagnosed patients with type 2 diabetes in the fasting state at beginning and end of the 24 week trial period. The patients were educated for a low energy diet and treated with metformin (2000 mg/day). Glycaemic control was determined by blood glucose and HbA1C. Plasma MG was detected by HPLC. The glyoxalase system was measured by enzyme assays in peripherial blood mononuclear cells and red blood cells. TPINT was determined by endpoint enzymatic assays. Plasma N-Epsilon-(Carboxymethyl)-Lysine (CML) modified protein concentration was determined by ELISA.
Results: At baseline MG levels correlated with Glo1, but not glyoxalase 2 (Glo2) activity. Metformin treatment in addition to low energy diet reduced significantly fasting glucose from 7.7 to 6.3 mmol/l (p=0.04), HbA1c from 7.02 to 6.23% (p=0.02) while body weight and BMI was only marginally reduced during the 24 week trail. Treatment reduced significantly MG, from 653.3 to 451.1 nM (p=0.015) and subsequently also levels of CML, from 283.37 to 197.8 ng/ml (p=0.016). The reduction of MG was paralleled by a significant increase in the activity of Glo1 in peripherial blood mononuclear cells, from baseline 0.51 to 1.02 mM-1min-1mg-1 (p=0.002) and red blood cells from 0.22 to 0.39 mM-1min-1mg-1 (p=0.03), while no effect was observed on Glo2 activity. In addition no effect was found on triosephosphate intermediates such as glyceraldehyde-3-phosphate (GAP), dihydroxyacetonephosphate (DHAP), fructose-1,6-bisphosphate (FDP) or the TPI-pool. Most importantly multivariate analysis showed that the changes in MG were dependent upon the metformin treatment.
Conclusion: Metformin might beneficial influence on the glycotoxic intermediate-methylglyoxal-metabolism in type 2 diabetes. Our results supports previously findings that metformin can reduce plasma MG in type 2 diabetic patients. However, given the observed increase in Glo1 activity, this reduction is due not to the scavenging properties of metformin, but restoration of Glo1 activity.
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Gastrointestinal tolerability with twice-daily or once-weekly exenatide formulations was not predicted by concentration with long-term treatment

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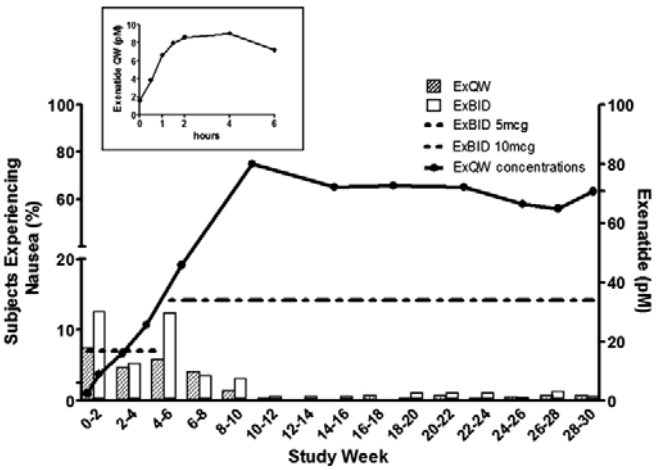
Background and aims: The most frequent adverse events (AEs) associated with GLP-1 receptor agonists (GLP-1RA) are gastrointestinal (GI) eg nausea and vomiting. It has been hypothesized that GI AEs are related to GLP-1RA concentration (conc). To decrease GI AEs for exenatide (Ex) twice daily (EBID), a dose titration paradigm was employed (5 mcg for 4 weeks; 10 mcg maintenance dose). We examined Ex conc and GI AEs for the once-weekly 2 mg (EQW) sustained-release formulation (apparent $t_{1/2}$ = 15 d) and EBID immediate-release formulation ($t_{1/2}$ = 2.4 h).
Materials and methods: Ex conc and/or tolerability data from 2 clinical trials of EQW vs EBID (24 or 30 weeks) were evaluated, including extension data to 4 y. The conc of Ex (pM) in plasma was measured by ELISA and geometric means ± SE were calculated.
Results: With EQW there is an initial release of Ex within hours of injection (peak mean conc 9.0 pM at 4 hours; Figure inset), which is less than the 5 mcg dose of EBID (17 pM). The overall incidence of nausea was low on day 1 for EQW (1.8%) and EBID (4.9%). The lower incidence of nausea for EQW is consistent with lower peak conc. With repeated dosing $C_{steady\ state}$ for EQW (8 to 30 weeks) was 69.4 ± 2.9 pM (n = 217) and C_{max} for 10 mcg EBID was 34.0 ± 3.5 pM (n = 72). Despite the higher sustained conc of Ex with EQW, the incidence of nausea over all 30 weeks was lower for EQW than for EBID (22% vs 37%) and fewer patients discontinued due to GI AEs (1% vs 4%, respectively). Overall, the time course of GI AEs differed from the time course of Ex conc with both Ex formulations. For EQW, Ex conc gradually increased over time (steady state conc at 8-10 weeks), peak nausea occurred before week 6 and then declined markedly and remained low (only 1% reported between 3.5 and 4 y, n = 94). For EBID, the incidence of nausea decreased over time except for a transient increase at weeks 4-6, which coincides with dose titration from 5 to 10 mcg. The decreased incidence was not due to patient discontinuation for either formulation. In patients switched from EBID to EQW, no increase in GI AEs was apparent despite sustained and increased Ex conc; only 1 patient who switched withdrew due to nausea. Furthermore, there was no meaningful difference in steady-state Ex conc between patients who did and did not report nausea and/or vomiting with EQW (74.1 ± 6.5 pM [n = 31] vs 68.7 ± 3.2 pM [n = 186], respectively).
Conclusion: Ex conc was not an important predictor of GI AEs following the development of tolerance to Ex, which occurs within weeks of initiating Ex therapy, regardless of formulation. The lower incidence of GI AEs in subjects receiving EQW occurred despite higher sustained exenatide conc compared with EBID. For EQW, the gradual release of Ex from the microspheres results in a slow increase in exenatide conc over the first 6-8 weeks, to achieve optimal steady state levels of Ex for glycemic response, without the need for dose titration to mitigate GI AEs.



Clinical Trial Registration Number: NCT00308139, NCT00877890

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Chronic palmitate excess results in reduced exendin-4 action and signalling via CREB and Akt in pancreatic beta cells

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Background and aims: The incretin effect is attenuated or markedly diminished in type 2 diabetic and obese subjects. The aim of this study was to investigate the mechanisms of incretin resistance induced by prolonged exposure to high concentrations of saturated fatty acids in pancreatic beta-cells.
Materials and methods: Studies were carried out in rat INS-1E cells treated with 0.5 mM palmitate for 24 h and in pancreatic islets from mice fed a high fat diet for 21 days. Mouse islets were isolated by bile duct perfusion and col-

lagenase digestion. Expression and phosphorylation levels of specific signaling molecules were assessed by immunoblotting techniques. Gene expression was evaluated by real-time RT-PCR. Insulin secretion was measured using an insulin ELISA.
Results: Prolonged exposure of INS-1E cells to palmitate reduced the ability of exendin-4 to augment insulin mRNA levels and to induce insulin release by 50% and 60%, respectively ($p<0.05$). In addition, palmitate markedly impaired exendin-4-stimulated CREB and Akt phosphorylation, whereas phosphorylation of MEK and Erk-1/2 was not altered; moreover, palmitate did not interfere with the ability of IGF-I to activate CREB and Akt, indicating that the inhibitory effects of palmitate are specific for exendin-4 signaling via CREB and Akt. Both islets from mice fed a high fat diet and INS-1E cells exposed to palmitate showed increased SREBP-1c mRNA (by 2-fold, $p<0.05$), reduced PDX-1 mRNA levels (by 45%; $p<0.05$), and reduced GLP-1 receptor mRNA and protein levels (by 30% and 50%, respectively; $p<0.05$) compared to control. Furthermore, in INS-1E cells, RNAi-mediated silencing of the GLP-1 receptor prevented exendin-4-induced CREB and Akt phosphorylation ($p<0.05$), but did not impair the ability to activate MEK and Erk-1/2. In addition, when INS-1E cells were pretreated with 1,10-phenanthroline, an inhibitor of SREBP-1c, the palmitate-induced reduction in PDX-1 and GLP-1 receptor levels was abrogated ($p<0.05$), and the ability of exendin-4 to stimulate CREB and Akt phosphorylation was restored ($p<0.05$). In contrast to palmitate, exposure of INS-1E cells to oleate for 24 h led to reduced SREBP-1c mRNA, and increased PDX-1 and GLP-1 receptor mRNA and protein levels. Consequently, oleate enhanced the ability of exendin-4 to stimulate CREB and Akt phosphorylation ($p<0.05$) and to induce insulin mRNA levels ($p<0.05$).
Conclusion: Prolonged exposure of pancreatic beta-cells to elevated concentrations of palmitate results in reduced exendin-4 action on insulin expression and secretion. The mechanisms underlying the resistance to the insulinotropic effect of exendin-4 appears to involve palmitate-mediated induction of SREBP-1c, which results in diminished GLP-1 receptor expression and specific reduction of signaling via CREB and Akt.

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MK0626, a DPP-4 inhibitor for use in mice, in combination with low-dose systemic anti-CD3 stably reverses new-onset diabetes in non-obese diabetic mice

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Background and aims: Immunotherapy can temporarily arrest loss of beta-cell function, but stable, permanent insulin independence is not achieved. Today, combining immune interventions with regenerative medicine or beta-cell replacement is considered as the way forward in type 1 diabetes cure. Dipeptidyl peptidase-4 (DPP-4) inhibitors increase circulating levels of incretin hormones, which can enhance insulin secretion and beta-cell function. We tested whether MK0626, a sitagliptin analog, enhances diabetes remission in new-onset diabetic non-obese diabetic (NOD) mice treated with low-dose systemic anti-CD3 and studied the effects of combination therapy (CT) on immune system as well as cellular and metabolic responses of beta-cells.
Materials and methods: Recent-onset diabetic NOD mice were given anti-CD3 for 5 consecutive days (clone 145-2C11, 2.5 μg/d, i.v.) together with MK0626 (3 mg/kg p.o.) for 3 weeks.
Results: Low-dose anti-CD3 plus MK0626 cured diabetes in 52% of animals (n=23), better than the anti-CD3 alone which cured diabetes in 35% (n=23), while MK0626 alone cured diabetes in 25% (n=4). Interestingly, anti-CD3 with MK0626 cured 69% of mice starting with glycaemia levels 350 mg/dl in contrast to 38% of mice treated with anti-CD3 alone. Anti-CD3 with MK0626 increased beta-cell volume as well as total insulin content in pancreas when compared to anti-CD3 alone. Moreover the CT induced recovery of GLUT-2 in beta-cells, although to the same extent as the anti-CD3 therapy alone. Splenic CD4+/CD8+ ratio for CT and anti-CD3 alone decreased temporarily compared to new-onset diabetic NOD mice; this normalised after 7 weeks of therapy discontinuation. CD4+CD25+FoxP3+ T-cell frequencies in pancreatic draining lymph nodes were significantly increased by CT and anti-CD3 alone compared to new-onset diabetic mice and this persisted long-term. As VEGF mRNA levels were clearly increased in pancreas of CT cured mice compared to anti-CD3 alone, we are investigating whether CT improved islet vasculature, which could increase the insulin content of recovered degranulated beta-cells.
Conclusions: MK0626 in combination with anti-CD3 modulates both the immune system and pancreatic beta-cells, which can lead to normalization of hyperglycaemia in diabetic NOD mice.
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Renal safety and outcomes with linagliptin: meta-analysis of individual data for 5466 patients with type 2 diabetes

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Background and aims: Long-term glycaemic control in diabetes is associated with reduced risk of renal microvascular complications. Linagliptin has been associated with significantly reduced albuminuria in patients with type 2 diabetes mellitus (T2DM) and renal dysfunction. As this effect was not directly related to short-term glycaemic improvements, it has been speculated that linagliptin may have beneficial renal effects. The aim of this study was to evaluate renal outcomes with linagliptin in completed Phase 3, randomised, double-blind, placebo-controlled trials (≥12 wks).
Materials and methods: Predefined events from 13 trials were analysed using a composite primary endpoint: new onset of a) microalbuminuria, b) macroalbuminuria, c) new onset chronic kidney disease (CKD; serum creatinine increase ≥250 μmol/L), d) worsening CKD (loss in estimated glomerular filtration rate [eGFR] >50% vs. baseline), e) acute renal failure (standardised Medical Dictionary for Regulatory Activities query), and f) death by any cause. Statistical analyses included hazard ratio (HR) using cox regression.
Results: Of 5466 participants (mean baseline HbA1c, 8.2%; eGFR, 91.5 mL/min/1.73 m^2), 3505 received linagliptin 5 mg qd and 1961 received placebo. Cumulative exposure was 1756 and 1057 person years, respectively. Events in the composite endpoint occurred in 448 (12.8%) patients receiving linagliptin vs. 306 (15.6%) with placebo, resulting in an HR in favour of linagliptin of 0.84 (95% confidence interval [CI]: 0.72, 0.97; $P<0.05$). For white patients and Asian patients, HRs (95% CI) were 0.82 (0.68, 0.98) and 0.89 (0.68, 1.15), respectively. Among patients <65 years and ≥65 years, HRs (95% CI) were 0.77 (0.64, 0.92) and 1.04 (0.80, 1.35), respectively. HRs (95% CI) for individual renal endpoints were: microalbuminuria, 0.82 (0.69, 0.98); macroalbuminuria, 0.86 (0.61, 1.20); new onset of CKD, 0.32 (0.10, 1.01); worsening of CKD, 1.42 (0.24, 8.52); acute renal failure, 0.94 (0.61, 1.45); and death by any cause, 1.09 (0.29, 4.09).
Conclusion: This large meta-analysis of the linagliptin global Phase 3 development programme supplements the overall safety evidence for linagliptin and supports its potentially favourable renal safety profile. These combined findings support the hypothesis of a potential direct effect of linagliptin on the onset and progression of renal disease in T2DM.
Clinical Trial Registration Number: NCT00328172; NCT00309608; NCT00641043; NCT00621140; NCT00601250; NCT00602472; NCT00654381; NCT00819091; NCT00954447; NCT00800683; NCT00798161; NCT00740051; NCT01084005
Supported by: Boehringer Ingelheim

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Linagliptin versus placebo followed by glimepiride in type 2 diabetes patients with moderate to severe renal impairment

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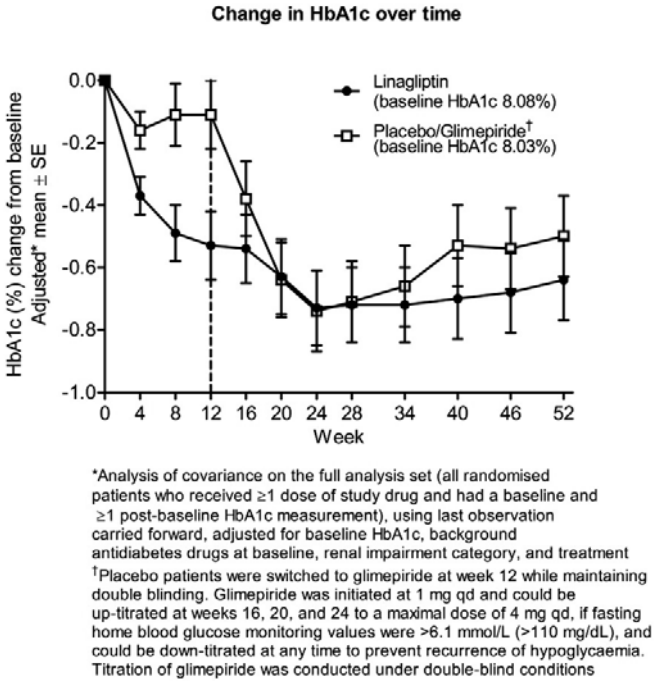
Background and aims: Renal impairment is a serious complication of type 2 diabetes mellitus (T2DM) that restricts options for managing hyperglycaemia and associated increased cardiovascular risk.

Materials and methods: This randomised double-blind trial evaluated the efficacy and tolerability of the dipeptidyl peptidase-4 inhibitor linagliptin in T2DM patients (HbA1c 7-10%) with moderate to severe renal impairment (estimated glomerular filtration rate [eGFR] <60 mL/min/1.73m^2; not on dialysis). Patients received linagliptin 5 mg once daily (qd) (n=113) or placebo (n=122) for 12 weeks, then placebo patients were switched to glimepiride 1-4 mg qd and treatment continued to week 52. The primary endpoint was HbA1c change from baseline at week 12.
Results: At baseline, 63.4% were male, mean±SD age 67±9 years, HbA1c 8.1±0.9% and eGFR 37±13 mL/min/1.73 m^2. Most patients had T2DM for >10 years (76.4%) and were on insulin (85.8%). At week 12, adjusted mean±SE HbA1c change with linagliptin was -0.50±0.06% (change with placebo -0.08±0.07%: difference -0.42%, 95% CI -0.60 to -0.24; *P*<0.0001). In the 40-week extension, HbA1c was lower with linagliptin than glimepiride (Figure). The incidence of drug-related adverse events was similar in the first 12 weeks (linagliptin 23.9%, placebo 24.6%), and lower with linagliptin in the extension (linagliptin 38.3%, glimepiride 46.5%). Hypoglycaemia was less frequent with linagliptin (linagliptin 57.9%, glimepiride 69.3%). Mean adjusted weight increase after 52 weeks was 0.06 kg (linagliptin) and 1.74 kg (placebo/glimepiride).
Conclusion: Linagliptin was efficacious and well tolerated in T2DM patients with moderate to severe renal impairment, with less hypoglycaemia and relative weight loss compared with glimepiride.



Clinical Trial Registration Number: NCT01087502
Supported by: Boehringer Ingelheim

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Efficacy and safety of alogliptin in subjects with type 2 diabetes: a randomised, double-blind, placebo-controlled, phase 3 study in mainland China, Taiwan and Hong Kong

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Background and aims: This multicenter clinical trial in Mainland China, Taiwan and Hong Kong was done to determine the efficacy and safety of once-daily oral Alogliptin (ALO) as monotherapy, add-on to ongoing metformin (MET) therapy, and add-on to pioglitazone (PIO) therapy (with or without MET) in patients with type 2 diabetes.
Materials and methods: A total of 506 subjects (185 in the monotherapy group, 197 in the add-on to MET therapy group, and 124 in the add-on to PIO group) were randomized to receive ALO 25mg QD or placebo (PBO) QD for 16 weeks. The primary endpoint was change from baseline in HbA1c at week 16. The secondary endpoints were change in fasting plasma glucose (FPG), incidence of marked hyperglycemia, clinical HbA1c response, and change in body weight from baseline.
Results: For monotherapy, the decrease of HbA1C from baseline to week 16 in the ALO and PBO arms were 0.99% and 0.42%, respectively (P <0.001). For the add-on to MET group, ALO decreased HbA1c by 0.91 % compared to PBO 0.22% (P<0.001). For add-on to PIO group, ALO decreased HbA1c by 0.76% vs PBO 0.25% (P<0.001). The decreases in FPG were greater in the ALO arms than in the PBO arms. The percentage of subjects able to achieve an HbA1c target of ≤ 6.5%, ≤7.0% and ≤7.5% were all significantly higher (P<0.001) in the ALO arms than in the PBO arms. ALO treatment showed lower incidence of hyperglycemia than PBO in all groups. No weight gain was observed in either the ALO or PBO arms. Pooled analysis showed that the percentage of subjects who experienced hypoglycemia was 1.6% in the ALO arm vs 0.8% in PBO arm. All episodes of hypoglycemia were considered mild or moderate. The percent of subjects who experienced at least one drug related treatment-emergent adverse events was 9.1% in the ALO arm vs. 8.7% in the PBO arm. The percentage of subjects who experienced treatment-emergent AEs leading to discontinuation of study drugs was1.6% in ALO arm vs. 2.0% in the PBO arm. No significant changes were observed between the ALO and PBO arms in laboratory parameters compared with baseline.
Conclusion: ALO 25mg QD significantly reduced HbA1c and FPG, and enhanced clinical HbA1c response compared to PBO when used as monotherapy, add-on to MET, and add-on to PIO. ALO 25mg QD showed a safety profile comparable to PBO.
Clinical Trial Registration Number: NCT01289119

916

Continuous infusion of lixisenatide or exenatide in mice stimulates calcitonin and GLP-1 receptor gene expression but does not induce thyroid C-cell proliferation

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Background and aims: Lixisenatide is a novel once-daily glucagon-like peptide-1 (GLP-1) receptor agonist, recently approved in Europe for the treatment of adults with type 2 diabetes mellitus. Long-term exposure to GLP-1 receptor agonists has been associated with the development of thyroid C-cell hyperplasia and tumours in rodents. Because calcitonin is synthesized in large amounts by thyroid C-cell tumours, it is frequently used as a marker for C-cell neoplasia. The current study evaluated the effect of a 3-month continuous infusion of lixisenatide or exenatide on thyroid C-cell proliferation in mice.
Materials and methods: CD1 mice were administered aqueous solution of lixisenatide or exenatide at 75 µg/animal/day or vehicle (control) by continuous infusion with subcutaneous mini-pump implants for 12 weeks. Blood samples were collected on Day 10 and/or Day 84 for evaluation of drug exposure and plasma calcitonin. At study end, histomorphometry analysis of C-cell numbers and/or volume was performed on standardized thyroid cross-sections stained for calcitonin by immunohistochemistry. Statistical comparisons between treated and control groups were conducted using pairwise two-sided Wilcoxon's tests. Gene expression of proliferative markers *Ccnd1, Ccnd2, Ccnd3, C-myc, Bcl-xl, p21* and *p27* was evaluated with quantitative real-time transcription polymerase chain reaction, as was gene expression for the *calcitonin* and *GLP-1 receptor* genes.
Results: Pharmacokinetic evaluation indicated good systemic drug exposure in both lixisenatide- and exenatide-treated mice (Table). Plasma calcitonin on Day 84 was elevated in both treatment groups vs controls (p≤0.05 for both, Table). Elevated gene expression for *calcitonin* and *GLP-1 receptor* was recorded in both treatment groups vs controls (Table). Changes in expression of other proliferative markers were minimal and generally not considered to be treatment-related or biologically relevant. No macroscopic or microscopic treatment-related changes were observed in the thyroid glands and the histomorphometric analysis of C-cells failed to demonstrate any difference in the volume and number of C-cells between either treatment group and negative controls.

Conclusion: In CD1 mice, a 3-month continuous subcutaneous infusion of lixisenatide stimulated calcitonin and GLP-1 receptor expression but did not induce C-cell proliferation.

Drug exposure, calcitonin and gene expression: 12 weeks of sc infusion with lixisenatide/exenatide

Drug exposure C_{mean} (pg/mL)			
		Lixisenatide†	Exenatide†
Day 10	Male Female	15,400 17,700	14,800 24,900
Day 84	Male Female	357,000 325,000	102,000 61,100
Plasma calcitonin concentration (pg/mL), mean (±SD)			
	Vehicle n=60	n=58	=58
Day 84, pooled male and female	42.0 (56.7)	136.9 (74.2)*	135.4 (61.6)*
Gene expression (fold increase vs vehicle)			
Calcitonin	Male (n=14) Female (n=15)	2.9 5.9	4.0 4.8
GLP-1 receptor	Male (n=14) Female (n=15)	3.0 4.5	4.5 4.5

C_{mean}=mean plasma concentration; SD=standard deviation; GLP-1=glucagon-like peptide-1; *p=0.05 vs control; †n=3 for each sex, drug and sampling day

Clinical Trial Registration Number: TXC1505
Supported by: Sanofi

917

GLP-1 receptor expression in human C-cell carcinomas and human thyroid glands without C-cell pathology

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Background and aims: In C-cells isolated from human thyroid gland tissue, only marginal glucagon-like peptide-1 (GLP-1) receptor expression has been observed. This study assessed the expression of the GLP-1 receptor in human thyroid tissue with proliferative C-cell findings compared with tissue without C-cell pathology.
Materials and methods: A total of 49 tissue samples were examined in three separate studies: human thyroid tissue without C-cell pathology (n=19; Group A), C-cell carcinoma (sporadic; n=10; Group B), C-cell carcinoma (multiple endocrine neoplasia; n=10; Group C), and non-neoplastic and neoplastic C-cell hyperplasia (Groups D1 [n=5] and D2 [n=5]). C-cells and follicular cells were evaluated separately for GLP-1 receptor expression after laser capture microdissection, with calcitonin expression levels determined to ensure proper separation of cell fractions.
Results: Calcitonin expression levels in the follicular cells were low or undetectable in Groups A (cycle time [CT]=32.1) and D (CT=28.4), and were higher in Groups B (CT=20.1) and C (CT=23.9). In the C-cell fractions, the calcitonin expression levels were consistently higher than in follicular cell fractions, with CT values of 21.5-26.6 for Group A, 23.7 for Group B, 25.0 for Group C, and 29.2 for Group D1 and 27.0 for Group D2. In both C-cell and follicular cell samples, GLP-1 receptor expression levels were low or undetectable in all groups. Calcitonin RNA was expressed in the C-cell fractions and, to a much lower extent, in the follicular cell samples, demonstrating that they had been successfully separated.
Conclusion: No difference in GLP-1 receptor expression was observed between thyroid tissue samples with or without proliferative C-cell findings, neither in follicular nor C-cell fractions.

Expression of the GLP-1 receptor in human thyroid tissue

	Expression level, mean CT value ± SD (range)							
	β-actin*		Thyroglobulin*		Calcitonin†		GLP-1R	
	Follicular cells	C-cells	Follicular cells	C-cells	Follicular cells	C-cells	Follicular cells	C-cells
Tissue without C-cell pathology (n=10)‡	26.4 ± 3.2 (22.0-29.9)	24.6 ± 3.8 (19.3-30.6)	25.4 ± 3.7 (20.3-30.0)	25.0 ± 4.0 (18.9-29.0)	32.1 ± 3.34§	26.6 ± 2.8 (22.8-30.4)	ND	ND
Tissue without C-cell pathology (n=5)‡	23.8 ± 2.0 (20.9-26.0)	24.3 ± 1.3 (22.2-25.8)	21.0 ± 2.3 (18.1-24.1)	22.3 ± 1.8 (19.8-24.6)	ND	21.5 ± 1.1 (19.9-22.6)	ND	ND
Tissue without C-cell pathology (n=4)¶	21.4 ± 1.2 (20.0-22.8)	23.2 ± 0.4 (23.0-23.5)**	18.7 ± 0.8 (17.6-19.6)	21.5 ± 0.7 (21.0-21.9)**	ND	20.7 ± 1.0 (20.0-21.4)**	ND	33.6 ± 0.6 (33.2-34.0)**
C-cell carcinoma (sporadic) (n=10)‡	26.2 ± 6.2 (18.5-36.2)	25.4 ± 5.0 (18.9-36.5)	25.7 ± 6.9 (20.8-36.9)	29.3 ± 3.2 (24.1-33.5)	20.1 ± 5.2	23.7 ± 4.9 (21.5-36.4)	ND	ND
C-cell carcinoma (MEN2) (n=10)‡	25.5 ± 1.5 (24.3-27.8)	28.7 ± 2.0 (25.7-32.1)	26.4 ± 4.1 (22.9-34.8)	32.0 ± 1.9 (28.9-34.1)	23.9 ± 4.9	25.0 ± 2.8 (22.9-31.4)	ND	ND
Non-neoplastic C-cell hyperplasia (n=5)‡	27.1 ± 2.3 (24.4-29.4)	28.6 ± 1.2 (26.8-29.9)	25.2 ± 1.7 (23.3-27.6)	28.1 ± 2.0 (26.2-30.9)	28.4 ± 5.4	29.2 ± 1.0 (28.0-30.7)	ND	ND
Neoplastic C-cell hyperplasia (n=5)‡	25.9 ± 3.5 (22.3-30.4)	28.4 ± 2.4 (25.1-31.5)	24.4 ± 4.8 (19.3-30.1)	28.2 ± 4.2 (23.7-34.8)	29.7 ± 3.3††	27.0 ± 3.3 (22.6-30.9)	ND	ND

CT=cycle time (Note: higher CT value=lower gene expression); SD=standard deviation; GLP-1R= glucagon-like peptide-1 receptor; ND=undetected; MEN=multiple endocrine neoplasia; *Expression of β-actin and thyroglobulin was used for quality control; †Calcitonin gene expression was used as a marker for C-cells; ‡Study 1; §Detectable in only 5/10 samples; ¶Study 2; ‖Study 3; **n=2; ††Detectable in only 3/5 samples

Supported by: Sanofi

918

Occurrence of spontaneous pancreatic lesions in normal and diabetic rats may confound the non-clinical assessment of glucagon-like peptide (GLP)-1-elevating therapies

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Background and aims: GLP-1 therapeutics, including GLP-1 receptor agonists and dipeptidyl peptidase (DPP) 4 inhibitors, have glycemic and potential non-glycemic benefits for Type 2 diabetes, but recent literature reports raise concern for a potentially increased risk of pancreatitis and pancreatic cancer with these new therapies. Preclinical reports have attributed a variety of findings (including ductular metaplasia, exocrine pancreas degeneration and pancreatic duct abnormalities) to GLP-1 therapeutics in normal rats and rodent models of diabetes and/or pancreatitis. However, extensive non-clinical toxicology experience with the saxagliptin (DPP4 inhibitor) and exenatide (GLP-1 analog) has not demonstrated drug-related exacerbation of pancreatitis or neoplasia in any species (mouse, rat, dog, or monkey) compared to controls, with hundreds of animals being dosed for up to 2 years at exposure multiples up to 2300x therapeutic levels.
Materials and methods: We hypothesized that the lesions attributed to GLP-1 therapeutics in previous studies are commonly observed in the absence of drug treatment, similar to those observed in control groups in our studies. Therefore, we endeavored to characterize the incidence of spontaneous pancreatic lesions in 3 rat strains (Sprague-Dawley [SD], Zucker diabetic fatty [ZDF] and HIP rats [rats expressing human islet amyloid polypeptide]; n=36/group) under different feeding conditions (standard [std] or high fat [HF] diet) over a 4-month period.

Results: Pancreatic findings in all groups included focal exocrine degeneration, atrophy, inflammation, ductular cell proliferation and/or observations in large pancreatic ducts similar to those described in the literature with an incidence of exocrine atrophy/inflammation in SD (42% [HF]-72% [std] of rats) > HIP (39%) > ZDF (6%).
Conclusion: These data indicate that "pancreatitis" is a common background finding in rats that is independent of diet or glycemic status. Thus, many of the preclinical pancreatic lesions that are described as resulting from GLP-1 elevating therapies occur regularly in untreated normal and diabetic animals.

PS 072 Incretin-based agents: assessing the risk-benefit ratio

919

Efficacy and safety of dulaglutide versus placebo and exenatide in type 2 diabetes (AWARD-1)

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Background and aims: This Phase 3, randomised, parallel-arm study compared efficacy and safety of 2 doses of dulaglutide (DU), a long-acting GLP-1 receptor agonist, with exenatide (EX) or placebo (PL) in patients (pts) with type 2 diabetes treated with metformin (1500-3000 mg) and pioglitazone (30-45 mg).
Materials and methods: Pts (N=976; mean baseline [BL] characteristics: age, 55.7 years; HbA_{1c}, 8.1%; weight, 96.0 kg) were randomised (2:2:2:1 ratio) openly to twice-daily EX 10 µg or in a double-blind fashion to once-weekly DU 1.5 mg, DU 0.75 mg, or PL. After 26 weeks (wks), pts on PL were randomly assigned to either DU 1.5 mg or 0.75 mg for 26 additional wks. All others continued in originally assigned treatments for the study duration (52 wks). Primary hypothesis was that DU 1.5 mg is superior to PL for change in HbA_{1c} from BL to 26 wks.
Results: Both DU doses were superior to PL at 26 wks and to EX at 26 and 52 wks as measured by HbA_{1c} change. Incidence of serious adverse events (AEs) was similar among PL and DU groups. The rank order of incidence of gastrointestinal-related AEs among groups was: EX ~ DU 1.5 mg > DU 0.75 mg > PL. Documented symptomatic hypoglycaemia (<3.9 mmol/L) incidence was 3.2%, 4.3%, 12.3%, and 1.4% for DU 1.5 mg, DU 0.75 mg, EX, and PL, respectively.
Conclusion: Both once-weekly DU doses demonstrated superior glycaemic control compared to PL or EX and were well tolerated.

Table 1. Primary and Final Time Point Results (HbA_{1c} and Weight Change)

Primary Time Point (26 Weeks, ITT, LOCF)	DU 1.5 mg (N=279)	DU 0.75 mg (N=280)	EX 10 µg (N=276)	PL (N=141)
HbA_{1c} change (%), LS Mean (SE)	-1.51 (0.06)††·‡‡	-1.30 (0.06)††·‡‡	-0.99 (0.06)*	-0.46 (0.08)
% of patients with HbA_{1c} <7%	78.2*·#	65.8*·#	52.3*	42.9
Weight change (kg), LS Mean (SE)	-1.30 (0.29)*	0.20 (0.29)*·#	-1.07 (0.29)*	1.24 (0.37)
Final Time Point (52 Weeks, ITT, LOCF)				
HbA_{1c} change (%), LS Mean (SE)	-1.36 (0.08)††	-1.07 (0.08)††	-0.80 (0.08)	NA
% of patients with HbA_{1c} <7%	70.8#	59.1#	49.2	NA
Weight change (kg), LS Mean (SE)	-1.01 (0.38)	0.54 (0.38)#	-0.71 (0.38)	NA

Abbreviations: ITT = intent-to-treat; LOCF = last observation carried forward; LS = least squares; SE = standard error
††·‡‡ multiplicity adjusted 1-sided p<0.001 for superiority, vs EX or PL, respectively, for HbA_{1c} change only
*2-sided p<0.05 vs PL at 26 wks
#2-sided p<0.05 vs EX

Clinical Trial Registration Number: NCT01064687
Supported by: Eli Lilly and Company

920

Safety and efficacy of dulaglutide vs sitagliptin after 104 weeks in type 2 diabetes (AWARD-5)

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Background and aims: This Phase 3, adaptive, double-blind, parallel arm trial compared dulaglutide (DU), a once-weekly, long-acting glucagon-like

peptide 1 receptor agonist, with sitagliptin (sita) and placebo (PL) in metformin-treated type 2 diabetes patients.
Materials and methods: Primary time point was 52 weeks (wks); final time point was 104 wks. Patients (N=1098; mean baseline age 54 years; HbA_{1c} 8.1%; weight 86.4 kg; diabetes duration 7 years) were randomized to DU 1.5 mg or 0.75 mg, sita 100 mg, or PL (up to 26 wks).
Results: Similar proportions of DU and sita patients completed 104 wks. Adverse event (AE) was the most common reason for discontinuation. Both DU doses reported higher incidence of treatment-emergent AEs vs sita due to gastrointestinal (GI) events. The incidence of the most common GI treatment-emergent AEs for DU 1.5 mg, DU 0.75 mg and sita 100 mg, respectively, were nausea (17%, 15%, 7%), vomiting (14%, 8%, 4%) and diarrhoea (16%, 12%, 6%). Incidence of GI AEs peaked during the first 12 wks, with no difference after 26 wks. Both DU doses were superior vs sita for change in HbA_{1c} (Table 1).
Conclusion: The AE profile and superior HbA_{1c} vs sita after 104 wks indicate an acceptable benefit/risk profile of DU over a longer time.

Table 1: Summary of Efficacy Measures at 104 Weeks

Final Time Point Measures (104 Weeks, ITT, LOCF)	DU 1.5 mg (N=304)	DU 0.75 mg (N=302)	Sita 100 mg (N=315)
HbA_{1c} change (%), LS Mean (SE)	-0.99 (0.06)††	-0.71 (0.07)††	-0.32 (0.06)
% of patients with HbA_{1c} <7%	54.3#	44.8#	31.1
Weight change (kg), LS Mean (SE)	-2.88 (0.25)#	-2.39 (0.26)	-1.75 (0.25)

††multiplicity adjusted 1-sided p<0.001 for superiority vs sita for HbA_{1c} change only.
#2-sided p<0.001 vs sita.
ITT, intent to treat; LOCF, last observation carried forward.

Clinical Trial Registration Number: NCT00734474
Supported by: Eli Lilly and Company

921

Efficacy and safety of dulaglutide vs sitagliptin after 52 weeks in type 2 diabetes (AWARD5)
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Background and aims: This Phase 3, adaptive, double-blind, parallel-arm trial compared dulaglutide (DU), a once-weekly, long-acting glucagon-like peptide-1 receptor agonist, with sitagliptin (sita) and placebo (PL) in poorly-controlled metformin-treated patients with type 2 diabetes. The primary objective was to show noninferiority of DU 1.5 mg vs sita on change in HbA_{1c} at 52 weeks.
Materials and methods: Patients (N=1098, mean baseline age 54 years, HbA_{1c} 8.1% [65 mmol/mol], weight 86.4 kg, diabetes duration 7 years) were randomised to DU 1.5 mg, DU 0.75 mg, sita 100 mg, or PL (replaced with sita after 26 weeks) in a 2:2:2:1 ratio. The statistical analysis plan allowed for subsequent superiority testing once the noninferiority criterion for each of the doses relative to sita had been met. The study continued until 104 weeks.
Results: Both DU doses were superior to sita in reducing HbA_{1c} after 52 weeks (Table 1, Summary of Efficacy Measures at 26 Weeks and 52 Weeks). Rates of self-monitored hypoglycaemia (plasma glucose ≤3.9 mmol/L and/or symptoms; events/patient/year) were 1.6 (DU 1.5 mg), 1.7 (DU 0.75 mg), 1.6 (sita), and 1.2 (PL). No severe hypoglycaemia was reported. The most common gastrointestinal treatment-emergent adverse events for DU 1.5 mg and 0.75 mg, respectively, were nausea (17.4%, 13.9%), vomiting (12.8%, 7.6%), and diarrhoea (14.5%, 9.9%). Incidence of discontinuations due to adverse event or death was 11.2% (DU 1.5 mg), 7.6% (DU 0.75 mg), 10.2% (sita), and 16.4% (PL), most commonly due to hyperglycaemia.
Conclusion: In conclusion, both once-weekly DU doses demonstrated superior glycaemic control vs sita after 52 weeks with an acceptable tolerability and safety profile.

Table 1: Summary of Efficacy Measures at 26 Weeks and 52 Weeks

Placebo-Controlled Period (26 Weeks, ITT, LOCF)	DU 1.5 mg (N=304)	DU 0.75 mg (N=302)	Sita 100 mg (N=315)	PL (N=177)
HbA_{1c} change (%), LS Mean (SE)	-1.22 (0.05)‡‡,#	-1.01 (0.06)‡‡,#	-0.61 (0.05)*	0.03 (0.07)
% of patients with HbA_{1c} <7%	60.9*,#	55.2*,#	37.8*	21.0
Weight change (kg), LS Mean (SE)	-3.18 (0.18)*,#	-2.63 (0.19)*,#	-1.46 (0.18)	-1.47 (0.24)
Primary Time Point (52 Weeks, ITT, LOCF)				
HbA_{1c} change (%), LS Mean (SE)	-1.10 (0.06)††	-0.87 (0.06)††	-0.39 (0.06)	NA
% of patients with HbA_{1c} <7%	57.6#	48.8#	33.0	NA
Weight change (kg), LS Mean (SE)	-3.03 (0.22)#	-2.60 (0.23)#	-1.53 (0.22)	NA

ITT = Intent-to-treat, LOCF = last observation carried forward, LS = least squares, N = number of patients in a specified category, NA = not applicable, SE = standard error.
†† and ‡‡ multiplicity adjusted (based on tree-gatekeeping) 1-sided p<0.001 for superiority vs sita at 52 weeks or PL at 26 weeks, respectively, for HbA_{1c} change only.
*2-sided p<0.001 vs PL at 26 weeks.
#2-sided p<0.001 vs sita.

Clinical Trial Registration Number: NCT00734474
Supported by: Eli Lilly and Company

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Efficacy and safety of lixisenatide in elderly type 2 diabetes mellitus patients: subanalysis from the GetGoal programme
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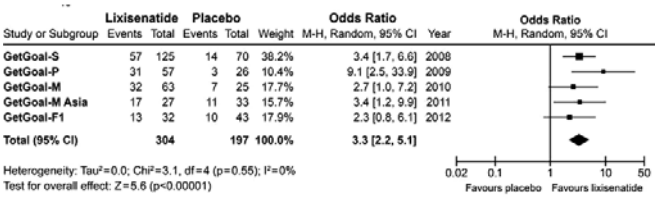
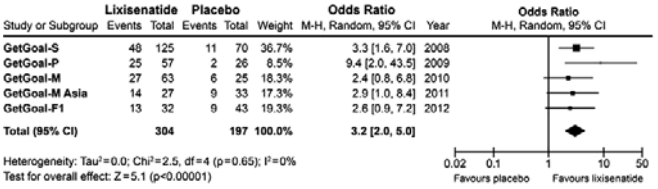
Background and aims: The number of elderly people with Type 2 diabetes mellitus (T2DM) is increasing. Therefore, the efficacy and safety of antidiabetic drugs for vulnerable patients is a key question for individualized treatment. Lixisenatide is a novel once-daily prandial glucagon-like peptide-1 (GLP-1) receptor agonist, which was granted marketing authorization in Europe in February 2013 for the treatment of T2DM.
Materials and methods: This meta-analysis of five Phase III lixisenatide randomized controlled trials (GetGoal-M, -P, -S, -M-Asia and -F1) evaluated lixisenatide as add-on to oral antidiabetic drugs compared with placebo in 501 elderly patients (≥65 years) with T2DM.
Results: At baseline, patients were aged 69.4 years; had BMI of 29.4 kg/m^2; mean diabetes duration of 10.6 years; HbA_{1c} of 8.0% (lixisenatide) and 7.9% (placebo); 90.0% of patients were treated with metformin, 44.7% with sulfonylurea and 16.6% with thiazolidinediones. After 24 weeks, lixisenatide significantly reduced HbA_{1c} compared with placebo (odds ratio [95% CI]: -0.5% [-0.7, -0.4], p<0.00001) and was statistically better than placebo in each of the following composite endpoints: proportion of patients achieving HbA_{1c} <7% (49.3 vs 22.8%, p<0.0001); HbA_{1c} <7% and no weight gain (41.8 vs 18.8%, p<0.0001); HbA_{1c} <7% and no documented symptomatic hypoglycaemia (44.4 vs 22.3%, p<0.0001); HbA_{1c} <7%, no documented symptomatic hypoglycaemia and no weight gain (37.2 vs 18.8%, p<0.0001). A higher trend for more frequent documented symptomatic hypoglycaemia was seen with lixisenatide compared with placebo (odds ratio [95% CI]: 2.1 [0.9, 5.0] p=0.09), mainly driven by sulfonylurea as background medication. There were no cases of severe hypoglycaemia in either group.
Conclusion: This *post hoc* analysis suggests that, in elderly patients with long-term T2DM inadequately controlled on oral antidiabetic drugs, lixisenatide significantly improved glycaemic control, particularly in composite assessments that indicate limited risk of weight gain or hypoglycaemia.

Figure. Meta-analysis of composite endpoints

A. HbA_{1C} <7%



B. HbA_{1C} <7% and no weight gain



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Effectiveness and tolerability with liraglutide among patients with type 2 diabetes. 1-year data from EVIDENCE: a 2-year, prospective, follow-up, post-marketing study

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Background and aims: Here we report 1-year data on the effectiveness and tolerability of the GLP-1 analogue liraglutide in the EVIDENCE observational, post-marketing study.
Materials and methods: EVIDENCE is a 2-year multicentre, observational, post-marketing outpatient study requested by the French National Health Authority in order to evaluate the efficacy and safety of liraglutide in routine clinical practice. The primary objective is to determine the percentage of patients still taking liraglutide and at HbA_{1c} target (<7%) after 2 years. EVIDENCE will also record data on characteristics of patients treated with liraglutide, how closely physicians follow instructions for use, treatment adherence, reasons for treatment discontinuation, change in HbA_{1c} and weight from baseline and long-term safety (over 2 years). Diabetologists and general practitioners in France recruited patients starting treatment with liraglutide. Patients and physicians completed questionnaires at study entry, 3 months and 6 months, then at 6-month intervals for a further 18 months. Statistical analyses were performed by Wilcoxon test on rank for paired quantitative variables. For quantitative variables a normality Kolmogorov-Smirnov test was used. For paired qualitative variables the McNemar test was used.
Results: Baseline data (mean±SD) were collected from 3137 patients (53% male, age 59±11 years, BMI 34±7 kg/m², duration of diabetes 10±6 years, HbA_{1c} 8.5±1.5%); 2433 (77.6%) patients were still in the study at 1 year. HbA_{1c} levels exceeded 7.0% (ADA/EASD target) in the majority of patients (n=2761, 88%) at baseline. The proportion of patients with HbA_{1c} <7% was significantly higher after 1 year's liraglutide treatment (n=850, 36.5%) vs. baseline (n=303, 9.8%; p<0.001). Following 1 year of liraglutide treatment, significant reductions in mean±SD HbA_{1c} (-1.04±0.98%, p<0.001), fasting plasma glucose (-0.36±0.60 g/L, p<0.001) and body weight (-3.56±5.78 kg, p<0.001) were observed from baseline. Gastrointestinal disorders (nausea, vomiting and diarrhoea) were the most frequent adverse events, reported by 247 patients (7.9%) treated with liraglutide; they were also the most common reason for withdrawal.
Conclusion: These results suggest that the efficacy of liraglutide in real world clinical practice is similar to that observed in randomised clinical trials (RCTs) (up to -1.5% HbA_{1c} reductions and -3.24 kg weight loss). However, the absence of a control arm makes it difficult to evaluate whether observed improvements are attributable to liraglutide alone. The slightly lower mean HbA_{1c} in this study may be due to differences in background therapies: in contrast with the RCTs there was no washout period prior to switching from an existing therapy to liraglutide in EVIDENCE. The incidence of gastrointestinal events was considerably lower than that reported in RCTs (up to 26.5%). In summary, 1-year results from the EVIDENCE study suggest that clinical trial data for liraglutide translate into therapeutic benefits in routine clinical practice.
Clinical Trial Registration Number: NCT01226966
Supported by: Novo Nordisk

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Quantification of the benefit-risk relationship between glycaemic control and hypoglycaemia: a comparison of exenatide once weekly with titrated insulin glargine

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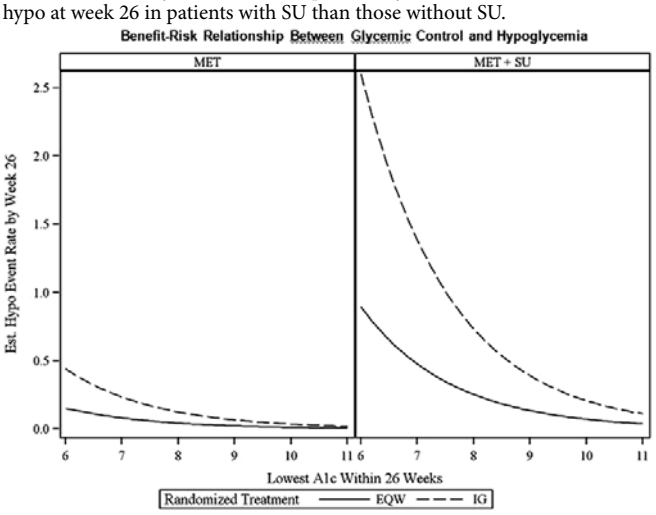
Background and aims: The DURATION-3 trial reported significantly greater HbA_{1c} reduction and lower risk of hypoglycemia (hypo) with the GLP-1 receptor agonist exenatide once weekly (EQW) compared with titrated once daily insulin glargine (IG) over 3 years. As the risk of hypo typically increases when better glucose control is achieved, these analyses further quantify the benefit-risk relationship between reduction in HbA_{1c} and incidence of hypo, as well as the impact of baseline characteristics.
Materials and methods: Exposure-adjusted event rates (EAER) of hypo were evaluated in relation to both the lowest and the weighted average HbA_{1c} over 26 weeks by Poisson regression models. Time to first hypo episode with EQW compared with IG, adjusted for end point HbA_{1c} (last observation carried forward), was evaluated by the Cox proportional hazard model. Baseline characteristics including age, gender, race, duration of diabetes, BMI, concomitant oral antihyperglycemic (OAH) use, and creatinine clearance, were examined as continuous covariates or categorical factors. Data beyond 26 weeks and up to 3 years were explored.
Results: The intent-to-treat (ITT) population comprised 233 EQW and 223 IG patients (mean baseline age 58 yrs, BMI 32.3 kg/m², duration of diabetes 7.9 yrs, HbA_{1c} 8.3%, OAH 70% metformin (MET) and 30% MET+sulfonylurea (SU). Results from the regression model showed that treatment (EQW or IG), concomitant OAH use (±SU), BMI, duration of diabetes, and HbA_{1c} (lowest or weighted average HbA_{1c} over 26 weeks) were statistically significant (P<0.05) factors for hypo EAER estimation. The EAER of hypo by week 26 with EQW, adjusted for the lowest HbA_{1c}, was 0.37 of that observed with IG. The same between-treatment risk ratio of hypo was reduced to 0.34 when OAH use was examined concurrently, with a 5.9 times hypo EAER in patients with SU compared with those without SU (figure). The risk of hypo in EQW to IG was 0.36 when BMI and duration of diabetes were further adjusted in the statistical model independently with higher risk in patients with lower BMI (1.38, <30 kg/m² vs ≥30 kg/m²) or longer duration of diabetes (1.35, ≥10 yrs of diabetes duration vs <10 yrs). Results from the hazard model showed that treatment, OAH use, and end point HbA_{1c} were statistically significant factors to predict the onset of the first hypo at week 26. The hazard ratio of first hypo between EQW and IG, adjusted for end point HbA_{1c}, was 0.33. The same hazard ratio was reduced slightly to 0.32 when OAH use was evaluated simultaneously with a 3.5 times probability on the occurrence of the first hypo at week 26 in patients with SU than those without SU.



Conclusion: Although the risk of hypo increased with the benefit of decreased HbA_{1c}, such risk was significantly reduced with EQW compared with IG. Further, regardless of EQW or IG, the EAER of hypo by week 26 was significantly higher in patients with concomitant SU use, lower BMI, or longer duration of diabetes, while incidence of hypo was significantly higher in patients with concomitant SU use.
Clinical Trial Registration Number: NCT00641056

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Efficacy and safety of low-dose sitagliptin (25 mg) in elderly (≥70 years old) Japanese patients with type 2 diabetes

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Background and aims: The standard dose of sitagliptin is 50 mg, but lower doses of 25 mg or 12.5 mg can be used in patients with decreased renal function. Low-dose administration of sitagliptin may be appropriate to improve blood glucose control in the elderly patients, who generally tend to have decreased renal function, but this approach has not been widely examined. Therefore, in this study, we examined the efficacy and safety of 25-mg sitagliptin in elderly patients with type 2 diabetes.
Materials and methods: The subjects were 39 Japanese outpatients (male: 23, female: 16) with type 2 diabetes who received administration of 25-mg sitagliptin for the first time at our hospital. All patients were aged ≥70 years old and their mean age was 78.5±4.82 years. The subjects included 13 patients who received 25-mg sitagliptin monotherapy and 26 who received 25-mg sitagliptin combination therapy with other anti-diabetic drugs. The control group included 39 younger Japanese outpatients (male: 27, female: 12) with type 2 diabetes who were matched for baseline HbA1c and BMI to those of the elderly subjects and received administration of 50-mg sitagliptin for the first time. All controls were aged ≤65 years old and their mean age was 54.9±6.99 years old. Among the 39 controls, 8 received 50-mg sitagliptin monotherapy and 31 received 50-mg sitagliptin combination therapy with other anti-diabetic drugs. Improvement of HbA1c from before to after 3 months of sitagliptin treatment and the incidence of adverse effects were compared between the two groups.
Results: HbA1c values before and after 3 months of sitagliptin treatment were 7.68% and 7.00%, respectively, in the 25-mg group, and 7.70% and 6.89%, respectively, in the 50-mg group, with both groups showing a significant improvement. There was no significant difference in improvement of HbA1c between the two groups. No serious hypoglycemia occurred in either group, but mild hypoglycemia occurred in 7 patients in the 25-mg group and 2 patients in the 50-mg group, with no significant difference between the groups. There were no changes in body weight in each group, no significant difference in digestive symptoms between the groups, and no changes in hepatic and renal functions in either group.
Conclusion: Administration of 25-mg sitagliptin in elderly Japanese patients (≥70 years old) with type 2 diabetes showed a similar effect on improvement of blood glucose control to that of 50-mg sitagliptin in patients aged ≤65 years old. There was a slight tendency for increased mild hypoglycemia in the elderly patients, but no significant difference in other side effects between the elderly and younger patients. Therefore, these results suggest that administration of 25-mg sitagliptin is effective and safe in elderly patients with type 2 diabetes. This low dosage may also be beneficial from a standpoint of medical costs.

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Renal safety of linagliptin in elderly patients with type 2 diabetes: analysis of pooled patient data from 7 phase 3 clinical trials

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Background and aims: Renal impairment (RI) is common in elderly patients with type 2 diabetes mellitus (T2DM), and progression of kidney disease is associated with increased morbidity and mortality. Renal safety is therefore an important consideration in the selection of appropriate treatments for this population. As several drugs are either contraindicated or require dose adjustment in patients with RI, it is important to identify well-tolerated treatments for elderly T2DM patients with or at risk of declining renal function. This analysis of pooled data from a large clinical trials programme investigated the efficacy and renal and overall safety of the DPP-4 inhibitor linagliptin in elderly patients with T2DM.
Materials and methods: This post hoc analysis evaluated data from 7 randomised, placebo-controlled Phase 3 trials of linagliptin 5 mg once daily as monotherapy or add-on to common glucose-lowering therapies for ≥24 weeks (including all subjects ≥65 years not receiving pioglitazone background therapy). Renal function was assessed by estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease formula.
Results: The pooled population comprised 1293 elderly patients randomised to either linagliptin (n=823) or placebo (n=470). Baseline characteristics (mean ± SD) were similar between the linagliptin and placebo groups: age, 71.1 ± 4.6 vs. 70.9 ± 4.7 years; HbA1c, 8.0 ± 0.8 vs. 8.1 ± 0.8%. In the linagliptin group, 56.4%, 14.8%, and 4.5% of patients had mild (eGFR 60 to <90 mL/min), moderate (eGFR 30 to <60 mL/min), and severe RI (eGFR <30 mL/min) with similar proportions found in the placebo group (51.3%, 18.7%, and 5.1%, respectively). Overall renal function was not significantly altered by treatment with linagliptin from baseline to Week 24 (adjusted mean ± SE eGFR [−1.8 ± 0.7 mL/min] vs. placebo [−1.1 ± 0.9 mL/min], resulting in a placebo-corrected difference of −0.7 ± 1.0 mL/min [95% CI: −2.6, 1.2; *P*=0.4912]). Changes in urine albumin-to-creatinine ratio trended toward improvement with linagliptin. In total, 71.3% and 72.8% of patients who received linagliptin or placebo, respectively, experienced adverse events (AEs). Drug-related AEs were less frequent with linagliptin (18.1%) than with placebo (20.0%). Renal and urinary AEs were experienced by 5.5% and 4.3% of linagliptin and placebo patients, respectively. Acute renal failure was a rare event and occurred in 0.5% and 0.2% of patients, respectively. Incidence of investigator-defined hypoglycaemia was lower in patients who received linagliptin (21.3%) compared with placebo (24.7%), with most events occurring in the trials that included a sulphonylurea or basal insulin as background therapy. Severe hypoglycaemic events were experienced by 1.0% and 1.7% of linagliptin and placebo patients, respectively. Linagliptin achieved placebo-corrected adjusted mean ± SE changes from baseline to Week 24 of −0.6 ± 0.1% (95% CI: −0.7, −0.5; *P*<0.0001) for HbA1c, and −0.8 ± 0.2 mmol/L (95% CI: −1.2, −0.5; *P*<0.0001) for fasting plasma glucose.
Conclusions: In an elderly patient population with renal function ranging from normal to severe RI, linagliptin was well tolerated, providing meaningful efficacy with a reassuring renal safety profile.
Clinical Trial Registration Number: NCT00621140; NCT00601250; NCT00602472; NCT00954447; NCT00800683; NCT00798161; NCT01084005
Supported by: Boehringer Ingelheim

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Clinical experience with vildagliptin in patients ≥ 75 years with type 2 diabetes mellitus and moderate or severe renal impairment

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Background and aims: Patients with type 2 diabetes (T2DM) are at increased risk for renal impairment (RI), and in addition there is an age-related decline in renal function. At the same time, T2DM treatment is more complex and treatment options are more limited in elderly patients as well as patients with RI, with the patient population ≥ 75 years with moderate or severe RI posing unique challenges, in particular the high risk and more severe consequences of hypoglycaemia. It was therefore of interest to assess the efficacy and tolerability of the dipeptidyl peptidase-4 (DPP-4) inhibitor vildagliptin in patients with T2DM ≥ 75 years who also have moderate or severe RI.
Materials and methods: This was a sub-analysis of data derived from a previously described randomised, double-blind, parallel-group, 24-week study (N=515) that included 105 patients (50 randomised to vildagliptin 50 mg qd and 55 to placebo) ≥ 75 years (mean age ~78 years) with T2DM and moderate or severe RI (baseline eGFR ~35 ml/min/1.73 m^2). Patients were treated mainly with either insulin (~55%) or OAD (~25%) monotherapy or a combination of insulin and OAD (~13%), and had long-standing disease (mean T2DM duration ~16 years).
Results: The adjusted mean change in HbA1c with vildagliptin was -1.0% from a baseline (BL) of 7.8% (between-group difference -0.8%; p<0.001). This improvement in glycaemic control was not associated with an increased risk

of hypoglycaemia; the rate of confirmed hypoglycaemia was 0.49 events per patient-year with vildagliptin and 0.96 events per patient-year with placebo (p=0.970). Weight remained stable with vildagliptin treatment (adjusted mean change from BL (81 kg) = 0.4 kg vs. -1.0 kg with placebo, p=0.015). Adverse events (AEs) (58.0 vs. 72.7%), serious AEs (14.0 vs. 16.4%), discontinuations due to AEs (4.0 vs. 9.1%) and deaths (0 vs. 5.5%) were reported at a comparable or lower frequency in patients receiving vildagliptin versus patients receiving placebo.
Conclusion: In this uniquely fragile elderly population ≥ 75 years with T2DM and moderate or severe renal impairment, vildagliptin was well tolerated and efficacious, with no increase in the rate of hypoglycaemia compared to placebo despite the marked improvement in glycaemic control.
Clinical Trial Registration Number: NCT00646542
Supported by:Novartis Pharma AG

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Treat 4 Ramadan trial: a randomised control trial comparing liraglutide vs a sulphonylurea as add-on to metformin in patients with established type 2 diabetes
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Background and aims: Treat 4 Ramadan was a randomised control trial comparing a sulphonylurea (SU) or Liraglutide (Lira) in combination with Metformin in patients on either mono or dual oral therapy with established Type 2 diabetes.
Materials and methods: 100 adults (50% male, mean age 52 years and BMI 32 Kg/m^2) were recruited from two UK sites (Leicester and Birmingham). Baseline data were collected ≥14 days prior to commencing their fast and follow-up at 4 (FU1) and 12 weeks (FU2) following Ramadan.
Results: Significantly more patients in the Lira compared to the SU group (FU1:37% vs. 7%, p=0.001; FU2:27% vs. 8%, p=0.03) achieved a composite endpoint of HbA1c <7%, weight reduction of ≥1 kg and no severe hypoglycaemia, defined as leading to hospitalisation. From a baseline of 7.7% there was no change in HbA1c at FU2 in the SU (+0.02%) compared to a 0.3% reduction in the Lira group (p=0.17). No significant differences in HbA1c were observed between prior mono (p=0.43) or dual therapy (p=0.14). Significant reductions were observed in body weight (-4.9 vs. +0.3kg, p=0.02) and diastolic BP (-6.2 vs. -0.06 mmHg, p=0.01) in the Lira compared to the SU group at FU2. More patients in the Lira group achieved the composites of weight reduction and improved HbA1c (p=0.04) and weight reduction and no severe hypoglycaemia (p=0.002) compared to SU. No severe hypoglycaemia was observed in any group.
Conclusion: Lira compared to SU appears to be a safe and well tolerated therapy in combination with Metformin during Ramadan with evidence of weight loss, no increase in severe hypoglycaemia and a trend towards improved HbA1c.
Clinical Trial Registration Number: 201100028427
Supported by: Novo Nordisk

PS 073 SGLT-2 clinical trials

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Verification of the efficacy and safety of tofogliflozin, a novel SGLT2 inhibitor, in Japanese patients with type 2 diabetes mellitus: results from a phase 2/3 clinical study
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Background and aims: Tofogliflozin, a highly selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, reduces blood glucose and body weight by inhibiting renal glucose reabsorption and promoting urinary excretion of excess blood glucose in type 2 diabetes mellitus (T2DM) patients. The characteristics of tofogliflozin, which include high selectivity toward SGLT2, a short half-life and an insulin-independent mode of action, mean that it exerts sustained efficacy with low hypoglycemic risk and can be used in combination with any existing T2DM therapy. This study was undertaken to verify the efficacy and safety of tofogliflozin in Japanese patients with T2DM with inadequate glycemic control on diet/exercise therapy.
Materials and methods: The study was designed as a randomized, double-blind, placebo-controlled, parallel-group comparison. Tofogliflozin 10, 20 or 40 mg (n=58 for each dose) or placebo (n=56) was orally administered once daily for 24 weeks to a total of 230 patients. The primary endpoint was the change from baseline in HbA1c.
Results: Tofogliflozin caused a statistically significant decrease in HbA1c at all doses tested (changes from baseline of 0.0%, −0.8%, −1.0%, and −0.9% in the placebo, 10 mg, 20 mg, and 40 mg groups, respectively; P<0.0001 vs. placebo). In addition, statistically significant decreases in fasting blood glucose (changes from baseline of -0.48, -1.77, -1.99, -1.80 mmol/L in the placebo, 10 mg, 20 mg, and 40 mg groups, respectively; P<0.0001 vs. placebo) and body weight (changes from baseline of −0.4, −2.2, −2.9, and −3.0 kg in the placebo, 10 mg, 20 mg, and 40 mg groups, respectively; P<0.0001 vs. placebo) were observed in the tofogliflozin groups, compared with the placebo group. Moreover, decreased blood pressure, improved HOMA-R and Matsuda index, reduced abdominal circumference, increased adiponectin and HDL-cholesterol levels, and decreased levels of uric acid, alanine aminotransferase and γ-glutamyl transferase were secondarily observed. Regarding safety, the incidences of adverse events were 44.6%, 60.3%, 53.4%, and 53.4% in the placebo, 10 mg, 20 mg, and 40 mg groups, respectively. Adverse drug reactions occurring at an incidence ≥5% higher than those in the placebo group included increased blood ketone bodies accompanying ketonuria, and pollakiuria. One patient (1.7%) in each of the 10 mg and 40 mg groups experienced symptoms suggesting hypoglycemia, while no patient in the placebo group and no patient in the 20 mg group experienced this adverse event. Tofogliflozin was well tolerated up to a dose of 40 mg.
Conclusion: In Japanese patients with T2DM with inadequate glycemic control on diet/exercise therapy, tofogliflozin, orally administered once daily for 24 weeks, exhibits significant blood glucose- and body weight-lowering effects and was well tolerated. Thus, the efficacy and safety of tofogliflozin has been verified.
Supported by: CHUGAI PHARMACEUTICAL CO.,LTD.

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Safety and efficacy of empagliflozin monotherapy in a 52-week study in Japanese patients with type 2 diabetes mellitus
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Background and aims: Empagliflozin (EMPA) is a potent and selective sodium glucose cotransporter 2 inhibitor in development for the treatment of type 2 diabetes mellitus (T2DM). A Phase II trial investigated the safety and efficacy of EMPA as monotherapy over 52 weeks in Japanese patients with T2DM.
Materials and methods: The study comprised a 12-week dose-finding period and a 40-week extension period. In the dose-finding period, patients (mean

age 57.5 [SD 9.9] years; mean BMI 25.5 [SD 3.9] kg/m^2) were randomised double-blind and treated with EMPA 5 mg (n=110), 10 mg (n=109), 25 mg (n=109) or 50 mg (n=110) qd or placebo (n=109) for 12 weeks. In the 40-week double-blind extension period, patients treated with EMPA 10 mg or 25 mg continued the same treatment and patients originally assigned placebo, EMPA 5 mg or 50 mg were re-allocated to EMPA 10 mg or 25 mg. Efficacy is reported for patients who took EMPA 10 mg or 25 mg in both treatment periods (i.e. for 52 weeks; n=109 each). Safety is reported for all patients who took at least one dose of EMPA 10 mg (n=267) or 25 mg (n=265) during the 52-week study.

Results: Treatment with EMPA for 52 weeks led to sustained reductions from baseline in HbA_{1c} and fasting plasma glucose (FPG) that were dose dependent, and in body weight, systolic BP (SBP) and diastolic BP (DBP) (Table). Adverse events (AEs) were reported in 70.8% of patients on EMPA 10 mg and 66.8% on EMPA 25 mg. Confirmed hypoglycaemic AEs (≤3.9 mmol/l and/or requiring assistance) was rare (1 patient in each of the EMPA 10 mg and 25 mg groups) and no patients required assistance. AEs consistent with urinary tract infection were reported in 7 patients (2.6%) on EMPA 10 mg and 3 patients (1.1%) on EMPA 25 mg. AEs consistent with genital infection were reported in 8 patients (3.0%) on EMPA 10 mg and 2 patients (0.8%) on EMPA 25 mg. AEs consistent with volume depletion were reported in 3 patients (1.1%) on EMPA 10 mg and 3 patients (1.1%) on EMPA 25 mg.

Conclusion: EMPA monotherapy led to sustained reductions in HbA_{1c}, FPG, body weight, SBP and DBP over 52 weeks in Japanese patients with T2DM, and was well tolerated.

Table. Efficacy (Adjusted Mean Difference From Baseline [SE]) and Adverse Event (%) Findings at 52 Weeks

	Albiglutide	Pioglitazone	Placebo
Participants (n)	269	273	115
HbA_{1c} (%)	−0.55 (0.06)	−0.80 (0.06)[a]	+0.33 (0.08)[b]
FPG (mg/dL)	−12.4 (2.9)	−31.4 (2.9)[b]	+11.5 (4.4)[b]
Weight (kg)	−0.4 (0.2)	+4.4 (0.2)[b]	−0.4 (0.4)[c]
Adverse events (% participants)			
Diarrhoea	8.9	5.4	2.6
Nausea/vomiting	9.6/2.6	4.3/1.8	3.5/0.9
Injection site reactions	12.9	3.2	3.5
Documented symptomatic hypoglycaemia[d]	13.7	25.3	7.0
Severe hypoglycaemia[d]	0.4	1.1	0.0

[a]Last observation prior to study discontinuation or hyperglycaemia rescue carried forward. Treatment comparison based on analysis of covariance model was adjusted for baseline HbA_{1c}, region, history of prior myocardial infarction, and age category; *p* (non-inferiority) not significant (albiglutide not noninferior to pioglitazone)
[b]*p* <.001 vs albiglutide; [c]Not significant; [d]Events prior to addition of hyperglycaemia rescue meds

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Empagliflozin as add-on to basal insulin for 78 weeks improves glycaemic control with weight loss in insulin-treated type 2 diabetes mellitus

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Background and aims: Empagliflozin (EMPA) is a selective SGLT2 inhibitor in development for the treatment of T2DM. We assessed the efficacy and safety of EMPA added-on to basal insulin in patients with T2DM.

Materials and methods: Patients with T2DM (mean age 58.8 yrs; HbA_{1c} 8.2%; BMI 32.2 kg/m^2) were randomized double-blind to receive EMPA 10 mg (n=169) or 25 mg qd (n=155) or placebo (PBO; n=170) for 78 weeks. Basal insulin dose remained constant for the first 18 weeks, then adjustments were allowed at investigator discretion. Primary endpoint was change from baseline in HbA_{1c} at week 18. Key secondary endpoints were changes from baseline in insulin dose and HbA_{1c} at week 78.

Results: EMPA significantly reduced HbA_{1c} at week 18 and 78, and insulin dose at week 78, vs PBO (Table). Further analyses showed reductions in FPG, body weight, and systolic BP (Table). Hypoglycemia (glucose ≤70 mg/dL and/or requiring assistance) was reported similarly in 36.1% of patients on EMPA 10 mg and 25 mg and 35.3% on PBO; 2 patients on EMPA 25 mg required assistance. AEs consistent with urinary tract infection were reported in 14.8%, 11.6% and 8.8% on EMPA 10 mg, EMPA 25 mg and PBO, respectively. AEs consistent with genital infection were reported in 7.7%, 5.2% and 1.8% on EMPA 10 mg, EMPA 25 mg and PBO, respectively.

Conclusion: EMPA 10 mg and 25 mg for 78 weeks as add-on to basal insulin improved glycemic control, led to reductions in body weight without increase in hypoglycemia risk, and were well tolerated except for increased genitourinary infections.

	Placebo	Empagliflozin 10 mg	Empagliflozin 25 mg
Baseline ±SE HbA_{1c} week 18 analysis set (%)	8.10±0.07	8.26±0.07	8.34±0.08
Change from baseline ±SE in HbA_{1c} at week 18 (%)	-0.01±0.07	-0.57±0.07***	-0.71±0.07***
Baseline ±SE HbA_{1c} week 78 analysis set (%)	8.09±0.07	8.27±0.07	8.29±0.08
Change from baseline ±SE in HbA_{1c} at week 78 (%)	-0.02±0.09	-0.48±0.08***	-0.64±0.09***
Baseline ±SE insulin dose (IU)	47.84±3.11	45.13±2.62	48.43±2.79
Change from baseline ±SE in insulin dose at week 78 (IU)	5.45±1.58	-1.21±1.48**	-0.47±1.59**
Baseline ±SE FPG (mg/dL)	142±4	138±4	146±4
Change from baseline ±SE in FPG at week 78 (mg/dL)	3±3	-10±3**	-15±3***
Baseline ±SE body weight (kg)	90.5±1.7	91.6±1.5	94.7±1.7
Change from baseline ±SE in body weight at week 78 (kg)	0.7±0.5	-2.2±0.5***	-2.0±0.5***

HbA_{1c} and insulin dose: Adjusted means based on ANCOVA in full analysis set of "completers" (patients who completed minimum treatment duration of 119 days [for week 18 data] and 532 days [for week 78 data]) with last observation carried forward imputation.
FPG and body weight: Adjusted means based on ANCOVA in full analysis set (all treated patients with baseline HbA_{1c}) with last observation carried forward imputation.
**p<0.01 vs placebo; ***p<0.001 vs placebo.

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932

Metabolic response to sodium-glucose transporter 2 (SGLT2) inhibition with empagliflozin in patients with type 2 diabetes

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Background and aims: SGLT2 inhibitors lower plasma glucose by enhancing urinary glucose excretion. The physiologic response to pharmacologically-induced glycosuria has not been investigated.

Materials and methods: We studied 66 T2D patients (62 ± 7 years, BMI = 31.6 ± 4.5 kg/m^2, HbA_{1c} = 7.2 ± 0.2%, mean ± SD) at baseline, after a single dose (25 mg, Study I), and following 4 weeks of treatment with empagliflozin (25 mg/day, Study II). For each study, patients received a mixed meal coupled with double-tracer glucose administration and indirect calorimetry.

Results: Compared to baseline, Study I caused glycosuria (7.8 [4.4] g over 3 hrs of fasting, median [IQR]), which led to an increase in endogenous glucose production (EGP, 13.8 [5.2] to 17.6 [4.8] $\mu mol \cdot kg_{FFM}^{-1} \cdot min^{-1}$, p<0.0001) matching the glycosuria. These fasting-state changes were maintained in Study II. Postmeal glycosuria rose to 29.0 [12.5] and 28.2 [15.4] g over 5 hrs in Study I and Study II, respectively. Correspondingly, postmeal glucose AUC (51 [11] and 51 [10] *vs* 57 [16] g/dL) and insulin AUC (80 [59] and 76 [59] *vs* 93 [68] nmol/L) dropped, whereas the glucagon response rose (6.5 [2.1] and 5.6 [1.8] *vs* 5.2 [1.6] nmol/L) (all p<0.001). While appearance of oral glucose was unchanged, postmeal EGP was increased (AUC = 40 [14] and 37 [11] *vs* 34 [11] g, both p<0.01). Tissue glucose disposal (= total glucose disposal minus glycosuria) was reduced (75 [16] and 70 [21] *vs* 93 [18] g, p<0.0001) due to a decrease in both glucose oxidation and non-oxidative glucose disposal, with a concomitant rise in lipid oxidation (all p<0.01). ß-cell glucose sensitivity improved (55 [35] and 55 [39] *vs* 44 [32] $pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$, p<0.0001), while insulin sensitivity was unchanged (9.1 [6.7] and 8.6 [8.0] *vs* 8.2[5.8] $ml \cdot kg_{FFM}^{-1} \cdot min^{-1} \cdot nM^{-1}$, p=ns).

Conclusion: In T2D patients empagliflozin lowers fasting and postprandial glycaemia by (a) increasing total glucose removal despite a compensatory in-

crease in endogenous glucose production, (b) improving ß-cell function, and (c) shifting substrate utilization from glucose to lipid.
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Canagliflozin added on to dipeptidyl peptidase-4 inhibitors or glucagon-like peptide-1 agonists with or without other antihyperglycaemic agents in type 2 diabetes mellitus

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Background and aims: Canagliflozin (CANA) is a sodium glucose co-transporter 2 (SGLT2) inhibitor developed for the treatment of patients with type 2 diabetes mellitus (T2DM). The efficacy and safety of CANA were evaluated in subjects with T2DM who were on dipeptidyl peptidase-4 inhibitors (DPP-4i) or glucagon-like peptide-1 (GLP-1) agonists as monotherapy or in combination with other antihyperglycaemic agents (AHAs).
Materials and methods: This post hoc analysis of data from the CANagliflozin cardioVascular Assessment Study (CANVAS; subjects with T2DM who had a history or high risk of cardiovascular disease) evaluated CANA 100 and 300 mg compared with placebo (PBO) in subsets of subjects who were on DPP-4i (N = 316; mean age, 63 years; HbA_{1c}, 8.1%; body mass index [BMI], 32.3 kg/m^2) or GLP-1 agonists (N = 95; mean age, 61 years; HbA_{1c}, 8.1%; BMI, 37.4 kg/m^2) over 18 weeks of therapy.
Results: At Week 18, CANA 100 and 300 mg reduced HbA_{1c} and body weight compared with PBO in both the DPP-4i and GLP-1 agonist subsets (Table). The overall incidence of adverse events (AEs) was higher with CANA than with PBO in the DPP-4i subset, and comparable or lower with CANA relative to PBO in the GLP-1 agonist subset. Rates of serious AEs were generally higher with CANA than with PBO in both subsets. The proportion of subjects with documented hypoglycaemia episodes was higher with CANA 100 and 300 mg than PBO among those who were on insulin, sulphonylurea, or meglitinide in the DPP-4i subset (17/70, 29/87, and 12/74, respectively) and the GLP-1 agonist subset (11/29, 11/22, and 4/26, respectively); only 2 and 1 CANA-treated subjects who were not on these concomitant agents reported documented hypoglycaemia episodes in the DPP-4i and GLP-1 agonist subsets, respectively.
Conclusion: CANA added on to DPP-4i or GLP-1 agonists (with or without other AHAs) lowered HbA_{1c}, reduced body weight, and was generally well tolerated in subjects with T2DM at 18 weeks.

Table. Summary of Efficacy (mITT, LOCF) and Safety (Safety Population) Endpoints at Week 18

	DPP-4 inhibitor subset			GLP-1 agonist subset		
	PBO (n = 102)	CANA 100 mg (n = 103)	CANA 300 mg (n = 111)	PBO (n = 30)	CANA 100 mg (n = 35)	CANA 300 mg (n = 30)
Efficacy Parameter						
HbA_{1c} baseline, %	8.1 (1.0)	8.1 (0.8)	8.0 (0.8)	7.9 (0.9)	8.2 (0.8)	8.3 (1.1)
Change	0.10	−0.46	−0.64	0.17	−0.83	−0.89
Difference vs PBO		−0.56 (−0.77, −0.35)	−0.75 (−0.95, −0.54)		−1.00 (−1.35, −0.65)	−1.06 (−1.43, −0.69)
Body weight baseline, kg	88.6 (19.1)	91.5 (18.3)	92.4 (18.0)	105.6 (19.3)	109.2 (23.3)	111.2 (23.1)
% change	−0.9	−3.2	−3.9	−0.4	−3.0	−3.7
Difference vs PBO		−2.3 (−3.1, −1.5)	−3.0 (−3.8, −2.2)		−2.5 (−3.7, −1.4)	−3.2 (−4.5, −2.0)
Safety Parameter, n (%)						
Any AE	60 (58.8)	66 (64.1)	70 (63.1)	22 (73.3)	22 (62.9)	22 (73.3)
AE leading to discontinuation	1 (1.0)	1 (1.0)	6 (5.4)	0	2 (5.7)	3 (10.0)
AE related to study drug	14 (13.7)	21 (20.4)	29 (26.1)	7 (23.3)	10 (28.6)	11 (36.7)
Serious AE	2 (2.0)	3 (2.9)	5 (4.5)	1 (3.3)	2 (5.7)	4 (13.3)

mITT, modified intent to treat; LOCF, last observation carried forward; SD, standard deviation; LS, least squares; ANCOVA, analysis of covariance; CI, confidence intervals. *Mean (SD) baseline value, LS mean change from baseline using ANCOVA, and PBO-subtracted LS mean (95% CI) values.

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Efficacy and safety of canagliflozin in subjects with type 2 diabetes mellitus inadequately controlled with metformin plus sulphonylurea over 52 weeks

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Background and aims: Canagliflozin (CANA) is a sodium glucose co-transporter 2 inhibitor developed for the treatment of patients with type 2 diabetes mellitus (T2DM). This 52-week study assessed the efficacy and safety of CANA in subjects with T2DM on background metformin (MET) plus sulphonylurea (SU) therapy.
Materials and methods: In this randomised, double-blind, placebo (PBO)-controlled, Phase 3 study, subjects with T2DM on MET + SU (N = 469) received CANA 100 or 300 mg or PBO once daily during a 26-week core period followed by a 26-week extension (n = 353). Changes from baseline in glycaemic and other efficacy parameters at Week 52 are reported. Adverse events (AEs) were recorded throughout the study.
Results: Mean baseline characteristics were similar across groups (age, 56.8 y; HbA_{1c}, 8.1%; fasting plasma glucose [FPG], 9.5 mmol/L; body mass index, 33.1 kg/m^2). Over 52 weeks, CANA 100 and 300 mg reduced HbA_{1c} and FPG compared with PBO (Table). More PBO-treated subjects received rescue therapy (24.4%) than those treated with CANA 100 or 300 mg (8.9% and 5.1%). Both CANA doses reduced body weight and systolic blood pressure (BP) and increased high-density lipoprotein cholesterol (HDL-C) compared with PBO; increases in low-density lipoprotein cholesterol (LDL-C) and non-HDL-C were seen with CANA 300 mg versus PBO. Overall incidence of AEs was slightly lower with CANA 100 mg than CANA 300 mg and PBO (67.5%, 73.1%, 71.2%). Serious AE rates were lower with CANA 100 mg (4.5%) and 300 mg (5.1%) than PBO (8.3%). Rates of AE-related discontinuations were higher with CANA 100 mg (7.0%) and 300 mg (7.7%) than PBO (4.5%). CANA 100 and 300 mg were associated with higher rates than PBO of genital mycotic infections in women (18.5%, 18.8%, 5.0%) and men (7.9%, 5.7%, 1.3%). Incidences of osmotic diuresis-related AEs (eg, pollakiuria, polyuria) were higher with CANA 100 and 300 mg than PBO (5.7%, 7.1%, 1.9%); none led to study discontinuation. Urinary tract infection rates were slightly higher with CANA 100 and 300 mg than PBO (8.3%, 8.3%, 7.7%). Incidences of AEs related to reduced intravascular volume (eg, postural dizziness, orthostatic hypotension) were 0.6%, 3.8%, and 1.9% with CANA 100 and 300 mg and PBO. Rates of documented hypoglycaemia were higher with CANA 100 and 300 mg than PBO (33.8%, 36.5%, 17.9%); the number of subjects with severe hypoglycaemia episodes was small (1 [0.6%] per group).
Conclusion: CANA 100 and 300 mg improved glycaemic control, reduced body weight, and were generally well tolerated in subjects with T2DM inadequately controlled with MET + SU over 52 weeks.

Table. Summary of Efficacy Endpoints at Week 52 (mITT, LOCF)

Parameter*	CANA 100 mg	CANA 300 mg	PBO
HbA_{1c} change, %	−0.74 (0.08)	−0.96 (0.08)	0.01 (0.08)
Difference vs PBO	−0.75 (−0.95, −0.55)	−0.97 (−1.17, −0.77)	
% of subjects reaching HbA_{1c} <7.0%†	39.4 (3.9)	52.6 (4.1)	18.7 (3.2)
Difference vs PBO	20.7 (10.1, 31.2)	34.0 (23.2, 44.7)	
FPG change, mmol/L	−1.1 (0.2)	−1.5 (0.2)	0.6 (0.2)
Difference vs PBO	−1.6 (−2.1, −1.1)	−2.1 (−2.6, −1.6)	
Body weight % change	−2.2 (0.3)	−3.2 (0.3)	−0.9 (0.3)
Difference vs PBO	−1.3 (−2.1, −0.5)	−2.2 (−3.0, −1.4)	
Systolic BP change, mmHg	−3.7 (1.0)	−2.9 (1.0)	0.1 (1.0)
Difference vs PBO	−3.7 (−6.2, −1.3)	−3.0 (−5.5, −0.5)	
Diastolic BP change, mmHg	−2.2 (0.6)	−1.7 (0.6)	−0.7 (0.6)
Difference vs PBO	−1.6 (−3.2, 0.1)	−1.1 (−2.7, 0.5)	
Triglycerides % change	8.5 (4.6)	6.7 (4.5)	4.7 (4.6)
Difference vs PBO	3.8 (−7.8, 15.4)	2.0 (−9.6, 13.6)	
HDL-C % change	6.6 (1.3)	8.2 (1.3)	3.3 (1.3)
Difference vs PBO	3.2 (−0.1, 6.5)	4.9 (1.6, 8.2)	
LDL-C % change	4.8 (2.8)	13.3 (2.8)	5.4 (2.8)
Difference vs PBO	−0.6 (−7.7, 6.5)	7.9 (0.8, 15.0)	
LDL-C/HDL-C % change	−0.3 (2.7)	5.1 (2.6)	3.7 (2.7)
Difference vs PBO	−4.0 (−10.8, 2.8)	1.4 (−5.4, 8.2)	
Non–HDL-C % change	2.5 (2.1)	7.5 (2.1)	3.9 (2.1)
Difference vs PBO	−1.5 (−6.9, 3.9)	3.6 (−1.8, 9.0)	

mITT, modified intent to treat; LOCF, last observation carried forward; LS, least squares; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. *LS mean (SE) change from baseline using ANCOVA and PBO-subtracted LS mean (95% CI) for all parameters except for % of subjects reaching HbA_{1c} <7.0%; †% (SE) and PBO-subtracted % (95% CI) of subjects reaching HbA_{1c} <7.0%.

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Efficacy and safety of canagliflozin monotherapy in subjects with type 2 diabetes mellitus inadequately controlled with diet and exercise over 52 weeks

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Background and aims: Canagliflozin (CANA) is an SGLT2 inhibitor developed for the treatment of type 2 diabetes mellitus (T2DM). This study assessed the efficacy and safety of CANA in subjects with T2DM who have inadequate glycaemic control with diet and exercise over 52 weeks.
Materials and methods: In this randomised, double-blind, Phase 3 study, subjects with T2DM (N = 584) received CANA 100 or 300 mg or PBO once daily during a PBO-controlled, 26-week core period followed by a 26-week extension (n = 451; blinded switch of PBO group to sitagliptin 100 mg [PBO/SITA; n = 119]). Changes from baseline in efficacy parameters at Week 52 are reported for CANA 100 and 300 mg (SITA was used only to maintain the double-blind and control group, not as an efficacy comparator). Safety data are reported for CANA and PBO/SITA at 52 weeks.
Results: Mean baseline characteristics were similar across groups (age, 55.4 y; HbA_{1c}, 8.0%; fasting plasma glucose [FPG], 9.5 mmol/L; body mass index, 31.6 kg/m^2). At Week 52, CANA showed numerical, dose-related reductions from baseline in HbA_{1c}, FPG, and body weight (Table). Both CANA doses were associated with decreases in systolic blood pressure (BP) and triglycerides, and increases in high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). From Weeks 26 to 52, changes in HbA_{1c}, body weight, and HDL-C with CANA were generally stable, with small increases observed in LDL-C. Over 52 weeks, overall AE rates with CANA 100 and 300 mg and PBO/SITA were 67.2%, 66.0%, and 64.1%, respectively. Serious AE rates were lower with CANA 300 mg than CANA 100 mg and PBO/SITA (2.5%, 5.6%, 5.7%). Rates of AE-related discontinuations were higher with CANA 100 and 300 mg than PBO/SITA (3.1%, 2.0%, 1.0%). CANA 100 and 300 mg were associated with higher rates than PBO/SITA of genital mycotic infections in women (11.4%, 9.3%, 4.8%) and men (6.2%, 9.0%, 0%). Urinary tract infection rates were slightly higher with CANA 100 and 300 mg than PBO/SITA (8.2%, 7.1%, 6.3%). Incidences of osmotic diuresis-related AEs (eg, pollakiuria, polyuria; 4.6%, 7.6%, 2.1%) and AEs related to reduced intravascular volume (eg, postural dizziness, orthostatic hypotension; 1.5%, 2.0%, 0.5%) were higher with CANA 100 and 300 mg than PBO/SITA, but generally did not lead to discontinuation. Hypoglycaemia rates were low across groups (CANA 100 mg, 5.1%; CANA 300 mg, 3.6%; PBO/SITA, 3.6%).
Conclusion: CANA 100 and 300 mg provided improvement in glycaemic control and body weight reduction, and were generally well tolerated in subjects with T2DM inadequately controlled with diet and exercise over 52 weeks.

Table. Summary of Efficacy Endpoints at Week 52 (Extension mITT*, LOCF)

Parameter†	CANA 100 mg	CANA 300 mg
HbA_{1c} change, %	–0.81 (0.07)	–1.11 (0.07)
% of subjects reaching HbA_{1c} <7.0%	52.4 (3.9)	64.5 (3.7)
FPG change, mmol/L	–1.5 (0.1)	–2.2 (0.1)
Body weight % change	–3.3 (0.3)	–4.4 (0.3)
Systolic BP change, mmHg	–1.4 (0.8)	–3.9 (0.8)
Diastolic BP change, mmHg	–0.4 (0.6)	–0.7 (0.6)
Triglycerides % change	–2.0 (3.0)	–2.1 (3.0)
HDL-C % change	11.1 (1.5)	14.7 (1.5)
LDL-C % change	6.3 (2.1)	11.2 (2.1)
LDL-C/HDL-C % change	–3.1 (2.1)	–0.1 (2.1)
Non–HDL-C % change	1.8 (1.7)	5.6 (1.7)

mITT, modified intent to treat; LOCF, last observation carried forward; ANCOVA, analysis of covariance; SE, standard error. *Including all subjects who were randomised to a treatment group on Day 1, took ≥1 dose of study drug, did not receive rescue medication in the core period, and entered the extension period; †Least squares mean (SE) change from baseline using ANCOVA for all parameters except for % of subjects reaching HbA_{1c} <7.0%.

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Durability of dapagliflozin vs glipizide as add-on therapies in type 2 diabetes inadequately controlled on metformin: 4-year data

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Background and aims: Dapagliflozin (DAPA), a selective SGLT2 inhibitor, lowers blood glucose by increasing urinary glucose excretion in an insulin-independent manner. Results from a randomised, double-blind trial of DAPA (≤10 mg/d, n=406) vs glipizide (GLIP; ≤20 mg/d, n=408) as add-on to metformin (MET; median 2000 mg/d) in T2DM, have been previously reported. DAPA was non-inferior to GLIP in HbA1c change at 52 weeks (primary endpoint; both −0.52%), with the added benefits of weight loss and reduced hypoglycaemia (hypo). Here we report 4-year data from this study, the longest duration of therapy studied for any SGLT2 inhibitor to date.
Materials and methods: In the double-blind extension of this study, patients continued to receive DAPA (n=204) and GLIP (n=188) added to MET. In Year 2, one up-titration was allowed if HbA1c ≥7%. In Years 3 and 4, one up-titration was allowed before rescue (DPP4-inhibitor, insulin and pioglitazone [only South Africa]); rescue may occur if HbA1c 7.0-<8.0% and must occur if HbA1c ≥8.0%. Efficacy was analyzed by a repeated measures mixed model, excludes data after rescue and are reported as adjusted mean changes (95% CI). Safety was assessed throughout and includes data after rescue.
Results: Mean baseline HbA1c was 7.72%. Of the 814 patients entering the study, 161 (39.7%) DAPA and 141 (34.6%) GLIP patients completed Year 4. Effect of therapy on HbA1c attenuated over time in both groups, but DAPA showed more persistent metabolic benefits vs GLIP up to Year 4 (change from baseline in HbA1c of −0.10 vs +0.20%): treatment difference of −0.30% (95% CI: −0.51, −0.09). Sustained and stable weight loss was seen with DAPA vs weight gain with GLIP (−3.95 vs +1.12 kg): difference of −5.07 kg (95% CI: −6.21, −3.93). More DAPA vs GLIP patients achieved a ≥5% weight loss (10.2% vs 1.8%) at Year 4. Mean systolic BP was reduced with DAPA but not with GLIP: difference of −3.7 mmHg (95% CI: −5.9, −1.4). Rate of patients with hypo was ~10-fold less with DAPA (5.4%) vs GLIP (51.5%); most patients first presented during Year 1. All major hypos (n=3) were with GLIP. There were no discontinuations due to hypo with DAPA. Overall frequencies of AEs and SAEs were similar between groups; 87.7% and 18.5% for DAPA vs 87.0% and 19.9% for GLIP. Discontinuation due to AEs was 13.3% for DAPA vs 11.3% for GLIP. Proportion of patients reporting urinary tract infections (UTI) was 13.5% for DAPA (8.8% male; 19.4% female) vs 9.3% for GLIP (5.8% male; 13.5% female). Upper UTI occurred in 1 patient receiving DAPA vs 3 patients receiving GLIP. Genital infections (GenI) occurred in 14.3% of DAPA patients (7.5% male; 22.8% female) vs 2.9% of GLIP patients (0.4% male; 5.9% female). The majority of patients with GenI and UTI first presented during Year 1. The majority of events were of mild/moderate intensity and resolved with standard treatment. There were no signs of deteriorating renal function as measured by eGFR over 4 years.
Conclusion: DAPA demonstrated sustained metabolic benefits, including stable weight loss and BP reductions with low rates of hypo, compared with GLIP over 4 years of treatment. DAPA was well-tolerated throughout, with no new safety signals identified.
Clinical Trial Registration Number: NCT00660907
Supported by: BMS/AZ

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Dapagliflozin improves glycaemic control and reduces body weight as add-on therapy to metformin plus sulphonylurea

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Background and aims: Dapagliflozin (DAPA) is a highly selective inhibitor of sodium glucose co-transporter 2 (SGLT2), the primary transporter responsible for mediating renal glucose reabsorption. DAPA has been shown to be well tolerated and effective in improving glycaemic control and promoting body weight loss in patients with type 2 diabetes (T2DM) both as monotherapy and in combination with other anti-hyperglycaemic medications. The aim of this 24 week randomised, double-blind, parallel-group phase III study, with

an on-going 28 week blinded extension period, was to evaluate the efficacy and safety of DAPA in patients with T2DM inadequately controlled with the combination of metformin (MET) plus sulphonylurea (SU).

Materials and methods: Men and women aged ≥18 years, receiving a maximum tolerated dose of SU (≥ half maximum dose) and MET (≥1500 mg QD), were randomised to receive double-blind DAPA 10 mg/d (N=108) or placebo (PBO, N=108) for 24 weeks.

Results: Demographic and baseline characteristics were generally balanced between DAPA and PBO groups: 55.6% and 42.6% were men, mean patient age was 61.1 and 60.9 years and mean T2DM duration was 9.28 and 9.62 years. At week 24, glycaemic measures (mean HbA1c and mean fasting plasma glucose [FPG]) were significantly improved with DAPA versus PBO (Table). In addition, a greater proportion of patients achieved a therapeutic glycaemic response, defined as the achievement of HbA1c <7.0%, following treatment for 24 weeks with DAPA (31.8%) versus PBO (11.1%). Total body weight was significantly reduced over 24 weeks with DAPA versus PBO. A significant reduction in seated systolic blood pressure (SBP) was observed at week 8 in patients treated with DAPA versus PBO. This change in SBP was maintained out to week 24. Overall, 48.6% of patients treated with DAPA and 51.4% with PBO experienced ≥1 adverse event that were mostly mild or moderate in intensity and unrelated to study treatment. Hypoglycaemic events were experienced by 12.8% of patients in the DAPA group versus 3.7% with PBO. In patients receiving DAPA, 6.4% had a down-titration of SU compared to 3.7% with PBO. Events of genital infection were experienced by 5.5% of patients receiving DAPA and none with PBO. Over 24 weeks, events of urinary tract infection were reported by 6.4% of patients in both groups. One event of pyelonephritis was observed in the DAPA group.

Conclusion: DAPA was well tolerated and effective over 24 weeks when administered as an add-on to MET therapy in the presence of SU.

Table

	PBO (N=108)	DAPA (N=108)
Mean BL HbA1c, % (SD), LA	8.24 (0.87)	8.08 (0.91)
Adj. mean change from BL to week 24 in HbA1c, % (95% CI), LA [*P* value vs placebo]	-0.17 (-0.31, -0.02) --	-0.86 (-1.00, -0.72) *P*<0.0001
Mean BL FPG, mg/dL [mmol/l] (SD), LOCF	180.49 (43.26) [10.02 (2.40)]	167.38 (43.32) [9.29 (2.40)]
Adj. mean change from BL to week 24 in FPG, mg/dL [mmol/l] (95% CI), LOCF [*P* value vs placebo]	-0.78 (-7.56, 6.01) [-0.04 (-0.42, 0.33)] --	-34.23 (-40.98, -27.48) [-1.90 (-2.27, -1.53)] *P*<0.0001
Mean BL BW, kg (SD), LOCF	90.07 (16.18)	88.57 (17.58)
Adj. mean change from BL to week 24 in BW, kg (95% CI), LOCF [*P* value vs placebo]	-0.58 (-1.09, -0.07) --	-2.65 (-3.16, -2.14) *P*<0.0001
Mean BL, SBP mmHg (SD), LOCF	136.31 (14.37)	134.70 (12.69)
Adj. mean change from BL to week 8 in SBP mmHg (95% CI), LOCF [*P* value vs placebo]	-0.27 (-2.60, 2.05) --	-4.04 (-6.36, -1.72) *P*=0.0250

LA, Longitudinal analysis; LOCF, Last observation carried forward; Adj., adjusted; BL, baseline; CI, confidence interval

Clinical Trial Registration Number: NCT01392677
Supported by: AZ, BMS

938

Dapagliflozin helps reduce HbA_{1c} and body weight in patients with type 2 diabetes as part of triple combination therapy: a subanalysis of four clinical studies

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Background and aims: Dapagliflozin (DAPA) is a highly selective inhibitor of sodium-glucose co-transporter 2, the primary transporter involved in mediating renal glucose re-uptake. DAPA has been shown to reduce hyperglycaemia in patients with type 2 diabetes mellitus (T2DM) through the urinary excretion of glucose. Due to an accompanying loss of calories, DAPA is also associated with a reduction in body weight (BW). DAPA has demonstrated efficacy as monotherapy in treatment-naïve patients and in those inadequately controlled with single oral antidiabetic drugs (OADs) or insulin (INS) (+/- OADs). In the progression of T2DM, second and even third agents are often needed to intensify therapy. To investigate the efficacy of DAPA when added to 2 stable medications, we performed subgroup analyses of four 24-week studies. The primary results from the full populations of these studies have been presented previously.

Materials and methods: Patients in the subgroups received placebo (PBO) or DAPA 10 mg for 24 weeks in the presence of either sitagliptin (SITA) plus metformin (MET) (prespecified stratification), or sulphonylurea (SU) plus MET (HbA1c analysis prespecified, BW analysis post hoc), or INS plus MET (analysis post hoc).

Results: DAPA reduced HbA1c compared with PBO at 24 weeks in each of the 4 subgroups (Table). A consistent clinically beneficial reduction in HbA1c was observed with DAPA when added to 2 prior antidiabetic therapies, including INS. BW was likewise reduced to a greater extent in the DAPA group compared with PBO across each of the studies.

Conclusion: DAPA is effective in reducing glycaemia and BW in patients with T2DM who are inadequately controlled on 2 different background antidiabetic medications. This observation is consistent with an INS-independent mechanism of action that works regardless of residual β-cell function.

Table

	HbA1c (%)		Body Weight (kg)	
	PBO	DAPA 10 mg	PBO	DAPA 10 mg
SITA + MET, n BL (SD) 24-week change from BL (95% CI)[a] Difference from placebo	113 7.87 (0.75) -0.02 (-0.15 to 0.10) --	113 7.80 (0.81) -0.43 (-0.55 to -0.30) -0.40 (-0.58 to -0.23)[b]	113 94.2 (20.9) -0.47 (-1.00 to 0.05) --	113 94.0 (19.8) -2.35 (-2.87 to -1.82) -1.87 (-2.61 to -1.13)[b]
SU + MET (Study 1), n BL (SD) 24-week change from BL (95% CI)[a] Difference from placebo	114 8.10 (0.77) -0.01 (-0.17 to 0.15) --	113 8.06 (0.78) -0.55 (-0.72 to -0.39) -0.54 (-0.73 to -0.36)[c]	114 89.0 (15.5) 0.0 (-0.6 to 0.6) --	112 87.6 (19.0) -2.2 (-2.8 to -1.6) -2.2 (-2.9 to -1.5)[c]
SU + MET (Study 2), n BL (SD) 24-week change from BL (95% CI)[a] Difference from placebo	109 7.96 (0.80) -0.07 (-0.26 to 0.12) --	105 8.03 (0.79) -0.55 (-0.75 to -0.36) -0.48 (-0.69 to -0.27)[c]	110 90.1 (15.2) -0.8 (-1.5 to -0.2) --	106 93.4 (19.5) -1.9 (-2.5 to -1.2) -1.1 (-1.8 to -0.3)[d]
INS + MET, n BL (SD) 24-week change from BL (95% CI)[a] Difference from placebo	77 8.43 (0.73) -0.31 (-0.47 to -0.16) --	83 8.52 (0.79) -0.93 (-1.08 to -0.78) -0.61 (-0.83 to -0.40)[c]	77 98.7 (17.5) -0.06 (-0.61 to 0.49) --	83 95.7 (16.2) -1.77 (-2.30 to -1.24) -1.71 (-2.47 to -0.95)[c]

[a]Adjusted mean change from baseline (LOCF); [b]prespecified *P* value <0.0001 for comparison of DAPA and PBO; [c]nominal *P* value <0.0001; [d]nominal *P* value = 0.0047; BL, baseline.

Clinical Trial Registration Number: NCT00984867; NCT01031680; NCT01042977; NCT00673231
Supported by: AZ, BMS

PS 074 SGLT-2 inhibitors: influence on cardiovascular risk factors

939

Combined HbA_{1c} and weight reduction is more frequent with dapagliflozin vs glipizide add-on treatment in patients with type 2 diabetes inadequately controlled on metformin

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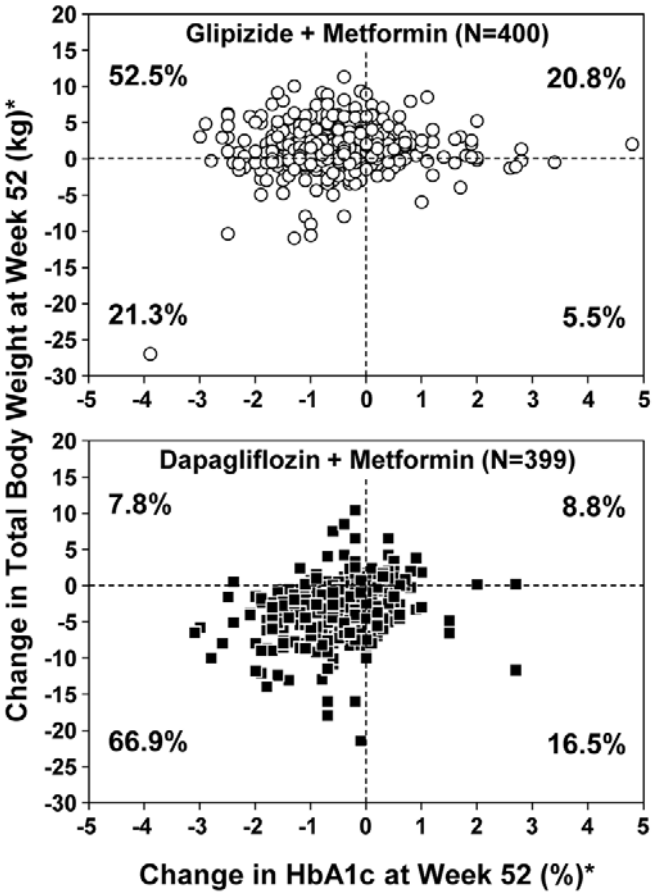
Background and aims: Sulfonylureas are often used as add-on therapy in type 2 diabetes when metformin fails to maintain glycaemic control, but risks include weight gain and hypoglycaemia. Dapagliflozin (DAPA), a sodium-glucose cotransporter 2 inhibitor, increases urinary glucose excretion and reduces hyperglycaemia independently of insulin secretion or action. The aim of this analysis was to compare the proportion of patients who had combined reduction in HbA1c and weight with DAPA therapy compared with glipizide (GLIP) after inadequate control on metformin alone.

Materials and methods: This 52-week, double-blind, active-controlled, non-inferiority trial randomised patients inadequately controlled on metformin (mean baseline HbA1c, 7.7%) to add-on DAPA (n=406, ≤10 mg/d) or GLIP (n=408, ≤20 mg/d) and maintained to Week 52 unless hypoglycaemia warranted down-titration. HbA1c non-inferiority at Week 52 has been reported previously. Here, we report the proportion of patients achieving combined HbA1c and weight reduction, and rates of hypoglycaemia. Combined HbA1c and body weight reduction was defined as any decrease in HbA1c of >0 % and any decrease in weight of >0 kg.



Results: At Week 52, three-fold more DAPA-treated patients achieved combined HbA1c and weight reduction (66.9%) vs 21.3% of GLIP-treated patients. Proportions achieving HbA1c reduction were 74.7% vs 73.8% and weight reduction were 83.5% vs 26.8% with DAPA vs GLIP, respectively. The distribution of these proportions was significantly different between treatments ($Chi^2 = 268.6$, df = 3, $p<0.0001$). Hypoglycaemic events were less frequent with DAPA (3.5%) vs GLIP (40.8%).

Conclusion: DAPA produced similar glycaemic efficacy to GLIP, but with the benefits of weight loss and low rates of hypoglycaemia.

Clinical Trial Registration Number: NCT00660907

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940

Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes

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Background and aims: Sodium-glucose co-transporter 2 (SGLT2) reabsorbs glucose and sodium in the renal proximal tubule. Dapagliflozin (DAPA), an SGLT2 transporter inhibitor, targets hyperglycaemia in type 2 diabetes mellitus (T2DM) by increasing renal glucose excretion. To investigate whether the parallel occurring sodium loss would have diuretic-like physiological effects, we compared DAPA and hydrochlorothiazide (HCTZ) effects on 24-h blood pressure (BP), body weight, plasma volume and glomerular filtration rate.

Materials and methods: In this randomised, placebo-controlled, double-blind trial, 75 subjects with T2DM aged 18-70 years, HbA1c 6.6%-9.5%, seated systolic/diastolic BP (130-165)/(80-105) mm Hg, on a stable dose of angiotensin-converting enzyme inhibitor or angiotensin receptor blockers and no other antihypertensive medications were randomly assigned to placebo (PBO), DAPA 10 mg/d, or HCTZ 25 mg/d. Changes from baseline in 24-h ambulatory BP, body weight, glomerular filtration rate measured by iohexol plasma clearance, and plasma volume measured by ^{125}I-labeled human serum albumin, were compared after 12 weeks of treatment.

Results: Patients' mean age was 56 years, T2DM duration was 6.3 years, and HbA1c was 7.5%. Treatment with PBO, DAPA, or HCTZ resulted in changes from baseline in 24-h ambulatory mean systolic BP of -0.9 (95% CI -4.2, +2.4), -3.3 (95% CI -6.8, +0.2), and -6.6 (95% CI -9.9, -3.2) mm Hg, respectively at week 12, adjusted for baseline systolic BP. Body weight decreased with DAPA and HCTZ. In a sub-study plasma volume appeared to decrease with DAPA but did not change with PBO or HCTZ treatment. DAPA was associated with a small reduction in glomerular filtration rate (-10.8%; 95% CI -14.6, -6.7) relative to PBO (-2.9%; 95% CI -6.9, +1.2) or HCTZ (-3.4%; 95% CI -7.3, +0.6).

Conclusion: DAPA-mediated SGLT2 inhibition is associated with reductions in 24-h BP, body weight and possibly plasma volume. Cumulatively, these effects suggest that DAPA may have a diuretic-like capacity to lower BP in addition to beneficial effects on glycaemic control.

Clinical Trial Registration Number: NCT00976495

Supported by: AZ, BMS

941

Weight loss related quality of life among type 2 diabetes mellitus patients treated with dapagliflozin

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Background and aims: Data related to the impact on health-related quality of life (HRQOL) from oral antidiabetic therapy-induced weight loss in T2DM patients are scarce. Dapagliflozin, a novel sodium-glucose cotransporter 2 (SGLT2) inhibitor, lowers blood glucose by increasing urinary glucose excretion and is associated with body weight reductions. This study evaluated HRQOL associated with weight change among T2DM patients treated with dapagliflozin.

Materials and methods: Patients with T2DM (BMI ≥25 kg/m^2; 56% men; mean age, 61 years) who had inadequate glycemic control on metformin (MET) alone were enrolled in a 24-week, international, double-blind, randomized, placebo-controlled study with an additional 78-week extension to evaluate the effect of dapagliflozin + MET. Primary end point was total body weight change at week 24, and weight loss was -2.96 kg for dapagliflozin and -0.88 kg for placebo ($p<0.0001$). Patients completed the SHIELD WQ-9, a weight change-related HRQOL survey, at baseline and week 102. Difference

of proportions for patients treated with dapagliflozin 10 mg + MET (n=89) or placebo + MET (n = 91) who reported improvement in each HRQOL domain and weight loss was analyzed with Fisher's exact test.

Results: Among patients with reported weight loss at week 102 (31.5% in dapagliflozin group; 29.7% in placebo group), baseline HRQOL (% reporting improvement in each of 9 domains) was similar for dapagliflozin and placebo groups (p>0.05). At week 102, among those who reported weight loss, more dapagliflozin-treated patients reported improvement in 8 of 9 HRQOL domains (14.3%-57.1%), compared with patients who received placebo (7.4%-37.0%). Significantly greater proportion of dapagliflozin patients than placebo patients with reported weight loss reported improvement in overall quality of life (57.1% vs. 22.2%, p=0.013) and self-esteem (57.1% vs. 25.9%, p=0.029).

Conclusion: Weight loss-related HRQOL was maintained or improved during 2 years of treatment with dapagliflozin, a novel mechanism for treatment of T2DM.

Clinical Trial Registration Number: NCT00855166

Supported by: Astra Zeneca LP

942

Empagliflozin improves blood pressure in patients with type 2 diabetes (T2DM) and hypertension

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Background and aims: A Phase III randomized placebo-controlled trial was conducted to investigate the efficacy, safety and tolerability of the SGLT2 inhibitor empagliflozin (EMPA) compared with placebo in patients with T2DM and hypertension.

Materials and methods: Patients with T2DM (mean [SD] age 60.2 [9.0] years and BMI 32.6 [5.1] kg/m^2) and hypertension (mean seated systolic blood pressure [SBP] 130-159 mmHg and diastolic blood pressure [DBP] 80-99 mmHg) were randomized double-blind and received EMPA 10 mg (n=276), EMPA 25 mg (n=276) or placebo (PBO; n=271) qd for 12 weeks. Co-primary endpoints were changes from baseline in HbA_{1c} and mean 24-hour SBP (ambulatory blood pressure monitoring [ABPM]) at week 12. The key secondary endpoint was change from baseline in mean 24-hour DBP (ABPM) at week 12. Other secondary endpoints included changes from baseline in office SBP and DBP, and the proportion of patients reaching controlled BP (<130/80 mmHg) at week 12.

Results: EMPA 10 and 25 mg significantly reduced HbA_{1c} and mean 24-hour SBP and DBP compared with placebo (Table). Changes in office SBP and DBP were consistent with the changes in ABPM results. More patients receiving EMPA 10 mg and 25 mg who had uncontrolled BP at baseline had controlled BP (<130/80 mmHg) at week 12 vs PBO (18% and 16% vs 8%, respectively; p<0.01). Adverse events (AEs) were reported by 48.9%, 51.4%, and 52.6% of patients on EMPA 10 mg, 25 mg, and PBO, respectively. Events consistent with volume depletion were reported in 1 patient (0.4%) on EMPA 10 mg, no patients on EMPA 25 mg and 1 patient (0.4%) on PBO. AEs consistent with urinary tract infection were reported in 4.0% of patients on EMPA 10 mg, 4.7% on EMPA 25 mg and 3.7% on PBO. AEs consistent with genital infection were reported in 5.1% of patients on EMPA 10 mg, 5.4% on EMPA 25 mg and 0.4% on PBO.

Conclusion: EMPA 10 mg and 25 mg qd were associated with significant and clinically meaningful reductions in BP compared with PBO, and were well tolerated in patients with T2DM and hypertension.

	Placebo	Empagliflozin 10 mg	Empagliflozin 25 mg
Baseline HbA_{1c}, % (SE)	7.90 (0.04)	7.87 (0.05)	7.92 (0.04)
Change from baseline in HbA_{1c}, % (SE)	0.03 (0.04)	-0.59 (0.04)***	-0.62 (0.04)***
Baseline 24-h SBP (ABPM), mmHg (SE)	131.72 (0.72)	131.34 (0.78)	131.18 (0.73)
Change from baseline in 24-h SBP (ABPM), mmHg (SE)	0.48 (0.49)	-2.95 (0.48)***	-3.68 (0.48)***
Baseline mean seated office SBP, mmHg (SE)	141.98 (0.75)	142.32 (0.73)***	141.87 (0.76)***
Change from baseline in mean seated office SBP, mmHg (SE)	-0.67 (0.70)	-4.60 (0.69)	-5.47 (0.69)
Baseline 24-h DBP, mmHg (SE)	75.16 (0.45)	75.13 (0.50)	74.64 (0.45)
Change from baseline in 24-h DBP, mmHg (SE)	0.32 (0.29)	-1.04 (0.28)***	-1.40 (0.28)***
Baseline mean seated office DBP, mmHg (SE)	83.67 (0.43)	84.13 (0.44)	83.82 (0.41)
Change from baseline in mean seated office DBP, mmHg (SE)	-1.13 (0.39)	-3.06 (0.39)***	-3.02 (0.39)***

Adjusted means based on ANCOVA in full analysis set with last observation carried forward (LOCF) imputation. For blood pressure parameters, values following a change in antihypertensive therapy were set to missing and imputed via LOCF. Values after start of antidiabetic rescue medication were set to missing and imputed via LOCF for all parameters. For each dose group, statistical testing of primary and key secondary endpoints was hierarchical at alpha = 0.05. Further endpoints were called statistically significant if p-values were smaller than nominal alpha = 0.05. *p<0.05 vs. placebo; **p<0.01 vs. placebo; ***p<0.001 vs. placebo.

Clinical Trial Registration Number: NCT01370005

Supported by: Boehringer Ingelheim

943

Empagliflozin improves glycaemic parameters and cardiovascular risk factors in patients with type 2 diabetes: pooled data from four pivotal phase III trials

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Background and aims: We analysed pooled data from 2477 patients with T2DM (mean [SD] age 55.6 [10.2] years, HbA_{1c} 7.99 [0.85], BMI 28.7 [5.5]) from four randomized, placebo-controlled Phase III trials that investigated empagliflozin (EMPA) 10 mg or 25 mg given for 24 weeks as monotherapy, add-on to metformin (MET), add-on to MET + SU, or add-on to pioglitazone ± MET.

Materials and methods: Effects on HbA_{1c}, fasting plasma glucose (FPG), weight, systolic and diastolic blood pressure (SBP and DBP) were evaluated in the full analysis set (placebo [PBO]: n=825, EMPA 10 mg: n=831, EMPA 25 mg: n=821). Effects on lipids and uric acid were evaluated in all treated patients (PBO: n=825, EMPA 10 mg: n=830, EMPA 25 mg: n=822). Effects on SBP and DBP were also evaluated in patients with uncontrolled BP (SBP ≥130 mmHg or DBP ≥80 mmHg) at baseline (PBO: n=501, EMPA 10 mg: n=517, EMPA 25 mg: n=506).

Results: EMPA significantly reduced HbA_{1c}, FPG, weight, SBP, DBP and uric acid at week 24 vs PBO. Reductions in SBP and DBP were more pronounced in patients with uncontrolled BP at baseline. Small increases in HDL- and LDL-cholesterol and small decreases in triglyceride levels were observed with EMPA vs PBO.

	Placebo	Empagliflozin 10 mg	Empagliflozin 25 mg
Baseline HbA_{1c}, % (SE) [¶‡]	8.02 (0.03)	7.98 (0.03)	7.96 (0.03)
Change from baseline in HbA_{1c} at week 24 (SE)[¶]	-0.08 (0.03)	-0.70 (0.03)***	-0.76 (0.03)***
Baseline FPG, mg/dL (SE)[†]	153.7 (1.3)	152.6 (1.2)	152.6 (1.2)
Change from baseline in FPG at week 24, mg/dL (SE)[†]	7.4 (1.0)	-20.5 (1.0)***	-23.2 (1.0)***
Baseline body weight, kg (SE)[†]	78.03 (0.66)	78.77 (0.65)	79.10 (0.66)
Change from baseline in body weight at week 24, kg (SE)[†]	-0.24 (0.09)	-2.05 (0.09)***	-2.25 (0.09)***
Baseline SBP, mmHg (SE)[†]	128.6 (0.5)	129.6 (0.5)	129.0 (0.5)
Change from baseline in SBP at week 24, mmHg (SE)[†]	-0.5 (0.4)	-3.9 (0.4)***	-4.3 (0.4)***
Baseline DBP, mmHg (SE)[†]	78.0 (0.3)	78.7 (0.3)	78.3 (0.3)
Change from baseline in DBP at week 24, mmHg (SE)[†]	-0.6 (0.2)	-1.8 (0.2)***	-2.0 (0.2)***

Adjusted means based on ANCOVA with last observation carried forward imputation; values on rescue medication were excluded from analysis of HbA1c, FPG, weight and BP.
[†]Full analysis set (all randomized and treated patients who had a baseline HbA_{1c} value).
[¶]Inclusion criteria: HbA1c ≥7.0% to ≤10.0% .
***p<0.001 vs placebo.

Conclusion: In a pooled analysis of data from four Phase III trials, 24 weeks' treatment with EMPA 10 mg or 25 mg provided clinically meaningful improvements in glycemic parameters, weight, and BP, with positive effects on uric acid and small effects on lipids.

Clinical Trial Registration Number: NCT01210001, NCT01177813, NCT01159600

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Design of the empagliflozin cardiovascular outcome event trial in type 2 diabetes mellitus

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Background and aims: A large number of diabetes drug classes are available for the management of type 2 diabetes mellitus (T2DM), but the impact of different glucose-lowering therapies on cardiovascular (CV) disease remains unclear.

Materials and methods: The empagliflozin CV outcome event trial is an ongoing multi-center, randomized, double blind, placebo-controlled trial designed to assess the impact of the SGLT-2 inhibitor empagliflozin 10 or 25 mg, compared with placebo (1:1:1), on CV events.

Results: 7000 patients with T2DM being treatment naive or receiving any glucose lowering therapy (≥18 years, body mass index ≤45 kg/m^2, estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73m^2) at elevated CV risk will be studied (TABLE). First patient was randomized September 2010 and the primary outcome is time-to-first occurrence of CV death, nonfatal myocardial infarction or nonfatal stroke. 691 events will be required to provide 90% power to yield the upper limit of the adjusted 95% confidence interval for hazard ratio <1.3 at a one-sided α level of 0.025, assuming equal risks. Hierarchical testing for superiority will follow for the primary and key secondary (time-to-first occurrence of CV death, nonfatal myocardial infarction, nonfatal stroke or hospitalization for unstable angina pectoris) outcomes where non-inferiority is achieved for the pooled doses vs. placebo. Other outcomes include a microvascular composite (laser therapy for retinopathy, vitreous hemorrhage, blindness, new/worsening nephropathy [macroalbuminuria, doubling of serum-creatinine and eGFR ≤ 45 mL/min/1.73m^2, renal replacement therapy, death due to renal disease]) and efficacy (glycaemia, weight, blood pressure).

Conclusion: The empagliflozin CV outcome event trial will complete its recruitment in 2013 and is powered to show CV safety with the option to demonstrate CV superiority for the SGLT-2 inhibitor empagliflozin in patients with T2DM at elevated risk for CV complications.

Table. Glycaemic and CV entry criteria for the empagliflozin CV outcome event trial

Glycemic entry criterion (HbA1c):	Drug-naive patients: 7.0 – 9.0%
	Stable pharmacological therapy: 7.0 – 10.0%
CV entry criteria - any of the following:	History of MI (>2 months prior to enrollment)
	Evidence of CAD* in ≥2 major vessels or left main coronary artery
	Evidence of single-vessel CAD* with no scheduled revasc./previously unsuccessful revasc. and: a) positive non-invasive, functional stress test for ischemia (ECG, echo or nuclear), or b) hospital-discharge due to unstable angina pectoris ≤12 months before enrollment
	Hospital-discharge due to unstable angina pectoris >2 months before enrollment with evidence of CAD* according to any of the following: a) left main coronary artery b) ≥2 major vessels c) single vessel with positive non-invasive, functional stress test for ischemia (ECG, echo or nuclear) and no scheduled revasc./previously unsuccessful revasc.
	History of stroke (>2 months prior to enrollment)
	Peripheral occlusive arterial disease according to any of the following: a) previous limb angioplasty, stenting or bypass surgery b) previous limb or foot amputation due to circulatory insufficiency c) significant peripheral artery stenosis (> 50%) in at least one limb (angiography or non-invasive) d) ankle brachial index < 0.9 in at least one limb

Abbreviations: CAD – coronary artery disease; revasc – revascularization. *CAD defined as ≥ 50% luminal narrowing detected on invasive coronary angiography or multi-sliced computed tomography angiography

Clinical Trial Registration Number: NCT01131676

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Effects of canagliflozin on blood pressure and adverse events related to osmotic diuresis and reduced intravascular volume in subjects with type 2 diabetes mellitus

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Background and aims: Hypertension is a common comorbidity of T2DM. The blood pressure (BP)-lowering effects of canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor developed for the treatment of type 2 diabetes mellitus (T2DM), were evaluated in Phase 3 studies.

Materials and methods: BP changes were assessed based on pooled analyses of data from 4 randomised, double-blind, placebo (PBO)-controlled studies in subjects with T2DM at Week 26 (N = 2,313; mean age, 56.0 y; HbA_{1c}, 8.0%; BMI, 32.1 kg/m^2; estimated glomerular filtration rate [eGFR], 88.1 mL/min/1.73 m^2).

Results: Relative to PBO, CANA 100 and 300 mg were associated with reductions in systolic BP (SBP; -4.0 and -4.7 mmHg) and diastolic BP (DBP; -1.9 and -1.9 mmHg) (**Table**). No notable changes in heart rate were seen with CANA 100 and 300 mg versus PBO (-0.6, -0.4, and 0.0 beats/min). In subjects with baseline SBP ≥140 mmHg (n = 453), SBP was reduced with CANA 100 and 300 mg and PBO (-12.8, -14.2, and -6.8 mmHg); PBO-subtracted decreases were -6.0 and -7.4 mmHg with CANA 100 and 300 mg (P <0.001). In subjects with baseline DBP ≥90 mmHg (n = 171), decreases in DBP were observed with CANA 100 and 300 mg and PBO (-5.9, -9.0, and -7.4 mmHg); a PBO-subtracted reduction was seen with CANA 300 mg (-1.6 mmHg) and a slight increase was seen with CANA 100 mg (1.5 mmHg). Absolute decreases in BP were larger in subjects with higher baseline BP, but PBO-subtracted decreases were generally similar across groups. PBO-subtracted decreases in BP with CANA 100 and 300 mg were similar among subjects on antihypertensives (n = 1,332; SBP, -4.4 and -4.6 mmHg; DBP, -1.8 and -1.7 mmHg) and those not on these agents (n = 981; SBP, -3.5 and -4.7 mmHg; DBP, -2.1 and -2.0 mmHg). Consistent with CANA's osmotic diuretic effect, an increase in related AEs was seen with CANA 100 and 300 mg compared with PBO (6.7%, 5.6%, 0.8%), the most common being pollakiuria (4.2%, 3.1%, 0.6%), thirst (1.3%, 1.9%, 0.2%), and polyuria (0.7%, 1.4%, 0%). Similar rates of AEs related to reduced intravascular volume were observed with CANA 100 and 300 mg and PBO (1.2%, 1.3%, 1.1%), including hypotension (0.7%, 0.2%, 0.6%), postural dizziness (0.4%, 0.5%, 0.3%), and orthostatic hypotension (0%, 0.5%, 0.2%). AEs related to osmotic diuresis and reduced intravascular volume were generally mild or moderate in severity and led to few discontinuations.

Conclusion: CANA was associated with BP lowering compared with PBO in subjects across a range of baseline BP; absolute reductions were greater in subjects with higher BP, but PBO-subtracted decreases were generally similar across groups. In this pooled analysis, CANA was associated with an increased incidence of AEs related to osmotic diuresis but the rates of AEs related to reduced intravascular volume were similar across groups.

Table. Glycaemic and CV entry criteria for the empagliflozin CV outcome event trial

Glycemic entry criterion (HbA1c):	Drug-naive patients: 7.0 – 9.0%
	Stable pharmacological therapy: 7.0 – 10.0%
CV entry criteria - any of the following:	History of MI (>2 months prior to enrollment)
	Evidence of CAD* in ≥2 major vessels or left main coronary artery
	Evidence of single-vessel CAD* with no scheduled revasc./previously unsuccessful revasc. and: a) positive non-invasive, functional stress test for ischemia (ECG, echo or nuclear), or b) hospital-discharge due to unstable angina pectoris ≤12 months before enrollment
	Hospital-discharge due to unstable angina pectoris >2 months before enrollment with evidence of CAD* according to any of the following: a) left main coronary artery b) ≥2 major vessels c) single vessel with positive non-invasive, functional stress test for ischemia (ECG, echo or nuclear) and no scheduled revasc./previously unsuccessful revasc.
	History of stroke (>2 months prior to enrollment)
	Peripheral occlusive arterial disease according to any of the following: a) previous limb angioplasty, stenting or bypass surgery b) previous limb or foot amputation due to circulatory insufficiency c) significant peripheral artery stenosis (> 50%) in at least one limb (angiography or non-invasive) d) ankle brachial index < 0.9 in at least one limb

Abbreviations: CAD = coronary artery disease; revasc = revascularization. *CAD defined as ≥ 50% luminal narrowing detected on invasive coronary angiography or multi-sliced computed tomography angiography

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Canagliflozin lowers HbA_{1c} and blood pressure through weight loss-independent and weight loss-associated mechanisms

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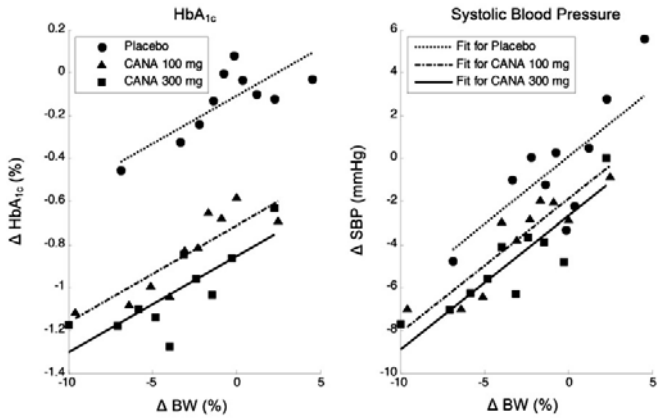
Background and aims: Canagliflozin (CANA) is a sodium glucose co-transporter 2 (SGLT2) inhibitor that increases urinary glucose excretion (UGE), leading to decreased plasma glucose and HbA_{1c} and weight loss (WL) in subjects with type 2 diabetes mellitus (T2DM). Systolic blood pressure (SBP) also decreases, possibly due in part to the osmotic diuresis with increased glucose excretion. Since WL can improve glycaemic control and lower BP, this analysis examined the WL-independent (WL-I) and WL-associated (WL-A) effects of CANA on HbA_{1c} and SBP in subjects with T2DM enrolled in Phase 3 studies.
Materials and methods: WL-I and WL-A effects of CANA were evaluated based on a pooled analysis of 4 previously reported, randomised, double-blind, 26-week, Phase 3 studies (N = 2,250). Each study had 3 groups: placebo (PBO), CANA 100 mg, CANA 300 mg. Mean baseline values of HbA_{1c} = 8.0%, body weight (BW) = 89 kg, and SBP = 128 mmHg were similar across groups. Within each group, mean changes in HbA_{1c} and SBP were calculated for each decile of WL, and ANCOVA analysis was done with ΔHbA_{1c} or ΔSBP as response and ΔBW (%) as covariate. Plots of ΔHbA_{1c} and ΔSBP versus ΔBW were generated to illustrate the changes observed over the different deciles of weight loss and the relationships obtained from the ANCOVA models. WL-I effects were defined as the vertical distance between lines (ie, difference in response observed when WL is the same in each group) and WL-A effects were determined as the total difference in group means minus the WL-I effects.
Results: Greater reductions in HbA_{1c} and SBP were seen with greater WL, with similar slopes in each group (Figure). Total PBO-subtracted mean reduction in HbA_{1c} was 0.72% with CANA 100 mg (0.60% [85%] of the effect was WL-I) and 0.90% with CANA 300 mg (0.75% [85%] of the effect was WL-I). Total PBO-subtracted reduction in SBP was 3.5 mmHg with CANA 100 mg (2.0 mmHg [57%] WL-I) and 4.7 mmHg with CANA 300 mg (2.7 mmHg [59%] WL-I).
Conclusion: CANA lowers both HbA_{1c} and BP through WL-I and WL-A mechanisms; a greater proportion of the reduction in HbA_{1c} is WL-I compared to SBP.



Supported by: Janssen Research & Development, LLC

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Changes in lipid profiles of patients with type 2 diabetes mellitus on dapagliflozin therapy

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Background and aims: Dapagliflozin (DAPA), a sodium-glucose cotransporter 2 inhibitor, increases urinary glucose excretion, and improves hyperglycaemia in patients with type 2 diabetes mellitus (T2DM). DAPA also favourably affects cardiovascular risk factors by reducing weight, blood pressure and uric acid in these patients. The effects of DAPA on lipids in patients with T2DM are reported here.
Materials and methods: Data from 3731 patients in 12 phase 2b/3 double-blind controlled trials receiving DAPA 5 or 10 mg or placebo (PBO) for up to 24 weeks were analysed. Changes in the plasma lipid profile were pre-specified for analysis in these studies. Adjustments in concomitant lipid-lowering medications were permitted.
Results: Demographic and baseline characteristics were balanced between DAPA and PBO groups. Improved glycaemic control with DAPA versus PBO has been previously reported in these studies. Changes in lipid parameters from individual trials showed variability across studies. In a 24-week pooled analysis, changes from baseline in HDL-C were +6.5% and +5.5% for DAPA 5 and 10 mg, respectively and +3.8% for PBO; LDL-C were +0.6% and +2.7% for DAPA 5 and 10 mg, respectively, and −1.9% for PBO; total cholesterol were +1.1% and +1.4% for DAPA 5 and 10 mg, respectively, and −0.4% for PBO. Changes from baseline to Week 24 in LDL-C/HDL-C ratio were −5.4% and −2.8% for DAPA 5 and 10 mg respectively, and −5.4% for PBO.
Conclusion: DAPA is associated with small mean changes in fasting lipid parameters versus PBO at 24 weeks.

Plasma lipid changes from baseline (%, 95% CI) in pooled DAPA studies at 24 weeks

	PBO (N=1393)	DAPA 5 mg (N=1145)	DAPA 10 mg (N=1193)
TC	.	.	.
n	989	888	834
BL mean	5.06 mmol/L	5.04 mmol/L	5.07 mmol/L
Mean change	-0.4%	+1.1%	+1.4%
(95% CI)	(-1.4, +0.6)	(0.0, +2.2)	(+0.2, +2.6)
HDL-C	.	.	.
n	990	889	834
BL mean	1.15 mmol/L	1.16 mmol/L	1.17 mmol/L
Mean change	+3.8%	+6.5%	+5.5%
(95% CI)	(+2.8, +4.8)	(+5.3, +7.8)	(+4.3, +6.7)
LDL-C	.	.	.
n	985	884	828
BL mean	2.97 mmol/L	2.93 mmol/L	2.95 mmol/L
Mean change	-1.9%	+0.6%	+2.7%
(95% CI)	(-3.5, -0.3)	(-1.2, +2.4)	(+0.8, +4.7)
TG	.	.	.
n	984	886	831
BL mean	2.12 mmol/L	2.15 mmol/L	2.19 mmol/L
Mean change	-0.7%	-3.2%	-5.4%
(95% CI)	(-3.0, +1.7)	(-5.8, -0.6)	(-7.9, -2.8)
FFA	.	.	.
n	838	732	694
BL mean	0.56 meq/L	0.58 meq/L	0.56 meq/L
Mean change	-5.7%	-0.5%	+1.2%
(95% CI)	(-8.9, -2.5)	(-3.7, +2.8)	(-2.4, +5.0)

Data include observations after rescue. N is the number of treated patients. n is the number of treated patients with non-missing baseline and week 24 values. BL=baseline, CI=confidence interval, DAPA=dapagliflozin, FFA=free fatty acids, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, PBO=placebo, TC=total cholesterol, TG=triglycerides.

Clinical Trial Registration Number: NCT00528372, NCT00528879, NCT00736879, NCT00263276, NCT00972244, NCT00643851, NCT00859898, NCT00855166, NCT00680745, NCT00683878, NCT00673231, NCT00357370
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LX4211, a dual inhibitor of sodium glucose transporters SGLT1 and SGLT2, reduces blood pressure in patients with type 2 diabetes

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Background and aims: Dual inhibition of sodium glucose transporter 1 (SGLT1) and SGLT2 by LX4211 is being evaluated as a potential treatment of diabetes. Since SGLT1 in the gastrointestinal tract is involved in sodium absorption and SGLT2 in the kidney is involved in urinary sodium excretion, we evaluated the impact of LX4211 on several measures of glycemic control and blood pressure (BP) in a dose-ranging study of 299 patients with type 2 diabetes mellitus.
Materials and methods: Patients with inadequately controlled type 2 diabetes mellitus on metformin were randomly assigned to receive LX4211 (doses of 75 mg qd, 200 mg qd, 200 mg bid, or 400 mg qd) or placebo for 12 weeks. Changes in trough sitting systolic and diastolic blood pressure (SBP and DBP) were secondary endpoints. Patients were allowed in the study both with and without hypertension, and there were no restrictions on the prescribing or adjustment of antihypertensive medications.
Results: At entry, mean age was 55.9 years, A1C was 8.1%, body mass index was 33.1 kg/m^2, and BP was 125/79 mmHg. At Week 12, LX4211 reduced both A1C and BP in a dose-dependent manner. SBP reductions were 0, 4, 4, 6, and 0 mmHg with LX4211 75 mg qd, 200 mg qd, 200 mg bid, 400 mg qd, and placebo, respectively ($p<0.05$ vs. placebo for LX4211 200 mg bid and 400 mg qd). Corresponding DBP reductions were 1, 3, 2, 2, and 0 mmHg (p=NS vs. placebo for all dose arms). In those with baseline SBP ≥130 mmHg, placebo-subtracted SBP reductions for LX4211 400 mg were 14 mmHg (p=0.002) as compared to 1 mmHg (p=0.6) for those with baseline SBP <130 mmHg. Placebo-subtracted DBP reductions for LX4211 400 mg were 3 mmHg (p=0.2) and 0 mmHg (p=0.9) in those with baseline DBP ≥80 mmHg and DBP <80 mmHg, respectively.
Conclusion: Dual inhibition of SGLT1 and SGLT2 by LX4211 was associated with marked BP reductions among hypertensive patients.
Clinical Trial Registration Number: NCT01376557

PS 075 SGLT-2 inhibitors: side effects, special populations

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Urinary tract infection with canagliflozin in subjects with type 2 diabetes mellitus

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Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor developed for the treatment of type 2 diabetes mellitus (T2DM), inhibits renal glucose reabsorption, resulting in increased glucosuria. The impact of this on urinary tract infection (UTI) was assessed in subjects with T2DM enrolled in placebo (PBO)-controlled, Phase 3 studies of CANA.
Materials and methods: UTIs were evaluated using a UTI case report form to characterise the presentation and severity of events. Findings are reported based on pooled analyses of subjects from four 26-week studies (DS1; N = 2,313) and subjects with moderate renal impairment (eGFR ≥30 and <60 mL/min/1.73 m^2) from four 18- or 26-week studies (DS2; N = 1,085), and for the CANagliflozin cardioVascular Assessment Study (CANVAS; 52-week interim safety analysis) in subjects with a history or high risk of cardiovascular disease (N = 4,327). Subjects with a history of UTI were not excluded from these studies.
Results: Across all datasets analysed, the proportion of subjects with UTI adverse events (AEs) was slightly higher with CANA than PBO, with no increase in recurrent or serious events or in UTI-related discontinuations (Table). Incidences of upper UTIs were low and similar across groups. The majority of UTI AEs were considered to be mild or moderate in severity, as assessed by the investigator. UTI was more common in women than men, but the increased incidence with CANA was consistent between genders. UTIs generally occurred within 26 weeks of initiating CANA therapy, with a subsequent attenuation in incidences. Median time to first symptomatic UTI tended to be earlier with CANA relative to PBO, but median duration and severity of symptoms of UTIs were similar with CANA and PBO.
Conclusion: Treatment with CANA was associated with a slight increase in UTI rates, with no increase in serious or upper UTIs, in subjects with T2DM.

Table. Summary of UTI Adverse Events

	Subjects,* n (%)			
	PBO	CANA 100 mg	CANA 300 mg	All CANA
Any UTI				
DS1†	26 (4.0)	49 (5.9)	36 (4.3)	85 (5.1)
DS2‡	23 (6.0)	21 (6.2)	27 (7.4)	48 (6.8)
CANVAS	63 (4.4)	72 (5.0)	82 (5.7)	154 (5.3)
Symptomatic UTI				
DS1†	17 (2.6)	32 (3.8)	27 (3.2)	59 (3.5)
DS2‡	13 (3.4)	16 (4.7)	15 (4.1)	31 (4.4)
CANVAS	45 (3.1)	59 (4.1)	60 (4.2)	119 (4.1)
Upper UTI§				
DS1†	0	1 (0.1)	1 (0.1)	2 (0.1)
DS2‡	1 (0.3)	1 (0.3)	1 (0.3)	2 (0.3)
CANVAS	5 (0.3)	9 (0.6)	3 (0.2)	12 (0.4)
UTI leading to discontinuation				
DS1†	1 (0.2)	1 (0.1)	0	1 (0.1)
DS2‡	2 (0.5)	1 (0.3)	0	1 (0.1)
CANVAS	1 (0.1)	4 (0.3)	2 (0.1)	6 (0.2)
Serious UTI				
DS1†	0	2 (0.2)	1 (0.1)	3 (0.2)
DS2‡	3 (0.8)	1 (0.3)	0	1 (0.1)
CANVAS	2 (0.1)	3 (0.2)	3 (0.2)	6 (0.2)

*DS1: PBO, n = 646; CANA 100 mg, n = 833; CANA 300 mg, n = 834; all CANA, n = 1,667; DS2: PBO, n = 382; CANA 100 mg, n = 338; CANA 300 mg, n = 365; all CANA, n = 703; CANVAS: PBO, n = 1,441; CANA 100 mg, n = 1,445; CANA 300 mg, n = 1,441; all CANA, n = 2,886.
†Including four 26-week, PBO-controlled studies of CANA as monotherapy, add-on to metformin plus sulphonylurea, add-on to metformin vs sitagliptin, and add-on to metformin plus pioglitazone.
‡Including the study in subjects with moderate renal impairment (eGFR >30 and ≤50 mL/min/1.73 m^2) and subsets of subjects from the monotherapy study, the study in older subjects (aged ≥55 to ≤80 y), and CANVAS who had eGFR in the >30 and ≤60 mL/min/1.73 m^2 range.
§Based on a pre-specified list of MedDRA preferred terms.

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Genital mycotic infections with canagliflozin in subjects with type 2 diabetes mellitus

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Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor developed for the treatment of type 2 diabetes mellitus (T2DM), lowers plasma glucose by increasing urinary glucose excretion. Genital mycotic infections (GMIs) associated with CANA were assessed in subjects with T2DM.
Materials and methods: GMIs were characterised based on pooled analyses of data from 4 randomised, double-blind, 26-week, placebo (PBO)-controlled studies in subjects with T2DM (N = 2,313; mean age, 56.0 y; HbA_{1c}, 8.0%; body mass index, 32.1 kg/m^2).
Results: The incidence of GMIs was 11% versus 3% (women) and 4% versus 1% (men) with CANA relative to PBO (**Table**). GMIs were generally considered mild or moderate in severity by investigators, none were serious, and few led to discontinuation; most events with CANA were treated with antifungal agents (women, 82%; men, 83%). CANA-treated women with GMIs were more likely to have a history of vulvovaginitis (29% vs 12%), be premenopausal (35% vs 27%), and reside in North America (60% vs 42%) than those without GMIs. CANA-treated men with GMIs were more likely to have a history of balanitis/balanoposthitis (25% vs 2%), have a slightly longer mean T2DM duration (9 vs 7 years), and to reside in Europe (44% vs 26%) than men without GMIs. With CANA treatment, 2% and 1% of females and males, respectively, had >1 GMI. In a larger dataset (N = 9,439) from 8 studies with longer mean exposure (CANA, 68 weeks; control, 64 weeks), a higher rate of male GMIs was seen (n = 5,493; 8% and 2% for CANA [n = 3,569] and control [n = 1,924]), more commonly in uncircumcised than circumcised men (11% vs 3%) and infrequently associated with phimosis or the need for circumcision.
Conclusion: Treatment with CANA was associated with modestly higher rates of GMIs relative to PBO that were generally manageable with standard treatments.

Table. Summary of Genital Mycotic Infection Adverse Events (AEs)

Female genital mycotic infection AEs[*,†]				
Subjects, n (%)	**PBO (n = 312)**	**CANA 100 mg (n = 425)**	**CANA 300 mg (n = 430)**	**All CANA (n = 855)**
Any AE	10 (3.2)	44 (10.4)	49 (11.4)	93 (10.9)
AEs leading to discontinuation	0	4 (0.9)	2 (0.5)	6 (0.7)
AEs related to study drug[‡]	8 (2.6)	33 (7.8)	45 (10.5)	78 (9.1)
Serious AEs	0	0	0	0
Male genital mycotic infection AEs[§,‖]				
Subjects, n (%)	**PBO (n = 334)**	**CANA 100 mg (n = 408)**	**CANA 300 mg (n = 404)**	**All CANA (n = 812)**
Any AE	2 (0.6)	17 (4.2)	15 (3.7)	32 (3.9)
AEs leading to discontinuation	0	2 (0.5)	2 (0.5)	4 (0.5)
AEs related to study drug[‡]	2 (0.6)	12 (2.9)	12 (3.0)	24 (3.0)
Serious AEs	0	0	0	0

*n = 1,167 females.
†Including genital infection fungal, vaginal infection, vulvitis, vulvovaginal candidiasis, vulvovaginal mycotic infection, and vulvovaginitis.
‡Possibly, probably, or very likely related to study drug, as assessed by investigators.
§n = 1,146 males.
‖Including balanitis, balanitis candida, balanoposthitis, and genital infection fungal.

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Efficacy and safety of canagliflozin in subjects with type 2 diabetes mellitus and chronic kidney disease over 52 weeks

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Background and aims: Canagliflozin (CANA), an SGLT2 inhibitor developed for the treatment of type 2 diabetes mellitus (T2DM), lowers plasma glucose by increasing urinary glucose excretion. This randomised, double-blind, Phase 3 study assessed the efficacy and safety of CANA in subjects with T2DM and chronic kidney disease (CKD) over 52 weeks.
Materials and methods: Subjects (N = 269) with T2DM and Stage 3 CKD (estimated glomerular filtration rate [eGFR] ≥30 and <50 mL/min/1.73 m^2) received CANA 100 or 300 mg or placebo (PBO) added to current therapy (96.3% on insulin and/or sulphonylurea [SU]) during a 26-week core period followed by a 26-week extension (n = 207). Changes from baseline in efficacy parameters at Week 52 are reported. Adverse events (AEs) were recorded throughout the study. Renal safety parameters were also evaluated.
Results: Mean baseline characteristics were similar across groups (age, 68.5 y; HbA_{1c}, 8.0%; fasting plasma glucose [FPG], 9.1 mmol/L; BMI, 33.0 kg/m^2; eGFR, 39.4 mL/min/1.73 m^2; median albumin/creatinine ratio [ACR], 30.0 µg/mg). At 52 weeks, CANA 100 and 300 mg reduced HbA_{1c}, FPG, body weight, and systolic BP relative to PBO; small increases in high-density lipoprotein cholesterol (HDL-C) and decreases in low-density lipoprotein cholesterol (LDL-C) were seen with both CANA doses versus PBO (**Table**).

Table. Summary of Efficacy Endpoints at Week 52 (mITT, LOCF)

Parameter*	CANA 100 mg	CANA 300 mg	PBO
HbA_{1c} change, %	–0.19 (0.10)	–0.33 (0.10)	0.07 (0.10)
Difference vs PBO	–0.27 (–0.53, 0.001)	–0.41 (–0.68, –0.14)	
% of subjects reaching HbA_{1c} <7.0%†	23.6 (4.5)	28.1 (4.8)	18.4 (4.2)
Difference vs PBO	5.2 (–7.9, 18.3)	9.7 (–3.8, 23.2)	
FPG change, mmol/L	–0.1 (0.3)	–0.3 (0.3)	0.5 (0.3)
Difference vs PBO	–0.7 (–1.5, 0.2)	–0.8 (–1.7, 0.1)	
Body weight % change	–1.3 (0.4)	–1.0 (0.4)	0.1 (0.4)
Difference vs PBO	–1.5 (–2.6, –0.4)	–1.1 (–2.2, 0.0)	
Systolic BP change, mmHg	–5.5 (1.5)	–6.7 (1.5)	–0.01 (1.5)
Difference vs PBO	–5.5 (–9.3, –1.7)	–6.7 (–10.5, –2.9)	
Diastolic BP change, mmHg	–2.0 (1.0)	–2.4 (1.0)	–1.4 (1.0)
Difference vs PBO	–0.7 (–3.1, 1.8)	–1.0 (–3.5, 1.5)	
Triglycerides % change	12.4 (4.8)	9.1 (4.7)	9.9 (4.9)
Difference vs PBO	2.5 (–9.9, 14.9)	–0.8 (–13.0, 11.4)	
HDL-C % change	3.3 (1.9)	4.1 (1.8)	1.3 (1.9)
Difference vs PBO	1.9 (–2.9, 6.8)	2.8 (–1.9, 7.6)	
LDL-C % change	6.4 (3.8)	2.5 (3.7)	9.4 (3.9)
Difference vs PBO	–3.0 (–12.7, 6.8)	–6.9 (–16.6, 2.8)	
LDL-C/HDL-C % change	4.7 (4.1)	–1.2 (4.0)	9.0 (4.2)
Difference vs PBO	–4.3 (–14.9, 6.3)	–10.2 (–20.7, 0.4)	
Non–HDL-C % change	6.1 (2.9)	3.5 (2.8)	6.4 (3.0)
Difference vs PBO	–0.3 (–7.9, 7.3)	–2.9 (–10.3, 4.6)	

mITT, modified intent to treat; LOCF, last observation carried forward; LS, least squares; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. *LS mean (SE) change from baseline using ANCOVA and PBO-subtracted LS mean (95% CI) for all parameters except for % of subjects reaching HbA_{1c} <7.0%; †% (SE) and PBO-subtracted % (95% CI) of subjects reaching HbA_{1c} <7.0%.

Overall AE rates were slightly higher with PBO (86.7%) than CANA 100 and 300 mg (85.6%, 80.9%). Serious AE rates were lower with CANA 100 and 300 mg than PBO (20.0%, 23.6%, 26.7%); AE-related discontinuation rates were low across groups. Rates of osmotic diuresis-related AEs (eg, pollakiuria, thirst) were higher with CANA 100 and 300 mg than PBO (3.3%, 6.7%, 2.2%). Higher rates were seen with CANA 300 mg than CANA 100 mg and PBO for urinary tract infections (14.6%, 5.6%, 10.0%) and AEs related to reduced intravascular volume (eg, postural dizziness, orthostatic hypotension; 12.4%, 5.6%, 5.6%). Genital mycotic infection rates were low in women and men (n ≤2 per group). Among subjects on background therapy associated with hypoglycaemia (insulin/SU), documented hypoglycaemia rates were higher with CANA 100 and 300 mg than PBO (61.2%, 57.0%, 43.2%); severe hypoglycaemia rates were low, but higher with CANA 100 and 300 mg than PBO (5.9%, 2.3%, 1.1%). No hypoglycaemia was reported in subjects not on insulin/SU. Increases in blood urea nitrogen (12.0%, 17.3%, 5.4%) and decreases in eGFR (-4.3%, -9.4%, -2.8%) were seen with CANA 100 and 300 mg versus PBO. Median ACR was decreased with CANA 100 and 300 mg versus PBO (-16.4%, -28.0%, 19.7%).
Conclusion: CANA 100 and 300 mg improved glycaemic control and were generally well tolerated in subjects with T2DM and Stage 3 CKD over 52 weeks.
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Empagliflozin in patients with type 2 diabetes mellitus and stage 3A, 3B and 4 chronic kidney disease (CKD)

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Background and aims: A Phase III trial investigated the efficacy and safety of empagliflozin (EMPA) as add-on to existing therapy for 52 weeks in patients with T2DM and CKD stage 3A, 3B and 4.
Materials and methods: Patients with CKD stage 3A (eGFR [MDRD equation] ≥45 to <60 ml/min/1.73 m^2; n=180; mean [SD] age 64.5 [8.0] years; mean [SD] BMI 30.5 [5.2] kg/m^2), CKD stage 3B (eGFR ≥30 to <45 ml/min/1.73 m^2; n=194; mean [SD] age 65.2 [9.0] years; mean [SD] BMI 30.0 [5.4] kg/m^2) or CKD stage 4 (eGFR ≥15 to <30 ml/min/1.73 m^2; n=74; mean [SD] age 64.1 [11.1] years; mean [SD] BMI 30.4 [5.6] kg/m^2) received EMPA 25 mg qd or PBO for 52 weeks. In exploratory analyses, we assessed the long-term efficacy and safety of EMPA, including changes from baseline in HbA_{1c}, fasting plasma glucose (FPG), weight and systolic and diastolic blood pressure (SBP and DBP) at week 52.
Results: EMPA 25 mg significantly reduced HbA_{1c} vs PBO at week 52 in patients with CKD stage 3A and 3B (Table). EMPA 25 mg did not reduce HbA_{1c} in patients with CKD stage 4 (Table). EMPA significantly reduced FPG, weight, SBP and DBP in patients with CKD stage 3A, and significantly reduced FPG and weight in patients with CKD stage 3B (Table). Reductions in FPG, weight and BP were observed in patients with CKD stage 4 (Table). During 52 weeks' treatment, adverse events (AEs) were reported by 86.8% and 79.8% of patients with CKD stage 3A on EMPA 25 mg and PBO, respectively, by 80.2% and 86.7% of patients with CKD stage 3B on EMPA 25 mg and PBO, respectively, and by 91.9% and 83.8% of patients with CKD stage 4 on EMPA 25 mg and PBO, respectively. AEs consistent with volume depletion were reported by 4.4% and 2.2% of patients with CKD stage 3A on EMPA 25 mg and PBO, respectively, by 3.1% of patients with CKD stage 3B on EMPA 25 mg or PBO, and by 5.4% patients with CKD stage 4 on EMPA 25 mg or PBO. AEs consistent with urinary tract infection were reported by 16.5% and 18.0% of patients with CKD stage 3A on EMPA 25 mg and PBO, respectively, by 16.7% and 13.3% of patients with CKD stage 3B on EMPA 25 mg and PBO, respectively, and by 18.9% and 8.1% of patients with CKD stage 4 on EMPA 25 mg and PBO, respectively. Treatment with EMPA for 52 weeks resulted in small decreases in eGFR in each group; eGFR returned to near baseline values within 3 weeks of last drug intake.
Conclusion: EMPA 25 mg for 52 weeks was associated with significant and clinically meaningful reductions in HbA_{1c} compared with placebo in patients with T2DM and CKD stage 3A or 3B. EMPA led to favourable reductions in body weight and BP in patients with T2DM and CKD stage 3A, 3B or 4. EMPA was well tolerated, and showed small changes in eGFR that were reversible after the end of treatment.

	CKD stage 3A Placebo (n=89)	CKD stage 3A EMPA 25 mg (n=91)	CKD stage 3B Placebo (n=98)	CKD stage 3B EMPA 25 mg (n=96)	CKD stage 4 Placebo (n=37)	CKD stage 4 EMPA 25 mg (n=37)
Baseline HbA_{1c},% (SE)[†]	8.08 (0.09)	8.12 (0.08)	8.01 (0.08)	7.95 (0.08)	8.18 (0.16)	8.06 (0.17)
Change from baseline in HbA_{1c} at week 52, % (SE)	0.06 (0.07)	-0.48 (0.07)***	0.19 (0.08)	-0.18 (0.08)**	-0.37 (0.13)	0.11 (0.24)
Baseline FPG, mg/dL (SE)	154.1 (4.6)	144.6 (4.14)	134.4 (5.3)	141.1 (3.4)	147.3 (9.6)	157.1 (8.8)
Change from baseline in FPG at week 52, mg/dL (SE)	2.6 (4.3)	-10.0 (4.3)*	9.0 (4.4)	-4.6 (4.5)*	6.8 (11.5)	-4.7 (12.1)
Baseline body weight, kg (SE)	83.2 (1.8)	84.9 (2.2)	81.8 (1.9)	81.6 (1.9)	84.1 (3.5)	77.9 (2.7)
Change from baseline in body weight at week 52, kg (SE)	-0.1 (0.3)	-1.4 (0.3)**	0.1 (0.3)	-1.0 (0.3)*	-0.0 (0.6)	-1.0 (0.5)
Baseline SBP, mmHg (SE)	137.3 (1.9)	135.0 (2.0)	135.6 (1.9)	138.2 (1.7)	146.2 (3.6)	145.0 (3.4)
Change from baseline in SBP at week 52, mmHg (SE)	-0.9 (1.4)	-6.6 (1.4)**	-0.7 (1.4)	-3.6 (1.4)	1.0 (2.9)	-11.2 (2.6)
Baseline DBP, mmHg (SE)	76.5 (0.9)	75.0 (1.1)	72.8 (1.0)	75.4 (1.0)	78.0 (2.0)	77.2 (1.5)
Change from baseline in DBP at week 52, mmHg (SE)	-0.4 (0.7)	-2.9 (0.7)*	-0.2 (0.6)	-0.7 (0.6)	1.4 (1.4)	-4.3 (1.5)

Adjusted means based on ANCOVA in full analysis set with last observation carried forward imputation (CKD stage 3A and 3B). Results in CKD stage 4 are descriptive statistics with last observation carried forward imputation. [†]Inclusion criteria: HbA_{1c} ≥7.0% to ≤10.0%. **p<0.01 vs placebo; ***p<0.001 vs placebo.

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Canagliflozin is effective and generally well tolerated in subjects with type 2 diabetes mellitus and Stage 3 chronic kidney disease

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Background and aims: The efficacy and safety of the sodium glucose co-transporter 2 (SGLT2) inhibitor, canagliflozin (CANA), were evaluated in subjects with type 2 diabetes mellitus (T2DM) and Stage 3 chronic kidney disease (CKD) using a pooled analysis of data from four Phase 3 clinical studies.
Materials and methods: Data were pooled from subjects with T2DM from 4 randomised, placebo (PBO)-controlled studies (at Week 18 [1 study] and Week 26 [3 studies]) who had a baseline estimated glomerular filtration rate (eGFR) ≥30 and <60 mL/min/1.73 m^2 (N = 1,085), and in subgroups of subjects with baseline eGFR ≥45 and <60 mL/min/1.73 m^2 (n = 721) or ≥30 and <45 mL/min/1.73 m^2 (n = 364).
Results: CANA 100 and 300 mg reduced HbA_{1c}, body weight, and systolic blood pressure compared with PBO in the overall population and in subjects with eGFR ≥45 or <45 mL/min/1.73 m^2 (Table); HbA_{1c} and body weight changes with CANA relative to PBO were larger in subjects with eGFR ≥45 mL/min/1.73 m^2 than in those with eGFR <45 mL/min/1.73 m^2. For the pooled CANA group, the incidence of overall AEs was higher than with PBO across populations (eGFR ≥30 to <60: 74.7% vs 70.4%; ≥45: 71.0% vs 66.9%; <45: 81.5% vs 78.4%); serious AE rates were higher with PBO than CANA and AE-related discontinuation rates were low across populations. Incidences of osmotic diuresis-related AEs (eg, pollakiuria [increased urine frequency], polyuria [increased urine volume]) were higher with CANA than PBO in subjects with eGFR ≥30 and <60 mL/min/1.73 m^2 (4.0% vs 3.7%) and in those with eGFR <45 mL/min/1.73 m^2 (4.8% vs 2.6%). Incidences of AEs related to reduced intravascular volume (eg, orthostatic hypotension, postural dizziness) were higher with CANA than PBO across populations (eGFR ≥30 to <60: 6.8% vs 2.6%; ≥45: 5.9% vs 3.4%; <45: 8.9% vs 1.7%). Rates of serious renal-related AEs were low and similar across groups.
Conclusion: In subjects with T2DM and Stage 3 CKD, CANA reduced HbA_{1c}, with a greater effect in subjects with eGFR ≥45 than <45 mL/min/1.73 m^2, and was generally well tolerated.

Table. Summary of Efficacy Parameters

Parameter[*,†]	Baseline eGFR, mL/min/1.73 m^2		
HbA_{1c}	≥30 and <60[‡]	≥45 and <60[§]	≥30 and <45[‖]
PBO			
Change, %	−0.14 (0.06)	−0.10 (0.07)	0.05 (0.19)
CANA 100 mg			
Change, %	−0.52 (0.06)	−0.57 (0.07)	−0.18 (0.19)
Difference vs PBO	−0.38 (−0.50, −0.26)[¶]	−0.47 (−0.61, −0.32)	−0.23 (−0.45, −0.01)
CANA 300 mg			
Change, %	−0.62 (0.06)	−0.62 (0.07)	−0.34 (0.19)
Difference vs PBO	−0.47 (−0.60, −0.35)[¶]	−0.52 (−0.67, −0.38)	−0.39 (−0.61, −0.17)
Body weight	**≥30 and <60[‡]**	**≥45 and <60[§]**	**≥30 and <45[‖]**
PBO			
Change, kg	−0.5 (0.2)	−0.5 (0.2)	0.7 (0.6)
% change	−0.5 (0.2)	−0.6 (0.2)	0.9 (0.6)
CANA 100 mg			
Change, kg	−1.9 (0.2)	−2.1 (0.2)	−0.3 (0.6)
% change	−2.0 (0.2)	−2.3 (0.2)	−0.3 (0.6)
Difference vs PBO	−1.6 (−2.0, −1.1)[¶]	−1.8 (−2.3, −1.2)	−1.2 (−1.9, −0.5)
CANA 300 mg			
Change, kg	−2.3 (0.2)	−2.4 (0.2)	−0.8 (0.6)
% change	−2.4 (0.2)	−2.5 (0.2)	−0.9 (0.6)
Difference vs PBO	−1.9 (−2.3, −1.5)[¶]	−2.0 (−2.5, −1.5)	−1.8 (−2.5, −1.1)
Systolic BP	**≥30 and <60[‡]**	**≥45 and <60[§]**	**≥30 and <45[‖]**
PBO			
Change, mmHg	−1.6 (0.9)	−2.6 (1.0)	5.7 (3.2)
CANA 100 mg			
Change, mmHg	−4.4 (0.9)	−4.4 (1.1)	0.8 (3.2)
Difference vs PBO	−2.8 (−4.7, −0.8)	−1.8 (−4.1, 0.5)	−4.8 (−8.5, −1.2)
CANA 300 mg			
Change, mmHg	−6.0 (0.9)	−6.8 (1.1)	0.8 (3.3)
Difference vs PBO	−4.4 (−6.3, −2.4)	−4.3 (−6.5, −2.0)	−4.9 (−8.5, −1.2)

LS, least squares; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. [*]LS mean (SE) change from baseline using ANCOVA and PBO-subtracted LS mean (95% CI) values; [†]*P* values are reported for pre-specified comparisons only; [‡]Mean baseline age, 67.1 y; HbA_{1c}, 8.1%; eGFR, 48.2 mL/min/1.73 m^2; body weight, 90.9 kg; [§]Mean baseline age, 66.3 y; HbA_{1c}, 8.1%; eGFR, 53.3 mL/min/1.73 m^2; body weight, 90.4 kg; [‖]Mean baseline age, 68.6 y; HbA_{1c}, 8.1%; eGFR, 38.2 mL/min/1.73 m^2; body weight, 91.7 kg; [¶]*P* <0.001 vs PBO.

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Safety and efficacy of the SGLT2 inhibitor dapagliflozin in older patients with type 2 diabetes

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Background and aims: Given the high prevalence of type 2 diabetes (T2DM) and its co-morbid conditions in the elderly, this report summarises efficacy and safety observations in patients aged ≥65 years treated with dapagliflozin (DAPA) 10 mg daily. DAPA, a first-in-class inhibitor of the sodium–glucose co-transporter 2 (SGLT2), reduces renal glucose reabsorption and increases glucuresis, resulting in improved glycaemic control and weight loss. DAPA efficacy is dependent on the filtered glucose load, a function of the plasma glucose concentration and the glomerular filtration rate (GFR).
Materials and methods: Efficacy variables were analysed using pooled data from 9 phase 3 studies of DAPA (n=4047; placebo [PBO]=1257, DAPA=2790) up to 24 weeks. Safety variables were analysed using pooled data from 12 phase 2b/3 studies of DAPA (n=4684; PBO=1393, DAPA=3291), including studies as monotherapy or in combination with metformin, pioglitazone, sulphonylurea, or insulin.
Results: Approximately 20% of patients in both efficacy (776/4047) and safety (907/4684) analyses were aged ≥65 years, with equal gender distribution. Treatment with DAPA was associated with HbA1c reductions across baseline age subgroups (<65 years and ≥65 years). PBO-corrected reductions in HbA1c appeared to be greater in patients aged <65 years (-0.62% [95% CI -0.71, -0.54]) than in patients aged ≥65 years (-0.41% [-0.58, -0.22]). Efficacy was assessed in different estimated GFR (eGFR) categories and did not appear to be age dependent in patients with eGFR ≥60 mL/min/1.72 m^2. After controlling for eGFR, no conclusive evidence was found that age affects efficacy as an independent factor (interaction *P*-value=0.2903). PBO-corrected reductions in body weight were similar in patients aged <65 years (-1.77 kg [-2.05, -1.48]) and ≥65 years (-1.73 kg [-2.32, -1.41]). Overall, the proportions of patients with adverse events (AEs) were similar in both age populations. Events of special interest are presented in Table 1. Hypoglycaemia, volume depletion, and renal AEs occurred more often in patients aged ≥65 years. Orthostatic hypotension occurred infrequently in both age groups (<0.1%). The majority of renal AEs were patients reaching pre-defined thresholds of serum creatinine. AEs of urinary tract and genital infection were similar for both DAPA-treated age groups.
Conclusion: These results demonstrate that DAPA is efficacious and safe as monotherapy or combination therapy in patients aged ≥65 years when compared with placebo. Findings of somewhat reduced efficacy and slightly increased renal AEs in patients aged ≥65 years reflect the lower eGFR in this population. DAPA's efficacy and safety observations indicate a practical, new approach to the treatment of T2DM patients aged ≥65 years.

Table 1

	Patients aged <65 years		Patients aged =65 years	
N (%)	PBO N=1117	DAPA 10 mg N=989	PBO N=276	DAPA 10 mg N=204
All AEs	638 (57.1)	611 (61.8)	154 (55.8)	123 (60.3)
Hypoglycaemia	72 (6.4)	95 (9.6)	26 (9.4)	27 (13.2)
Urinary tract infection	45 (4.0)	41 (4.1)	7 (2.5)	10 (4.9)
Genital infection	10 (0.9)	50 (5.1)	2 (0.7)	7 (3.4)
Hypotension/hypovolemia/dehydration (volume depletion)	4 (0.4)	6 (0.6)	1 (0.4)	2 (1.0)
Renal impairment or failure	9 (0.8)	6 (0.6)	3 (1.1)	5 (2.5)

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Efficacy and safety of canagliflozin in older subjects with type 2 diabetes mellitus

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Background and aims: The efficacy and safety of canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor, were evaluated in older subjects with type 2 diabetes mellitus (T2DM) using a pooled analysis of data from four Phase 3 clinical studies.
Materials and methods: Data from subjects with T2DM were pooled from 4 randomised, placebo (PBO)-controlled, 26-week studies (N = 2,313) and were analysed by age: <65 years (n = 1,868; male, 49.1%; mean age, 52.8 years; HbA_{1c}, 8.0%; body weight, 90.1 kg; estimated glomerular filtration rate [eGFR], 90.8 mL/min/1.73 m^2) or ≥65 years (n = 445; male, 51.5%; mean age, 69.3 years; HbA_{1c}, 7.9%; body weight, 85.1 kg; eGFR, 76.9 mL/min/1.73 m^2).
Results: CANA 100 and 300 mg reduced HbA_{1c}, body weight, and systolic blood pressure compared with PBO in subjects <65 and ≥65 years of age (Table); similar lipid changes were seen in both age groups. The incidence of overall adverse events (AEs) was similar with CANA 100 and 300 mg and PBO in subjects <65 years (59.9%, 59.0%, 58.9%) and those ≥65 years (61.0%, 60.4%, 61.3%). Rates of serious AEs and AE-related discontinuations were similar with CANA 100 and 300 mg and PBO in subjects <65 years (serious AEs: 2.5%, 2.5%, 3.3%; AE-related discontinuations: 3.3%, 3.2%, 2.8%), and higher with CANA 100 mg than CANA 300 mg or PBO in subjects ≥65 years (serious AEs: 6.9%, 3.4%, 3.6%; AE-related discontinuations: 8.8%, 5.4%, 4.4%). As in subjects <65 years, those ≥65 years who received CANA had higher rates than PBO of genital mycotic infections in women and men and osmotic diuresis-related AEs (Table); the incidence of AEs related to reduced intravascular volume was low across treatment groups in both age groups. Incidences of urinary tract infections and renal-related AEs were similar across groups in subjects ≥65 years.
Conclusion: CANA 100 and 300 mg provided reductions in HbA_{1c} and body weight and were generally well tolerated in older subjects with T2DM.

Table. Summary of Efficacy Parameters and Selected AEs for Subjects <65 and ≥65 y

	Subjects <65 y			Subjects ≥65 y		
Efficacy Parameters[*,†]	**PBO (n = 509)**	**CANA 100 mg (n = 674)**	**CANA 300 mg (n = 685)**	**PBO (n = 137)**	**CANA 100 mg (n = 159)**	**CANA 300 mg (n = 149)**
HbA_{1c}, change, %	−0.15 (0.04)	−0.89 (0.03)	−1.08 (0.03)	−0.05 (0.07)	−0.69 (0.07)	−0.88 (0.07)
Difference vs PBO		−0.74 (0.05)	−0.93 (0.05)		−0.64 (0.10)	−0.82 (0.10)
Body weight % change	−0.6 (0.2)	−2.8 (0.1)	−3.4 (0.1)	−0.6 (0.3)	−2.9 (0.3)	−3.8 (0.3)
Difference vs PBO		−2.2 (0.2)	−2.9 (0.2)		−2.3 (0.4)	−3.2 (0.4)
Systolic BP change, mmHg	−0.3 (0.5)	−4.2 (0.4)	−4.8 (0.4)	−0.8 (1.1)	−4.6 (1.0)	−5.9 (1.0)
Difference vs PBO		−3.9 (0.6)	−4.5 (0.6)		−3.9 (1.4)	−5.1 (1.5)
Triglycerides % change[‡]	9.1 (2.2)	3.7 (2.0)	0.6 (2.0)	2.6 (3.0)	−2.4 (2.8)	−2.5 (2.9)
Difference vs PBO		−5.4 (2.9)	−8.5 (2.9)		−5.0 (4.1)	−5.2 (4.2)
LDL-C % change[‡]	1.5 (1.4)	6.3 (1.2)	9.8 (1.2)	0.7 (2.1)	3.5 (1.9)	6.8 (2.0)
Difference vs PBO		4.8 (1.8)	8.3 (1.8)		2.8 (2.9)	6.1 (2.9)
HDL-C % change[‡]	4.0 (0.8)	9.2 (0.7)	10.2 (0.7)	3.7 (1.4)	9.7 (1.3)	10.7 (1.4)
Difference vs PBO		5.2 (1.1)	6.2 (1.1)		6.0 (1.9)	7.1 (1.9)
LDL-C/HDL-C % change[‡]	−0.5 (1.4)	−0.7 (1.2)	1.3 (1.2)	−1.4 (2.3)	−4.2 (2.2)	−1.2 (2.3)
Difference vs PBO		−0.2 (1.8)	1.8 (1.8)		−2.8 (3.2)	0.2 (3.2)
Non–HDL-C % change[‡]	1.1 (1.0)	2.8 (0.9)	4.7 (0.9)	−0.5 (1.8)	0.8 (1.7)	2.9 (1.8)
Difference vs PBO		1.4 (1.4)	3.6 (1.4)		1.4 (2.5)	3.4 (2.6)
Selected AEs[§]	**PBO (n = 509)**	**CANA 100 mg (n = 674)**	**CANA 300 mg (n = 685)**	**PBO (n = 137)**	**CANA 100 mg (n = 159)**	**CANA 300 mg (n = 149)**
Genital mycotic infection						
Male[‖]	2 (0.8)	14 (4.3)	11 (3.4)	0	3 (3.7)	4 (5.2)
Female[¶]	10 (4.1)	37 (10.7)	44 (12.3)	0	7 (9.0)	5 (6.9)
Urinary tract infection	20 (3.9)	41 (6.1)	29 (4.2)	6 (4.4)	8 (5.0)	7 (4.7)
Osmotic diuresis-related AEs	4 (0.8)	44 (6.5)	39 (5.7)	1 (0.7)	12 (7.5)	8 (5.4)
Volume-related AEs	5 (1.0)	6 (0.9)	8 (1.2)	2 (1.5)	4 (2.5)	3 (2.0)
Renal-related AEs	2 (0.4)	2 (0.3)	12 (1.8)	2 (1.5)	3 (1.9)	2 (1.3)

LS, least squares; SE, standard error; ANCOVA, analysis of covariance. *LS mean (SE) change from baseline using ANCOVA and PBO-subtracted LS mean (SE) values; †Data are reported for prior to rescue therapy except for lipid parameters, which are for regardless of rescue therapy; ‡% changes determined based on changes in conventional units; §Number (%) of subjects; ‖Males <65 y: PBO, n = 263; CANA 100 mg, n = 327; CANA 300 mg, n = 327; males ≥65 y: PBO, n = 71; CANA 100 mg, n = 81; CANA 300 mg, n = 77; ¶Females <65 y: PBO, n = 246; CANA 100 mg, n = 347; CANA 300 mg, n = 358; females ≥65 y: PBO, n = 66; CANA 100 mg, n = 78; CANA 300 mg, n = 72.

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Luseogliflozin, a SGLT2 inhibitor, improves glycaemic control and reduces body weight as monotherapy up to 52 weeks in Japanese patients with type 2 diabetes mellitus

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Background and aims: Luseogliflozin is a highly selective sodium glucose co-transporter 2 (SGLT2) inhibitor. In a previous 12-weeks confirmatory dose finding study, 2.5 mg or higher doses of luseogliflozin showed amelioration of glycemic control in Japanese patients with type 2 diabetes mellitus (T2DM). The aims were to evaluate the efficacy and safety of luseogliflozin monotherapy compared with placebo over 24 weeks (Study-1, N = 158), and to evaluate the long-term efficacy and safety of luseogliflozin as monotherapy up to 52 weeks (Study-2, N = 299), in Japanese T2DM patients inadequately controlled with diet and exercise.
Materials and methods: In Study-1, a double-blind, placebo-controlled confirmatory study, a total of 158 Japanese patients with T2DM (HbA1c 6.9-10.4%) were randomized to receive luseogliflozin 2.5 mg (N = 79) or placebo (N = 79) once daily for 24 weeks. Change from baseline and difference versus placebo in HbA1c and other efficacy parameters were assessed at week 24 using last observation carried forward (LOCF). In Study-2, an open-label study, Japanese patients with T2DM (HbA1c 6.9-10.4%) received luseogliflozin 2.5 mg (N = 299) once daily for 52 weeks. The starting dose of luseogliflozin was 2.5 mg. Patients whose glycemic control was insufficient could be titrated from 2.5 mg to 5 mg at week 24. Change from baseline in HbA1c and other efficacy parameters were assessed at week 52. In both studies, adverse events, clinical laboratory tests, vital signs and 12-lead ECGs were recorded throughout the study.
Results: Luseogliflozin significantly reduced HbA1c, fasting plasma glucose (FPG), 2-hour postprandial plasma glucose (PPG), body weight and abdominal circumstance (Table). In addition, luseogliflozin reduced systolic blood pressure (difference versus placebo; -5.6 mmHg) and diastolic blood pressure (difference versus placebo; -2.5 mmHg) without relevant change in heart rate and showed trends toward improvement in plasma lipids (triglyceride and HDL-cholesterol) in both studies. Frequencies of adverse events were similar across groups (Luseogliflozin 2.5mg, 59.5%; placebo, 57.0%) in Study-1 and 75.3% in Study-2. Most adverse events were mild in severity. There were no major changes in clinical laboratory tests. Luseogliflozin was well tolerated throughout both studies.
Conclusion: Once daily treatment of luseogliflozin as monotherapy for 24 weeks significantly improved glycemic control and reduced body weight, with amelioration of abdominal circumstance compared with placebo. Glycemic control and body weight reduction were sustained for up to 52-weeks. Luseogliflozin showed trend toward improvement in blood pressures and plasma lipids, and was well tolerated in Japanese patients with T2DM.

Table. Summary efficacy endpoints

Study-1 (24 weeks, Double-blind, N = 158)

	Placebo (N = 79)	Luseogliflozin (N = 79)	Difference vs placebo
HbA1c, %			
Baseline	8.17	8.14	—
Change from baseline	0.13 (-0.04, 0.29)	-0.63 (-0.79, -0.46)	-0.75 (-0.99, -0.52)**
FPG, mg/dL			
Baseline	161.9	160.8	—
Change from baseline	-0.8 (-5.4, 3.7)	-28.3 (-32.9, -23.8)	-27.5 (-33.9, -21.1)**
2-hour PPG, mg/dL			
Baseline	262.0	257.4	—
Change from baseline	1.1 (-8.0, 10.1)	-55.8 (-64.7, -46.8)	-56.8 (-69.6, -44.1)**
Body weight, kg			
Baseline	66.7	70.2	—
Change from baseline	-0.9 (-1.3, -0.6)	-2.7 (-3.1, -2.3)	-1.8 (-2.3, -1.2)**
Abdominal circumstance, cm			
Baseline	88.7	90.5	—
Change from baseline	-0.92 (-1.51, -0.33)	-2.17 (-2.77, -1.58)	-1.26 (-2.09, -0.42)*

Study-2 (52 weeks, Open-label, N = 279†)

	Baseline	Week 52	Change from baseline
HbA1c, %	7.68	7.19	-0.50 (-0.6, -0.4)#
FPG, mg/dL	139.4	123.1	-16.3 (-19, -14)#
Body weight, kg	70.2	67.5	-2.7 (-2.9, -2.4)#

[Study-1]
Mean value for baseline, LS mean (95% CI) for change from baseline at week 24 using last observation carried forward; ANCOVA with baseline as covariate for HbA1c, FPG and 2-hour PPG, ANOVA for body weight and abdominal circumstance; *: P<0.05 vs placebo , **: P<0.001 vs placebo
[Study-2]
†: patients who completed 52-weeks trial period; Mean value for baseline and week 52, LS mean (95% CI) for change from baseline at week 52; 1-sample t-test #: P<0.05 vs baseline

Clinical Trial Registration Number: JapicCTI-111509, JapicCTI-111661

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Luseogliflozin, a SGLT2 inhibitor, as add-on therapy to 5 types of oral antidiabetic drugs improves glycaemic control and reduces body weight in Japanese patients with type 2 diabetes mellitus

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Background and aims: Luseogliflozin increases urinary glucose excretion by inhibiting renal sodium glucose co-tanspoter 2 (SGLT2). In a previous 12 weeks monotherapy study, once daily administration of luseogliflozin 2.5 mg or higher doses significantly improved glycemic control and showed a favorable safety profile in patients with type 2 diabetes mellitus (T2DM). In drug-drug interaction studies of luseogliflozin with other oral antidiabetic drugs (OADs), no significant pharmacokinetic interactions between luseogliflozin and OADs were observed. This study examined the long-term efficacy and safety of luseogliflozin added-on to OADs [metformin (MET), dipeptidyl peptidase-4 inhibitor (DPP4i), pioglitazone (PIO), Glinide, or alpha-glucosidase inhibitor (α-GI)] for 52 weeks in Japanese patients with T2DM inadequately controlled with OADs monotherapy. The effects of add-on luseogliflozin on glimepiride was examined in another study.
Materials and methods: In this phase 3, multicenter study, patients exhibiting insufficient glycemic control (HbA1c 6.9-10.4%), who had received OADs monotherapy with fixed dose over 12 weeks, were administered 2.5 mg luseogliflozin as starting dose [Total (N = 487); MET (N = 117), DPP4i (N = 111), PIO (N = 95), Glinide (N = 59), α-GI (N = 105)]. Patients received luseogliflozin once daily for 52 weeks. If their glycemic control was insufficient, dose of luseogliflozin could be increased to 5 mg at week 24. Changes from baseline in glycemic and other efficacy endpoints were assessed. Adverse events (AEs), clinical laboratory tests, vital signs and 12-lead ECGs were recorded throughout the study.
Results: Mean baseline characteristics in each group were 7.8-8.0% in HbA1c, 142-152 mg/dL in fasting plasma glucose (FPG), and 25.1-26.9 kg/m^2 in BMI. HbA1c, FPG, and body weight were significantly decreased from baseline at week 52 in all groups (shown in Table). Luseogliflozin decreased blood pressure and showed trends toward improvement in plasma lipids (triglyceride and HDL-cholesterol) at week 52 compared with baseline in all groups. The frequency of AEs, serious AEs, AEs leading to discontinuation were comparable across treatment groups. In AEs of special interest, hypoglycemia, pollakiuria or urine output increase and events suggestive of urinary tract/genital infection were observed in all groups, but most of these events were mild in severity. Luseogliflozin was well tolerated throughout the study.
Conclusion: Luseogliflozin combination therapy with other OADs improved glycemic control and showed a favorable safety profile without dose adjustments. Luseogliflozin may provide a new treatment option in patients with T2DM inadequately controlled with currently available OADs.

Table. Mean change from baseline of the efficacy parameters after 52-weeks dosing of luseogliflozin

		MET	DPP4i	PIO	Glinide	α-GI
		N = 109	N = 103	N = 83	N = 49	N = 94
HbA1c * (%)	Baseline	7.82	7.86	7.99	8.03	7.84
	Week 52	7.21	7.34	7.39	7.43	7.16
	Difference from baseline	-0.61 **	-0.52 **	-0.60 **	-0.59 **	-0.68 **
FPG * (mg/dL)	Baseline	143.6	151.2	142.3	148.1	147.9
	Week 52	125.8	132.7	123.7	128.7	126.5
	Difference from baseline	-17.8 **	-18.5 **	-18.6 **	-19.4 **	-21.4 **
Body weight * (kg)	Baseline	69.3	68.6	71.7	67.7	66.0
	Week 52	66.5	66.6	69.4	64.8	63.2
	Difference from baseline	-2.9 **	-2.0 **	-2.3 **	-2.9 **	-2.8 **

* The data from patients who completed 52-weeks trial period
1-sample t-test **: P < 0.05 vs baseline

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PS 076 Metformin: old agent - novel perspectives

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Metformin and berberine promote glucose metabolism via inhibiting mitochondrial respiratory chain complex I independently of AMPK activation

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Background and aims: Metformin is a well-known hypoglycemic agent for treatment of type 2 diabetes. Berberine, a plant alkaloid, has also been found to possess anti-diabetic action. The mechanisms of both medicines were proposed to activate AMP-activated protein kinase (AMPK) pathway. This study aimed to examine whether AMPK activation was necessary for their glucose-lowering effect, and tried to explore the exact mechanism.
Materials and methods: Glucose consumption was used to evaluate the glucose-lowering effect of metformin and berberine on HepG2 hepatocytes and C2C12 myotubes. Compound C, an AMPK inhibitor, or small interference RNA (siRNA) was administrated to block the activity or expression of AMPKα in the cells. AMPK phosphorylation and ACC phosphorylation were measured with western blot to assess the activity of AMPK pathway. Anaerobic glycolysis was evaluated through determining lactate concentration in the medium. Oxygen consumption rate of C2C12 myotubes was determined in Seahorse XF24 analyzer to estimate the mitochondrial respiratory function.
Results: In this study, we found that in HepG2 hepatocyte and C2C12 myotubes, metformin and berberine significantly increased glucose consumption in a dose-dependent manner. Meanwhile, AMPK and ACC phosphorylation was stimulated by 10 mmol/L metformin or 20 μmol/L berberine. Nevertheless, the inhibition of AMPK activity by Compound C, or suppression of AMPKα expression by siRNA, failed to diminish the promotion in glucose utilization of both medicines. On the other hand, the lactate production was largely enhanced by metformin or berberine in the cells in the same way that they affect glucose consumption. Both drugs deeply inhibited the mitochondrial respiratory function in C2C12 myotubes. The activity of respiratory chain complex I was significantly reduced. All these changes could not be blocked by Compound C.
Conclusion: These results suggest that metformin and berberine promote glucose metabolism by stimulation of glycolysis, which is probably resulted from inhibition of mitochondrial respiratory chain complex I, independent of AMPK activation.
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Early phase of metformin action in dietary-obese mice: lack of involvement of AMPK and possible interaction with *n-3* fatty acids

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Background and aims: Long-chain *n*-3 polyunsaturated fatty acids (omega-3) act as natural hypolipidaemics and prevent development of cardiovascular disease in humans. We have shown that omega-3 could prevent impairement of hepatic insulin resistance in mice fed high-fat diet (HFD), depending on functional AMP-activated protein kinase (AMPK). Metformin is a first-line oral antidiabetic drug with blood glucose-lowering effect and beneficial impact on lipid metabolism. The precise mechanism of metformin action still remains unknown. Existing data suggest that metformin lowers glycaemia via mild suppression of mitochondrial complex I activity, leading to a suppression of hepatic glucose production and concomitant AMPK activation. However, AMPK-independent mechanisms of metformin action may also exist. We sought to learn whether pre-treatment of dietary-obese mice with omega-3 could enhance early phase of metformin action, and whether AMPK is involved.
Materials and methods: Adult male B6 mice were fed a corn oil-based HFD (35 % lipids wt/wt) for 6 weeks. Mice were then randomly divided into two groups and fed for 2 weeks either the HFD or HFD-based diet containing omega-3 concentrate replacing 15 % (wt/wt) of dietary lipids (HFD-F). At the end of dietary intervention, mice were treated with a single dose of metformin (400 mg/kg body weight) or NaCl (placebo) administered by oral gavage, and 30 min later some mice were subjected to oral glucose tolerance test (OGTT), while the remaining mice were killed in order to collect liver and skeletal muscle for the AMPK activity assay and western blot analysis. A similar experimental setup, with a lower metformin dose (60 mg/kg), was used to investigate the involvement of AMPK in metformin action using transgenic mice with a whole-body inactivation of α2 subunit of AMPK (AMPKα2-KO).
Results: Glucose levels at 30th and 60th minute after glucose administration during OGTT, as well as an incremental area under the glycaemic curve (AUC; marker of glucose intolerance), were decreased (1.7-fold, 2.0-fold and 2.9-fold, respectively; $p<0.05$) after the single dose of metformin as compared to the saline-treated mice. Two-week consumption of HFD-F diet lowered AUC as compared to the HFD diet-fed mice (1.5-fold, $p<0.05$), the omega-3 treatment tended to augment effect of metformin, but the effect of the interaction between omega-3 and metformin was not statistically significant (3.2-fold vs. the HFD group; 1.1-fold vs. the HFD + metformin group). No differences in AMPK activity between the subgroups were detected. There were no significant differences in the OGTT in the response to metformin between wild type and AMPKα2-KO mice.
Conclusion: We demonstrated acute dose-dependent hypoglycaemic effect of metformin during OGTT in dietary-obese mice. Although two-week-supplementation by omega-3 lowered the response to glucose challenge, significant synergistic effect of omega-3 and metformin was not found in this experimental setup. There was also no difference between wild type and AMPKα2 deficient mice suggesting that AMPK was not essential for the acute blood glucose-lowering effect of metformin. Possible AMPK-independent interaction between omega-3 and metformin in their effects on glucose homeostasis is likely and requires further characterization.
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Metformin extended release compared to metformin immediate release on glycaemic control and patients' satisfaction in type 2 diabetic patients

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Background and aims: Metformin is usually the first line therapy for the treatment of type 2 diabetes mellitus; metformin is available in two formulations: immediate release (IR) as tablets, and extended release (XR) in powder form. The aim of this study was to evaluate, using international questionnaires, validated in Italian, if metformin XR formulation can be more effective than metformin IR in improving patients' quality of life and satisfaction towards treatment in a self-controlled clinical trial.
Materials and methods: Two hundred patients were enrolled. All patients were taking metformin IR at different dosages, and they were instructed to take metformin XR at the same dosage for 6 months. The following questionnaires were self-administered to each patient at the baseline and after 6 months since the beginning of treatment: the SF-36 Health Survey, the Diabetes Quality Of Life Modified questionnaire (DQOL/Mod) and the Diabetes Treatment Satisfaction Questionnaire (DTSQ). In particular the DTSQ was developed to assess the total diabetes treatment satisfaction and can be used in patients with type 2 diabetes mellitus. It consists of eight items; six of them (item 1: satisfaction with current treatment; item 4: treatment convenience; item 5: flexibility of treatment; item 6: understanding of diabetes; item 7: continuity of treatment, and item 8: recommending treatment to others with diabetes), are summed to get treatment satisfaction score with a possible range of 0 (very dissatisfied) to 36 (very satisfied). Item 2 evaluates perceived frequency of hyperglycemia and item 3 perceived frequency of hypoglycemia. All items are rated on a seven point scale (0-6). We also evaluated the following parameters, at the baseline and after 3, and 6 months: fasting plasma glucose (FPG), post-prandial glucose (PPG), glycated hemoglobin (HbA_{1c}), fasting plasma insulin (FPI), HOMA-index (HOMA-IR).
Results: We did not record any significant differences between metformin IR and metformin XR on glycemic control. We did not observe any differences in SF-36 Health Survey and the DQOL/Mod questionnaires scores between the two treatments. However, there was a greater satisfaction towards met-

formin XR according to the DTSQ questionnaire, in particular there was a greater score at the DTSQ in items 1, 4-8 with metformin XR compared to metformin IR. No differences in items regarding "Perceived Hyperglycemia, and "Perceived Hypoglycemia" were recorded.
Conclusion: Metformin XR showed to be as effective as metformin IR in maintaining glycemic control, and to be more effective in improving patients satisfaction toward anti-diabetic treatment.

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Association between metformin therapy and quantity and quality of sleep in type 2 diabetic patients referred for potential sleep disorders

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Background and aims: Since sleep disorders are linked with insulin resistance, and since metformin bears favorable effects on insulin resistance and also exerts direct effect on ventilator drive in non-obese rats, we tested the hypothesis of a favorable impact of metformin therapy on sleep parameters (in quality and quantity) in type 2 diabetic patients.
Materials and methods: We systematically searched in our university hospital's database for patients referred for potential sleep disorders. From the selected patients, we compared metformin-treated patients with those not treated with this drug. All study patients underwent the same procedure of polysomnography. Multivariate analysis was conducted to establish whether there was, or not, an independent relationship between metformin use and sleep parameters when controlling for age, gender, body mass index, neck circumference, cumulated risk factors, and insulin use.
Results: We studied 387 patients (58.4 ± 10.8 yr, sex ratio M/F 0.64), of whom 314 were treated with metformin. The metformin user and non-user groups did not differ significantly in terms of the main demographic characteristics and the use of hypnotic and sedative drugs. Total sleep time and sleep efficiency (total sleep time/total recording time) were higher in metformin-treated patients (6 hrs 39 min *vs.* 6 hrs 03 min, p = 0.002; and 77.9±12.3 % *vs.* 71.5±17.2 %, p = 0.003, respectively). These differences were persisting after control for study variables (p =0.0002 for both). Patients with or without metformin did not differ significantly in terms of sleep apnea syndrome (although BMI tended to be higher in metformin-treated patients: 38.4±7.9 *vs*. 36.7±8.2 kg/m^2, NS).
Conclusion: We showed for the first time that metformin therapy was associated with longer sleep duration and better sleep efficiency. Prospective studies are needed for confirming such a favourable effect of metformin on sleep disorders.

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Metformin therapy improves pregnancy outcome in women with polycystic ovary syndrome

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Background and aim: Previous studies have shown that many risk factors associated with polycystic ovary syndrome (PCOS) such as obesity, insulin resistance, hyperandrogenemia and polycystic ovaries may predict more frequent poor pregnancy outcomes and congenital malformations. Since when insulin resistance and hyperinsulinemia may play a critical role in the pathogenesis of PCOS, metformin, the insulin-sensitizing drug, has been introduced as a therapeutic option in PCOS, stirring a better control of glucose uptake. However, the role of metformin therapy on pregnancy outcome in women with PCOS is still controversial. This study was aimed to evaluate the effect of metformin on (a) the rate of first trimester spontaneous abortion (SA), (b) incidence of gestational diabetes (GD) and (c) outcome of pregnancies in women with the PCOS.
Materials and methods: The study included 83 oligo-amenorrheic women with PCOS conceived on metformin 1500 mg/day+lifestyle changes therapy. 43 of them continued metformin+lifestyle changes therapy throughout pregnancy (group A: BMI: 30,12 +/- 0.78 kg/m^2, age: 27,32+/-2,54 years) and 40 were treated without metformin and with lifestyle changes only (group B: BMI: 27,83 +/- 0.96 kg/m^2, age: 28,13+/-2,24 years). Insulin resistance was estimated using homeostatic model assessment, HOMA-IR, calculated from basal insulinemia and glycaemia. Outcome measures included number of first trimester SA, GD, live births, congenital defects (CD), gestational age, birthweight, height and APGAR score.
Results: HOMA-IR was significantly higher in metformin group then in group B before conception (A: 4,65 +/- 0.78 vs B: 3,08+/- 0.57, p< 0.05). SA rate was practically the same in both groups (A: 4.67% vs B: 5.03%, p=ns). The incidence of GD was significantly lower in group A vs group B (A: 6,8% vs B: 24% (p<0.01). Simultaneously, there was no significant difference in gestational age at the delivery in the two groups (A: 37,45+/- 0.64 vs B: 37,23+/- 0.58, p=ns). In addition the percent of live births was the same in women on or without metformin therapy. However, average birthweight was higher in the group without metformin (A: 3,025+/- 0.14 vs B: 3,197+/- 0.213, p< 0.05), while the height and APGAR score were not significantly differ between the groups. No congenital anomaly was found in either group, as well.
Conclusion: Our results have demonstrated that metformin therapy during pregnancy was associated with more favorable results in pregnancy outcome in women with PCOS by its insulin-lowering effect. These results also imply that use of metformin throughout pregnancy is responsible for a fourfold reduction of gestational diabetes.

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Metformin treatment is associated with increased serum levels of methylmalonic acid in patients with type 2 diabetes treated with insulin: a placebo-controlled 4-year trial

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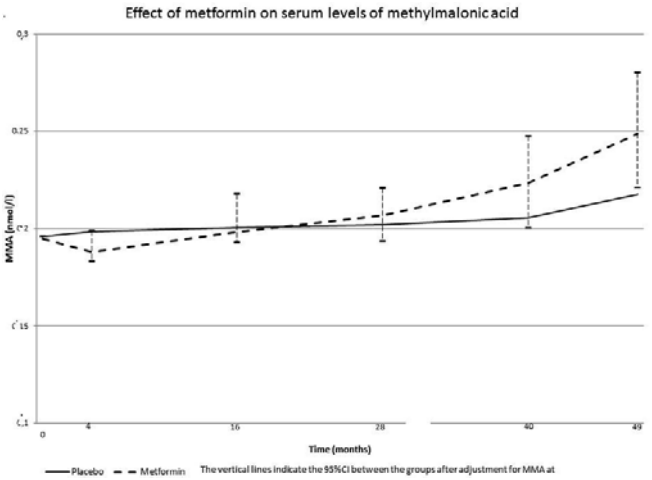
Background and aims: Metformin is a key treatment option in type 2 diabetes (T2D), but is associated with lowering of vitamin B12 (B12) levels. Although it is commonly assumed that lower B12 levels reflect biological B12 deficiency, this is in fact not certain. Accumulation of methylmalonic acid (MMA) may reflect a biologically important B12 deficit. We investigated whether treatment with metformin during 52 months affects serum levels of MMA.
Materials and methods: In this placebo-controlled trial, 390 patients with T2D were randomized to metformin or placebo added to insulin therapy. Baseline characteristics have been described previously (BMJ 2010). MMA levels were measured by ultra performance liquid chromatography tandem mass spectrometry (UPLCMSMS).
Results: Multivariate regression showed that metformin treatment significantly raised MMA serum concentrations. Compared to placebo, metformin increased MMA by 0.03 nmol/l (95% Confidence Interval 0.003 to 0.063; p=0.03; after adjustment for MMA at baseline). Renal function and age did not affect this increase. In addition, this effect appeared to be dose-related (P<0.001).
Conclusion: This long-term placebo controlled trial is the first study to show that in patients with T2D metformin not only reduces serum levels of B12, but also increases serum MMA, thus supporting the view that metformin-associated lowering of B12 levels reflects biological B12 deficiency.



Clinical Trial Registration Number: NCT00375388

964

Metformin use in diabetics with reduced kidney function: prescription pattern in 4,414 subjects with type 2 diabetes and different eGFR levels

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Background and aims: Evaluation of eGFR is important to guide drug prescription, because most hypoglycaemic medications require dose adjustment and/or are contraindicated according to the degree of kidney dysfunction. Metformin is traditionally considered to be contraindicated when eGFR falls below 60 ml/min; however, recent guidelines and expert recommendations propose to extend metformin use to lower eGFR classes, with specific cautions. Therefore, the objective of this cross-sectional study was to evaluate the distribution of eGFR, calculated by the abbreviated MDRD equation, in a cohort of type 2 diabetic patients, and to investigate the use of diabetes medications and specifically metformin in patients with different eGFR.
Materials and methods: A total of 4,414 type 2 diabetic patients (2,330 M; 2,084 F) were sub-grouped according to eGFR values >60 ml/min (n=3,357; 76%), 30-60 ml/min (n=967; 22%), or <30 ml/min (n=88; 2%), respectively.
Results: Mean ± SE BMI and waist were progressively higher in subjects with decreasing kidney function, from eGFR >60 (29.3 ± 0.1 kg/m^2; 101.9 ± 0.3 cm) to eGFR <30 (31.5 ± 0.7 kg/m^2; 109.6 ± 2.2 cm) (p<0.05). HbA1c was higher in the intermediate kidney function patients (7.5 ± 0.1%) as compared to the eGFR >60 (7.3 ± 0.1%) and eGFR <30 groups (7.4 ± 0.1%) (p<0.05). The proportion of patients with CKD using oral diabetes medications was lower as kidney disease progressed, and decreased from 55.3% in the eGFR >60 group to 43.5% in the eGFR 30-60 group and to 19.3% in the eGFR <30 group (p<0.05). Conversely, the proportion of patients using insulin alone was 13.2% in the eGFR >60 group, 21.7% in the eGFR 30-60 group, and 54.4% in the eGFR <30 group (p<0.001). The proportion of patients using association of oral diabetes medications and insulin was higher in the eGFR 30-60 group (21.4%) compared to the eGFR >60 (15.5%) and eGFR <30 groups (12.5%) (p<0.05). The proportion of drug-naive patients was comparable among groups (~15.0%). Metformin resulted to be prescribed in 43.4% of the eGFR 30-60 group and in 4.5% of the eGFR <30 group. In the eGFR 30-60 group, patients using metformin showed significant differences compared to patients not on metformin, in terms of age (70.1 ± 0.4 vs 73.9 ± 0.4 yrs), BMI (30.8 ± 0.3 vs 29.9 ± 0.3 kg/m^2), and eGFR (51.0 ± 0.3 vs 47.1 ± 0.3 ml/min) (p<0.05).
Conclusion: Almost 25% of patients with type 2 diabetes in this cohort show some degree of CKD. Use of oral medications declines and insulin prescription increases as eGFR falls. Metformin appears to be widely prescribed also in patients with eGFR between 30-60 ml/min. Younger age, higher BMI and eGFR in the upper half of the 30-60 ml/min range appear to be indicators of a more liberal use of metformin in type 2 diabetic patients with moderate kidney dysfunction.

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Metformin reduces serum CA199 levels in type 2 diabetic patients with time-effect and gender difference

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Background and aims: The antineoplastic effect of metformin has been reported. This study was to clarify the influence of metformin on serum carbohydrate antigen 199 (CA199) levels and its associated factors in Chinese type 2 diabetic patients with normal renal and hepatic function.
Materials and methods: Serum CA199 levels was measured and compared in 1253 Type 2 diabetic patients with or without metformin treatment. Non-metformin group consists of 616 patients (male : female=347:269) and metformin-treated group has 637 patients (male : female=332:305). The latter was further divided into short-term metformin group (n=325, male: female=163:162), and long-term metformin group (n=312, male : female=169:143) based on whether the treatment duration exceeds two years. Association between CA199 and other variables were assessed with Spearman or Pearson correlation and multiple stepwise regression analysis.
Results: In overall patients, CA199 levels in females was significantly higher than that of males [(22.89 &plusmn 15.03 *vs* 15.97&plusmn18.89) U/ml, *P*=0.001]. The correlation and multiple stepwise regression analysis revealed that HbA1c, metformin, gender, TC and FSH were independent risk factors of CA199 concentrations (all *P*& it0.05). Serum CA199 levels in the short-term metformin group (17.62&plusmn10.87 U/ml) was lower than that in the non-metformin group (25.60&plusmn13.68 U/ml, *P*=0.000), and the long-term metformin group showed the lowest concentration of CA199 (10.54&plusmn8.14 U/ml) (*P*& it0.01). After 1-year of follow-up, CA199 in the long-term metformin group was decreased to the greatest extent compared with short-term and non-metformin controls (-17% *vs* -4.9% *vs* 3%,*P*=0.000). The change in females was more apparent than that of males (-18% *vs* -5%, *P*=0.000). Binary logistic regression revealed that the risk of abnormal CA199 concentrations of short-term metformin group and long-term metformin group decreased 11% (OR=0.89, 95%CI 0.87-0.92, *P*& it0.01) and 30% (OR=0.70, 95%CI 0.65-0.75,*P*& it0.01) respectively after adjusting for HbA1c, TC and sex.
Conclusion: Metformin therapy reduced serum CA199 levels of T2DM patients, and its greater decline range occurred in women with longer therapeutic time.
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966

Improved resistance to exercise in mice treated with metformin

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Background and aims: Skeletal muscle insulin resistance is a key development in diabetes pathogenesis and induces functional, metabolic and structural changes leading to muscle weakness and muscle atrophy. Metformin (MET) is a first-line anti-diabetic therapy exhibiting potent antihyperglycemic and insulin-sensitizing properties. Its ability to regulate blood glucose has been attributed to a suppression of hepatic gluconeogenesis and increased glucose uptake in peripheral tissues such as skeletal muscle. Its action mechanism in skeletal muscle still remains unclear, although a number of studies have suggested that MET may act to stimulate glucose uptake independently of insulin or may potentiate insulin-stimulated glucose uptake. Skeletal muscle differentiation is a process in which proliferative myoblasts break free from the cell cycle and fuse to form multinucleated myotubes. These events are orchestrated by early Myogenic Regulator Factors (MRFs: MyoD, Myf-5, myogenin and Myf-6) and late myogenic protein MyHC (Myosin Heavy Chain), through p38 MAP kinase/ERK pathway modulation. Our previous data suggested that MET induces ERK pathway activation and MyHC synthesis in *vitro* muscle model (C2C12). Aim of this work is to confirm in C2C12 in vitro model and to study *in vivo,* in a rodent model, the action of MET during myogenesis process and in hypertrophy genesis.
Materials and methods: We studied muscle proteosynthesis and morphological features in the late differentiation. After 72 h of differentiation, cells were treated with 400 μM MET for 4, 8 and 24 h. We used a positive control with 0.1 nM insulin added to medium and a negative control in which MET and insulin were not added. MRFs protein expression levels and morphological characteristics were evaluated by Western Blot and Immunofluorescence. We investigated *in vivo* the action of MET on exercise performance in adult C57BL6 mice through an endurance performance treadmill running test. Mice were injected intra abdominally with MET (250 mg/kg) and the control mice with 0.9% saline for 30 days.
Results: During late differentiation, MRFs, cytoskeletal and muscle marker protein expression levels were higher in cells treated with MET in respect to control. Furthermore, MET treatment is able to increase cell mass and fusion competence indicating that MET may regulate myogenesis progression and hypertrophy genesis. Endurance performance treadmill running test, made at the beginning and at the end of this study, revealed that MET treated mice exhibit an enhanced performance respect to the control mice. Statistical evaluation was performed using an unpaired *t*-test. Data are presented as means ± SD. Results were considered statistically significant if p≤0.05.
Conclusion: Data shown the role of MET in myogenesis promotion and in neo-formed myotubes hypertrophy induction. Our findings revealed a novel therapeutic indication of MET for muscle hypotrophy conditions in chronic muscle impairment (diabetic sarcopenia and cachexia) and a new potential role as integrator in exercise performance.

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Effect of acarbose compared with metformin on incretins of GIP, PYY, Ghrelin in patients of newly diagnosed type 2 diabetes mellitus

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Background and aims: Compare the effect of Acarbose and Metformin on serum incretins of GIP, PYY, Ghrelin levels in patients of newly diagnosed T2DM for 1 year.
Materials and methods: 70 T2DM patients were randomized into two groups and received treatment of Acarbose or Metformin respectively for 1 year. Diet style, BMI, waist circumferences, hip circumferences, plasma glucose, lipids, serum GIP, PYY, Ghrelin were followed up after 6 and 12 months treatment.
Results: 1. The 0h, 1/2h, 2h serum PYY levels in standard meal test and the mean area under the curve (AUC) of PYY(PYY_{AUC}) in T2DM group were lower than those in NC group(P <0.05). The 0h, 1/2h, 2h serum Ghrelin levels in standard meal test and $Ghrelin_{AUC}$ in T2DM group were higher than those in NC group(P<0.05). The fasting serum GIP level was positively correlated with 2-hour postprandial plasma glucose (2hPG)(P <0.05) in NC group. In T2DM group the fasting serum GIP level was correlated positively with BMI (P <0.05), PYY_{AUC} and triglycerides (TG) were negatively correlated(P <0.05), the fasting serum Ghrelin level and total cholesterol (TC) were negatively correlated(P <0.05), $Ghrelin_{AUC}$ was correlated negativly with the low-density lipoprotein cholesterol (LDL-C) and fasting plasma glucose (FPG) (P <0.05). 2. In Acarbose group, after treatment of 6 months, FPG, 2hPG, BMI, waist circumference and hip circumference were lower than those at baseline (P<0.05), the 0h, 1/2h, 2h serum PYY levels and PYY_{AUC} increased comparing with those at baseline (P<0.05), the 2h serum Ghrelin level in standard meal test was lower than that at baseline (P<0.05). After reatment of 1 year, high-density lipoprotein cholesterol (HDL-C) was higher than that at baseline (P <0.05), waist circumference and HDL-C increased and 2hPG decreased comparing with those after treatment of 6 months(P<0.05). In Metformin group, after treatment for of 6 months and 1 year, FPG, 2hPG, BMI, waist circumference, hip circumference, WHR, TC and LDL-C were lower than those at baseline (P<0.05), HDL-C, the 0h, 1/2h, 2h serum PYY levels in standard meal test, and PYY_{AUC} were higher than those at baseline (P<0.05). No significant differences were found in these indicators between treatment of 6 months and treatment of 1 year.3. After treatment for 6 months and 1 year, no significant differences of the changes of PYY, PYY_{AUC}, plasma glucose, BMI between Acarbose group and Metformin group were found.
Conclusion: Patients of T2DM had decreased fasting and postprandial PYY levels and increased levels of Ghrelin. Both Acarbose and Metformin can comparably increase serum PYY levels in newly diagnosed T2DM, which may play a role in glucose control. In addition, Acarbose can reduce the 2h Ghrelin level, and Metformin had no effect on Ghrelin.

PS 077 Oral antidiabetic agents

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SLC47A1 gene rs2289669 G>A variant influences the hypoglycaemic effect of metformin in type 2 diabetes patients

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Background and aims: The SLC47A1 gene encodes for multidrug and toxin extrusion 1(MATE1) which plays a pivotal role in the metabolism and excretion of metformin. The present study is to investigate whether the SLC47A1 gene polymorphism influence the therapeutic effect of metformin in Chinese Hans who suffered from type 2 diabetes mellitus (T2DM).
Materials and methods: The SLC47A1 single nucleotide polymorphism (SNP) rs2289669 G/A was genotyped in 497 T2DM patients, including a metformin treated group (n = 224) and a non-metformin-treated group (n = 288) with PCR-restriction fragment length polymorphism method. HbA1c was followed up for 6 months after metformin treatment.
Results: Three SLC47A1 genotypes, GA, GG and AA were found and the frequency of the SLC47A1 rs2289669 G/A and A allele was 46.99% in T2DM patients with the similar frequency in metformin group(47.1%) and non-metformin group(46.9%). The plasma lactate levels was significantly higher in AA carrier patients(1.18±0.47mmol/L) than that in patients who carried GA (1.04±0.39mmol/L) and GG (1.04±0.34mmol/L, *P*=0.008), and the incidence of hyperlactatemia increased significantly in patients with SLC47A1 rs2289669 AA genotype (5.9%) compared to GA (3.4%) and GG genotype (1.5%, *P*<0.01). The 6-month follow-up of 250 patients revealed that there was greatest reduction of HbA1c in patients with SLC47A1 rs2289669 AA genotype than that of GA and GG genotype (ΔHbA1c -2.3% in AA genotype vs. -1.3% in GA, and -1.1% in GG genotype, *P*<0.01).
Conclusion: The SLC47A1 gene rs2289669 G>A variant influences the hypoglycemic effect of metfotmin in Chinese T2DM patients, and the patients with AA genotype got better HbA1c reduction.
Clinical Trial Registration Number: NSFC 81070650

969

SNPs related to the glucose lowering effect of metformin: do they affect plasma concentrations of metformin and their effects on insulin requirements?

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Background and aims: Several genes involved in the pharmacokinetics and pharmacodynamics of metformin may influence the glucose lowering effect of metformin. However, the clinical importance is yet unknown. In the population of the HOME trial (a placebo-controlled, randomised trial with patients with type 2 diabetes, treated with insulin) we genotyped four single nucletide polymorphisms (SNPs) of such genes to detect their potential influence on metformin plasma concentrations. Furthermore, we studied the potential of these SNPs and the metformin plasma concentrations to predict the reduction of the daily dose of insulin (DDI), an important action of metformin in insulin therapy.
Materials and methods: We genotyped 164 metformin users of the HOME trial for polymorphisms in the genes coding for organic cation transporter 1 (OCT1, rs12208357 and rs622342), multidrug and toxin extrusion 1 transporter (MATE1, rs2289669) and Ataxia Telangiectasia Mutated (ATM, rs11212617). The daily dose of metformin and DDI have been reported previously (Arch Int Med 2009). Metformin plasma concentrations were measured using HPLC-UV.
Results: Stepwise linear analysis showed that the SNP rs11212617 (ATM) had a significant effect on metformin plasma concentrations (p < 0.001), but not on DDI. Other SNPs studied did not affect metformin plasma concentrations or DDI (P values ranging between 0.30-0.92 and 0.40-0.72, respectively). Overall, we found a significant relationship between metformin plasma concentrations and DDI (most pronounced after adjustment for renal clearance): an increase of metformin plasma concentrations with 1mg/ml was associ-

ated with a decrease of DDI with 3.51 IU (95% CI: -6.70 to -0.32; p=0.01). The daily dose of metformin predicted the metformin plasma concentrations (p<0.001) and was strongly correlated with a reduction of DDI: an increase of the daily dose of metformin with 1 gram/day was associated with a mean decrease of DDI with 10.57 IU (95% CI: -17.20 to -3.94; p<0.001).
Conclusion: Of the SNPs studied, only the ATM SNP has an effect on metformin plasma concentrations, but its clinical relevance is unclear, since this SNP has no effect on DDI. The daily dose of metformin is a strong predictor of the effect of metformin on DDI, independent of all SNPs studied.
Clinical Trial Registration Number: NCT00375388

970

Efficacy and safety of acarbose plus metformin fixed-dose combination versus acarbose monotherapy for type 2 diabetes: a randomised, double-blind, parallel-group study

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Background and aims: To compare the efficacy and safety of acarbose plus metformin fixed-dose combination (FDC) versus acarbose monotherapy for type 2 diabetes (T2D).
Materials and methods: Subjects were T2D patients aged 20-80 years, with body mass index <35 kg/m2, glycosylated haemoglobin (HbA1c) 7.0-10.0%, and being treated with diet control only or oral antidiabetic medications. Eligible patients were run-in with acarbose 50 mg thrice-daily for 4 weeks, then randomised either to acarbose 50 mg plus metformin hydrochloride 500 mg FDC (acarbose/metformin FDC), or to acarbose 50 mg monotherapy, both thrice-daily for 16 weeks. The primary endpoint was HbA1c change from baseline.
Results: Acarbose/metformin FDC therapy significantly reduced HbA1c, fasting plasma glucose (FPG), and 2-hour postprandial plasma glucose (PPG) relative to baseline (all p<0.0001) with antihyperglycaemic efficacy superior to acarbose monotherapy (between-group differences HbA1c -1.35%, FPG -29.5 mg/dl, 2-hour PPG -41.6 mg/dl, all p<0.0001). A greater proportion of patients treated with acarbose/metformin FDC (47.8% vs. 10.7%, p<0.0001) achieved HbA1c target (<7.0%). Both treatments reduced bodyweight from baseline (both p<0.0001), with a significant between-group difference (-0.6 kg, p<0.01) favouring acarbose/metformin FDC. Hypoglycaemia was not reported in either treatment group, and incidences of other adverse events did not differ significantly between them.
Conclusion: Compared with acarbose monotherapy, acarbose/metformin FDC has superior antihyperglycaemic efficacy, enables proportionally more T2D patients with inadequate glycaemic control to achieve HbA1c goal, and further reduces bodyweight. Acarbose/metformin FDC is well-tolerated without significant risk of hypoglycaemia. Collectively, these attributes make acarbose/metformin FDC a potentially advantageous combination therapy for T2D.
Clinical Trial Registration Number: NCT01245166
Supported by: Lotus Pharmaceutical Co. Ltd.

971

Sulfonylureas use among US veterans with type 2 diabetes mellitus at high risk for cardiovascular disease (CVD) or existing CVD-CREST study: comparison to current treatment guidelines

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Aims: Increased risk of cardiovascular disease (CVD) and all-cause mortality is associated with sulfonylureas (SUs). This retrospective cohort study examined the treatment patterns of SUs in US veterans with different levels of cardiovascular risk or existing CVD.
Materials and methods: Electronic medical and pharmacy records were obtained for 149,053 patients with at least 2 records of T2DM diagnosis from the Veterans Integrated Service Network (VISN) 16 data warehouse from 01/01/2004 to 06/30/2010. All patients were required to be ≥ 18 years and have their first diagnosis of T2DM (ICD-9-CM codes: 250.x0 and 250.x2) after 01/01/2005 to ensure the one-year baseline information. Patients (n=18,099) with type 1 diabetes were excluded (ICD-9-CM codes: 250.x1 and 250.x3). Group 1: Patients with existing CVD (n=16,913) were defined by at least one of the following, ischemic heart disease, peripheral vascular disease, and ischemic stroke; Group 2: Patient with multiple CVD risk factors (n=30,348) were defined by, age ≥ 55 (male) or 60 (female), plus at least 1 additional risk factor (treated or non-treated): dyslipidemia or hypertension. Group 3: The remaining patients were considered as low risk patients (n=13,014). Frequency of SU use was reported as use rate per 1,000 patient years and by percentages (%). Chi-square tests were used to test the cross-cohort difference (Group 1 vs. 3 and Group 2 vs. 3) in the % of SU use. The results of SU use were further adjusted by within age-group comparisons to address the age differences resulting from the study cohort definition. Logistic regression models were used to explore the associations between baseline characteristics and use of SUs in adjusted odds ratio with 95% confidence interval (aOR [95% CI]) within each of the study groups.
Results: Across the three cohorts, approximately one quarter of patients reported the use of SUs in the study period (Group 1: 24.9%, Group 2: 25.4%, Group 3: 26.3%). The SU use over the study period was 104.8/1,000 person-year for Group 1, 106.5/1,000 person-year for Group 2, and 114.6/1000 person-year for Group 3. The adjustment of within-age-group comparisons further showed that the use frequencies of SUs for Group 1 or 2 were significantly higher (p<0.05) than Group 3 within most of age groups: 18-54, 55-64, 65-74, and >75 years old. Logistic regression models found statistically significant factors associated with the current use of SUs (aOR[95%CI]): Group 1: chronic kidney disease (CKD) (1.24 [1.05-1.47]), congestive heart failure (CHF) (1.14 [1.02-1.27]), and depression (0.87 [0.79-0.97]); Group 2: CHF (1.45, [1.25-1.67]), hypertension (1.10 [1.04-1.17]), and osteoporosis (0.68 [0.49-0.96]); Group 3: hypertension (1.17 [1.07-1.27]), obesity (0.90 [0.82-0.99]), and arthritis (0.81 [0.72-0.91]).
Conclusion: The use of SUs among veterans with either a history of cardiovascular disease or multiple risk factors for cardiovascular disease were still quite prevalent and similar to those at low risk for CVD, despite recommendations against such use in the ADA/EASD guidelines.
Supported by: Bristol-Myers Squibb

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Change in body weight after 24 weeks of vildagliptin therapy as a function of baseline fasting plasma glucose levels in patients with type 2 diabetes

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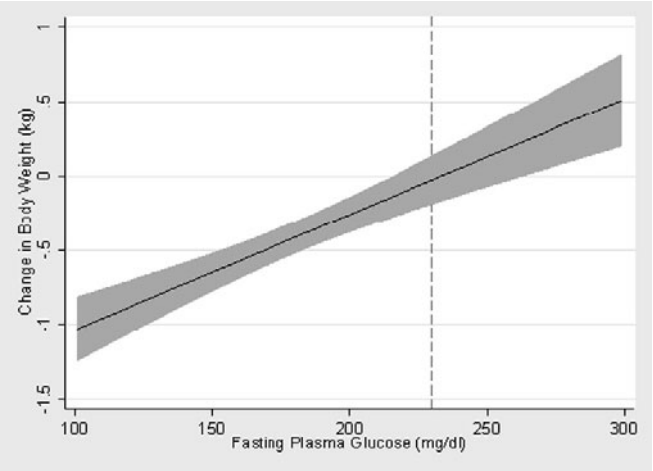
Background and aims: Overall, treatment with DPP-4 inhibitors is weight neutral. However, from lower baseline levels of glycaemia it was reported that vildagliptin therapy results in >1 kg weight loss over a 2-year treatment period. Reducing glucose levels from above the renal threshold to below the renal threshold with any therapy can lead to increased caloric balance by less calories lost in the urine. We hypothesise that when weight change is correlated with baseline fasting plasma glucose (FPG), patients with FPG predicted

to be above the renal threshold would be associated with positive caloric balance and those predicted to be below the renal threshold would be associated with negative caloric balance.
Materials and methods: Utilising the vildagliptin (50 mg bid and qd) database of clinical monotherapy studies, we used a linear regression model to analyse the body weight changes from baseline to week 24 as a function of baseline FPG.
Results: We included 2863 vildagliptin-treated patients of which 55% were male, average age was 54±11 years, BMI was 30±5 kg/m^2 and mean duration of type 2 diabetes was 2±3 years. The analysis confirms that there is a positive slope of 0.0085 kg/mg (95% CI: 0.005, 0.011; p<0.001). Neutral caloric balance (no weight change) was observed at FPG of 230 mg/dL. Baseline FPG values below and above this threshold were associated with weight loss and weight gain, respectively.
Conclusion: Extra-pancreatic effects of vildagliptin to reduce fat absorption and increase energy expenditure during meals may explain this negative caloric balance when glycosuria is considered. Overall the data support the conclusion that when loss of glycosuria is taken into consideration, treatment with vildagliptin results in negative caloric balance. This may explain why vildagliptin is associated with a modest reduction in fasting lipids and blood pressure.



Supported by: Novartis Pharma AG

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Saxagliptin improves glycaemic control and is well tolerated in adult diabetes patients with glutamic acid decarboxylase antibodies
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Background and aims: Latent autoimmune diabetes in adults (LADA) is recognized by the presence of glutamic acid decarboxylase (GAD) antibodies, and there is a need for effective therapies for these patients.
Materials and methods: Patients from 5 placebo-controlled studies from the saxagliptin phase 3 program were pooled and classified by GAD status (GAD-positive: values ≥ lower limit of quantitation [LLOQ]; GAD-negative: values <LLOQ). To assess similarity in saxagliptin efficacy by GAD status, we analyzed treatment effects (HbA_{1c}, fasting plasma glucose [FPG], 120-min postprandial glucose [PPG], adverse events [AEs], serious AEs, hypoglycemia) using analysis of covariance, with test for treatment by subgroup interaction.
Results: The GAD-positive group included 98 saxagliptin patients and 35 placebo patients; the GAD-negative group included 1849 saxagliptin and 727 placebo patients. Age, sex, and race were balanced across subgroups. For GAD-positive and GAD-negative patients, baseline mean HbA_{1c} was 8.2% in both subgroups, mean FPG was 177 and 170 mg/dL, and mean C-peptide was 3.3 and 3.4 nmol/L. Saxagliptin showed greater decreases from baseline than placebo, with consistent treatment effects in GAD-positive and GAD-negative patients: mean change from baseline HbA_{1c} was −0.64% and −0.62%, respectively (P=0.93) and PPG was −39.1 and −39.7 mg/dL, respectively (P=0.97). Differences in achieving HbA_{1c} <7% were also consistent between GAD-positive and GAD-negative patients. There was a larger treatment effect for FPG for GAD-positive vs GAD-negative patients (−33.9 vs. −13.8 mg/dL; P<0.01). General similarity was shown with saxagliptin vs placebo for both GAD categories for incidence of AEs (65% vs 80% for GAD-positive; 73% vs 70% for GAD-negative), serious AEs (2.0% vs 5.7% and 3.5% vs 3.4%, respectively), and hypoglycemic events (5.1% vs 5.7% and 7.4% vs 6.5%, respectively).
Conclusion: SAXA was effective and generally well tolerated in patients with type 2 diabetes, regardless of GAD status. These data suggest that SAXA is an effective glycemic treatment in patients with LADA
Clinical Trial Registration Number: NCT00121641, NCT00316082, NCT00121667, NCT00295633, NCT00313313
Supported by: Bristol-Myers Squibb, AstraZeneca

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Use of pioglitazone and risk of bladder cancer among diabetic patients in Korea: a multicentre retrospective cohort study supported by a nested case-control analysis
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Background and aims: Although an increased risk of bladder cancer with pioglitazone exposure has been suggested, it has not been determined whether chronic exposure to low dose of pioglitazone also exhibits such risk. We aimed to assess the risk of bladder cancer associated with pioglitazone in Korea, where the approved dosage of pioglitazone is 15mg per day.
Materials and methods: Using electronic medical record of the four tertiary referral hospitals in Korea, we extracted a clinical database of diabetic patients with ≥2 visit to the clinic during November 2005 and June 2011. Cox regression-generated age-, sex-adjusted hazard ratios (HRs) compared the incidence rate of bladder cancer between never- and ever-user of pioglitazone. A nested case-control study was followed to further adjust other confounders.
Results: After exclusion of patients who had diagnosis of bladder cancer before onset of diabetes, 113,193 patients were included. Among these patients, 11,240 patients was ever-user of pioglitazone. We found 237 and 30 cases of incident bladder cancer (rate 64.9 and 54.9 per 100000 person years) in never-users and ever-users of pioglitazone. Ever-use of pioglitazone was not significantly associated with bladder cancer incidence (adjusted HR 1.135, 95% confidence interval [CI] 0.769-1.677). In the nested case-control analysis, use of pioglitazone with observed duration of >6 months was associated with an increased rate of bladder cancer (rate ratio 2.143, 95% CI 1.041-4.414).
Conclusion: Ever-use of pioglitazone was not significantly associated with incidence of bladder cancer. However, association between longer-term use of pioglitazone and risk of bladder cancer could be suggested.
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Pioglitazone increases bone marrow fat content
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Background and aims: Use of the thiazolidinedione pioglitazone is associated with an increased risk of fractures. Proposed mechanisms based on animal studies include increased bone marrow adiposity, decreased bone formation, and/or accelerated bone resorption, although the exact impact of pioglitazone on these parameters in humans remains unclear.
Materials and methods: In this double-blind placebo-controlled clinical trial, we randomized 30 obese volunteers with the metabolic syndrome to pioglitazone (PIO, 45 mg/day) or matching Placebo (PLA) for 1 year. Liver and femoral neck bone marrow fat content was measured by ^{1}H-magnetic reso-

nance spectroscopy at baseline, 6 and 12 months. The bone turnover markers serum C-telopeptide (CTX, bone resorption) and bone-specific alkaline phosphatase (BSAP, bone formation), and beta-cell function and insulin sensitivity (SI, by an insulin-modified frequently sampled intravenous glucose tolerance test) were measured at baseline, 1, 2, 6 and 12 months.
Results: Subjects included 13 women and 17 men, with mean age of 52.2 years, mean weight of 94.5 Kg, and mean BMI of 33.3 Kg/m^2. PIO use was associated with a significant decline in liver fat content and a significant increase in bone marrow fat content and insulin sensitivity (SI) at 6 and 12 months (Table). The change in bone marrow fat content was inversely and significantly associated with the change in liver fat at 12 months (Spearman R= -0.41, p=0.026). There was a trend towards increased bone resorption based on serum CTX elevation at 6 months vs. baseline in the pioglitazone group.
Conclusion: PIO use is associated with a significant increase in bone marrow fat content at the femoral neck. This increase in bone marrow fat was significantly correlated with the decrease in liver fat content, a finding compatible with the proposed model of fat redistribution from liver (and other ectopic tissues) to the bone marrow compartment.

Table:

Group	ΔLiver Fat (Month 6 vs. 0)	ΔLiver Fat (Month 12 vs. 0)	ΔBone Fat (Month 6 vs. 0)	ΔBone Fat (Month 12 vs. 0)	ΔSI (Month 6 vs. 0)	ΔSI (Month 12 vs. 0)	ΔCTX (Month 6 vs. 0)
Placebo	-1.8±4.2	+0.7±6.7	-1.7±5.6	-3.1±5.9	-0.4±1.1	-0.2±1.0	+0.04±0.09
Pioglitazone	-7.7±8.9	-6.3±6.4	+4.3±9.2	+3.8±9.8	+1.3±1.4	+1.5±1.7	+0.11±0.20
p-value*	0.01	0.001	0.02	0.01	<0.0001	0.001	0.26

* p-value for group x visit interaction by Mixed Model Repeated Measures

Clinical Trial Registration Number: NCT00855010
Supported by: NIH K23 to IL

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Effect of pioglitazone on thyroid stimulating hormone and insulin resistance in hypothyroid patients
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Background and aims: Mechanism of insulin resistance in hypothyroidism is still not fully understood. Thyroid hormone receptor and PPAR-gamma are members of the same nuclear receptors superfamily and it is speculated that it could be a crosstalk between PPAR-gamma and thyroid hormone signaling pathways. Accordingly, it has been reported that intracerebral injection of PPAR-gamma agonists modify transcription of thyroid release hormone in the hypothalamus and increase serum thyroxine levels. The aim of this study is to estimate if PPAR-gamma agonist pioglitazone has an influence on thyroid stimulating hormone (TSH) and its relationship with insulin resistance parameters in hypothyroid patients treated with levothyroxine.
Materials and methods: 17 women and 8 men with overt hypothyroidism and insulin resistance without diabetes were included in the crossover study. After three months period when patients received levothyroxine (first line approach), treatment was changed to combination of levothyroxine and pioglitazone for next three months (second line approach). We measured: TSH, free triiodothyronine (fT3), free thyroxine (fT4), fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance index (HOMA-IR), HOMA-B%, glycated hemoglobin (HbA1c) lipid profile at baseline and after each treatment approach.
Results: We observed significant decrease of TSH level following combining levothyroxine with pioglitazone in comparison with levothyroxine treatment alone (1.57±0.15 vs. 3.52±0.28 μIU/ml, p<0.001). Significant difference of fT3 and fT4 levels were observed between two treatment approaches (3.17±0.09 vs. 2.81±0.08 pg/ml, P<0.001 and 1.65±0.08 vs. 1.23±0.09 ng/dl, P<0.001 respectively). HbA1c decreased after second line treatment approach in comparison to the first one (5.44±0.05 vs. 5.85±0.06, P<0.001). Significant change in glucose (from 6.07±0.08 to 4.91±0.08 mmol/l (P<0.001) and insulin (from 15.94±0.57 to 8.57±0.15 μIU/ml (P<0.001) between first and second treatment lines was also established. HOMA-IR improved from 4.29±0.16 to 1.87±0.04 (P<0.001) after course with pioglitazone in comparison to levothyroxine treatment alone. Our investigation shows significant increase of high density lipoproteins (1.42±0.06 to 1.62±0.06 mmol/l, P<0.05) and significant decrease of atherogenic index (3.42±0.33 to 2.42±0.20, P<0.05) after second line approach in comparison to baseline. There was no significant difference between two treatment approaches in HOMA-B%, total cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, very-low-density lipoproteins.
Conclusion: To our knowledge, it was discovered for the first time that pioglitazone administration is characterized by elevation of thyroid hormone and by diminishing of thyroid stimulating hormone. We also present novel data indicating pioglitazone as a drug with ability to decrease HbA1c, to improve HOMA-IR index by lowering insulin and glucose without influence on HOMA-B% in hypothyroid patients. Therefore our study suggests that PPAR-gamma receptors play a role in pathogenesis of hypothyroidism and PPAR-gamma agonist pioglitazone may have new indication to be used.

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The glucagon receptor antagonist LY2409021 significantly lowers HbA_{1c} and is well tolerated in patients with type 2 diabetes mellitus: a 24-week phase 2 study
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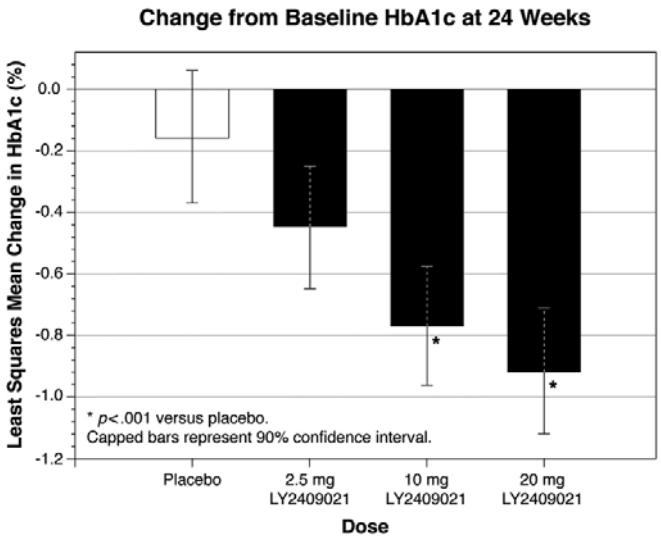
Background and aims: LY2409021 (LY), a potent, selective antagonist of the human glucagon receptor (GRA), is being investigated as a treatment for type 2 diabetes mellitus (T2DM). This phase 2, multicenter, double-blind, placebo-controlled, parallel-group study examined the efficacy of once-daily LY, compared with placebo, as measured by the mean change in HbA1c from baseline to the end of the 24-week active treatment period.
Materials and methods: A total of 254 participants (aged 18-70 years) with T2DM (HbA1c 7.0% to 10.5%) who were naive to antidiabetic medications or taking stable doses of metformin were randomized to LY 2.5 mg (n=63), 10 mg (n=64), 20 mg (n=64), or placebo (n=63), each administered once daily.
Results: Once-daily doses of 10-mg and 20-mg LY were associated with statistically and clinically significant reductions in HbA1c. The least squares mean differences for HbA1c change from baseline to the 24-week endpoint were −0.15%, −0.45%, −0.78%, and −0.92% for the placebo, LY 2.5-mg, LY 10-mg, and LY 20-mg groups, respectively (p<.001 vs placebo for the LY 10-mg and LY 20-mg groups) (Figure). The frequency of hypoglycemia was low: 17 subjects (7%) reported a total of 20 events (none was severe or required hospitalization), and the overall incidence did not differ significantly between placebo and active treatment groups. The LY fasting glucose response relative to placebo was dose-dependent and was statistically significant versus placebo (p=.035) in the 20-mg group. The safety and efficacy findings were consistent with those from previous studies of LY in subjects with T2DM. There were no dose-dependent effects of LY on lipids, weight, or blood pressure. Small increases in hepatic aminotransferases (mean alanine aminotransferase elevation of about 10 U/L from baseline for the 10-mg and 20-mg groups) were observed and reversed during treatment or following cessation of treatment. Dose-dependent increases in fasting glucagon were observed; they returned to pre-LY levels after cessation of treatment.
Conclusion: GRA treatment significantly lowered HbA1c with good overall tolerability and a low frequency of hypoglycemia. Small increases in hepatic aminotransferases that were observed were reversible.



Clinical Trial Registration Number: NCT01241448

PS 078 Effects of glucose-lowering therapy on cardiovascular risks

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Effects of pioglitazone vs glyburide on endothelial function in type 2 diabetic patients with vascular complications: the SPLENDOR study

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Background and aims: In patients with type 2 diabetes mellitus (T2DM), pioglitazone treatment has been shown to reduce the risk for cardiovascular disease (CVD). This effect is most likely due to improved insulin sensitivity and the beneficial effects on lipids, blood pressure, and inflammation. We now report results of a double-blind, randomized 24-week trial examining the effect of pioglitazone (PIO) vs. glyburide (GLY) as add-on to metformin on the endothelial function of T2DM patients with CV disease.
Materials and methods: T2DM patients were randomized to 30 mg/day PIO (n=18; age 62±6 years, BMI 30.1±3.4 kg/m2, HbA1c 8.0±0.4%) or 10 mg/day GLY (n=17; age 60±9 years, BMI 31.5±4.1 kg/m2, HbA1c 7.9±0.5%). All randomized patients completed the study with a mean exposure to study drug of 184.5±8.0 days (range 167-197) for PIO and 184.6±10.2 days (range 172-211) for GLY and with a compliance rate of 96.9±2.7% and 96.3±5.4% for the two treatments, respectively. Before randomization and at end of treatment, brachial artery endothelium dependent flow-mediated dilation (FMD) and non-endothelium dependent glyceryl trinitrate-mediated dilation (GTN) were determined by high-resolution ultrasound and computerized edge detection system along with measurement of markers of lipid metabolism and inflammatory response and insulin sensitivity (OGIS).
Results: Glycemic control improved to a similar extent with no difference in HbA1c reduction at week 24 between PIO (-0.76±0.19%) and GLY (-0.77±0.18%). Basal brachial artery diameter (BAD; 4.3±0.2 vs. 4.3±0.3 mm) increased by week 24 with PIO (+0.19±0.10 mm) compared to no changes in GLY (-0.04±0.10 mm, p=0.098). Maximal BAD in response to reactive hyperemia was greater with PIO than with GLY (+0.22±0.10 vs. -0.05±0.10 mm, p=0.034). Consensually, FMD increase from baseline (4.04±0.63 vs. 3.65±0.60%) was greater with PIO (+0.72±0.88 vs. +0.32±0.77%; p=0.574) though not statistically different. There was no difference between groups as far as GTN% was concerned. With PIO, insulin sensitivity improved (OGIS, +36±12 vs. +5±7; p=0.04) and adiponectin levels increased (+2.5±1.0 vs. +0.2±0.4 ng/ml; p=0.0005) along with a greater reduction in serum levels of triglycerides (-60±20 vs. +3±14 mg/dl; p=0.008) and an increase in HDL-cholesterol (+6±2 vs. -3±1 mg/dl; p=0.0002) and Apo-A1 (+0.11±0.04 vs. -0.05±0.05 g/l; p=0.029). No differences were apparent for other lipid parameters as well as ox-LDL levels. Compared to GLY, PIO treatment was associated with a slight reduction in serum levels of hs-CRP (-21±11 vs. +2.74±10 mg/l; p=0.084), PAI-1 (-16.3±9.2 vs. +4.4±18.1 ng/ml; p=0.056), with no differences for IL-6, sICAM-1, sVCAM-1, and MCP-1.
Conclusion: In T2DM with CVD already on metformin failure, 24 week addition of pioglitazone as compared to glyburide, was not associated with significant changes in endothelial function in spite of improved insulin sensitivity, better lipid profile, and marginal improvement in inflammatory response.
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The comparative safety and effectiveness of sitagliptin in patients with type 2 diabetes and heart failure

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Background and aims: Sitagliptin belongs to a new class of antidiabetic agents called incretins, which are hypothesized to have pleiotropic effects on the cardiovascular system as well as heart failure specific effects. The objective of this study was to determine if the use of sitagliptin was associated with any

benefit or risk on clinical outcomes in a population-based cohort of patients with type 2 diabetes and incident heart failure.
Materials and methods: Using a large commercially insured US claims and integrated laboratory database, 11,967 subjects with type 2 diabetes and incident heart failure were identified through physician claims, hospital discharge abstracts, and/or ambulatory care visits based on ICD-9 CM codes. Our population-based cohort was followed from January 1, 2004 until death, termination of medical insurance, or December 31, 2010. Time-varying multivariable Cox proportional hazards models were used to asses differences in all-cause mortality.
Results: Average age of subjects was 56 years, 39.6% were female, and median duration of follow-up was 1.94 years. In total, 653 subjects died (5.5%). No association between all-cause death and sitaglitpin use was observed compared to other glucose lowering agents. After adjustment for demographics, clinical & laboratory data, pharmacy claims, health care utilization and time-varying propensity scores, any sitagliptin use was not associated with a statistically significant increase in mortality (adjusted HR 0.64, 95% CI 0.37-1.12) compared to no sitagliptin use. Similarly, compared to metformin/sulfonylurea combination therapy, sitagliptin combination therapy was not associated with an increase in mortality (adjusted HR 1.01, 95% CI 0.40-2.56).
Conclusion: Sitagliptin therapy was not associated with excess risk of all-cause death among patients with type 2 diabetes and heart failure.

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Efficacy and safety of exenatide once weekly over 5 years
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Background and aims: In the 30-week controlled DURATION-1 trial patients with T2DM on a range of background therapies treated with the once weekly formulation of the GLP-1 receptor agonist (GLP-1 RA) exenatide (EQW) exhibited a greater reduction in HbA_{1c} compared with the twice daily (BID) formulation of exenatide (ΔHbA_{1c} -1.9% vs -1.5%). The controlled period of the trial was followed by an open-ended, open-label period in which all patients either continued EQW or switched from exenatide BID to EQW. The current analyses examined the safety and efficacy of treatment with EQW over 5 years, the longest assessment of a GLP-1RA reported to date.
Materials and methods: Study end points included change in HbA_{1c}, fasting plasma glucose (FPG), weight, lipid profile, and systolic blood pressure. Efficacy data are reported for the completer population. Safety data are reported for the intent-to-treat (ITT) population comprised of individuals who received at least one dose of exenatide.
Results: Approximately 54% (n=158) of the 295 ITT patients completed 5 years of treatment. Completer baseline characteristics (mean±SD) were HbA_{1c} 8.2±0.9%; FPG 9.18±2.24 mmol/l; weight 100±18 kg; duration of diabetes 7±6 years. Significant HbA_{1c} improvement (LS mean [95%CI]: 1.6% [-1.8, -1.3]) was observed with long term EQW treatment. The mean±SE HbA_{1c} was 7.1±1.0% at the 5 year end point, with 32% achieving $HbA_{1c} \leq 6.5\%$. Significant improvements in FPG (LS mean 1.66 mmol/l [-2.04, -1.27]) and weight (2.8kg [-4.3, -1.2]) were also observed. At 5 years of treatment the following changes in fasting metabolic markers were observed: total cholesterol (0.31 mmol/l [0.47, -0.14]), HDL cholesterol (+0.06 mmol/l [+0.02, +0.10]), LDL cholesterol (0.25 mmol/l [-0.38, -0.11]), and triglycerides (geometric LS mean [95%CI]: -13% [-20%, -5%]). Change (LS mean [95%CI]) in systolic blood pressure was +0.63 mmHg [-2.29, 3.56]. Within the ITT population nausea (predominantly mild) was the most common adverse event with EQW during the initial controlled period with a treatment exposure-adjusted annual event rate of 0.85 which notably decreased over time (0.08 from week 30 to year 5). The exposure-adjusted annual event rates of injection site pruritus and erythema lessened considerably from the controlled period compared with the open-ended period (0.51 and 0.14 vs 0.02 and 0.01, respectively). Treatment-emergent adverse events leading to withdrawal from week 30 to year 5 were also infrequent (5.8%). One case of pancreatitis and 3 cases of acute renal failure were reported during the 5 year treatment period. No severe hypoglycemia was observed; minor hypoglycemia in this treatment period occurred predominantly with concomitant sulfonylurea use.
Conclusion: Greater than 50% of patients continued treatment with EQW for 5 years. Long-term treatment with EQW was well-tolerated and associated with sustained improvement in glycemic control in patients with T2DM on an array of oral therapies with no unexpected safety findings. This outcome was associated with improvements in broader metabolic measures, including body weight and lipids.
Clinical Trial Registration Number: NCT00308139

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Long-term cardiovascular outcomes with exenatide twice daily compared to insulin: a retrospective observational study
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Background and aims: Treatment with exenatide has shown some beneficial effects on cardiovascular (CV) risk factors, including weight loss, in patients with T2DM. However, data on the long-term CV outcomes of treating T2DM patients with glucagon-like peptide-1 (GLP-1) receptor agonists remain limited. Using the GE Healthcare database, we evaluated the risk of heart failure (HF), myocardial infarction (MI) and stroke in patients initiating exenatide twice daily (EBID; n=2795) or any insulin (INS; n=51,547) in routine clinical practice.
Materials and methods: A cohort of 54,342 patients who received a first prescription of EBID or INS between June 2005 and May 2009, combined with oral antidiabetes agents (OADs), was selected for this retrospective study under the following conditions: a minimum follow-up period of 3 years, a minimum of 6 months under the same drug regimen, no crossover between EBID and INS during the follow-up, no cardiovascular event within 6 months of start of the follow-up period, and age-matched between the two drug regimens. The possible effects of EBID vs INS on CV events in this age-matched cohort were evaluated using a stratified Cox regression model, adjusted for age, sex, race, history of CV disease, OADs, and cardio-protective medications.
Results: Demographics of the EBID and INS-treated patients were: 39% vs 47% men; 56 vs 59 years of age, 54% vs 48% white, 11% vs 12% with a history of CV disease, 39 vs 34 kg/m^2 BMI, respectively). Medication use for the two groups differed for some drug classes (Metformin: 89% vs 61%; SFU: 55% vs 46%; TZDs: 47% vs 33%; ACE-ARB: 74% vs 82%; β blocker: 43% vs 56%; statins: 85% vs 84% for EBID vs INS, respectively). During a median follow-up of 4.3 years for EBID and 4.2 years for INS, the rate of CV events were 2.1% vs 5.8% for HF, 0.5% vs 0.9% for MI, and 0.9% vs 2.1% for stroke, respectively. CV event rates per 1000 person-years were significantly lower among patients treated with EBID vs INS (HF: 4.8 vs 13.6, MI: 1.1 vs 2.1, stroke: 2.0 vs 4.9). Compared to INS, patients treated with EBID had significantly lower risk of HF by 53% (HR CI: 0.36, 0.61) and MI or stroke by 48% (HR CI: 0.38, 0.72). Patients with a history of CV disease had a 47% (HR CI: 1.35, 1.60) increased risk of HF and 70% (HR CI: 1.50, 1.91) increased risk of MI or stroke compared to patients without a history of CV disease. Potential limitations of this analysis include misclassification of outcome and residual confounding.
Conclusion: In this retrospective database study, treatment with EBID was associated with significantly lower risk of HF and MI or stroke during 4 years of follow-up compared with INS.
Supported by: Amylin Pharmaceuticals, LLC

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Impact on cardiovascular risk factors of exenatide BID vs insulin lispro TID added to titrated insulin glargine QD in metformin-treated type 2 diabetes mellitus patients: the 4B trial
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Background and aims: Many patients with type 2 diabetes mellitus (T2DM) who fail to achieve adequate glycaemic control on oral antidiabetic agents plus insulin have elevated cardiovascular (CV) risk factors including excess weight, hypertension and hyperlipidaemia. Therefore, add-on therapies should be evaluated for their effect on these and related characteristics. The 4B Trial prospectively assessed the effect of Basal insulin glargine (IG) + Exenatide BID Treatment (BET) versus Basal IG + mealtime Bolus insulin lispro (IL) 3 times daily (TID) Treatment (BBT) in patients (pts) with insufficient glycaemic control despite intensified IG. Secondary outcomes included the evaluation of CV risk factors.
Materials and methods: After a 2-wk screening period and 12-wk basal insulin optimization (BIO) phase to titrate IG dose (INITIATE algorithm) to achieve the lowest possible FBG (target of 5.6 mmol/L) without hypo, pts

unable to achieve HbA_{1c} ≤7% (53 mmol/mol) despite IG intensification were randomised in the 30-wk intervention phase comparing BET to BBT. Secondary outcomes assessed change from randomisation to 30-wk endpoint of body weight, BMI, waist circumference, blood pressure (BP), pulse rate, fasting lipids (total-, HDL- and LDL-cholesterol, TG), high sensitivity C-reactive protein (hs-CRP), adiponectin and urinary albumin excretion (UACR).

Results: 1036 pts were screened and 917 pts entered the 12-wk BIO phase. 637 pts (concomitant medications: statins 53%, fibrates 11%, antihypertensives 76%) were randomised 1:1 to BET (n=316, PP n=247) or BBT (n=321, PP n=263). HbA_{1c} reduction with BET was non-inferior to BBT for mean change in HbA_{1c} from randomisation to wk 30. Body weight decreased significantly in the BET arm from randomisation to wk 30 and increased significantly in the BBT arm (BET vs BBT treatment difference -4.6 kg [95% CI, -5.2 to -3.9], *P*<.0001). LS mean change in waist circumference for BET was -1.98 cm and for BBT was 0.72 cm (between group comparison *P*<.0001). In the BET arm LS mean change for SBP was -4.1 mm Hg, in the BBT arm the LS mean change was +0.4 mm Hg (between group comparison *P* = .0004). LS mean changes in DBP were -0.6 for BET and -0.1 mm Hg for BBT. Analyses did not identify significant treatment group differences in hs-CRP, adiponectin and UACR.

Conclusion: There was a significant difference between treatment groups for weight change, and changes in waist circumference and systolic BP. Other changes in both groups were not significant.

	At Randomisation		Change At 30 wk		
Characteristic or Variable	BET* n = 247	BBT* n = 263	BET**	BBT**	BET-BBT** (95% CI)
BMI (kg/m²)	32.6 (4.7)	32.3 (4.7)	-0.89 (0.1)	+0.77 (0.1)	-1.7, (-1.9, -1.4)
Body weight (kg)	91.1 (16.6)	89.4 (17.0)	-2.4 (0.3)	+2.1 (0.2)	-4.6 (-5.2, -3.9)
Systolic BP (mm Hg)	137.1 (15.9)	134.7 (15.2)	-4.1 (1.0)	+0.4 (0.9)	-4.5 (-7.0, -2.0)
Diastolic BP (mm Hg)	79.3 (9.7)	78.3 (9.2)	-0.6 (0.6)	-0.1 (0.6)	-0.5 (-2.0, 1.0)
Pulse rate (beats per min)	74.1 (10.6)	73.0 (9.6)	+1.5 (0.6)	+0.9 (0.6)	+0.6 (-1.0, 2.2)
Total cholesterol (mmol/L)	4.55 (1.0)	4.59 (1.0)	-0.14 (0.05)	-0.03 (0.05)	-0.11 (-0.23, 0.01)
LDL cholesterol (mmol/L)	2.53 (0.8)	2.60 (0.9)	-0.12 (0.04)	-0.03 (0.04)	-0.09 (-0.19, 0.01)
HDL cholesterol (mmol/L)	1.24 (0.3)	1.20 (0.3)	-0.04 (0.01)	+0.03 (0.01)	-0.07 (-0.1, -0.04)
Triglycerides (mmol/L)	1.75 (0.8)	1.74 (0.9)	+0.06 (0.06)	-0.05 (0.06)	+0.11 (-0.03, 0.24)

*Mean (SD), **LS mean (SE) change by MMRM, per protocol population (ITT analyses yielded comparable results)

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Cardiovascular effects of antidiabetic drugs: exendin-4 and liraglutide

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Background and aims: Exendin-4 (exe4) postconditioning has been shown to limit reperfusion injury (RI) in experimental [1], [2], [3] and clinical settings [4]. Left ventricle hypertrophy (LVH) may be associated with increased RI. Our objective was to study exe4 and liraglutide postconditioning (PostC) in hearts with LVH, isolated from hypertensive SHR-SP (hypertensive LVH) rats.

Materials and methods: Hearts isolated from WKY (control) and SHR-SP rats (11-15 weeks old) were subjected to 35 min LAD occlusion-2 hrs reperfusion, with exe4 0.3 nM or liraglutide 0.3 nM present during the first 15 min in treated hearts. Evans blue/TTC method was used to determine area-at-risk (AAR) and infarct size (% of AAR). Akt phosphorylation (Akt-P) was measured on western blots after 3 min of reperfusion. Arterial blood pressure (BP) was measured in conscious animals by tail cuff method.

Results: BP and heart/body weight ratio were increased in SHR-SP compared to WKY rats (p<0.0001 for both parameters). Infarcts were larger in SHR-SP than in WKY (65.7 +/-3.2, N=7 vs 37.1 +/-3.4, N=12 respectively; P<0.05). Exe-4 and liraglutide PostC decreased infarct size (IS) after 35 min ischemia in WKY (p<0.05). Liraglutide and preconditioning, but not Exe-4, decreased IS after 35 min in SHR-SP (p<0.05). Exe4 PostC decreased IS after 15 min ischemia in SHR-SP (p<0.05). In WKY hearts, exe4 treatment significantly decreased diastolic contracture and increased left ventricle developed pressure. Liraglutide, but not exe4, decreased diastolic pressure in SHR hearts. Degree of Akt phosphorylation was smaller in LVH hearts compared to normal hearts.

Conclusion: These data suggest that 0.3 nM liraglutide was more effective than 0.3 nM exe4 in limiting reperfusion injury in both WKY and SHR-SP. In both WKY and SHR-SP hearts there was a loss of response to PostC by exe4 with increasing ischemia time and infarct size. This loss of response to PostC occurs earlier in hypertrophy hearts.

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Effects of lixisenatide on survival and cardiac function in a mouse model of chronic myocardial infarction

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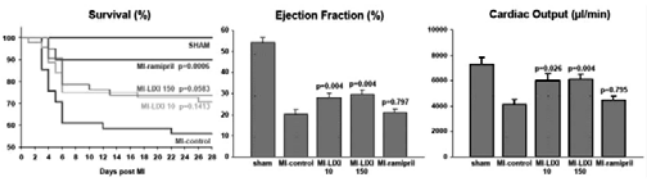
Background and aims: Cardioprotection by the GLP-1 receptor agonist lixisenatide (LIXI) has recently been described in *ex vivo* and *in vivo* ischaemia/reperfusion rat studies. Here, we assessed the cardioprotective activity of LIXI in a more severe mouse model of myocardial infarction (MI), induced by permanent ligation of the left anterior descending coronary artery.

Materials and methods: The protective effects of once-daily treatment with LIXI for 4 weeks were compared with placebo and to sham-operated controls without MI. The ACE inhibitor ramipril served as reference. Treatment was started 3 days prior to induction of ischaemia. Male C57BL/6 mice (6-7 weeks of age) were randomized into 5 groups: Sham, MI-control (placebo injection QD); MI-LIXI 10 (10 µg/kg s.c. QD), MI-LIXI 150 (150 µg/kg s.c. QD) and MI-ramipril (2.5 mg/kg*day in drinking water). Post-MI survival was monitored throughout the study and cardiac function was assessed by pressure-volume loop analysis at study end.

Results: Four weeks after infarct induction, MI-control animals showed dramatically reduced global cardiac parameters with significantly depressed systolic and diastolic function. This resulted in a significantly increased mortality (survival <60%). The survival rate with ramipril was, however, ~90%. Chronic daily injection with LIXI (at 10 and 150 µg/kg) significantly improved (40 and 47%) cardiac function, as indicated by an increase in ejection fraction, stroke volume and cardiac output compared with MI-control, translating into an increased survival rate of >70% (see figure).

Conclusion: Cardioprotection by LIXI following ischaemia/reperfusion has already been demonstrated in previous studies in the rat. With the present study, we could confirm and even extended the cardioprotective effects of LIXI towards a more aggravated MI-induced heart failure mouse model with permanent coronary artery occlusion.

Cardioprotective effects of lixisenatide treatment in mice*



*p values are versus MI-control; MI=myocardial infarction; LIXI=lixisenatide

Supported by: Sanofi

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Patient-reported outcomes with dulaglutide, exenatide, or placebo (AWARD-1)

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Background and aims: Patient-reported Outcomes (PRO) were secondary objectives in this study designed to compare the safety and efficacy of a long acting GLP-1 receptor agonist dulaglutide (DU) to exenatide (EX) and placebo (PL) in patients with type 2 diabetes.

Materials and methods: This was a 52-week (wk), Phase 3, randomised study where patients received once-weekly DU 1.5 mg or 0.75 mg, twice daily EX, or PL (26 wks only). PRO measures for treatment satisfaction (DTSQ), weight-related self-perception (IW-SP), ability to perform physical activities of daily living (APPADL), and health status (EQ-5D) were administered at baseline (BL), 26, and 52 wks.

Results: Both DU doses were superior to PL (26 wks) and EX (26 and 52 wks) in HbA_{1C} change from baseline (BL; p<0.001). Weight reductions were observed in DU 1.5 mg and EX. Significant (p<0.05) improvements from BL were observed in IW-SP (26 and 52 wks, all groups) and total DTSQ scores

(26 wks, both DU doses and EX; 52 wks, both DU doses). A decrease in frequency of perceived hyperglycaemia (all groups) was observed at 26 and 52 wks, but an increase in perceived hypoglycaemia was observed with EX at 26 and 52 wks. EQ-5D visual analog scale (VAS) score showed significant improvements for both DU doses and EX at 26 and 52 wks. No significant changes were observed in APPADL and EQ-5D index scores for any group. Some significant between group differences were observed (Table 1).

Conclusion: Both once weekly DU doses and EX showed improvements from BL for IW-SP, total DTSQ, perceived hyperglycaemia, and EQ-5D VAS scores. Improvement in total DTSQ and perceived hyperglycaemia scores were greater in both DU doses vs EX.

Table 1. Change from BL score ITT, LOCF

Change from BL score ITT, LOCF	DU 1.5 mg (N=279) 26 wks	DU 0.75 mg (N=280) 26 wks	EX (N=276) 26 wks	PL (N=141) 26 wks	DU 1.5 mg (N=279) 52 wks	DU 0.75 mg (N=280) 52 wks	EX (N=276) 52 wks
DTSQ, total	2.40 (0.34)‡·*·*	2.56 (0.33)‡·*·*	0.85 (0.33)‡	0.49 (0.45)	2.05 (0.36)‡·*	2.11 (0.36)‡·*	0.69 (0.36)
IW-SP, total	0.56 (0.15)‡	0.47 (0.15)‡	0.46 (0.15)‡	0.45 (0.20)‡	0.50 (0.15)‡	0.47 (0.15)‡	0.64 (0.15)‡
APPADL, total	0.18 (0.27)	0.12 (0.27)	0.47 (0.27)	0.03 (0.36)	0.18 (0.29)	-0.18 (0.29)	0.35 (0.29)
EQ-5D Index	0.01 (0.01)	0.01 (0.01)	0.00 (0.01)	0.00 (0.02)	0.02 (0.01)	0.01 (0.01)	0.00 (0.01)
EQ-5D VAS	4.50 (0.85)‡·*	2.41 (0.85)‡	3.94 (0.85)‡·*	0.71 (1.15)	5.15 (0.89)‡	3.52 (0.89)‡	3.51 (0.89)‡
DTSQ (Hyperglycaemia)	-1.93 (0.13)‡·*	-1.77 (0.13)‡·*	-1.10 (0.13)‡·*	-0.47 (0.18)‡	-1.89 (0.14)‡·*	-1.63 (0.14)‡·*	-1.13 (0.14)‡
DTSQ (Hypoglycaemia)	0.11 (0.11)*	0.16 (0.11)*	0.48 (0.11)‡	0.27 (0.15)	-0.05 (0.11)*	0.03 (0.11)*	0.34 (0.11)‡

Data presented are LS mean (SE). ‡·*·* 2-sided p<0.05 vs BL, EX, and PL, respectively. Abbreviations: ITT: intent-to-treat; LOCF: last observation carried forward

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Safety and tolerability of linagliptin in 7400 patients with type 2 diabetes: a pooled comprehensive analysis of prospective safety reporting in placebo-controlled studies

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Background and aims: A large number of diabetes drugs are available for the management of type 2 diabetes (T2D) and drug safety is a key consideration. We aimed to assess the safety and tolerability of the dipeptidyl peptidase-4 inhibitor linagliptin involving patients with T2D in a large global development program.

Materials and methods: Safety data were pooled from a comprehensive trial data base comprising all available randomized, double-blind, placebo controlled, clinical trials with linagliptin lasting up to 52 weeks. Incidences of predefined adverse events (AE) were calculated with descriptive statistics and results are summarized by treatment group (linagliptin, placebo) overall and by age category (≤ 65 years, 65-74 years, ≥ 75 years). Hypoglycaemia was assessed descriptively and with an exploratory analysis based on the risk ratio, the respective 95% confidence interval (CI) and Fisher's exact test.

Results: In total, 22 trials were included in the analysis involving 7400 patients (age ≥ 65 years: 69.7%) whereof 4810 received linagliptin 5 mg once daily or placebo (n= 2590). The overall incidence of AE or serious AE (SAE) by various system organ classes including cardiac disorders, vascular disorders, infections and infestations, gastrointestinal disorders (including pancreatitis) with linagliptin was similar to placebo (AE 56.5% vs. 61.2%; SAEs 4.8% vs. 6.3%, respectively). As expected, with advancing age, aggregated incidence of AE and SAE numerically increased, but the incidences comparing linagliptin to placebo remained similar (TABLE). Overall hypoglycaemia incidence was significantly lower for linagliptin (11.5%) as compared to placebo (14.0%) with a risk ratio of 0.82 (95% CI: 0.73 - 0.93; p=0.0021). A low hypoglycaemia risk with linagliptin was seen in all three subgroups of advancing age.

Conclusion: This pooled comprehensive safety analysis supports previous evidence that linagliptin is well tolerated overall and across all age groups, with a low incidence of hypoglycaemic events. Ongoing large outcome trials will provide further insights into the long-term safety profile of linagliptin.

Table. Frequency of investigator reported AEs, SAEs and hypoglycaemic events for linagliptin vs. placebo in 7400 patients included in a global clinical development program.

	Age ≤ 65		Age 65-74		Age ≥75	
	Placebo	*Lina*	*Placebo*	*Lina*	*Placebo*	*Lina*
N	1789	3389	647	1153	154	268
Overall Aes	1047 (58.5%)	1844 (54.4%)	426 (65.8%)	695 (60.3%)	112 (72.7%)	179 (66.8%)
Overall SAEs	78 (4.4%)	141 (4.2%)	59 (9.1%)	68 (5.9%)	27 (17.5%)	21 (7.8%)
Overall hypoglycaemia	187 (10.5%)	322 (9.5%)	140 (21.6%)	172 (14.9%)	35 (22.7%)	59 (22.0%)
Adverse events for selected System Organ Class categories						
Infections and infestations	412 (23.0%)	714 (21.1%)	184 (28.4%)	243 (21.1%)	50 (32.5%)	61 (22.8%)
- nasopharyngitis	99 (5.5%)	182 (5.4%)	47 (7.3%)	82 (7.1%)	9 (5.8%)	17 (6.3%)
Gastrointestinal disorders	212 (11.9%)	368 (10.9%)	89 (13.8%)	147 (12.7%)	29 (18.8%)	29 (10.8%)
- Pancreatitis	0 (0.0%)	2 (0.1%)	1 (0.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Musculoskeletal and connective tissue disorders	195 (10.9%)	350 (10.3%)	98 (15.1%)	148 (12.8%)	22 (14.3%)	39 (14.6%)
Nervous system disorders	147 (8.2%)	259 (7.6%)	75 (11.6%)	96 (8.3%)	24 (15.6%)	30 (11.2%)
Eye disorders	51 (2.9%)	104 (3.1%)	26 (4.0%)	43 (3.7%)	10 (6.5%)	11 (4.1%)
Blood and lymphatic disorders	30 (1.7%)	72 (2.1%)	16 (2.5%)	19 (1.6%)	8 (5.2%)	5 (1.9%)
Neoplasms	9 (0.5%)	18 (0.5%)	11 (1.7%)	10 (0.9%)	4 (2.6%)	1 (0.4%)
Hepatobiliary disorders	16 (0.9%)	31 (0.9%)	6 (0.9%)	10 (0.9%)	1 (0.6%)	0 (0.0%)
Vascular disorders	58 (3.2%)	116 (3.4%)	39 (6.0%)	51 (4.4%)	15 (9.7%)	13 (4.9%)
- Hypertension	39 (2.2%)	74 (2.2%)	27 (4.2%)	28 (2.4%)	7 (4.5%)	10 (3.7%)
Cardiac disorders	46 (2.6%)	88 (2.6%)	28 (4.3%)	52 (4.5%)	9 (5.8%)	12 (4.5%)
- palpitations	5 (0.3%)	11 (0.3%)	3 (0.5%)	7 (0.6%)	0 (0.0%)	2 (0.7%)

Supported by: Boehringer-Ingelheim

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Metformin safety in the very elderly

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Background and aims: With the increasing life expectancy more and more subjects with type 2 diabetes are aged over 80, and they often have a high quality of life. According to DECODE study, over 40% of octogenarians suffer from diabetes. However, there are no specific guidelines for the treatment of this group of the very elderly patients, and metformin use in this population is controversial. The aim of our study was to assess metformin use, with a special reference to its safety, in the very elderly individuals with type 2 diabetes mellitus.

Materials and methods: We have assessed cross-sectionally modes of type 2 diabetes therapy used in randomly selected subjects aged 80-90 (n=158) - and retrospectively analyzed 3-year safety of thereof. The comparative group comprised gender- and BMI-matched type 2 diabetes subjects aged 60-70 years (n=112).

Results: The treatment modes and cardiovascular risk factors distribution is presented in the table (mean±SD or %). Symptomatic hypoglycemia was recorded in 21% of the very elderly and 45% of the controls (p<0.01) in the 3-year period preceding the cross-sectional analysis. No case of ketoacidosis or lactic acidosis was noted in the studied subjects.

Conclusion: Majority of the very elderly type 2 diabetes patients are treated safely with metformin. Moreover, glucose control and cardiovascular risk profile is more favorable in these subjects than in the younger ones, suggesting the effective use of available therapies. The very elderly subjects with type 2 diabetes, despite differing at many instances from the over-a-decade younger patients, can be effectively and safely treated with traditional therapies, including metformin.

	The Very Elderly	*Controls*
Age (yrs)	83.2±2.9*	64.8±3.5
Diabetes duration (yrs)	11.8±10.9*	8.2±3.4
Metformin (%)	62*	82
Daily metformin dose (mg)	1600±700*	1850±1000
Sulfonylureas (%)	47*	63
Any insulin (%)	45*	32
Insulin + metformin/sulfonylureas (%)	33*	25
Daily insulin dose (IU)	41±26*	56±29
Insulin therapy duration (yrs)	5.5±2.9*	4.1±1.6
BMI (kg/m^2)	30.6±4.4	30.9±3.7
Systolic blood pressure (mmHg)	138±14	139±17
Diastolic blood pressure (mmHg)	75±8*	89±13
HbA_{1c}(%)	7.1±0.9*	7.8±1.3
Total Cholesterol (mg/dl)	185±39*	215±43
Triglycerides (mg/dl)	146±73*	186±83
HDL cholesterol (mg/dl)	50±12*	41±16
LDL cholesterol (mg/dl)	108±33*	132±21
Serum creatinine (mg/dl)	1.19±0.31*	1.02±0.25
**p<0.05 vs controls*		

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PS 079 Novel approaches to treat type 1 and type 2 diabetes

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Metabolic effects of cell therapy based GLP-1 treatment in streptozotocin diabetic mice

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Background and aims: The use of long-acting glucagon-like peptide-1 (GLP-1) mimetics has generated significant therapeutic interest for diabetes. Despite their obvious attractiveness, timing of injections and circulating drug levels remain an issue. As such, *in situ* production of GLP-1 by cellular delivery could circumvent these problems. We have investigated the effects of GLP-1 delivery by cell therapy using subscapular implantation of the GLP-1 secreting cell line, GLUTag, in streptozotocin-induced diabetic mice.
Materials and methods: Diabetes was induced in female severe combined immunodeficient mice (15-28 g) by intraperitoneal injection of streptozotocin (150 mg/kg bw). GLUTag cells were suspended at a density of 50 x 10^6 cells/ml and 100 µl injected subcutaneously at the subscapular region of diabetic mice. Control mice received 100 µl vehicle (cell culture media alone). All diabetic mice were given insulin (15 U/kg bw) from days 7 to 29 after streptozotocin treatment. Food intake, water intake, body weight and blood glucose were monitored every 2-3 days. In addition a glucose tolerance test (18 mmol/kg bw) was carried out 30 days post transplantation. At termination, plasma insulin, glucagon and GLP-1 levels were assessed. Additional terminal analyses included assessment of GLP-1 intestinal content, pancreatic morphology and Ki-67 and TUNEL staining of beta-cells.
Results: Cell implantation was well tolerated and over the 30 day study period, transplantation gave rise to encapsulated and well vascularised growths which contained immunoreactive GLP-1. These weighed between 0.21 g to 0.81 g with cell mass sizes between 9 x 8 mm^2 to 21 x 19 mm^2. Transplantation significantly (P<0.001) countered excessive food and fluid intake in diabetic mice and maintained normal control body weight. Circulating glucose was significantly (P<0.01) lowered from day 9 post-implantation onwards, and plasma insulin was 1.9 ± 0.7 ng/ml on day 30, compared with undetectable levels of insulin in diabetic controls. In addition, glucagon levels were significantly (3.7-fold; P<0.05) reduced and GLP-1 (6.2-fold; P<0.05) increased in transplanted mice compared to controls. This was associated with significantly (P<0.05 to P<0.01) lower blood glucose levels in GLUTag cell implanted mice following exogenous glucose administration. The improvement of glucose tolerance was corroborated by significantly (1.5-fold; P<0.01) decreased AUC values when compared to controls. GLP-1 content of intestines of implanted mice was substantially (8.7-fold; P<0.05) higher than controls. Histological examination of the pancreata of transplanted mice revealed significant elevations in islet (2.0-fold; P<0.001) and beta-cell (2.3-fold; P<0.001) area, with reduced (1.5-fold; P<0.001) alpha-cell area. Ki-67 and TUNEL staining demonstrating that increased beta-cell mass was due to enhanced proliferation relative to apoptosis, with approximate 2:1 ratio favouring enhanced beta-cell mass.
Conclusion: GLP-1 delivery by cell therapy has significant beneficial effects on metabolic control in streptozotocin-induced diabetic mice. The positive effects in diabetes were associated with enhanced beta-cell mass expansion and insulin-secretion. The results represent proof of concept that GLP-1 delivery by cell therapy affords a possible means of improving metabolic control in diabetes.

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Molecular mechanism of protective effect by pioglitazone and/or liraglutide on beta cell damage with the progression of diabetic morbidity

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Background and aims: We previously reported that pioglitazone(PIO) and liraglutide(LIRA) preserved pancreatic beta-cell mass and function in obese

diabetic *db/db* mice. The aim of this study is to examine the molecular mechanism of protective effects of these drugs on beta-cell damage with the progression of diabetic morbidity.
Materials and methods: Seven week-old male *db/db* mice, as early stage model, and 16 week-old male *db/db* mice, as advanced stage model, received PIO (25mg/kg QD, PO), LIRA (0.2mg/kg BID, *s.c.*), PIO and LIRA combination, or vehicle for 2 days (*study 1*: for acute effect) or 2 weeks (*study 2*: for chronic effect) (n=5 for each group). Body weight, fasted blood glucose (FBG), plasma insulin (FIRI), and plasma triglyceride (TG) levels were measured before and after the intervention. The beta-cell mass was assessed after 2weeks intervention. Gene expressions specific for the core area of pancreatic islet were analyzed by LCM method and real time RT-PCR. Primer pairs encoding genes associated with pancreatic hormones, cell differentiation/proliferation, cell cycle, apoptosis, oxidative/endoplasmic reticulum(ER) stress, inflammation and fibrosis were prepared, and real-time RT-PCR with Sybr Green was applied. Each genes expression was relatively quantified by the comparative Ct method with each result in the 18 SrRNA as a control.
Results: Study 1: After 2-days intervention, when the deranged biochemical markers did not ameliorate completely, gene expressions of *NeuroD*, *PDX-1*, and *ERK1* in early stage model were significantly up-regulated by PIO and/or LIRA treatment, and the most significant effect was observed in the combination group, but those effects were not demonstrated in advanced stage. The *bcl-2* gene expression was increased, and *caspase8* and *caspase3* gene expressions were decreased by PIO and/or LIRA in both models. The combination treatment showed more significant effect on those gene expressions. The short-term intervention did not alter the oxidative/ ER stress and inflammation related gene expressions in both early and advanced model of mice. Study 2: In early stage, LIRA significantly lowered FBG with an increment of insulin level. PIO also decreased FBG compared with the control. FBG reduction was further enhanced by combination treatment. In advanced stage, FBG was lowered only in combination group. The beta-cell mass was greater in LIRA and/or PIO groups than in the control in early stage, and the most significant effect was observed in combination group. All drug groups preserved the beta-cell mass also in advanced stage, but effects were not so much significant compared with those in early stage model. Gene expression profiles related with cell proliferation and apoptosis were not different from those shown in *Study 1* (2 days intervention) in both early and advanced stage models. Furthermore, the long-term treatment with PIO and/or LIRA down-regulated gene expressions associated with fibrosis. The combination of 2 drugs also showed the most significant effect.
Conclusion: Both PIO and LIRA protect beta-cell damage directly through promotion of cell differentiation/proliferation and inhibition of apoptosis in early stage, but those effects attenuate in advanced stage. The present results also suggest that action mechanisms of PIO and LIRA on beta-cell survival differ each other.

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GLP-1 attenuates the acceleration of gastric emptying induced by hypoglycaemia in healthy subjects

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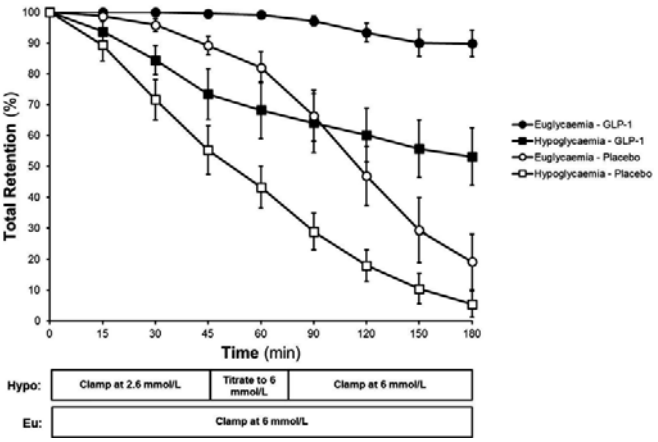
Background and aims: Acute administration of glucagon-like peptide-1 (GLP-1) and 'short acting' GLP-1 agonists markedly slow gastric emptying in health and type 2 diabetes, which is likely the major mechanism responsible for the reduction in postprandial glycaemic excursions. Acute changes in glycaemia also modulate gastric emptying, such that gastric emptying is slower during hyperglycaemia and markedly accelerated by insulin-induced hypoglycaemia. The latter is likely to be an important mechanism underlying the counter-regulation of hypoglycaemia by increasing nutrient availability. The aim of this study was to determine whether GLP-1 attenuates the acceleration of gastric emptying induced by hypoglycaemia in healthy subjects.
Materials and methods: Ten healthy subjects (5M:5F, age 69±1.7y, BMI 26±1 kg/m^2), were studied on 4 days in a randomised double-blind fashion. Following an overnight fast, blood glucose was stabilised using a glucose/insulin clamp at either hypoglycaemia (hypo; blood glucose 2.6mmol/L) or euglycaemia (eu; 6.0mmol/L) between T=-15 to 45min before clamping at 6.0mmol/L until 180min. During hypoglycaemia and euglycaemia each subject received intravenous GLP-1 (1.2pmolkg/min) or placebo between T=-60 to 180min. At T=0min subjects ingested 100g of minced beef (25g protein, 21g fat, ~270Kcal), labelled with 20MBq ^{99m}Technetium-sulphur-colloid. Gastric emptying was measured scintigraphically with 1 min images acquired until T=180min. The area under the curve (AUC) for gastric emptying (T=0-180min) was analysed using one-way RM-ANOVA with Bonferroni-Holm adjusted posthoc tests.
Results: Results are shown in the figure as mean ± SE. The studies were well tolerated and blood glucose concentrations were stabilised at the proposed levels. Gastric emptying was accelerated during hypoglycaemia (hypo/placebo vs eu/placebo; P<0.001), and administration of exogenous GLP-1 markedly slowed gastric emptying during euglycaemia (eu/placebo vs eu/GLP-1; P<0.001). During GLP-1 infusion gastric emptying was faster during hypoglycaemia than euglycaemia (hypo/GLP-1 vs eu/GLP-1; P<0.008). However, the hypoglycemia-induced acceleration of gastric emptying on placebo was attenuated by GLP-1 (hypo/placebo vs hypo/GLP-1; P<0.008) and there was no significant difference between gastric emptying during euglycaemia on placebo compared with hypoglycaemia after administration of GLP-1 (eu/placebo vs hypo/GLP-1; P = 0.52).
Conclusion: In health the acceleration of gastric emptying induced by hypoglycaemia is attenuated by exogenous GLP-1. This observation may be of particular relevance to the counter-regulatory response to hypoglycaemia with the combined use of GLP-1 agonists and basal insulin.



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GLP-1 and liraglutide increase the platelet inhibitory effects of nitric oxide

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Background and aims: Glucagon-like peptide-1 (GLP-1) exerts metabolic and cardiovascular actions. After its secretion, GLP-1(7-36) amide is rapidly truncated by dipeptidyl peptidase-4 to GLP-1(9-36) amide, the major circulating form of GLP-1, unable to interplay with GLP-1 receptor (GLP-1R). Defective GLP-1 synthesis and action occur in type 2 diabetic patients and incretin drugs are increasingly used in their therapy. GLP-1 exerts beneficial effects on heart and endothelium: its effects on platelets -which play a major role in diabetes vascular complications- are unknown. Aim of the study is to investigate the effects of GLP-1(7-36), GLP-1(9-36) amides and GLP-1 analogue Liraglutide on platelet sensitivity to agonists and to the anti-aggregating pathway nitric oxide (NO)/cGMP/Vasodilator-Stimulated Phosphoprotein (VASP).
Materials and methods: In platelet-rich plasma and washed platelets from 38 healthy subjects (M/F 23/15; age: 22.7±6.6 years; body mass index: 21.6±2.3 kg/m2) we measured the effects of a 10-min pre-incubation with 100 nmol/l GLP-1(7-36), or GLP-1(9-36) or Liraglutide on: i) platelet aggregation to ADP (10 micromol/l) and to collagen (4 mg/l), ii) cGMP synthesis (ELISA), and iii) expression of VASP phosphorylated (pVASP) at ser-239 (western blot), in the absence or in the presence of the NO donor sodium nitroprusside (SNP) (1-5 micromol/l, 5min). Experiments were repeated with GLP-1R antagonist Exendin (9-39) (100 nmol/l).
Results: GLP-1(7-36), GLP-1(9-36) and Liraglutide did not influence platelet aggregation to agonists, cGMP synthesis and pVASP ser-239 expression.

They increased, however, the inhibitory effects of SNP. In particular, the percent inhibition of the ADP-induced aggregation in the presence of SNP alone, SNP+GLP-1(7-36), SNP+GLP-1(9-36) and SNP+Liraglutide were 34.7±10.3, 56.1±11.9, 55.6±11.6 and 53.6±13.0, respectively (p<0.0001 vs SNP alone for all the incretin preparations); the percent inhibition of the collagen-induced aggregation in the presence of SNP alone, SNP+GLP-1(7-36), SNP+GLP-1(9-36) and SNP+Liraglutide was 35.1±12.6, 62.1±13.6, 60.7±12.4 and 50.7±21 (p<0.0001 vs SNP alone for all the incretin preparations). The increased platelet response to SNP was attributable, at least in part, to the increased cGMP synthesis/action: actually, i) the percent increase of cGMP concentrations with SNP alone, SNP+GLP-1(7-36), and SNP+GLP-1(9-36) were 92.6±42.8, 171.1±80.2 (p<0.01 vs SNP alone) and 197.3±92.9 (p<0.04 vs SNP alone); ii) the percent increase of SNP-induced pVASP ser-239 expression with SNP alone, SNP+GLP-1(7-36), SNP+GLP-1(9-36) and SNP+Liraglutide were 198.6±74.5, 371.1±94.4 (p<0.001 vs SNP alone), 423±93.9 (p<0.0001 vs SNP alone), and 398.6±75.7 (p<0.005 vs SNP alone), respectively. Pre-incubation with Exendin(9-39) did not modify GLP-1(7-36), GLP-1 (9-36) and Liraglutide influence on the platelet inhibitory effects exerted by SNP.

Conclusion: In platelets from healthy subjects GLP-1 increases platelet sensitivity to NO via GLP-1R-independent mechanisms. This beneficial effect of GLP-1 induces to hypothesize that its defective synthesis/action play a role in the impairment of platelet sensitivity to NO observed in type 2 diabetes, and that this impairment could be attenuated by incretin therapy.

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Exploring the potential of dapagliflozin in type 1 diabetes: phase 2a pilot study

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Background and aims: Intensive insulin therapy for type 1 diabetes (T1D) is associated with weight gain and is often insufficient in maintaining glycaemic control. Dapagliflozin (DAPA), an insulin-independent sodium glucose cotransporter 2 inhibitor that increases urinary glucose excretion, has shown antihyperglycaemic efficacy in type 2 diabetes. This 2-week randomised, double-blind, placebo-controlled, Phase 2a study evaluated DAPA added to insulin in patients with suboptimally controlled T1D.

Materials and methods: Adult patients on stable insulin ≥12 months with HbA_{1c} 7-10% (baseline mean 8.5%) were randomised to receive DAPA (1, 2.5, 5, or 10 mg) or placebo once daily for 14 days (Days -3 to 7 as inpatients). DAPA pharmacokinetics/pharmacodynamics, including continuous glucose monitoring (CGM) and 24-hour urine glucose, was assessed at Day 7.

Results: Seventy patients were randomised to treatment; 62 (88.6%) completed the trial. As expected, there was a dose-dependent increase in urine glucose with DAPA. CGM data suggested a potential for reduced glycaemic levels and diminished glycaemic variability with DAPA. Marked reductions in total daily insulin dosing at Day 7 were reported for DAPA 5 mg (-19%) and 10 mg (-16%). Hypoglycaemia was common in all treatment groups; 1 event (DAPA 10 mg) was major and led to discontinuation. The incidence of adverse events (AEs) was 38.5% to 61.5%; there was 1 non-treatment-related serious AE (DAPA 5 mg) and no deaths.

		Placebo + insulin (n=10 to 13)	DAPA 1 mg + insulin (n=10 to 13)	DAPA 2.5 mg + insulin (n=11 to 15)	DAPA 5 mg + insulin (n=13 to 14)	DAPA 10 mg + insulin (n=9 to 15)
Hypoglycaemia (%)	No. pts (%) with =1 event	8 (61.5)	12 (92.3)	9 (60.0)	11 (78.6)	10 (66.7)
24 h urine glucose excretion	Baseline, g/24h ± SD	30.7 ± 52.0	6.6 ± 6.5	12.1 ± 12.3	11.2 ± 10.9	11.9 ± 14.3
	Day 7, g/24h ± SD	9.0 ± 7.5	48.5 ± 20.8	60.8 ± 38.2	83.6 ± 47.9	99.7 ± 58
	Mean change from baseline, g/24h (95% CI)	-21.6 (-54.4, 11.1)	41.9 (27.6, 56.1)	48.5 (28.1, 69.0)	72.4 (47.0, 97.9)	88.8 (55.2, 122.5)
24 h glucose/CGM daily average	Baseline, mmol/L ± SD	9.6 ± 2.4	9.0 ± 1.7	9.7 ± 2.3	9.6 ± 1.8	9.7 ± 2.5
	Day 7, mmol/L ± SD	8.5 ± 2.3	8.1 ± 1.1	9.1 ± 2.1	8.0 ± 2.8	7.7 ± 1.9
	Mean change from baseline, mmol/L (95% CI)	-1.1 (-3.6, 1.4)	-0.9 (-2.1, 0.4)	-0.8 (-2.6, 1.1)	-1.6 (-2.6, -0.7)	-2.3 (-3.7, -0.9)

Conclusion: DAPA was generally well tolerated in this T1D population. Further studies to determine the potential benefit of DAPA as treatment of T1D are warranted.

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Effects of one-year treatment with canagliflozin or sitagliptin on glycaemic control and beta cell function measures in subjects with type 2 diabetes on metformin + sulfonylurea

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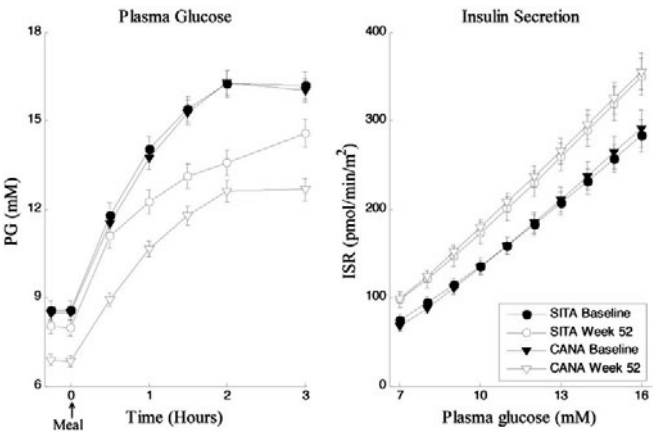
Background and aims: Canagliflozin (CANA), an SGLT2 inhibitor, and sitagliptin (SITA), a DPP-4 inhibitor, lower plasma glucose (PG) by different mechanisms. CANA lowers PG by increasing urinary glucose excretion, whereas SITA increases active concentrations of the incretin hormones GLP-1 and GIP. The aim of this analysis was to compare the effects of CANA and SITA treatment on fasting and postprandial glucose (PPG) control and measures of β-cell function (βCF) in subjects with type 2 diabetes mellitus (T2DM) with inadequate glycaemic control on metformin + sulfonylurea (MET + SU).

Materials and methods: Data were obtained during a 52-week Phase 3 study comparing the effects of CANA 300 mg and SITA 100 mg as add-on therapy in subjects with T2DM with inadequate glycaemic control on MET + SU (mean baseline characteristics: age = 57 y; HbA_{1c} = 8.1%; body weight = 88 kg; BMI = 32 kg/m^2). A subset of subjects (n = 249) in the study underwent a 3-hr frequently-sampled mixed-meal tolerance test (FS-MMTT), scheduled at baseline and Week 52. During the FS-MMTT, PG, insulin, and C-peptide were measured at several time points. PG control was assessed using fasting PG (FPG), mean PG during the FS-MMTT (MPG; calculated over t = 0-3 hr), and mean incremental (above fasting) PG during the FS-MMTT (MiPG). βCF was assessed using a model-based approach that calculates the insulin secretion rate (ISR) from deconvolution of measured C-peptide and relates ISR to PG. Calculated βCF parameters included ISR at 9 mM glucose and β-cell glucose sensitivity (the slope of the ISR vs PG relationship). Subjects with FS-MMTT data at both visits (n = 135) were included in the analysis. Statistical comparisons were made using ANCOVA with baseline value as a covariate.

Results: Both CANA and SITA reduced FPG and PPG compared to baseline (Figure). Greater reductions in both FPG (mean [SE] ΔFPG = -1.6 [0.2] mM with CANA vs -0.6 [0.3] mM with SITA; p<0.001) and MPG (ΔMPG = -3.1 [0.3] mM with CANA vs -1.8 [0.4] mM with SITA; p<0.001) were observed with CANA than with SITA, whereas both treatments had generally similar reductions in incremental PG excursions (ΔMiPG = -1.5 [0.2] mM with CANA vs -1.2 [0.2] mM with SITA; p=0.2). Both treatments shifted the ISR vs PG relationship (Figure) and provided similar increases in ISR at 9 mM (ΔISR at 9 mM = 41 [7] pmol/min/m^2 with CANA vs 31 [11] pmol/min/m^2 with SITA; p=0.4). Small numerical increases in mean β-cell glucose sensitivity were observed with both treatments, but these changes were not statistically significant.



Conclusion: After one year of treatment with CANA or SITA in subjects with T2DM who have inadequate glucose control with MET + SU, CANA provided greater reductions in FPG and MPG than observed with SITA. Both treatments provided similar improvements in measures of βCF.
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Synergistic glucose-lowering effects of SGLT1- and ASBT-inhibitor combinations in ZDF rats
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Background and aims: The profound, rapid, and weight-independent antidiabetic effects of bariatric surgery affirm the promise of modulating gastrointestinal function for therapeutic purposes. We have previously demonstrated separate glucose-lowering effects of inhibitors of the sodium-glucose cotransporter 1 (SGLT1) and the apical sodium-dependent bile acid transporter (ASBT) in human and/or animal studies.
Materials and methods: A potential synergy between SGLT1 inhibition and ASBT inhibition was tested in male Zucker-fatty diabetic rats using a novel dose-ratio scanning method. First, equi-effective (ED30) glucose-lowering doses of an SGLT1 inhibitor (KGA2727; 0.5 mg/kg bid) and an ASBT inhibitor (GSK2299027; 0.14 mg/kg bid) were determined by dose-response analysis of each agent.
Results: At high doses, both KGA2727 and GSK2299027 significantly decreased blood glucose. In subsequent studies, a maximally effective dose of an ASBT inhibitor (264W94) reduced plasma [glucose] to 196 ± 33 mg/dL versus 341 ± 24 mg/dL in vehicle controls. A series of 9 combination treatments in ZDF rats (n=16 rats each), the subtraction of one agent was made up by the addition of the other, were conducted at the same time. With a 50%-of-ED30 + 50%-of-ED30 mixture (KGA2727, 0.25 mg/kg; GSK2299027, 0.07 mg/kg), maximal effect was attained (plasma [glucose] 192 ± 29 mg/dL) without evidence of correlated increases in cecum weight, fecal water content, or fecal bile acid excretion.
Conclusion: In summary, we demonstrate synergy of the glucose-lowering, but not the adverse effects of distinct gastrointestinal mechanisms (ASBT and SGLT1 inhibition), each capable of being invoked by agents restricted to the gut lumen.

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LX2761, an SGLT1 inhibitor restricted to the intestine, improves glycaemic control in mice
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Background and aims: LX2761, an orally available small molecule with IC50 = 2 nM vs both hSGLT1 and hSGLT2, was designed to improve glycemic control by remaining in the intestine (Int) to inhibit SGLT1-mediated glucose (G) absorption and to trigger release of glucagon-like peptide 1 (GLP1). Studies were performed to test the ability of LX2761 to achieve these endpoints.
Materials and methods: Study 1 asked if LX2761 is absorbed by the Int, by measuring 24-hr urine G excretion (UGE) in healthy wild type (WT) mice after 1 oral dose of LX2761. Studies 2-5 asked how multiple once-daily oral doses of LX2761 affect glycemic control: study 2 asked if a meal challenge containing 9.2 g G raises cecal G and serum GLP1 in WT mice 7 hr after their 4th LX2761 dose; study 3 asked if G tolerance (GT) during an oral GT test (oGTT; 4 g/kg G) is improved in WT mice 15 hr after their 4th LX2761 dose; study 4 asked if GT (2 g/kg G) and HbA1c are improved in KKA^y diabetic mice after 30 days of LX2761; and study 5 asked if GT (2 g/kg G) and HbA1c are improved, and if an oral G bolus raises cecal G and serum GLP1, in STZ diabetic mice treated with LX2761 for 7 weeks.
Results: Data from studies 1-4 are shown in the Table below. Study 5 found that STZ diabetic mice receiving 3 mg/kg/day LX2761: 1) showed improved GT (G AUC of 38 ± 10 g/dL•min vs vehicle G AUC of 65 ± 3 g/dL•min, p<0.001); 2) had lower HbA1c (decrease from baseline of 0.9 ±1.6 % vs vehicle increase from baseline of 0.9 ±1.4 %, p<0.05); and 3) showed, 2 hours after a 2 g/kg oral G bolus, higher postprandial cecal G (1.4 ± 0.8 mg vs vehicle 0.2 ± 0.1 mg, p<0.01) and higher postprandial serum tGLP1 (103 ± 28 pM vs vehicle 66 ± 5 pM, p<0.05).
Conclusion: 1) In LX2761-treated WT mice, low UGE suggests that Int absorption of LX2761 is trivial; 2) In LX2761-treated WT and STZ diabetic mice, oral G raises cecal G and serum GLP-1 levels, and improves GT, suggesting that LX2761 inhibits Int SGLT1; and 3) In LX2761-treated KKA^y and STZ diabetic mice, improved GT and lower HbA1c suggest that LX2761 significantly improves glycemic control. Overall, these data demonstrate that LX2761 significantly improves glycemic control via local inhibition of Int SGLT1.

	Study 1	Study 2		Study 3	Study 4	
LX2761 Dose, mg/kg	UGE, mg	Cecal G, mg	tGLP1, pM	oGTT AUC, g/dL•min	oGTT AUC, g/dL•min	HbA1c, %
Vehicle	1±1 (6)	0.1±0.03 (5)*	78±28 (5)*	3.2±0.4 (8)*	53±7 (10)*	12.0±0.5 (10)^
0.05	---	---	---	1.3±0.9 (9)	---	---
1.5	1±1 (5)	12.1±6.70 (5)	196±50 (5)	---	38±7 (10)	11.2±1.0 (10)

Data are mean ± SD; (N), mouse number; tGLP1, total GLP1; oGTT, oral glucose tolerance test.
Different from LX2761. ^ p<0.05; * p<0.001

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LX4211, a dual SGLT1/SGLT2 inhibitor, decreases body weight and triglycerides in patients with type 2 diabetes mellitus and elevated baseline values
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Background and aims: LX4211 reduced intestinal glucose absorption and enhances GLP-1 and PYY release in the gastrointestinal tract through inhibition of SGLT1. In addition, LX4211 increases urinary glucose excretion through inhibition of SGLT2 in the kidney.
Materials and methods: In this dose-ranging study, 299 patients with type 2 diabetes mellitus (T2DM) and inadequate glycemic control on stable dose metformin monotherapy were treated with LX4211 (75 mg qd, 200 mg qd, 200 mg bid or 400 mg qd) or placebo for 12 weeks. The primary endpoint was hemoglobin A1C (A1C). Secondary endpoints included body weight (BW) and triglycerides (TG). LX4211 produced significant decreases in A1C, fasting plasma glucose, blood pressure, and BW. Patients were subgrouped based on baseline BW and TG to further explore the effect of LX4211 on these parameters.
Results: For patients with a baseline BMI (body mass index) of ≥30 kg/m^2, LX4211 produced a significant reduction in BW at Week 12 relative to baseline (-0.91 kg, -1.84 kg, -2.89 kg, -1.97 kg, and -0.44 kg for 75 mg qd, 200 mg qd, 200 mg bid, 400 mg qd, and placebo arms, respectively; p<0.001 for the top 3 dose arms vs. placebo). For subjects with elevated baseline TG (≥2.26 and <5.65 mmol/L), LX4211 treatment resulted in significant reductions in TG relative to baseline in 3 of the treatment arms (-0.76 mmol/L -0.55 mmol/L, and -0.92 mmol/L for 75 mg qd, 200 mg bid, and 400 mg qd arms, respectively; p<0.05 for all 3 arms). A reduction of -0.41 mmol/L (NS) was observed in the placebo arm.
Conclusion: Treatment with LX4211 reduced BW and TG in T2DM patients with elevated baseline values, and these results merit further investigation in future trials.
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The effect of ipragliflozin on the total glucose turnover after OGTT in healthy subjects and patients with type 2 diabetes mellitus
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Background and aims: Sodium Glucose co-Transporter 2 (SGLT2) accounts for about 90% of renal of glucose reabsorption. Ipragliflozin is a novel selective SGLT2 inhibitor, which increases urinary glucose excretion (UGE), thereby reducing plasma glucose levels in patients with Type 2 diabetes mellitus (T2DM). This study investigated the effect of ipragliflozin on the total glucose turnover in healthy subjects (HS) and patients with T2DM.
Materials and methods: Twelve HS (age 46.1 yrs (sd 8.69)) and 12 T2DM patients (age 59.0 (6.16)) received ipragliflozin 100 mg or placebo once daily for 6 days in a 2 period crossover design. In each period an OGTT with a

double-tracer methodology (oral administration of [$_{13}C_6$]-labeled glucose during a [6,6-$^2H_2$]-glucose intravenous infusion) was performed at baseline (Day -1) and on Day 6. The amounts of glucose absorbed from the gut and disappearing from the circulation, as well as the UGE and the endogenous glucose production (EGP) were determined.
Results: Baseline EGP (EGP_{basal}) was lower in HS compared to T2DM patients (11.2 (1.0) vs 12.7 (1.6) µmol/min/kg). The baseline EGP increased after ipragliflozin compared to placebo in HS and in T2DM patients (P<0.05; +0.68 (1.5) and +0.91 (1.4) µmol/min/kg, respectively). During the OGTT the mean $EGP_{0\text{-}6h}$ in HS and in T2DM patients was 6.0 (1.2) and 4.1 (0.5) µmol/min/kg, respectively. The OGTT-related decrease from baseline was less with ipragliflozin than with placebo (P< 0.01) without any difference between the subject groups. At baseline in HS the total amount of glucose appearing in the circulation in 6 hours after the OGTT was 107.2 g of which 80.6 g resulting from the uptake via the gut and 26.7 g resulting from EGP. During the same time period 107.1 g disappeared from the circulation. There was no relevant UGE. In T2DM patients 107.1 g appeared in the circulation of with 88.6 g resulting from uptake from the gut, and 18.4 g from EGP. 107.5 g of glucose disappeared from the circulation of which 99 g was distributed into tissues and only a small portion excreted via urine (8.5 g). After 6 days of ipragliflozin treatment, in HS the total amount of glucose appearing in the circulation in 6 hours after the OGTT was 110 g, with 81.4 g resulting from the uptake via gut and 28.6 g from EGP. During the same time period, 108.1 g of glucose disappeared from the circulation. The majority of disappearing glucose (92.3 g) was distributed into the tissues while a smaller portion (15.8 g) was excreted via urine. In T2DM patients, the total amount of glucose appearing in the circulation in 6 hours after the OGTT was 110.7 g, with 87.9g resulting from the uptake via gut and 22.7g from EGP. During the same time period, 111.4 g of glucose disappeared from circulation. The majority of disappearing glucose (78.5 g) was distributed into the tissues and a considerable portion (32.9 g) was excreted via urine.
Conclusion: Ipragliflozin increased EGP_{basal} both in HS and patients with T2DM as a result of an increased UGE. After an OGTT the total amount of glucose absorbed from the gut and the EGP did not change through ipragliflozin, neither in HS nor in T2DM patients. Ipragliflozin increased UGE-which led to a reduced tissue glucose distribution.
Clinical Trial Registration Number: NCT01611363
Supported by: Astellas Pharma Europe BV

PS 080 Novel agents: novel mechanisms

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A novel GLP-1/glucagon co-agonist peptide exhibits improved glucose-lowering and insulinotropic actions in diet induced obese diabetic mice
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Background and aims: Patients with type 2 diabetes frequently require a range of anti-hyperglycaemic agents to successfully maintain glycaemic control and thus there is demand to develop novel and effective combination strategies. One approach is to generate incretin-glucagon co-agonist peptides combined within a single molecule to improve stability and maximise therapeutic efficacy. In this study we developed a novel GLP-1/glucagon peptide based on [DA^2]GLP-1 comprising mid-chain substitution with human glucagon at residues S^{12}-F^{22}. Biological properties of [DA^2]GLP-1/GcG were assessed *in vitro* and in a mouse model of diet induced obesity-diabetes.
Materials and methods: GLP-1, glucagon, exendin-4 and [DA^2]GLP-1/GcG (95% purity) were incubated (0, 2, 4 and 8 h; 5 g) with DPP-IV (5 mU; n=3) to determine enzyme stability and with BRIN-BD11 cells to evaluate insulin secretion and intracellular Ca^{2+} (20 min; n=8). cAMP production (n=4) was examined using GLP-1-R and glucagon-R transfected cells. Acute and persistent effects of peptides (25 nmol/kg bw; *ip*) on plasma glucose and insulin concentrations were examined in NIH *Swiss* high-fat fed (HFF) and GLP-1-R and GIP-R knockout mice (n=8). For longer-term studies, groups of HFF mice (n=8) received twice-daily injections (09:00 and 16:00 hr) of saline vehicle (0.9% w/v NaCl), exendin-4 or [DA^2]GLP-1/GcG (each at 25 nmol/kg bw; *ip*) for 21 days. Food intake, body weight, non-fasting plasma glucose and insulin concentrations were monitored every 2 to 4 days. Glucose tolerance (18 mmol/kg bw; *po*) and insulin sensitivity (25 U insulin/kg bw; *ip*) tests were performed at the end of the study.
Results: Native peptides were rapidly degraded by DPP-IV, however [DA^2] GLP-1/GcG remained intact during the incubation (half-life > 8h). [DA^2] GLP-1/GcG concentration-dependently (10^{-12} to 10^{-6}M) stimulated insulin secretion (1.2 to 1.7 fold; P<0.001), intracellular Ca^{2+} (1.5-fold; P<0.05) and cAMP production. Acute administration of [DA^2]GLP-1/GcG significantly lowered plasma glucose (14% reduction; P<0.05) and increased plasma insulin concentrations (1.4-fold; P<0.05) in HFF mice. Furthermore, [DA^2] GLP-1/GcG elicited a significantly protracted glucose-lowering effect (29% reduction; P<0.05) and insulinotropic response (1.4 to 1.8-fold; P<0.001) when administered 8 hours prior to a glucose load in HFF mice. Twice-daily administration of [DA^2]GLP-1/GcG for 21 days decreased body weight (4% reduction; P>0.05), non-fasting plasma glucose (40% reduction; P<0.05) and increased non-fasting plasma insulin concentrations (1.2 to 1.3-fold; P<0.05 to P<0.001) compared to saline-treated controls. Furthermore, [DA^2]GLP-1/GcG improved oral glucose tolerance (21% reduction; P<0.05), glucose-mediated insulin secretion (1.2-fold; P<0.01) and insulin sensitivity (46% reduction; P<0.001). Food intake remained unchanged during the study. Interestingly, [DA^2]GLP-1/GcG treatment resulted in significantly improved insulinotropic responses (1.2-fold; P<0.05) compared to exendin-4. Studies in GLP-1R and GIP-R knockout mice confirmed primary action of [DA^2] GLP-1/GcG via the GLP-1-R.
Conclusions: Ability of [DA^2]GLP-1/GcG to improve glucose homeostasis and insulin secretion in HFF mice suggests significant promise for novel GLP-1/glucagon hybrid peptides as therapies in obesity-diabetes.
Supported by: DRWF

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Apical sodium-dependent bile acid transport inhibitors (ASBTi) exhibit potent antidiabetic activity in ZDF rats
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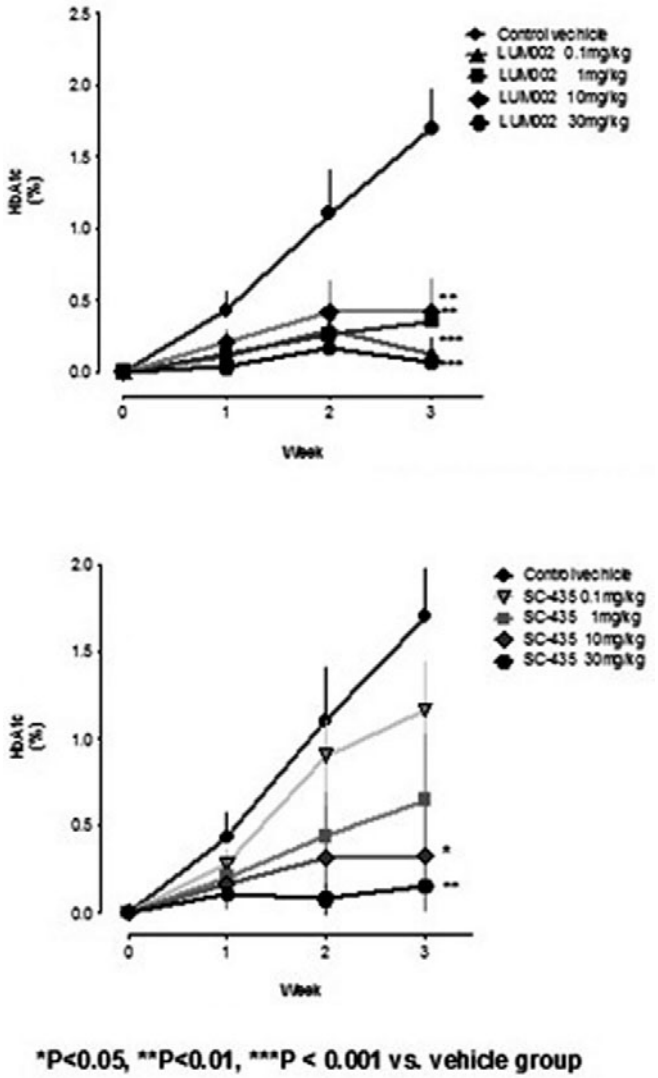
Background and aims: Bile acids (BAs) are recognized as important regulators of metabolism. Free BAs in the distal gut bind TGR5 and stimulate secretion of GLP-1 and other active peptides from enteroendocrine L-cells. One approach to increase free BAs in the distal gut is to block BA recycling with an ASBT inhibitor. In the present study, we used the Zucker Diabetic Fatty (ZDF) rat model of Type 2 diabetes to test the hypothesis that ASBTi treatment will

increase BAs in the large intestine, stimulate GLP-1 release and elicit an anti-diabetic response as measured by markers of glucose metabolism.

Materials and methods: Two structurally distinct, minimally-absorbed ASBTi's, LUM002 and SC-435 (in vitro IC50 =0.6 nm and 12.2 nm respectively), were administered to 8-wk old ZDF rats by twice daily oral gavage at doses of 0, 0.1, 1, 10 and 30 mg/kg. The rats (n=3-8/group) were initially randomized by plasma HbA1c and glucose values. Feces were collected (24 hr) on day 10 for fecal BA analysis and weekly blood samples were drawn for analysis of plasma markers. At 3 wks, OGTTs were performed on fasted animals in the 0, 0.1 and 10 mg/kg groups.

Results: Oral dosing of LUM002 and SC-435 increased 24hr fecal BA concentrations up to 4-fold (P<0.01) and decreased total plasma BAs by 30-60% (P<0.05) across all doses compared to the vehicle group. Steady-state non-fasting plasma total GLP-1 was increased up to 50% by LUM002 (P<0.05); SC-435 showed a trend for increases in GLP-1 that did not attain statistical significance. During the 3-wk study, vehicle-treated rats developed progressive hyperglycemia with increases in both plasma glucose (55.6±5.03%) and HbA1c (1.70±0.27%). Treatment with both ASBTi's caused dose-dependent reductions in plasma glucose up to 57.6% (P<0.01). The compounds also significantly attenuated the increase in percent HbA1c observed in the vehicle animals (see figure). Insulin secretion stimulated during OGTT was enhanced 3- and 2-fold with LUM002 and SC-435, respectively (P<0.05 for both) and resulted in up to 57% reduction in glucose excursion vs. vehicle when calculated as AUC. (P<0.05 for 0.1 and 10 mg/kg doses of LUM002; P<0.05 for 10 mg/kg SC-435.) Both in vitro and in vivo data suggest that LUM002 is about 10-fold more potent than SC-435.

Conclusion: Our results confirm that two distinct structural classes of ASBT's increase BA flux into the distal gut, promote GLP-1 release and improve glycemic control in ZDF rats. Thus, ASBTi may offer a new therapeutic approach for treating diabetes/metabolic disease.



1000

HM11260C, a new generation long acting GLP-1R agonist with a unique pharmacokinetic profile improves glucose control and GI tolerability; a phase IIa clinical trial in type 2 diabetes mellitus

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Background and aims: A very long T1/2(~ 180 hrs) and no burst absorption (Tmax:: ~144 hrs) of HM11260C, a novel long acting GLP-1R agonist, was confirmed in previous single ascending dose study in T2DM. The aim of study is to investigate safety, tolerability, PK and PD of Langlenatide when treated with multiple doses.

Materials and methods: Patients with T2DM who participate in this double-blinded, randomized, placebo controlled trial are on stable Metformin monotherapy at study start. Subjects were randomized 3:1 to HM11260C or placebo injections. HM11260C was administered subcutaneously over an 8-week for weekly regimens or a 9-week for monthly regimens. PK was assessed up to 35 days after the last dose. PD endpoints include the markers of metabolic control, and changes in body weight (BW). Tolerability and safety are determined with AEs, vital signs, laboratory and ECG.

Results: Data through Day 57 from 48 patients in W1 (1 mg/wk), W2 (2 mg/wk), W3 (4 mg/wk), M1 (8 mg/mo) and M2 (12 mg/mo) are reported. Key demographics were (active vs placebo; mean [SD]): age, 53.3 [7.0] vs 52.9 [8.7] years; HbA1c, 8.4 [1.0] vs 8.1 [0.9] %. At Day 57, patients treated with weekly regimens or monthly regimens experienced clinical significant improvements from baseline HbA1c, fasting plasma glucose, body weight compared with placebo. Most common AEs were nausea, vomiting and diarrhea and were mild or moderate. Weekly regimen showed fewer GI AEs and most events were reported after first injection. No notable safety signal was apparent for laboratory parameters, vital sign or ECG. At weekly cohorts, absorption rate was indicated by a T_{max} of 109 ~ 128 hrs and elimination rate was indicated by a $T_{1/2}$ of 153~ 156 hrs. The accumulation ratio (around 3.5) was consistent with elimination kinetics and did not show relevant differences between the cohorts. The peak-to-trough ratio was 1.4. At M1 cohort, the T_{max} was 129 hrs and the $T_{1/2}$ was 162 hrs. The accumulation ratio was 1.1 and the peak-to-trough ratio was 10.4.

Conclusion: From this study, with the demonstrated unique pharmacokinetic profiles, HM11260C shows meaningful improvements in blood glucose control and good tolerability after repeated treatment in all weekly and monthly cohorts. Further development of HM11260C is warranted to explore its full potential as mono and combination therapy in patients with T2DM.

	Weekly Regimens (Day57)				Monthly Regimens (Day57)		
	Weekly Placebo	W1 Cohort	W2 Cohort	W3 Cohort	Monthly Placebo	M1 Cohort	M2 Cohort
ΔHbA1c (%, LS mean [p-value])	-0.09	-0.92 (p=.021)	-1.02 (p=.008)	-1.24 (p=.002)	-0.22	-1.32 (p=.006)	-1.05 (p=.018)
ΔFPG (mg/dL, LS mean [p-value])	1.63	-36.16 (p=.036)	-26.43 (p=.103)	-65.73 (p<.001)	-1.18	-23.92 (p=.322)	-8.05 (p=.742)
ΔBody weight (kg, LS mean [p-value])	0.27	-1.16 (p=.275)	-1.29 (p=.206)	-2.42 (p=.030)	-0.79	-0.18 (p=.638)	0.29 (p=.376)
Nausea # Patient (%) [#AEs]	3 (33%) [3]	0	1 (11%) [1]	2 (22%) [2]	2 (29%) [5]	5 (56%) [10]	4 (44%) [7]
Vomiting # Patient (%) [#AEs]	1 (11%) [1]	0	1 (11%) [1]	1 (11%) [1]	2 (29%) [4]	2 (22%) [4]	1 (11%) [3]
Diarrhea # Patient (%) [#AEs]	1 (11%) [1]	2 (22%) [2]	0	1 (11%) [1]	1 (14%) [1]	2 (22%) [2]	1 (11%) [1]

Clinical Trial Registration Number: NCT01452451
Supported by: Korea Drug Development Fund R&D Project (KDDF-201204-03)

1001

Sitagliptin improves blood glucose control by suppressing glucagon secretion in insulin-treated type 2 diabetic patients

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Background and aims: Amelioration of blood glucose control by DPP-4 inhibitors is reportedly accompanied by increased insulin release and suppressed glucagon secretion in drug naïve diabetic patients and patients treated with oral hypoglycemic agents. DPP-4 inhibitors are also demonstrated to improve blood glucose control in patients supplemented with insulin by multiple daily insulin injection. However, islet hormone changes in response

to DPP-4 inhibitors and thus glucose lowering mechanisms of DPP-4 inhibitors are not fully examined in insulin-treated patients.
Materials and methods: Sixteen patients with type 2 diabetes (age: 61.4 ± 10.9 years old, duration of diabetes 18.7 ± 8.1 years, BMI 23.8 ± 3.2 kg/m^2, HbA1c:8.35 ± 0.74%) treated with multiple daily injections of insulin (total daily dose of insulin: 33.8 ± 12.2 units) without any hypoglycemic reagents were randomly assigned to 750 mg metformin or 50 mg sitagliptin addition. Insulin dose would be fixed unless hypoglycemia occurred and other drugs, such as antihypertensive and antilipidemic agents, were not changes throughout the study. At 0, 4, 8 and 12 weeks of the study, patients were subjected to meal (450 Kcal, 17.2 g protein, 16.6 g fat, 57.6 g carbohydrate)-tolerance tests (MTT). Blood glucose, C-peptide and glucagon levels were determined at 0,15, 30, 60, 90 and 120 min after the meal loads.
Results: Dose of insulin has not changed since there was no hypoglycemia event during the study. Both sitagliptin and metformin reduced HbA1c levels after the 12-week treatment (sitagliptin: -0.81% (95% CI -0.35 ~ -1.28%), metformin: -0.86% (-0.40 ~ -1.33), P < 0.001 for both). After 12 weeks, fasting glucose, C-peptide and glucagon levels were unaltered in both groups. Glucose excursion during MTT was reduced in both groups: AUC of plasma glucose was lowered from 620.8 mg*h/dl to 491.0 for sitagliptin (P < 0.05) and 497.4 to 446.0 for metformin (P < 0.05). C-peptide responses were unaltered by sitagliptin addition but slightly increased by 12.9% (P = 0.035) in insulin-treated patients with additional metformin. AUC for glucagon responses were reduced by -15.34% (P <0.001) after 12 weeks in the sitagliptin group but showed minimal changes in the metformin group (-6.3%, P = 0.07). When data from patients of the two groups were combined, analysis of covariance revealed that suppression of glucagon secretion was a determinant of reduced HbA1c levels. There were no changes in body weight in both groups.
Conclusion: Our findings indicate that sitagliptin and metformin achieve blood glucose control though different effects on islet function in type 2 diabetes patients treated with multiple daily insulin injections: sitagliptin suppresses glucagon secretion while metformin exerts minimal effects on islet function. Our data also suggest that reduction of glucagon secretion is one of factors contributing plasma glucose control in insulin-treated patients, highlighting glucagon secretion as a therapeutic target of diabetes mellitus.
Clinical Trial Registration Number: UMIN000008155

1002

Exenatide suspension, a novel exenatide formulation, improves insulin sensitivity 28 days after a single injection in diabetic fatty Zucker rats
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Background and aims: Extended release (once-weekly) exenatide (EQW) is approved for the treatment of T2DM. Exenatide suspension uses the same extended-release microspheres as EQW but is reformulated with a medium chain triglyceride vehicle with a history of safe use in humans. This formulation was developed to 1) yield a presuspended product not requiring reconstitution of microspheres just prior to injection as with EQW, 2) be compatible with an easier-to-use pen device. The present study assessed the pharmacodynamic effects of a single SC injection of exenatide suspension (ExS) in diabetic fatty Zucker (ZDF) rats, a model of type 2 diabetes.
Materials and methods: 10-week-old male ZDF rats with matched initial HbA1c values (n=8-9/group) received a single SC injection of ExS (0, 0.003, 0.03, 0.3, 1 and 3 mg/rat) or 1mg/rat EQW. Food intake was measured daily; body weight, HbA1c and PK exposure were measured on days 0, 10, 20 and 28; overnight fasting glucose on days 0, 10 and 28; glomerular filtration rate, creatinine and glucose excretion on day 20; insulin sensitivity, assessed as the glucose infusion rate required to maintain euglycemia during a hyperinsulinemic-euglycemic clamp, and glucose-stimulated β-cell secretion, assessed by changes in plasma C-peptide after an IVGTT, on day 28.
Results: Single injections of ExS evoked dose-dependent reductions in total food intake (756±40, 757±74, 733±49, 667±61, 578±34, 534±36, 526±39 g; *P*<0.0001 ANOVA) and weight gain (101±16, 94±23, 92±13, 68±15, 57±9, 49±9, 50±13 g; *P*<0.0001 ANOVA) for up to 28 days after administration when compared to vehicle controls. The effect of ExS treatment to improve glycemic endpoints was dose-dependent, despite very slight progression of diabetes in most rats, but did not achieve significance in this study. Glomerular filtration rate, plasma creatinine, urine volume or urine creatinine remained unchanged from controls with long term exposure to ExS. Plasma exenatide concentrations were sustained for at least 28 days (1.4±0.7, 42±29, 290±182, 1099±327, 3993±1278, 1787±486 pM) and increased with time in all but the 0.003 mg dose. During the euglycemic-hyperinsulinemic clamp, dose-dependent and significant increases in insulin sensitivity were observed with ExS at 0.3 mg and above (glucose infusion rate required to maintain euglycemia over 60-180 min: 7.5±0.8, 7.6±2.4, 7.4±1.8, 9.9±1.5, 10.4±1.8, 11.4±1.6, 11.7±1.2 mg/kg/min; *P*<0.0001 ANOVA). ExS at doses of 0.03 mg and above significantly decreased C-peptide responses compared to controls (485±196, 369±244, 263±164, 73±87, 4.6±3.9, 5.1±8.9, 4.9±5.3 pM; *P*<0.001 ANOVA), reflecting a decrease in insulin demand due to improved insulin action. Importantly, these changes were comparable to the responses observed with EQW treatment in this model.
Conclusion: The significant metabolic improvements seen in diabetic ZDF rats with exenatide suspension further elucidate the mechanism of action and prolonged duration of action of this novel exenatide formulation. Together with the beneficial glycemic actions observed with once-weekly injection of ExS in a clinical trial, these data lend further support to the ongoing development of exenatide suspension for the treatment of Type 2 diabetes, administered as both weekly and monthly regimens.

1003

Dose-finding results in an adaptive trial of dulaglutide combined with metformin in type 2 diabetes (AWARD-5)
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Background and aims: The AWARD-5 study was an adaptive, double-blind, placebo (PL)- and sitagliptin (sita)-controlled Phase 3 trial with dose-finding, efficacy, and safety objectives to assess dulaglutide (DU), a long-acting glucagon-like peptide-1 receptor agonist, in type 2 diabetes mellitus (T2DM). We present the dose-finding results from this study that selected doses for the DU clinical development plan.
Materials and methods: Patients with T2DM (HbA_{1c} ≥7.0% to ≤9.5%), treated with diet and exercise only, oral antihyperglycaemic medication (OAM) monotherapy, or metformin (MET) combination with OAM, were randomised 1:1:3 to PL, sita 100 milligrams (mg) once daily, or 1 of 7 once-weekly DU doses (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 mg) after stabilisation on MET monotherapy. A Bayesian algorithm was used to adaptively randomise patients to the DU doses based on accumulated data (updated bi-weekly) on HbA_{1c}, weight, pulse rate, and diastolic blood pressure. Predicted outcomes of these measures relative to the comparators (predicted HbA_{1c} at 52 weeks relative to sitagliptin and predicted outcomes at 26 weeks relative to placebo for the other 3 measures) were combined into a single metric, a clinical utility index (CUI), to assess relative benefit/risk for each DU dose. When sufficient data accumulated, DU dose selection would occur or the trial would terminate, based on prespecified decision rules. Dose selection would occur if the maximum CUI dose provided clinically meaningful benefit. This dose would then continue in the study and the program. One additional, lower DU dose would also be selected if it had an acceptable CUI and was ≤50% of the maximum CUI dose (to ensure pharmacokinetic separation). If dose selection occurred, patients randomised to the selected DU doses or the comparator arms would continue seamlessly on treatment; randomisation to the selected DU doses and the comparator arms would continue until the planned total sample size was reached.
Results: Of 1202 randomised patients, the initial 230 participated in dose-finding. A Data Monitoring Committee discontinued DU 3.0 mg before dose selection due to concerns about increased incidence of gastrointestinal adverse events and elevated pulse rate. The DU 1.5 mg (maximum CUI) and 0.75 mg doses were selected (Table 1).
Conclusion: The adaptive algorithm facilitated inclusion of a large number of doses during dose-finding, efficiently allocated patients to doses of greater clinical value, and exposed fewer patients to less-desirable doses for the minimum time necessary to make a dose decision. DU 1.5 mg was selected as the optimal dose and DU 0.75 mg was selected as the lower dose to be continued for the purposes of confirmation of long-term safety and efficacy of dulaglutide in AWARD-5 and the entire Phase 3 program.

Table 1. Model-Predicted Adjusted Mean (95% CI) Changes from Baseline and Mean Utility Values

Treatment	HbA_{1c} (%) 52-week sitagliptin-adjusted	Weight (kg) 26-week placebo-adjusted	Pulse Rate (beats/min) 26-week placebo-adjusted	Diastolic Blood Pressure (mmHg) 26-week placebo-adjusted	Mean utility (95% credible interval)
0.25 mg dulaglutide	-0.12 (-0.38; 0.20), n=13	-0.49 (-1.36; 0.44), n=16	-1.16 (-4.40; 1.90), n=16	0.35 (-2.60; 3.60), n=16	0.733 (0, 1.639)
0.50 mg dulaglutide	-0.24 (-0.44; 0.02), n=16	-0.97 (-1.80; -0.12), n=22	-1.32 (-4.30; 1.80), n=22	0.11 (-2.40; 3.20), n=22	1.362 (0, 2.326)
0.75 mg dulaglutide	-0.23 (-0.44; 0.08), n=20	-0.44 (-1.16; 0.44), n=20	-1.80 (-5.50; 2.10), n=20	-0.27 (-3.00; 2.50), n=20	1.054 (0.090, 1.923)
1.00 mg dulaglutide	-0.30 (-0.58; 0.08), n=7	-1.53 (-2.44; -0.60), n=9	-0.71 (-4.00; 2.50), n=9	-0.55 (-3.90; 2.20), n=9	2.021 (0.415, 3.267)
1.50 mg dulaglutide	-0.63 (-0.98; -0.20), n=18	-1.67 (-2.48; -0.92), n=22	-0.07 (-3.20; 2.70), n=22	-0.62 (-3.40; 2.30), n=22	3.052 (0.721, 4.037)
2.00 mg dulaglutide	-0.58 (-0.76; -0.32), n=23	-1.99 (-2.88; -1.20), n=29	0.78 (-2.10; 3.80), n=29	-0.41 (-2.60; 3.20), n=29	2.996 (0, 3.702)
3.00 mg dulaglutide	-0.29 (-0.62; -0.02), n=9	-3.93 (-4.84; -2.88), n=13	2.98 (-0.60; 8.70), n=13	-0.19 (-2.70; 2.80), n=13	Not Applicable

n = number of patients with at least one post-baseline measurement in the specified category

Clinical Trial Registration Number: NCT00734474
Supported by: Eli Lilly and Company

1004

Consistent reduction of postprandial glucagon and insulin by lixisenatide in the GetGoal clinical trial programme

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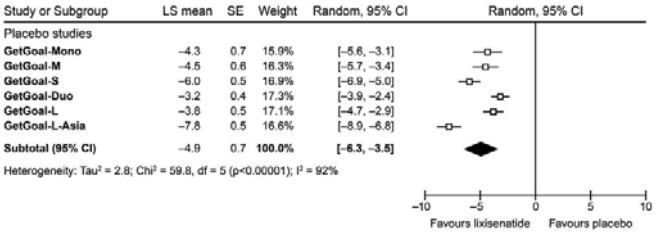
Background and aims: Glucagon-like peptide-1 (GLP-1) receptor agonists improve islet function and delay gastric emptying in subjects with type 2 diabetes mellitus. We evaluated 2-hour glucose, glucagon and insulin changes following a standardized mixed-meal tolerance test before and after 24 weeks of treatment with the once-daily prandial GLP-1 receptor agonist lixisenatide (approved for a therapeutic dose of 20 µg once daily) in six randomized, placebo-controlled studies within the lixisenatide Phase III GetGoal programme. In the studies, the mixed-meal test was conducted before and after: (1) lixisenatide treatment in patients insufficiently controlled despite diet and exercise (GetGoal-Mono), (2) lixisenatide treatment in combination with oral antidiabetic drugs (OADs) (GetGoal-M and GetGoal-S), or (3) lixisenatide treatment in combination with basal insulin ± OAD (GetGoal-Duo 1, GetGoal-L and GetGoal-L-Asia).
Materials and methods: A meta-analysis was performed (lixisenatide n=1124 vs placebo n=707) combining ANCOVA least squares (LS) mean values using an inverse variance weighted analysis.
Results: Lixisenatide significantly reduced 2-hour postprandial glucose from baseline (LS mean difference vs placebo: -4.9 mmol/L, p<0.0001, Figure) and glucose excursions (LS mean difference vs placebo: -4.5 mmol/L, p<0.0001). As measured in two studies, lixisenatide also reduced postprandial glucagon (LS mean difference vs placebo: -19.0 ng/L, p<0.0001) and insulin (LS mean difference vs placebo: -64.8 pmol/L, p<0.0001), although the glucagon/insulin ratio was increased (LS mean difference vs placebo: 0.15, p=0.02) compared with placebo.
Conclusion: The results show that lixisenatide potently reduces the glucose excursion after meal ingestion in subjects with type 2 diabetes, in association with marked reductions in glucagon and insulin levels. It is suggested that diminished glucagon secretion and slower gastric emptying contribute to reduced hepatic glucose production and delayed glucose absorption, enabling postprandial glycaemia to be controlled with less demand on beta-cell insulin secretion.

Figure. Meta-analysis* of standardized meal test: 2-hour postprandial glucose



*Lixisenatide (recently granted marketing authorization in Europe for the treatment of type 2 diabetes mellitus) versus placebo

Clinical Trial Registration Number: NCT00688701; NCT00712673; NCT00713830; NCT00975286; NCT00715624; NCT00866658
Supported by: Sanofi

1005

Once-daily lixisenatide in combination with basal insulin ± OADs in patients with type 2 diabetes selectively reduces postprandial hyperglycaemic daytime exposure

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Background and aims: Basal insulin reduces basal hyperglycaemia (BHG), but HbA_{1c} may remain high due to persisting postprandial hyperglycaemia (PPHG). Lixisenatide is a new once-daily prandial glucagon-like peptide-1 (GLP-1) receptor agonist, which was granted marketing authorization in Europe in February 2013 for the treatment of Type 2 diabetes mellitus (T2DM). Lixisenatide has been demonstrated to reduce PPHG and HbA_{1c} with no weight gain, or with weight loss, and thus has properties that are complementary to those of basal insulin.
Materials and methods: Patient-level data were pooled from three randomized Phase III studies to evaluate once-daily lixisenatide + standard of care (SOC; basal insulin ± oral agents) versus placebo + SOC, to quantify the effects of lixisenatide on BHG and PPHG exposures. BHG (24-hr area above 5.6 mmol/L and under the fasting level) and incremental PPHG (area above fasting and under self-monitored 7-point plasma glucose profiles [AUC_{24h}]) exposures were calculated.
Results: The 753 eligible patients with evaluable profile data had a mean age of 57 years, BMI of 29.8 kg/m², diabetes duration of 11.5 years, HbA_{1c} of 8.2% and a fasting glucose of 7.5 mmol/L. At baseline, mean daytime BHG and PPHG exposures were 49.2 and 55.1 mmol/L/hr, respectively. Mean BHG and PPHG contributions to hyperglycaemia were 42% and 58%, respectively. After 24 weeks of treatment, adjusted least squares (LS) mean HbA_{1c} change in the lixisenatide + SOC group was -0.8% compared with -0.3% in the placebo + SOC group (p<0.0001). The BHG exposure values for lixisenatide + SOC and placebo + SOC were similar (adjusted LS mean change for AUC_{24h} -13 vs -11 mmol/L/hr [NS]), but PPHG exposure was reduced more with lixisenatide + SOC compared with placebo + SOC (adjusted LS mean change -21 vs -10 mmol/L/hr, p<0.0001). The mean BHG and PPHG contributions to hyperglycaemia were 46% vs 54% with lixisenatide + SOC and 39% vs 61% with placebo + SOC, respectively.
Conclusion: This analysis suggests that once-daily lixisenatide complements the effects of basal insulin on glycaemic control in T2DM, decreasing HbA_{1c} mainly by reducing PPHG.
Supported by: Sanofi

1006

Liraglutide demonstrates similar pharmacokinetic properties in patients with type 1 and type 2 diabetes

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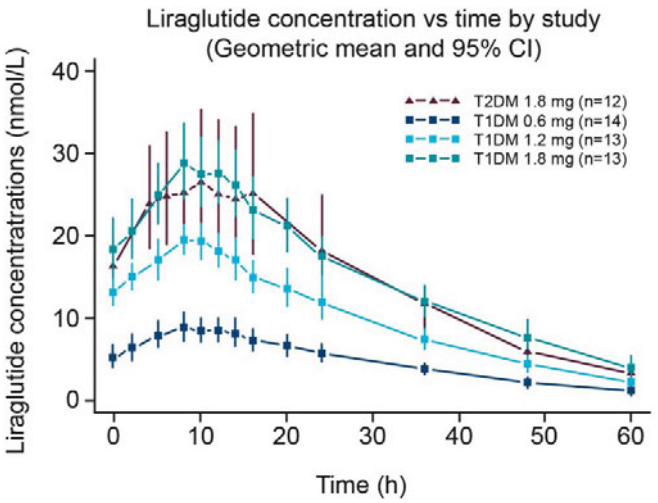
Background and aims: Liraglutide is a well established treatment in patients with type 2 diabetes (T2D) and is currently under investigation as a treatment option in patients with type 1 diabetes (T1D) as adjunct to insulin. The aim of the following pharmacokinetic (PK) analysis was to compare PK properties of liraglutide in T1D and T2D.
Materials and methods: We assessed the PK properties of liraglutide in a randomised, placebo controlled, double blind, crossover trial investigating effects of liraglutide as adjunct to insulin on counter regulatory responses to hypoglycaemia in T1D. 45 adults with T1D were allocated to 1 of 3 dose groups of liraglutide (0.6, 1.2 or 1.8 mg/day) or placebo for 4 weeks in a cross-over design with a washout period of 2-3 weeks. Steady state PK profiles over 60 hours were obtained at the end of each treatment period. The PK analysis was conducted via both noncompartmental and population PK analyses. Data from this trial were compared with PK data from a randomised, double blind, crossover trial in subjects with T2D evaluating the effect of 1.8 mg liraglutide/placebo on postprandial lipid response. The liraglutide concentration-time profile was adequately described by a linear one compartment model with first order absorption and elimination parameterised with apparent clearance (CL/F), apparent volume of distribution (V/F) and absorption rate constant (K_A). Effects of body weight and sex on CL/F and body weight on V/F were estimated to account for body weight and male/female ratio differences in the two trials.
Results: 52 PK profiles (T1D:40, T2D:12) were used for the analysis. Mean body weight [minimum; maximum] was 74.2 kg [55.5; 92.2] in T1D and 82.4 kg [71.6; 104.1] in T2D. The percentage of males was 65% (26 males vs 14 females) in T1D and 50% (6/6) in T2D. The exposure of 1.8 mg liraglutide was similar in T1D and T2D ($AUC_{ss,0\text{-}24h}$ = 569771(26%) pmol*h/l in T1D vs 561404(36%) pmol*h/l in T2D; C_{max} = 29751(28%) pmol/l in T1D vs 29820(39%) pmol/l in T2D [geometric mean(CV%)]). The mean PK profiles following liraglutide doses of 0.6, 1.2 and 1.8 mg in T1D and 1.8 mg in T2D are shown in figure. The population PK analysis demonstrated linear (dose independent) PK properties of liraglutide in T1D. The covariate analysis revealed comparable CL/F and V/F, and hence exposure, between T1D and T2D, with a lower between subject variability of CL/F in T1D (CV% = 21) compared to T2D (CV% = 39).
Conclusion: In conclusion, similar liraglutide PK properties were observed in T1D and T2D.

Figure: Liraglutide concentration time profiles for 0.5, 1.2 and 1.8 mg dose groups in T1D and for 1.8 mg liraglutide in T2D.



Clinical Trial Registration Number: NCT01536665
Supported by: Novo Nordisk

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Treatment with liraglutide as adjunct to insulin in type 1 diabetes; effects on counter regulatory response to hypoglycaemia: a randomised, double blind, crossover trial

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Background and aims: The counter regulatory hormone (CRH) response to hypoglycaemia is important in type 1 diabetes (T1D). This trial aimed to investigate if liraglutide as adjunct to insulin treatment blunts the counter regulatory response during hypoglycaemia in subjects with T1D compared to placebo as adjunct to insulin.
Materials and methods: 45 adults with T1D were randomised to 1 of 3 dose groups of liraglutide (0.6, 1.2 or 1.8 mg/day) and placebo as add on to insulin for 4 weeks in a placebo controlled, crossover design with a wash out period of 2-3 weeks between treatments. At the end of each treatment period, a stepwise hypoglycaemic clamp was performed via controlled i.v. insulin/glucose infusion. The successive plasma glucose (PG) levels were: 5.5, 3.5, 2.5 mmol/L (nadir) and 4.0 mmol/L (recovery). The evening before the clamp, sampling for glucagon and plasma glucose was performed (ambient hyperglycaemic PG level). During the clamp, CRHs (glucagon, adrenalin, noradrenalin, cortisol and growth hormone) and vital signs (pulse, blood pressure) were measured at each of the successive PG levels. Glucose infusion rate (GIR) was assessed, and a hypoglycaemic symptoms questionnaire and cognitive function tests were carried out.
Results: Baseline characteristics were similar between groups: age 34.5±11.2 years, BMI 23.9±2.4 kg/m², HbA_{1c} 7.6±0.8%, T1D duration 16.6±9.4 years [mean±SD]. At nadir PG, no significant differences in glucagon and no systematic differences in other CRHs were seen between treatments (see Table). However, a trend towards lower glucagon level at increasing liraglutide dose was seen at nadir PG levels. Change in glucagon from PG 5.5 mmol/L to nadir PG demonstrated little glucagon response to hypoglycaemia in all groups with no difference between treatments, consistent with the duration of T1D in the trial population. Increased concentrations of other CRHs in response to hypoglycaemia were seen with no differences in change from PG 5.5 mmol/L to nadir PG between treatments. Pulse was higher for liraglutide at nadir PG, but with similar change from PG 5.5 mmol/L to nadir PG across groups. AUC_{GIR} indicated less glucose was needed to obtain PG levels with liraglutide treatment with a similar trend during recovery from hypoglycaemia. Cognitive tests and hypoglycaemic symptoms questionnaire showed no systematic differences in responses between groups at all PG levels.

	Liraglutide 0.6 mg/ placebo	Liraglutide 1.2 mg/ placebo	Liraglutide 1.8 mg/ placebo
Glucagon (pg/mL) at nadir[a] *(primary endpoint)*	34.7/38.1 R=0.91 95% CI=[0.66;1.25]	28.8/37.2 R=0.77 95% CI=[0.56;1.08]	28.4/37.5 R=0.76 95% CI=[0.55;1.05]
Glucagon change (pg/mL) from PG 5.5 mmol/L to nadir[a] *(post hoc)*	3.9/4.1 ETD=-0.2 95% CI=[-4.0;3.6]	3.6/2.5 ETD=1.1 95% CI=[-2.8;5.1]	4.3/6.0 ETD=-1.7 95% CI=[-5.7;2.2]
Glucagon (pg/mL) at ambient[b]	40.9/49.7 R=0.82 95% CI=[0.61;1.11]	39.0/51.2 R=0.76 95% CI=[0.56;1.04]	28.8/43.4 R=0.66* 95% CI=[0.49;0.90]
Ambient[b] PG (mmol/L) (mean±SD)	14.5±6.5/ 11.4±5.4	11.4±4.1/ 13.4±5.9	12.8±5.4/ 14.5±5.0
Adrenaline (pg/mL) at nadir[a]	133.8/139.9 R=0.96 95% CI=[0.54;1.70]	100.2/61.4 R=1.63 95% CI=[0.90;2.96]	127.1/122.1 R=1.04 95% CI=[0.58;1.87]
Noradrenaline (pg/mL) at nadir[a]	151.3/141.9 R=1.07 95% CI=[0.67;1.71]	148.9/100.5 R=1.48 95% CI=[0.91;2.42]	133.3/136.7 R=0.97 95% CI=[0.60;1.58]
Cortisol (ng/mL) at nadir[a]	143.2/159.2 R=0.90 95% CI=[0.78;1.04]	174.4/148.2 R=1.18* 95% CI=[1.01;1.37]	166.3/159.8 R=1.04 95% CI=[0.90;1.21]
Growth hormone (ng/mL) at nadir[a]	9.48/11.35 R=0.84 95% CI=[0.36;1.92]	5.74/4.17 R=1.38 95% CI=[0.58;3.27]	4.09/6.68 R=0.61 95% CI=[0.26;1.44]
AUC_{GIR} (mg/kg) at nadir[a]	42.6/58.5 R=0.73* 95% CI=[0.55;0.97]	57.8/69.4 R=0.83 95% CI=[0.62;1.12]	44.0/68.3 R=0.64** 95% CI=[0.48;0.86]

Note: Data are presented as estimated means unless otherwise stated.
[a]Nadir: PG=2.5 mmol/L. [b]Ambient =glucose level the evening before clamp. *$p<0.05$; **$p<0.01$
AUC=area under the curve; CI=confidence interval; ETD=estimated treatment difference; GIR=glucose infusion rate; PG=plasma glucose; R=ratio; SD=standard deviation.

Conclusion: Counter regulatory responses to hypoglycaemia were unchanged with liraglutide as adjunct to insulin treatment in T1D compared to placebo as adjunct to insulin.
Clinical Trial Registration Number: NCT01536665
Supported by: Novo Nordisk

PS 081 Innovative treatments of type 2 diabetes

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Safety and efficacy of lobeglitazone monotherapy in patients with type 2 diabetes mellitus over 52 weeks: a randomised open-label trial
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Background: Lobeglitazone is a novel PPAR-γ agonist with substituted pyrimidine derivatives containing TZD, and is expected to improve insulin sensitivity and blood lipid profile with a lower effective dose. We recently reported that lobeglitazone 0.5 mg monotherapy improved glycemic control over 24-week in patients with type 2 diabetes mellitus (T2DM) inadequately controlled on diet and exercise. In addition, lobeglitazone treatment significantly improved lipid profiles compared to placebo. This 52-week extension study was to assess the long-term safety and efficacy of lobeglitazone.
Methods: Following a 24-week, multicenter, randomized, double-blind, parallel-group, placebo controlled study, patients entered a 28-week extension study. In the open-label extension study, patients treated with placebo were switched to lobeglitzone 0.5 mg (the group switched, Group S) while the patients who were randomly assigned to lobeglitazone 0.5 mg at baseline continued the same treatment (the group maintained, Group M) for the entire 52 weeks.
Results: Among 173 patients who were randomly assigned (a 2:1 ratio) to lobeglitazone 0.5 mg (n=115) or matching placebo (n=58), 144 patients completed the 24-week treatment period. Of them, 94 patients (Group M; 65 vs Group S; 29) entered the extension study. The mean reduction from baseline in HbA1c at the end of the base study (week 24) were - 0.53% (P<0.001) in the Group M and the benefits seen with lobeglitazone treatment were sustained during the 52-week extension period (from 7.79% to 7.3%, mean difference -0.5%, P<0.001). Also, in the Group S, HbA1c was significantly decreased to -0.53% (from 8.01% at 24 weeks to 7.48% at 52 weeks, P<0.001). In addition, lobeglitazone treatment in both groups significantly improved HDL cholesterol, triglycerides, small dense LDL cholesterol and free fatty acid. As expected, weight gain was observed in the Group M (+ 1.48 kg) over 52-weeks and in the Group S (+ 1.65 kg) during the extension period (from 24 weeks to 52 weeks). The other safety profile was good in the two groups and lobeglitazone treatment was also well tolerated.
Conclusion: Lobeglitazone 0.5 mg showed a favorable balance in the efficacy and safety profile in Korean patients with type 2 diabetes, and clinical benefits were maintained for 52 weeks.
Clinical Trial Registration Number: NCT01001611
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A novel oral dual-action amylin and calcitonin receptor agonist (UGP302) rebalances insulin and glucagon action to attenuate diabetic hyperglycaemia in Zucker diabetic fatty rats
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Background and aims: Oral delivery of the peptide hormone salmon Calcitonin (sCT) possesses weight and glucoregulatory effects in obese type 2 diabetic rats by dual-action targeting the amylin and calcitonin receptor. UGP302 is a novel peptide mimetic with an enhanced in vitro pharmacological profile to native sCT. In here, we investigated the anti-diabetic efficacy of oral UGP302 vs oral sCT in a proof of concept study using Zucker diabetic fatty (ZDF) rats, a golden standard animal model of type 2 diabetes.

Materials and methods: Male ZDF rats were treated with oral sCT or UGP302 (0.25, 0.5, 1 or 2 mg/kg) or oral vehicle twice daily for 7 weeks. Fasting and non-fasted plasma glucose and HbA1c were determined to evaluate glucose homeostasis. Furthermore, levels of pancreatic glucoregulatory hormones (insulin and glucagon) were measured to examine insulinotropic and glucagonostatic actions, respectively. Additionally, OGTT and insulin tolerance test (ITT) were performed to investigate glucose tolerance and insulin sensitivity. Finally, pancreatic insulin and glucagon content were analyzed to estimate pancreatic islet beta-cell and alpha-cell mass.
Results: Oral UGP302 attenuated diabetic hyperglycemia during the intervention period. At study end, oral UGP302 at doses of 1 mg/kg and 2 mg/kg reduced blood glucose by approx 8 mmol/l and HbA1c by 1.6% compared to vehicle ($p < 0.001$). Furthermore, oral UGP302 at doses of 1 mg/kg and 2 mg/kg improved glucose intolerance during OGTT by reducing incremental area under the curve by ~ 50% when compared to vehicle ($p < 0.001$). Additionally, at doses of 1 mg/kg and 2 mg/kg, oral UGP302 exerted insulin sensitizing effects and enhanced blood glucose disposal during ITT by approx 23% compared to vehicle ($p < 0.001$). Lastly, at study end compared to vehicle, oral UGP302 at 1 mg/kg and 2 mg/kg doses sustained hyperinsulinemia ($p < 0.01$), and ameliorated hyperglucagonemia ($p < 0.01$), in conjunction with preserved pancreatic insulin and glucagon content. In contrast, only at highest dosing regimen (2 mg/kg) did oral sCT induce a comparable anti-hyperglycemic effect, alleviation of insulin resistance and impact on pancreatic glucoregulatory hormones.
Conclusion: Oral UGP302 is a superior dual-action amylin and calcitonin receptor agonist to native salmon Calcitonin. Oral UGP302 exerts anti-hyperglycemic efficacy by rebalancing insulin and glucagon action and preserving pancreatic islet glucoregulatory hormonal mass and secretory function. These data indicate the clinical usefulness of oral UGP302 as anti-diabetic treatment.
Supported by: Danish Research Foundation

1010

Dual-action targeting the amylin and calcitonin receptor by oral salmon calcitonin improves glucose homeostasis and insulin sensitivity in an animal model of type 2 diabetes
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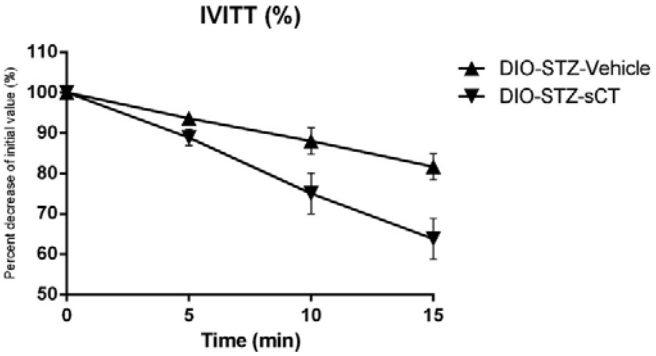
Background and aims: Oral salmon calcitonin (sCT), a dual-action amylin and calcitonin receptor agonist, has previously been demonstrated to attenuate diabetic hyperglycemia in obese diabetic rats, possibly through enhanced insulin action. The present study investigated oral sCT as anti-diabetic intervention in combined diet-induced obese and streptozotosin-type 2 diabetic animals, focusing on the effect on insulin sensitivity.
Materials and methods: Diet-induced obese (DIO) rats were given a low dose (25 mg/kg) of streptozotocin (STZ) resulting in diabetic hyperglycemia without hypoinsulinemia. The DIO-STZ rats were orally dosed with either 0.5 mg/kg salmon calcitonin (sCT) or its oral vehicle 5-CNAC once daily for 21 days. The effects of chronic treatment with sCT were evaluated with regards to fasting and non-fasting blood glucose, glycated hemoglobin (HbA1c), insulin sensitivity, glucose tolerance, and circulating glucagon levels and both circulating insulin and insulin content extracted from pancreas isolated at study end.
Results: Oral sCT improved hyperglycemia during the intervention period resulting in 5 mM reduction of PPG at study end, when compared to vehicle ($p<0.01$). During an oral glucose tolerance test, oral sCT improved glucose intolerance by reducing the area under the curve with ~35 % when compared to vehicle ($p<0.001$). Furthermore, oral sCT reduced fasting blood glucose and HbA1c at study end by ~7 mM and 1 % respectively ($p<0.05$). Furthermore sCT exerted a glucagonostatic effect. During OGTT sCT reduced the area under the curve of glucagon with ~75 % ($p<0.05$). Both the vehicle and sCT groups lost weight after injections with STZ with no significant difference between groups. Oral sCT did not exert an insulinotropic action; neither the basal fasting insulin nor insulin levels during OGTT were significantly different in the treatment group. In contrast, compared to vehicle group, oral sCT caused a 2-fold increase in glucose disappearance rate (K_{ITT}) during intravenous insulin tolerance test ($p<0.01$), indicating increased insulin sensitivity (Fig. 1). Finally there was as indication towards β-cell preservation when measuring insulin content in pancreas isolated at study end where the sCT group.
Conclusion: In conclusion, oral sCT, as interventional therapy and irrespective of inducing weight loss, improved glucose homeostasis and enhanced insulin action in diet-induced obese type 2 diabetic animals. Furthermore, oral sCT improved glucose intolerance by partly influencing gastric emptying and exerting glucagonostatic action and, importantly, by increasing insulin sensitivity. These non-insulinotropic systemic effects and preservation of insulin-producing β-cells could hold great promise for future treatment and demonstrates oral sCTas a therapeutic agent in type 2 diabetes.



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Effect of exogenous glucagon vs spontaneous recovery from hypoglycaemia in subjects with type 2 diabetes treated with a novel glucokinase activator AZD1656 and metformin
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Background and aims: We studied the effect of exogenous glucagon on recovery from insulin-induced hypoglycaemia in subjects with type 2 diabetes treated with the novel glucokinase activator AZD1656 in combination with metformin.
Materials and methods: Single centre randomized, open, two-way cross-over phase I, automated (Biostator®) glucose clamp study (NCT00817271) of 8 subjects (7 men and 1 woman, mean age 58.6 years, body mass index 28.1 kg/m^2). A 2-day titration phase commenced with 40 mg AZD1656 bid escalating to 80 mg bid if tolerated. Three subjects received 40 mg AZD1656 bid and 5 subjects 80 mg bid for 4 days, followed by a single dose of 80 mg or 160 mg AZD1656 respectively on day 5 and day 8. All subjects received a stable dose of metformin bid ranging from 1000 to 2250 mg daily. After an overnight fast on days 5 and 8 controlled hypoglycaemia was induced using an exogenous i.v. insulin infusion. Plasma glucose was lowered in a stepwise fashion over 3 hours to attain a target nadir of 2.7 mmol/L sustained for 30 minutes, at the end of which the hypoglycaemic clamp was released. In random sequence subjects either received an intramuscular injection of 1 mg glucagon or were allowed to recover from hypoglycaemia without exogenous glucagon.
Results: Mean plasma glucose at 20 minutes after release of the hypoglycaemic clamp was 3.1 ± 0.3 v 4.9 ± 0.8 mmol/l for AZD1656 alone and AZD1656+glucagon, respectively. Plasma glucagon AUC0-6h was lower (-72%, 95% CI [-75% to -67%], $p<0.001$) for AZD1656 alone. Catecholamine and cortisol responses were similar for both treatments. Growth hormone (GH) response was lower for AZD1656 alone (-18%, 95% CI [-29% to -7%], $p=0.01$) consistent with the physiological effect of glucagon on GH secretion. No safety or tolerability concerns were observed.
Conclusion: Exogenous glucagon was effective as a rescue treatment for hypoglycaemia during treatment with AZD1656 in combination with metformin in subjects with type 2 diabetes.

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Evidence that two divergent mechanisms contribute to blood glucose lowering by imidazoline compounds in mice
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Background and aims: Evidence suggests that imidazoline derivatives can directly amplify glucose-induced insulin secretion by β-cells via at least two

mechanisms, which are closing of K_{ATP} channels and antagonism at adrenergic α_{2A}-receptors. The relative importance of these two mechanisms for the antihyperglycaemic effects *in vivo* is a matter of debate. This study was to clarify this matter for two classic imidazoline compounds, phentolamine (PHE) and idazoxan (IDA).
Materials and methods: The effects of PHE and IDA on insulin secretion of perifused islets from C57BL-mice were examined in the presence of inhibitors of insulin release which oppose the mechanisms putatively addressed by imidazolines: 250µmol/l of the K_{ATP} channel opener diazoxide (DIAZ), or 1µmol/l of the α_2-adrenoceptor agonist UK14,304 (UK). To unmask the mechanisms predominately responsible for the antihypergycaemic effect, the minimal doses of PHE and IDA needed to counteract hyperglycaemia triggered by administration of the inhibitors were compared in C57BL-mice (250mg/kg DIAZ, p.o.; 100µg/kg UK, i.p.).
Results: In isolated islets, PHE and IDA (100µmol/l) were equally strong as amplifiers of glucose-stimulated insulin release in the presence of the α_2-adrenoceptor agonist (fmol insulin released per islet during 30min of stimulation with high glucose: UK alone, 1.7±0.3; UK+PHE, 177.2±32.1; UK+IDA, 176.4±29.7; PHE vs IDA, ns), but PHE was superior in counteracting inhibition of insulin secretion by the K_{ATP} channel opener (DIAZ alone, 2.2±0.7; DIAZ+PHE, 161.2±19.1; DIAZ+IDA, 11.7±6.4; PHE vs IDA, p<0.001). Our results suggest similar effects via antagonism at the α_{2A}-adrenoceptor, but superiority of PHE via another mechanism involving closure of K_{ATP} channels. The assumption underlying the *in vivo*-experiments - that an α_{2A}-antagonist would be stronger in counteracting UK, whereas a K_{ATP} channel blocker would be superior in counteracting DIAZ - was confirmed with the non-imidazoline α_2-antagonist yohimbine and with the K_{ATP} channel-blocking sulfonylurea gliclazide. In line with the observations made *in vitro* and resembling the effects of yohimbine, a low dose of 0.1mg/kg IDA counteracted hyperglycaemia induced by the α_2-agonist but not by the K_{ATP} channel-opener (incremental $AUC_{glucose}$ in mol/l*min: UK alone, 1.60±0.06, vs UK+IDA, 1.06±0.15, p=0.007; DIAZ alone, 1.16±0.16, vs DIAZ+IDA, 1.12±0.08, ns). At variance to this, the same minimal dose of PHE was required to counteract hyperglycaemia caused by UK and DIAZ, with no effects obtained at 2mg/kg but robust effects at 4mg/kg PHE (UK alone, 1.40±0.10, vs UK+2mg/kg PHE, 1.19±0.12, ns, and vs UK+4mg/kg PHE, 0.72±0.11, p<0.001; DIAZ alone, 1.11±0.12, vs DIAZ+2mg/kg PHE, 0.99±0.09, ns, and vs DIAZ+4mg/kg PHE, 0.58±0.06, p<0.001).
Conclusion: The results indicate that α_{2A}-antagonism as exerted by IDA is sufficient for an antihyperglycaemic effect *in vivo*, whereas closure of K_{ATP}-channels makes a major contribution to glucose lowering induced by PHE. Hence, at least two divergent mechanisms account for the antihypergycaemic activities of imidazolines, whereby the relative contributions of each mechanism can markedly differ between individual imidazoline compounds.

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MTBL0036, a promising antidiabetic candidate, impacts both insulin action and insulin secretion in type 2 diabetic rats
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Background and aims: The objective of the present study was to establish the characteristics of the effect of MTBL0036, a new optimized and patented antidiabetic drug candidate, on glucose metabolism and insulin secretion in the STZ-N0 rat, a good animal model of type 2 diabetes.
Materials and methods: MTBL0036 was administered per os as a suspension in 0.5% methylcellulose 60 min before an oral glucose load (2g/kg), and control 17h-fasted animals received only the vehicle. The glycemia was measured using a glucometer (Lifescan one Touch Ultra, Lifescan, J&J company, USA)and, after the glucose load, the Areas Under the Curves (AUCs) were calculated using the GraphCalc software. The plasma concentration of insulin was measured by an immunoassay. Precision-cut liver slices from 48h-fasted STZ-N0 rats were also incubated in the presence of 5 mM L-lactate to study the effect of MTBL0036 on lactate gluconeogenesis.
Results: Administration of MTBL0036 (50 and 200 mg/kg) did not cause hypoglycemia in fasted rats but reduced in a statistically significant manner by 51 % and 64-80%, respectively, the AUCs for glucose after the oral glucose load. The administration of metformin (200mg/kg), the gold standard for the treatment of type 2 diabetic patients, reduced by 64% the AUC for glucose after the same oral glucose load. The administration of MTBL0036 (200 mg/kg) also augmented in a statistically significant manner the plasma concentration of insulin both before and after the glucose load. MTBL0036 (0.5 and 2 mM) reduced lactate gluconeogenesis in precision-cut liver slices with an IC_{50} which was less than that of metformin.
Conclusion: MTBL0036, is a very promising antidiabetic drug candidate because, unlike current antidiabetic drugs on the market, it has the potential to impact the three major defects observed in type 2 diabetes: (i) decreased peripheral glucose utilization, (ii) increased gluconeogenesis and (iii) deficit of insulin secretion.

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A one-week phase 1 trial with the DGAT1 inhibitor AZD7687: lipid handling, hormone secretion and adverse effects in the gut
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Background and aims: Inhibition of diacylglycerol acyltransferase 1 (DGAT1) has been proposed as a treatment for type 2 diabetes and obesity, and preclinical data have suggested insulin sensitization and weight loss. Proof of mechanism with regards to gut DGAT1 inhibition in man has recently been provided for the reversible, selective DGAT1 inhibitor AZD7687. This clinical study explored the tolerability and pharmacodynamic effects of different dose levels of AZD7687 during one week treatment.
Material and methods: Randomized, single-blind, placebo-controlled study in 62 overweight/obese male subjects. AZD7687 (1, 2.5, 5, 10, 20 mg per day n=6 or 12 for each) or placebo (n=20) was administered during 8 days in total. Plasma AZD7687 exposure was measured repeatedly. Postprandial serum triacylglycerol (TAG) excursion was measured during 8 hours after a standardized mixed meal with fat energy content of 45%, before and during treatment, to assess effects on gut DGAT1 activity. Gut hormone profiles (GLP-1, PYY) and paracetamol challenge to assess gastric emptying were performed.
Results: A consistent dose-dependent reduction of post-prandial TAG excursion was demonstrated with AZD7687 at doses of ≥5 mg as compared to placebo (p<0.01) indicating inhibition of gut DGAT1. Significant increases (p<0.05) of plasma GLP-1 and plasma PYY were seen at these dose levels, but no significant sustained effect on gastric emptying was demonstrated. There were no consistent effects on plasma glucose, insulin or lipids. Marked gastrointestinaI side effects, mainly diarrhoea, occurred at drug dose levels that inhibited TAG excursions by ≥50% (i.e. doses ≥5 mg per day). At doses higher than 5 mg, 11/18 subjects discontinued treatment due to diarrhoea. Exploratory data suggest that increased free fatty acid exposure and inflammation in the gut may contribute to the GI side effects.
Conclusion: Effects on lipid handling and hormone secretion in the gut were demonstrated during one-week treatment of overweight/obese men with the DGAT1 inhibitor AZD7687. However, frequent gastrointestinal side effects occurred and were dose-limiting. The utility of DGAT1 inhibition as a novel treatment modality in diabetes and obesity can be questioned due to lack of therapeutic window.
Clinical Trial Registration Number: NCT01119352

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Metabolic effects and safety of two selective 11β-HSD1 inhibitors (RO5093151 (RO151) and RO5027838 (RO838) in metformin-treated patients with type 2 diabetes
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Background and aims: The metabolic effects of two selective 11β-HSD1 inhibitors (RO151 and RO838) were assessed in a randomised, controlled study in patients with type 2 diabetes.
Materials and methods: Patients either received placebo (PBO) (n=21), RO151 5mg BID (n=24) or 200mg BID (n=20), or RO838 50 mg QD (n=21) or 200mg QD (n=24) for 28 days plus a stable dose of metformin. Metabolic assessments (including oral glucose tolerance and/or standardized meal tests) and safety assessments (including ACTH stimulation test) were done at baseline and around 14 and 28 days of treatment.
Results: Key demographics at baseline were similar between groups (mean ranges: age 53-57 yrs, BMI 32-33 kg/m², diabetes duration 6.5-9.4 yrs, FPG

9.4 - 9.7 mmol/l). Despite the short treatment duration, both RO151 and RO838 showed trends for improved HbA_{1c}. Improvements in several insulin sensitivity parameters were seen with 200 mg RO151 but were not significant. While there was a slight decrease in body weight with PBO (-0.28 kg), greater reductions (-0.86 to -1.67 kg) occurred in all active treatment groups reaching statistical significance vs.PBO in the RO151 200mg BID group only (LS mean -1.39 kg, 95% CI -2.54, -0.23 kg; p=0.019). Lipid parameters did not consistently improve with either compound; mild increases in triglycerides and VLDL cholesterol (vs. PBO) with RO838 were not significant. Both compounds were well tolerated. Slight non-significant changes in systolic blood pressure were seen with 5 mg RO151 (decreases in day, night and 24h values) and 200 mg RO838 (increases in semi-supine values which were not repeated in the 24 hour ABPM). Increased concentrations of ACTH and adrenal androgen precursors were found with RO151, but not with RO838.
Conclusion: Modest metabolic improvements were seen, particularly with the higher dose of RO151. Longer studies are needed to investigate potential benefits and risks of these compounds.

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Pharmacological characterisation of 55P0251, a derivative from a novel class of molecules with distinct antihyperglycaemic activity in rodents
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Background and aims: In an effort to develop plant-derived molecules, we discovered a whole class of novel compounds with distinct anti-hyperglycaemic activity. An extensive derivatisation program was governed by OGTTs in orally treated mice, which ascertained that anti-hypergylcaemic activity was accompanied by reasonable oral bioavailability and absence of severe acute toxicity. We here report pharmacological characteristics of one selected derivative, 55P0251.
Materials and methods: Development of new derivatives and analysis of structure-activity relationship (SAR) were guided by a standard OGTT (3g/kg) in healthy male C57BL/6J-mice performed 45min after oral administration of the respective compound. Further procedures used for pharmacological characterisation are addressed in the results section.
Results: In the standard OGTT in mice, 55P0251 had a minimal effective dose of 0.7mg/kg and an ED_{50} of 1.6mg/kg (e.g. total AUC in mol/l*min: vehicle, 1.58±0.06; 9mg/kg 55P0251: 1.02±0.05, p<0.0001). An intravenous glucose tolerance test in rats (1g/kg) revealed that improvement of glucose tolerance by 55P0251 (total AUC in mol/l*min: vehicle, 1.25±0.04; 22.5mg/kg, 1.06±0.07, p=0.04; 90mg/kg, 0.87±0.03, p<0.0001) was related to elevated plasma insulin (AUC in nmol/l*min: vehicle, 15±2; 22.5mg/kg, 31±3, p=0.001; 90mg/kg, 44±5, p<0.0001). A parallel increase in circulating C-peptide indicated that this was due to higher insulin release (AUC in nmol/l*min: vehicle, 33±6; 90mg/kg, 180±22, p<0.0001). 55P0251 did not bind to the sulfonyurea receptor and when directly compared to gliclazide in the standard OGTT in mice, it exhibited an attractive ratio of hypoglycemic activity (change in basal glucose after 45min, mmol/l: vehicle, +0.75±0.21; 8mg/kg gliclazide, -2.00±0.24; 10mg/kg 55P0251, +0.27±0.37; gliclazide vs P550251, p=0.0002) to anti-hyperglycaemic activity (incremental AUC, mmol/l*min: vehicle, 373±39; gliclazide, 229±47; 55P0251, -16±45; gliclazide vs 55P0251, p=0.002), suggesting a favourable therapeutic profile over sulfonylurea drugs. Although glucose-dependent amplification of insulin release is reminiscent of drugs that interfere with the GLP-1 system, we neither obtained evidence for 55P0251-induced activation of the GLP-1 receptor (no signal transduction in vitro), nor for inhibition of dipeptidyl peptidase-4 (no direct effect on enzyme, no loss of activity with parenteral glucose administration). Pharmacokinetic attributes (ADME) were examined in orally and intravenously dosed rats. After oral administration of 22.5mg/kg, 55P0251 (given as hydrochloride salt) had a plasma half-life ($t_{1/2}$) of 3.2h, a bioavailability (F) of 58%, and a peak plasma concentration (c_{max}) of 4.3µmol/l reached after 2h (t_{max}).
Conclusion: 55P0251, which belongs to a newly discovered class of molecules, has potent anti-hyperglycaemic action in rodents. While 55P0251 seems to act via amplification of glucose-stimulated insulin release, its molecular mechanism of action obviously differs from those of drugs in clinical use. The compound has attractive pharmacokinetic properties and shows less hypoglycaemic activity than a sulfonylurea.
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The effect of diiodothyronine mimetic on insulin sensitivity in male cardiometabolic patients: a double-blind randomised trial
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Background and aims: Obesity and its associated cardiometabolic co-morbidities are increasing worldwide. Since thyroid hormone mimetics are capable of uncoupling the beneficial metabolic effects of thyroid hormones from their deleterious effects on heart, bone and muscle, this class of drug is considered as adjacent therapeutics to weight-lowering strategies. This study investigated the safety and efficacy of TRC150094, a thyroid hormone mimetic.
Materials and methods: This 4-week, randomised, placebo-controlled, double-blind trial was conducted in India and The Netherlands. Forty subjects were randomised at a 1:1 ratio to receive either TRC150094 dosed at 50 mg or placebo once daily for 4 weeks. Hyperinsulinaemic euglycaaemic clamp and ^{1}H-Magnetic Resonance Spectroscopy were performed before and after treatment.
Results: At baseline, subjects were characterised by markedly impaired hepatic and peripheral insulin sensitivity, compared to reference values observed in healthy, non-obese control subjects. TRC150094 dosed 50mg once daily was safe and well tolerated. Hepatic nor peripheral insulin sensitivity did not improve after TRC150094 treatment, expressed as the suppression of Endogenous Glucose Production from 59.5 to 62.1%; p = 0.477, and as the rate of glucose disappearance from 28.8 to 26.4 $\mu mo/kg^{-1}min^{-1}$, p = 0.185. Although T2's mechanism of action is expected to stimulate lipolysis and fatty acid oxidation, TRC150094 administration did not result in differences in fasting plasma free fatty acids from 0.51 to 0.51 mmol/L, p= 0.887 or in insulin-mediated suppression of lipolysis from 57 to 54%, p = 0.102. Also, intrahepatic triglyceride content was unaltered. To evaluate whether the response differed between subjects with mild and severe metabolic derangement, we analyzed the subjects with a mean triglyceride level above 1.64 mmol/L, however, the small subgroup did not reach statistical significance.
Conclusion: Collectively, these data show that, in contrast to the potent metabolic effects in experimental models, TRC150094 at a dose of 50mg daily does not improve the metabolic homeostasis in subjects at an increased cardiometabolic risk. Further studies are needed to evaluate whether TRC150094 has beneficial effects in patients with more severe metabolic derangement, such as overt diabetes mellitus and hypertriglyceridaemia.
Clinical Trial Registration Number: NCT01408667
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PS 082 Treatment intensification in type 2 diabetes

1018

How many OADS before initiating insulin? A retrospective study of outcomes associated with initiation of basal insulin after failing oral antidiabetic drugs

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Background and aims: Current ADA/EASD guidelines recommend timely insulin initiation among patients with type 2 diabetes mellitus (T2DM) after failing oral antidiabetic drugs (OADs). However, research on the association between timing of insulin initiation and health outcomes in a real-world setting is limited. This study assessed clinical, utilization, and cost outcomes associated with timing of basal insulin initiation among T2DM patients previously treated with 1, 2, or 3+ OADs.
Materials and methods: This retrospective study, using the linked MarketScan® Commercial and Medicare Supplemental Databases and GE Centricity EMR databases from 2004-2011, included adult T2DM patients who initiated basal insulin after being treated with OADs only, and had continuous medical and pharmacy health plan coverage for ≥ 6 months before (baseline) and ≥ 12 months after initiation (follow-up). The population was stratified by the number of baseline OADs (1, 2, or 3+ OADs) as a proxy for timing of insulin initiation. HbA1c reduction from baseline, proportion of patients achieving HbA_{1c} < 7.0%, hypoglycaemia rates, treatment persistence, changes in body weight and body mass index (BMI), and health care utilization and costs were evaluated during a 12-month follow-up period. Confounding was addressed using the inverse probability of treatment weighting method.
Results: A total of 1,830 patients were included (baseline mean age: 57.2 years; HbA_{1c}: 9.2%; body weight: 99.5 kg; BMI: 34.5; hypoglycaemia rate: 3.0%; 1 OAD: 24.6%; 2 OADs: 40.3%; 3+ OADs: 35.1%). At baseline, no significant differences were observed between the 3 groups except for gender (female: 52.0% vs 47.0% vs 42.5%, P = 0.008) and the proportion of patients with all-cause hospital/emergency department (ED) visits (hospital: 21.1% vs 18.0% vs 13.9%, P = 0.006; ED: 27.8% vs 21.0% vs 18.5%, P = 0.001). Weighted results from a 12-month follow-up showed that the 1 OAD group had the greatest HbA1c reduction (−1.7% vs −1.0% vs −0.9%, P < 0.0001), the highest proportion of patients achieving HbA1c < 7.0% (38.2% vs 26.7% vs 19.6%, P < 0.0001), and the lowest overall and hospital/ED hypoglycaemia rates (overall: 2.7% vs 6.6% vs 5.0%, P = 0.0002; hospital/ED: 0.9% vs 1.9% vs 3.7%, P = 0.0001), despite its lower treatment persistence rate (51.7% vs. 58.0% vs. 68.7%, P < 0.0001). No significant differences were observed in change in body weight (+1.2 kg vs +1.6 kg vs +1.0 kg, P = 0.541), change in BMI (+0.5 vs +0.6 vs +0.4, P = 0.593), or total health care costs (all-cause: $21,167 vs $21,060 vs $20,133, P = 0.921; diabetes-related: $6,574 vs $6,257 vs $6,581, P = 0.926). When stratified by baseline BMI, HbA1c reduction was greater in the 1 OAD group when baseline BMI was ≤ 30 (−2.7% vs −1.5% vs −1.5%, P = 0.001) and 31–35 (−1.9% vs −0.7% vs −0.7, P = 0.0009), but similar when BMI was > 35 (−0.9% vs −1.0% vs −0.7%, P = 0.584).
Conclusion: These data illustrated that initiating insulin therapy among patients previously treated with 1 OAD resulted in greater HbA_{1c} reductions and lower rates of hypoglycaemia than adding basal insulin to treatment with > 2 OADs. This data supports the call for timely initiation of insulin therapy for T2DM patients not maintaining glycemic control with OADs.
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Delay in starting insulin after failure of other treatments in patients with type 2 diabetes

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Background and aims: In type 2 diabetes mellitus (T2DM), therapies to maintain blood glucose control usually fail after several years. The aim of this study was to estimate the time to insulin initiation, the glycaemic burden that patients are exposed prior to conversion to insulin and their HbA_{1c} level at that time and a year later.
Materials and methods: Retrospective study. From our data base (Scottish Care Information-Diabetes Collaboration of Western General Hospital, Edinburgh Scotland) we identified patients with T2DM who started insulin therapy from 1-1-2002 to 30-6-2011. Exclusion criteria were conversion in insulin therapy less than 12 months from diagnosis, during pregnancy, acute illness or hospitalization. We also excluded patients with insufficient data for at least 3 visits. Finally, from 1221 files with sufficient data, 509 patients fulfilled the inclusion criteria. In these patients we estimated the duration of diabetes prior to conversion to insulin therapy, the months they spent with HbA_{1c} above 7%, 8% or 9% until starting insulin, HbA_{1c} and body weight (BW) at the time of conversion, at 6 and at 12 months before and after conversion.
Results: Our patients usually started insulin therapy 7.2+4.7 years (mean + SD) after diagnosis of T2DM. Mean HbA_{1c} was 10.3+1.6% at the time of conversion, 9.1+1.4% at -6 months, 8.7+1.6% at -12 months, 8.5+1.3% at +6 months and 8.3+1.4% at +12 months. Body weight (BW) was 89.7+21.6kg at conversion and 92.9+21.0kg at +12 months. Patients spent a mean period of 57.3+41.7 months with HbA_{1c} >7%, 32.1+31.7 months with HbA_{1c} > 8% and 14.9+17.7 months with HbA_{1c} > 9%. Insulin treatment resulted in a decrease in HbA_{1c} at 12 months of 2.0% (p<0.0001) but an increase in BW by 3.2kg (p<0.0001). After 12 months HbA_{1c} was <7% for 15.1% of the patients vs 8.8% 12 months before conversion and <9% for 7.4% vs 6.6%.
Conclusion: Healthcare professionals delay the initiation of insulin in patients with type 2 diabetes until their HbA_{1c} exceeds 10%. As a result patients are exposed to a significant glycaemic burden. Change in treatment improves their glycaemic control for the next 12 months.

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Durability of therapeutic intensification strategies in patients with uncontrolled type 2 diabetes

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Background and aims: Achieving desired glycaemic targets in type 2 diabetes (T2D) depends in part on medication efficacy and durability. A recent algorithm for pharmacologic intensification in patients with T2D uncontrolled on current medications recommends an individualised approach when choosing among the different classes of glucose lowering agents. There is little information available describing therapeutic durability and efficacy with different pharmacologic strategies in the clinical setting. To address this, we sought to identify therapeutic durability at one year following intensification with insulin, GLP-1 analogue or a third oral diabetes medication (ODM) in a class different from current therapy in patients with T2D with A1C ≥ 7% on one or two ODM.
Materials and methods: 1802 patients were identified between 2005-2011 from a US university based EMR and grouped according to intensification strategy with insulin (n=416), GLP-1 (n=68), or ODM (n=1408). Patients were grouped in one of 4 categories: intensification agent continued without additional therapy (Group1); intensification agent discontinued without additional therapy (Group 2); intensification agent discontinued with addition of another agent (Group 3); or intensification agent continued with addition of another agent (Group 4). The percent of patients in Group 1, identified as Durable Therapy, were compared with Groups 2-4 at one year following intensification (Table).
Results: Therapeutic durability was highest in those intensified with insulin and lowest in those intensified with GLP-1 agents (p<0.001) even after adjusting for baseline A1C, age, gender, race, and BMI (p<0.001). Compared to intensification with insulin, the adjusted OR for durability of therapy with GLP-1 agents was 0.17 (0.10-0.30), and with ODM 0.32 (0.23-0.43).
Conclusion: We conclude that in this observational study those patients with uncontrolled T2D who have intensification of glycaemic therapy with insulin are more likely to have this continued at one year than those who receive GLP-1 agents or an additional ODM. Further investigation into factors associated with persistence of different intensification strategies in patients with uncontrolled T2D is recommended.

	Intensification drug			
	Insulin	GLP-1	ODM	p value
	N = 416	N = 68	N = 1408	
Age	62 ± 14	56±12	63 ± 12	<0.001
% White	75	97	85	<0.001
% Female	53	58	47	0.02
Baseline A1C (%)	9.3 ± 2.0	8.3±1.2	8.3 ± 1.3	<0.001
Group 1 (% of patients)	84.9	52.9	66.0	<0.001
Group 2 (% of patients)	9.9	17.6	18.7	
Group 3 (% of patients)	1.2	17.6	6.0	
Group 4 (% of patients)	4.1	11.8	9.4	

Supported by: Sanofi

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Is assessment of postprandial hyperglycaemia useful in determining whether to initiate prandial therapy after basal insulin fails to achieve glycaemic control?

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Background and aims: We recently illustrated that after 24 weeks of basal insulin therapy in patients (pts) with type 2 diabetes mellitus (T2DM), HbA_{1c} ≥7.0% alone is a more inclusive indicator of postprandial glucose (PPG) defects than HbA_{1c} ≥7.0% and high fasting plasma glucose (FPG; ≥7.2 mmol/L) in patients with a high PPG (≥10 mmol/L) 2 hours after any meal. Here we investigated basal vs postprandial contributions to hyperglycaemic exposure (HE) in pts with high PPG and in pts with HbA_{1c} ≥7.0% but PPG <10 mmol/L after each meal. We also explored whether there is a threshold PPG that should be avoided, or if change between pre- and postprandial glucose levels (ΔPPG) is a better indicator of glycaemic control.
Materials and methods: Patient-level data from 6 randomized controlled trials in adult pts with T2DM collecting 7-point self-monitored blood glucose (SMBG) data and titrating insulin glargine for ≥24 weeks were pooled and analyzed (N=1,699). HE (>5.6 mmol/L) due to basal hyperglycaemia (BHG) and postprandial hyperglycaemia (PPHG) were calculated and compared at baseline and endpoint in pts with HbA_{1c} ≥7.0%, with or without a PPG ≥10 mmol/L after any meal, who had controlled or high FPG. Contributions to HE were also investigated in pts with HbA_{1c} ≥7.0% according to whether they had a ΔPPG ≥2.2 or 2.8 mmol/L around any meal.
Results: In total, 496 pts had HbA_{1c} ≥7.0% at endpoint: 340 with PPG ≥10 mmol/L after any meal and 156 with PPG <10 mmol/L after each meal (Table). At baseline, the relative contribution of BHG vs PPHG to HE was numerically higher in pts with HbA_{1c} ≥7.0% and PPG <10 mmol/L after each meal. After 24 weeks of treatment, across the whole patient population, the contribution of PPHG to HE in relation to BHG increased. Interestingly, regardless of whether a patient with HbA_{1c} ≥7.0% had a PPG ≥10 mmol/L after any meal or PPG <10 mmol/L after each meal, the contribution of PPHG to HE was >60%. Across both PPG groups, those pts with high FPG appeared to have a lower, but still substantial, relative contribution from PPHG compared with patients within target FPG levels (Table). When comparing contributions to HE according to whether pts had a ΔPPG ≥2.2 or 2.8 mmol/L around any meal, a similar pattern was observed (data not shown), though relative PPHG contributions were somewhat greater in magnitude than those observed with the PPG < or ≥10 mmol/L thresholds.
Conclusion: In pts systematically titrated with insulin glargine for 24 weeks, but not at target HbA_{1c}, there is a substantial contribution to HE from PPHG, regardless of FPG or PPG level. ΔPPG seems to be a stronger indicator of PPHG contribution than PPG level. This strong PPHG contribution to poor glycaemic control in pts titrated with basal insulin but not at target HbA_{1c} suggests an ideal therapy may be basal insulin plus a prandial agent.

Characteristic	PPG =10 mmol/L after any meal			PPG <10mmol/L after each meal		
	HbA_{1c} =7.0% at endpoint N=340	HbA_{1c} =7.0% and FPG <7.2 mmol/L at endpoint N=190	HbA_{1c} =7.0% and FPG =7.2 mmol/L at endpoint N=150	HbA_{1c} =7.0% at endpoint N=156	HbA_{1c} =7.0% and FPG <7.2 mmol/L at endpoint N=107	HbA_{1c} =7.0% and FPG =7.2 mmol/L at endpoint N=49
Age, years	60.3 (9.7)	60.5 (9.3)	60.1 (10.2)	59.6 (9.4)	60.4 (9.4)	57.6 (9.3)
Men, n (%)	169 (49.7)	95 (50.0)	74 (49.3)	102 (65.4)	66.4 (66.4)	63.3 (63.3)
T2DM duration, years	9.6 (6.0)	9.9 (6.3)	9.3 (5.7)	9.4 (6.9)	9.9 (7.3)	8.3 (5.8)
Baseline weight, kg	85.7 (16.2)	84.1 (15.1)*	87.8 (17.3)	86.2 (14.5)	85.9 (14.0)	87.0 (15.5)
Baseline HbA_{1c}, %	9.1 (1.0)	9.0 (0.9)	9.1 (1.0)	9.0 (0.9)	9.0 (0.8)	9.2 (1.0)
Endpoint HbA_{1c}, %	7.8 (0.8)	7.7 (0.6)*	8.0 (0.9)	7.7 (0.7)	7.6 (0.6)*	7.8 (0.8)
Endpoint insulin dose, IU/kg	0.47 (0.25)	0.45 (0.27)	0.49 (0.22)	0.41 (0.26)	0.41 (0.24)	0.40 (0.30)
BHG contribution, % Baseline Endpoint	76.0 (21.2) 36.3 (33.1)	73.9 (21.9) 23.7 (28.3)	78.7 (19.9) 52.3 (31.9)	81.3 (21.9) 37.7 (40.8)	80.5 (22.5) 28.4 (38.0)	83.1 (20.6) 58.2 (39.3)
PPHG contribution, % Baseline Endpoint	24.0 (21.2) 63.7 (33.1)	26.1 (21.9) 76.3 (28.3)	21.3 (19.9) 47.7 (31.9)	18.7 (21.9) 62.3 (40.8)	19.5 (22.5) 71.6 (38.0)	16.9 (20.6) 41.8 (39.3)
Any serious adverse event, n (%)	26 (7.6)	12 (6.3)	14 (9.3)	7 (4.5)	5 (4.7)	2 (4.1)
Any severe hypoglycemic events, n (%)	6 (1.8)	1 (0.6)	5 (3.4)	1 (0.7)	1 (1.0)	0

Table. Baseline and endpoint characteristics of patients with HbA_{1c} =7.0% at endpoint and a PPG =10 mmol/L after any meal or PPG <10 mmol/L after each meal. Data represent mean (standard deviation) unless otherwise specified.
*P<0.05 for FPG <7.2 mmol/L vs FPG =7.2 mmol/L

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Four-year evolution of insulin regimens, glycaemic control, hypoglycaemia and body weight after starting insulin therapy in type 2 diabetes across three continents

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Background and aims: Many different patterns of insulin therapy are used in the years after starting insulin treatment in people with type 2 diabetes. Our aim was to describe and understand these patterns and the associated outcomes over 4 years in diverse clinical environments.
Materials and methods: Data from people starting any insulin was collected in 12 countries in 3 continents. Baseline data were retrospective to avoid influence on insulin choice, follow-up data prospective, and both were extracted from regular clinical care records. Funding was provided only for data extraction.
Results: Of 2999 people enrolled in the study, 2272 people were followed over the 4 years. Baseline characteristics were typical of people starting insulin with diabetes duration 10.6 (mean, SD 7.8) yr, HbA1c 9.5 (1.9) % (80 (21) mmol/mol) and BMI 29.3 (6.2) kg/m^2. Starting insulin therapy was any basal for 52%, any premix 23%, meal-time (MT)+basal 14%, MT alone 8% and other insulin regimens 3%, and evolved at 4 years to 30%, 25%, 33%, 2%, and 5%, respectively, with 5% no longer taking insulin. Only 1258 people (53%) were on their baseline regimen at 4 years. Persistence differed by baseline insulin (Table), but was similar for premix and MT+basal. Baseline HbA1c (above) decreased to 7.7 (1.5) % (60 (16) mmol/mol) at 1 year and 7.6 (1.0) % (59 (11) mmol/mol) at 4 years. The change from baseline was similar by insulin regimen (Table), though modestly higher on more complex insulin regimens. Hypoglycaemia affected <20% of people taking any regimen in the last 6 months of the study, with rates indistinguishable for overall and nocturnal events, but too uncommon to draw conclusions for severe hypoglycaemia (Table). Hypoglycaemia did not vary by achieved HbA1c. Body weight increased by 2.7 (7.5) kg over 4 years, ranging from +4.2 (7.6) kg for MT+basal insulin to -2.2 (1.7) kg for no insulin therapy (Table).

Conclusion: Different insulin regimens were started in different populations, and evolved differently in terms of change of regimen and dose, but were associated with similar outcomes apart from body weight change.

	Insulin regimen at 4 years					
	Basal	Premix	MT+Basal	MT	Other	No insulin
n (%) N=2272	671 (30)	572 (25)	741 (33)	54 (2)	114 (5)	120 (5)
Baseline insulin (n (% baseline))						
Basal (n=1163)	605 (52)	142 (12)	341 (29)	7 (1)	25 (2)	43 (4)
Premix (n=536)	25 (5)	362 (68)	91 (17)	6 (1)	26 (5)	26 (5)
MT+basal (n=331)	32 (10)	35 (11)	222 (67)	7 (2)	9 (3)	26 (8)
MT (n=181)	9 (5)	26 (14)	73 (40)	33 (18)	18 (10)	22 (12)
Other (n=61)	0 (0)	7 (11)	14 (23)	1 (2)	36 (59)	3 (5)
HbA_{1c} (%)						
Baseline	9.2 (1.8)	9.8 (2.0)	9.6 (1.9)	9.1 (1.7)	9.7 (2.1)	9.8 (1.9)
Change	−1.8 (1.8)	−2.0 (2.0)	−1.8 (1.9)	−1.8 (1.9)	−2.0 (2.3)	−2.5 (2.1)
Weight (kg)						
Baseline	83.3 (18.4)	76.3 (18.2)	79.9 (17.3)	75.0 (24.8)	74.1 (18.5)	75.4 (21.9)
Change	+1.1 (7.8)	+3.4 (6.5)	+4.2 (7.6)	+0.6 (7.6)	+3.4 (6.0)	−2.2 (1.7)
Hypoglycaemia (last 6 months before 4-yr visit)						
People, n (%)	100 (14.9)	109 (19.1)	138 (18.6)	8 (14.8)	18 (15.8)	3 (2.5)
Overall (events/pt)	1.2 (4.7)	1.0 (3.6)	1.0 (3.6)	0.9 (4.2)	0.8 (2.2)	1.0 (3.9)
Nocturnal (events/pt)	0.2 (1.2)	0.2 (1.1)	0.2 (1.3)	0.3 (0.1)	0.2 (0.9)	0.2 (1.2)
Severe, n (%)	22 (3.3)	2 (0.3)	19 (2.6)	3 (5.6)	0 (0.0)	0 (0.0)
Insulin dose, U/day						
Baseline	15.7 (11.0)	20.9 (14.1)	23.3 (17.0)	18.4 (16.3)	22.8 (17.9)	17.2 (13.4)
4 years	32.6 (22.2)	44.7 (29.6)	59.8 (34.5)	27.2 (41.2)	46.2 (27.6)	0.0 (0.0)

Mean (SD) or n (%); MT, meal-time; pt, patient

Supported by: Sanofi

1023

Is FPG variability linked to dose escalation during basal insulin titration, leading to excess insulin, weight gain and hypoglycaemia in type 2 diabetes mellitus?

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Background and aims: Once attained, maintenance of target fasting plasma glucose (FPG) requires continued upward titration of once-nightly basal insulin in some patients with type 2 diabetes mellitus (T2DM), possibly due to increased FPG variability. This may lead to weight gain and hypoglycemia. A recent study showed that intra-day glycemic variability (both before initiation and during insulin treatment) is significantly associated with the risk of hypoglycemia during treatment.
Materials and methods: Data was analyzed from a 24-week trial, INITIATE. Starting from 10 IU once nightly, insulin glargine dose was increased 2-4 IU if FPG was >99 mg/dL for 3 days and decreased 2 IU/day if FPG was <72 mg/dL and symptomatic hypoglycemia occurred. Correlations between dose change after reaching goal, and variability of FPG (measured by SD), weight change, and hypoglycemia were assessed. Quartiles of SD (<20, ≥20 to <26, ≥26 to <35, and ≥35 mg/dL) were compared.
Results: Presented values are means. Of 101 patients with T2DM with daily self-monitored FPG and dose data (age 58 years; men 66%; weight 93 kg; HbA1C 8.8%), 95% (96 patients) achieved FPG goal (<100 mg/dL) during the study. FPG goal was first reached in 42 days at 0.39 IU/kg, increasing to 0.58 IU/kg by study end. FPG was 88 mg/dL at first goal achievement and 116 mg/dL at week 24 (paired t-test (P<0.0001). FPG SD correlated with dose change after reaching goal to study end (r=0.2837, (P=0.0051) and also with FPG SD for the first 3 days after insulin initiation but before titration (r=0.4339, P<0.0001). Dose change after reaching goal correlated weakly with weight gain from reaching goal to study end (r=0.2002, P=0.0505), but not with occurrence of any type of hypoglycemia after reaching goal. Dose change and weight gain were higher in the highest FPG SD group than in the lowest (ANOVA P<0.04), as was incidence of all types of hypoglycemia (except severe), but only numerically so (data not shown). No patient had severe hypoglycemia.
Conclusion: High SD after reaching FPG goal is associated with higher insulin dose, weight gain, and possibly increased hypoglycemia. Presence of high FPG SD during the first three days of insulin treatment suggests that FPG variability may be a patient characteristic that can be detected early. Investigations of causes for increased FPG variability are warranted.

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Two prandial insulin approaches for achieving adequate glycaemic control in patients not at goal HbA_{1c} on basal insulin plus oral agents alone: the AUTONOMY study

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Background and aims: There is limited evidence on optimal methods of prandial insulin dose adjustment, especially in the general practitioner setting. AUTONOMY evaluated two approaches to introduce lispro insulin therapy in patients with type 2 diabetes mellitus (T2DM) not achieving adequate glycaemic control on basal insulin plus oral agents. The study looked at changes in glycated hemoglobin (HbA1c), body weight, fasting blood glucose (FBG), and 1,5anhydroglucitol (1,5-AG) after 24 weeks of treatment with one of two patienttitrated basal-bolus algorithms. In addition, 7-point self-monitored blood glucose (SMBG) profile, hypoglycaemia, and adverse events data evaluation was conducted.
Materials and methods: Two independent multicentre, randomised, open-label, parallel studies (A and B) were performed under one protocol in 1106 patients. Inclusion criteria included patients aged 18 to 85 years currently treated with insulin glargine (GLA), NPH, NPL, or detemir (≥20 U/day) plus oral antihyperglycaemic agents for ≥3 months, with a HbA1c >7.0% and ≤12.0%, and who were considered appropriate candidates for prandial insulin therapy. Patients entering the trial on non-GLA basal insulins where converted to GLA and those needing basal optimization (based on goal fasting glucose) underwent a GLA 6-week optimization lead-in. At randomisation, patients were initiated on one of two bolus insulin treatment algorithms for a total of 24 weeks. Both algorithms added pre-meal lispro sequentially to a single meal and patients self-adjusted prandial insulin, with insulin management study diaries, either every 3 days (Q3D), based on previously published algorithm, or by 1 unit of insulin every day (Q1D).
Results: At Week 24, each individual study demonstrated that both algorithms resulted in a clinically equivalent and significant reduction in HbA1c and an increase in 1,5-AG. In addition, close to one-half of patients achieved a goal HbA1c of ≤7% with a low incidence and rate of nocturnal and severe hypoglycaemia (Table).
Conclusion: The AUTONOMY study is the first to demonstrate that in T2DM patients on basal insulin prandial therapy can be effectively and safely initiated in a clinical setting across a broad age range and that self-titration of mealtime lispro can be accomplished by using either of two simple algorithm without the complexity of carbohydrate counting or insulin correction factor.

	Study A (N=528)			Study B (N=578)		
Treatment	**Q1D (n=267)**	**Q3D (n=261)**	**Q3D vs Q1D p-value**	**Q1D (n=288)**	**Q3D (n=290)**	**Q3D vs Q1D p-value**
Baseline HbA1c % (Mean ± SD)	8.33 ± 0.92	8.39 ± 0.99	0.453	8.28 ± 0.99	8.40 ± 0.98	0.162
HbA1c % Change from baseline (LSM ± SE)[a]	-1.00 ± 0.08	-0.96 ± 0.08	0.706; 95% CI (-0.15,0.22)	-0.98 ± 0.07	-0.92 ± 0.07	0.515; 95% CI (-0.12,0.24)
Proportion achieving target HbA1c ≤7.0%:						
Overall—n (%)	133 (49.81)	111 (42.53)	0.128	142 (49.31)	123 (42.41)	0.162
Geriatric (≥65 yo)—n (%)	38 (58.46)	40 (57.97)	0.701	38 (67.86)	30 (46.15)	0.015
Weight (kg) change from baseline (LSM ± SE)	2.15 ± 0.27	2.96 ± 0.28	0.014	2.47 ± 0.24	1.97 ± 0.24	0.108
FBG (mmol/L) change from baseline (LSM ± SE)	0.08 ± 0.22	0.37 ± 0.23	0.238	-0.36 ± 0.21	0.45 ± 0.21	0.002
1,5-AG (µg/ml) change from baseline (LSM ± SM)	3.24 ± 0.35	3.09 ± 0.36	0.723	3.22 ± 0.32	2.95 ± 0.31	0.495
Hypoglycemia						
Overall						
Incidence—n (%)	231 (86.2)	218 (83.2)	0.435	238 (82.4)	231 (79.1)	0.351
Rate per 30 days—(LSM ± SE)	3.15 ± 0.23	3.33 ± 0.25	0.586	3.18 ± 0.26	3.33 ± 0.27	0.689
Nocturnal						
Incidence—n (%)	169 (63.1)	167 (63.7)	0.870	156 (54.0)	149 (51.0)	0.470
Rate per 30 days—(LSM ± SE)	0.71 ± 0.07	0.79 ± 0.08	0.404	0.59 ± 0.07	0.68 ± 0.07	0.358
Severe						
Incidence—n (%)	5 (1.9)	2 (0.8)	0.258	7 (2.4)	8 (2.7)	0.856
Rate per 30 days—(LSM ± SE)	0.00 ± 0.00	0.00 ± 0.00	0.756	0.00 ± 0.00	0.00 ± 0.00	0.934

[a] Primary object analysed through the classification method using mixed model, repeated measure (MMRM) approach.

Clinical Trial Registration Number: NCT01215955

1025

Insulin lispro mix 25/75 twice daily (LM25) vs basal insulin glargine once daily and prandial insulin lispro once daily (BP) in type 2 diabetes: insulin intensification

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Background and aims: Recent ADA/EASD consensus statements consider different approaches for intensifying insulin therapy in T2D. Head-to-head data comparing premixed insulin analogs vs addition of prandial insulin in patients inadequately controlled on a basal-only insulin regimen (BO) are lacking. We compared efficacy and safety of two insulin intensification strategies (LM25 vs BP) in patients inadequately controlled on once-daily basal insulin glargine + metformin and/or pioglitazone.
Materials and methods: This multinational, randomised, open-label, parallel-arm, phase IV trial compared efficacy and safety of LM25 and BP (+ metformin and/or pioglitazone) over 24 weeks in patients with T2D and HbA_{1c} 7.5-10.5% despite BO and fasting plasma glucose ≤6.7 mmol/L (>6.7 mmol/L if basal insulin could not be further titrated). Primary objective was to assess non-inferiority (NI) of LM25 vs BP (NI margin 0.4%, two-sided significance level 0.05, using likelihood-based mixed model repeated measures analysis).
Results: Patients [mean (SD) age 57.5 (9.52) years] from 11 countries were randomised to LM25 (n=236) or BP (n=242) [mean (SD) baseline HbA_{1c} 8.65 (0.79)% and 8.60 (0.75)%, respectively]. Estimated change [least squares (LS) mean (95% CI)] in HbA_{1c} at 24 weeks was -1.30 (-1.44, -1.16)% units with LM25 and -1.08 (-1.22, -0.94)% units with BP. NI was shown between the two treatment strategies [LS mean (95% CI) treatment difference -0.22 (-0.39, -0.05)]; gated superiority assessment showed a statistically significant advantage for LM25 (p=0.010). LS mean (95% CI) daily self-monitored blood glucose (SMBG) levels fell to 8.03 (7.82, 8.23) mmol/L with LM25 and to 8.14 (7.93, 8.35) mmol/L with BP at 24 weeks. Glycaemic variability, measured using SMBG, did not differ between treatments during the study. Overall, mean (SD) rates of documented symptomatic (≤3.9 mmol/l) and nocturnal hypoglycaemia were 7.21 (14.55)/year and 1.54 (4.58)/year with LM25 and 7.72 (15.67)/year and 1.82 (5.25)/year with BP, respectively; 2 patients experienced severe hypoglycaemia (both with LM25; neither required treatment discontinuation). LS mean (95% CI) bodyweight increase at 24 weeks was 1.13 (0.75, 1.52) kg with LM25 and 0.50 (0.11, 0.89) kg with BP (p=0.018). Total mean (SD) daily insulin doses were 53.1 (24.6) IU with LM25 and 50.8 (22.0) IU with BP at last visit. Insulin Treatment Satisfaction and Perception about Medications-Diabetes 21 questionnaires data at last visit showed no statistically significant differences between treatments.
Conclusion: In patients with T2D inadequately controlled on once-daily basal insulin glargine + metformin and/or pioglitazone, intensification with either LM25 or BP improved glycaemic control, although HbA_{1c} reduction was greater with LM25 than with BP. Both regimens were similarly tolerated. LM25 is therefore a valid strategy to intensify insulin treatment in patients inadequately controlled with a BO.
Clinical Trial Registration Number: NCT01175824
Supported by: Eli Lilly (authors thank C. Spencer for assistance)

1026

Intensification of basal insulin therapy with step-wise addition of insulin aspart boluses compared with basal-bolus therapy: the FullSTEP study

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Background and aims: When patients who are receiving basal insulin ± oral agents (OADs) fail to achieve a target HbA_{1c} of 7.0 %, one can transition to use of combined therapy with use of one, two or three boluses of a rapid-acting insulin analogue. The present randomised, open-label, parallel-group, multinational, 32-week trial compared the efficacy and safety of a step-wise insulin regimen with a basal-bolus insulin regimen in patients with type 2 diabetes inadequately controlled on basal insulin + OADs. Patients had used basal insulin (NPH, insulin detemir or insulin glargine), administered once or twice daily for a minimum of 6 months prior to the study. Patients had not regularly received prandial insulin prior to starting the trial, except during any hospitalisation.
Materials and methods: 590 subjects entered into an 8-week run-in period where they were switched from their previous basal insulin to once-daily insulin detemir at bedtime. Then, subjects with HbA_{1c} 7.0-9.0 % and fasting plasma glucose (FPG) ≤8.9 mmol/L (n=401, mean age 59.8 years; HbA_{1c} 7.9 %; diabetes duration 12.6 years) were randomised 1:1 to step-wise or basal-bolus treatment groups. The basal-bolus group received insulin aspart three-times daily for the duration of the trial (32 weeks). In the step-wise group, insulin aspart was introduced before the largest meal (period 1) and continued if HbA_{1c} remained <7.0 %. At 11 weeks (period 2) and again at 22 weeks (period 3), subjects with HbA_{1c} remaining ≥7 % were prescribed an additional insulin aspart bolus injection at the next larger meal.
Results: At 32 weeks, 17.4, 27.4 and 40.3% of the randomised step-wise subjects were receiving one, two or three daily boluses, respectively. The dropout rate was significantly lower for the step-wise (14%) than for the basal-bolus (26%) group. HbA_{1c} change from baseline (last observation carried forward) was −0.98% for the step-wise and −1.12% for the basal-bolus groups: Mean treatment difference (MTD) 0.14 [95% CI: −0.02;0.30]; NS. FPG was also comparable for the treatment groups (MTD 0.12 mmol/L; NS). Subjects in the step-wise group experienced fewer hypoglycaemic episodes compared with those on basal-bolus (rate ratio 0.58 [95% CI: 0.45;0.75]; $p<0.001$). Body weight was comparable for the groups (MTD −0.48 kg; NS), as was BMI (MTD −0.17 kg/m^2; NS). Patient-reported outcomes determined by DiabMedSat indicated better overall patient satisfaction with the step-wise regimen compared with the basal-bolus regimen ($p<0.01$). At 32 weeks, nearly half of all subjects (44.8%) randomised to the step-wise regimen required only one or two bolus injections per day. There were no differences in treatment-emergent adverse events between the two treatment regimens.
Conclusion: Step-wise intensification of prandial insulin therapy provides glycaemic control that is comparable to a basal-bolus regimen after 32 weeks, with a significantly lower risk of hypoglycaemia, improved patient satisfaction and no differences in weight change or treatment-emergent adverse events.
Clinical Trial Registration Number: NCT01165684
Supported by: Novo Nordisk

1027

Addition of liraglutide vs addition of a single dose of insulin aspart to insulin degludec plus metformin in patients with type 2 diabetes

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Background and aims: Previous studies have evaluated the efficacy and safety of adding a glucagon-like peptide-1 (GLP-1) analogue to basal insulin or vice versa in type 2 diabetes. The aim of this trial was to evaluate the efficacy and safety of intensifying treatment with ultra-long-acting insulin degludec (IDeg) plus metformin with the addition of a GLP-1 analogue (liraglutide [Lira]) or a single dose of mealtime insulin aspart (IAsp) in patients with type 2 diabetes.
Materials and methods: In this treat-to-target trial, subjects who remained inadequately controlled (i.e. HbA_{1c} ≥7%) after completing 104 weeks on IDeg once daily plus metformin (Met) were randomised to intensify treatment with either Lira once daily (N=88) or IAsp with largest meal once daily (N=89) for an additional 26 weeks. The primary endpoint was the difference in change in HbA_{1c} at 26 weeks between the two arms.
Results: Mean baseline HbA_{1c} was 7.7% in both arms. HbA_{1c} reduction was significantly greater with IDeg + Lira (−0.74%) than IDeg + IAsp (−0.39%) at Week 26 (estimated treatment difference [ETD] {IDeg + Lira}−{IDeg + IAsp}: −0.32% $[-0.53; -0.12]_{95\%CI}$; p=0.002). After 26 weeks, mean HbA_{1c} was 7.0% with IDeg + Lira and 7.3% with IDeg + IAsp. Significantly more subjects achieved the combined endpoint of HbA_{1c} <7%, without confirmed hypoglycaemia (plasma glucose <3.1 mmol/L or severe hypoglycaemia) and without weight gain with IDeg + Lira (49.4%) vs IDeg + IAsp (7.2%); estimated odds ratio {IDeg + Lira}/{IDeg + IAsp}: 13.8 [5.2; 36.3]; p<0.0001. With lower HbA_{1c} at end of trial, IDeg + Lira subjects experienced 87% less

confirmed hypoglycaemia vs IDeg + IAsp (1.00 and 8.15 events/patient-year, respectively; p<0.0001), 86% less nocturnal confirmed hypoglycaemia (0.17 and 1.11 events/patient-year, respectively; p=0.0002), and significantly greater weight loss (−2.8 kg) vs IDeg + IAsp (+0.9 kg); ETD {IDeg + Lira}−{IDeg + IAsp}: −3.8 kg [−4.7; −2.8]; p<0.0001. IDeg + Lira subjects initially reported more nausea (20.7% patients vs 1.2% for IDeg + IAsp) although incidence decreased within 2 weeks. No differences were seen between arms in adverse events or standard safety parameters.

Conclusion: Intensification with addition of liraglutide to insulin degludec once daily + metformin in patients with type 2 diabetes improved long-term glycaemic control, with weight loss and less hypoglycaemia compared with addition of a single daily dose of IAsp with the largest meal.

Clinical Trial Registration Number: NCT01388361

Supported by: Novo Nordisk A/S

PS 083 Novel insulin preparations: pharmakinetics and pharmacodynamics

1028

Novel formulations BIOD-238 and BIOD-250 result in more rapid absorption and declines from peak than Humalog®

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Background and aims: Currently available prandial insulins do not provide optimal postprandial glucose coverage when dosed with meals. Formulations of human insulin and insulin analogs containing citrate and sodium-EDTA (Na_2EDTA) have been shown to be more rapidly absorbed than the commercially available formulation of insulin lispro (Humalog®, HU). BIOD-238 and BIOD-250 are novel Na_2EDTA/citrate formulations of HU. BIOD-250 also contains magnesium sulfate which has previously been shown to mitigate Na_2EDTA-mediated local injection site discomfort.

Materials and methods: A single-center, randomized, double-blind three-period crossover study evaluating the pharmacokinetics of 0.2 unit/kg injections of BIOD-238 and BIOD-250 compared to HU was performed in 12 subjects with type 1 diabetes. Local injection site toleration measured with 100 mm visual analog scale (VAS), absolute and relative discomfort scores were also assessed after each test injection.

Results: Pharmacokinetic parameters are summarized in Table 1. Mean times to half maximal insulin concentrations ($T_{ins50\%Early}$), times to maximal insulin concentrations (T_{insMax}) and areas under the curve (AUC) for the first 30 and 45 minutes all indicated significantly increased early lispro absorption for both BIOD-238 and BIOD-250 compared to HU. Mean times to half maximal concentration after the peak ($T_{ins50\%Late}$) and $AUC_{120\text{-}480min}$ indicated that BIOD-238 and BIOD-250 were associated with more rapid declines from peak concentrations relative to HU. The mean VAS score was numerically lower, but not significantly different for BIOD-250 compared to HU (2.7 ± 1.6 mm for BIOD-250 and 8.2 ± 4.5 mm for HU). The mean VAS score for BIOD-238 was significantly higher than that associated with HU (24.2 ± 7.0 mm, p=0.029 vs. HU). Assessments of absolute and relative injection discomfort severity scores mirrored VAS results. Higher doses/injection volumes were associated with increased VAS discomfort scores for BIOD-238, BIOD-250, and HU. General safety results were comparable between treatments.

Conclusion: BIOD-238 and BIOD-250 are associated with more rapid absorption and more rapid declines from peak compared to HU. BIOD-250 and HU cause comparable injection site discomfort.

Table 1: Pharmacokinetic Parameters, Arithmetic Means ± SEM [Median]

Parameter	BIOD-238(n=10)	BIOD-250(n=11)	HU (n=10)	P-value BIOD-238 vs. HU	P-value BIOD-250 vs. HU
Tins 50%Early(min)	13.7±1.9 [13.6]	14.6±1.9 [12.9]	24.8±2.9 [22.6]	<0.001	0.001
TinsMax(min)	35.5±2.5 [37.5]	40.9±6.1 [40.0]	62.5±8.4 [60.0]	0.013	0.025
AUCins0-30(mU*min/L)	1278±164 [1105]	1186±133 [1260]	598±126 [654]	<0.001	0.002
AUCins0-45(mU*min/L)	2421±245 [2132]	2160±195 [2327]	1486±216 [1458]	<0.001	0.010
AUCins0-60(mU*min/L)	3476±326 [3197]	3081±245 [3125]	2505±280 [2358]	0.002	0.066
AUCins120-480(mU*min/L	4306±499 [4356]	5607±900 [4808]	5626±557 [6034]	<0.001	0.047
Tins 50%Late(min)	123.8±10.5 [125.3]	132.3±18.7 [117.0]	166.5±10.6 [183.4]	0.009	0.016
AUCins0-480(mU*min/L)	10952±889 [10465]	11747±1085 [11318]	11694±894 [12130]	0.143	0.162
Cmax(mU/L)	83.8±7.3 [86.2]	76.0±3.8 [76.9]	74.7±5.5 [77.0]	0.123	0.828

Clinical Trial Registration Number: NCT01811849

1029

New insulin glargine formulation has a flat and prolonged steady state profile in subjects with type 1 diabetes

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Background and aims: The new insulin glargine formulation with 300 U/mL (Gla-300) provides a prolonged duration of action compared with insulin glargine 100 U/mL (Gla-100) at single subcutaneous doses. This study investigated how these characteristics transfer into steady state.
Materials and methods: This randomized, double-blind, 2x2 crossover study in two parallel cohorts of subjects with type 1 diabetes (T1DM) compared the pharmacodynamic (PD) and pharmacokinetic (PK) properties, and the safety of once-daily administration of 0.4 U/kg (cohort 1, n=18) and 0.6 U/kg (cohort 2, n=12) Gla-300, with a standard dose of 0.4 U/kg Gla-100. Treatment was administered in an 8-day regimen with an automated euglycemic clamp over 36 h after the last injection.
Results: At steady state, the mean, smoothed, body-weight-standardized glucose infusion rate (GIR) of Gla-300 (0.4 U/kg once daily) showed a stable, plateau-like profile over 24 h after dosing, and a slow decline beyond, with activity until clamp end. Maximum GIR and fluctuation of the individual GIRs were lower, and euglycemia (duration until last smoothed blood glucose [BG] value ≤ 105 mg/dL) was maintained for longer with 0.4 U/kg Gla-300 compared with 0.4 U/kg Gla-100 (mean of 32 h and 29 h, respectively). Correspondingly, a daily dose of 0.6 U/kg Gla-300 resulted in a similarly flat GIR profile, but showed greater and longer activity (maintaining euglycemia for around 34 h) with a higher GIR compared with the lower dose. Serum insulin glargine concentrations corroborated the PD findings, with a flatter and more stable profile during the euglycemic clamp with Gla-300 compared with Gla-100. Exposure increased with dose for Gla-300 and was quantifiable until 32 h and 36 h with 0.4 and 0.6 U/kg Gla-300, respectively, compared with 28 h with 0.4 U/kg Gla-100. Both dose levels of Gla-300 and 0.4 U/kg Gla-100 were well tolerated.
Conclusion: This study in subjects with T1DM shows that, at steady state, the new insulin glargine formulation confers an even flatter and more stable PK/PD profile with longer and tighter BG control than Gla-100.
Clinical Trial Registration Number: NCT01349855
Supported by: Sanofi

1030

LY2605541 exhibits a flatter glucodynamic profile than insulin glargine at steady state in subjects with type 1 diabetes

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Background and aims: LY2605541 (LY) is a novel basal insulin analogue with prolonged duration of action. Euglycaemic clamp procedures were used to examine the pharmacodynamics of this new insulin, in comparison with insulin glargine (GL).
Materials and methods: In an open-label, randomised Phase 2 crossover type 1 diabetes mellitus substudy, subjects (n=23) underwent 24-hour Biostator-controlled euglycaemic clamps after 8 weeks treatment with GL or LY. Clinically titrated basal insulin doses (LY: 16 to 64 U, GL: 19 to 60 U) were administered the morning of the clamp. Local regression analysis (LOESS) was used to derive a glucose infusion rate (GIR) profile for each treatment group.
Results: At baseline, mean BMI was 26.8 kg/m^2 ± 4.2 (SD); mean HbA_{1c} was 7.7% ± 1.0. Endpoint dose was 0.43 U/kg ± 0.13 for LY and 0.42 U/kg ± 0.10 for GL. Daily mean blood glucose was 7.7 mmol/L ± 1.2 for LY and 7.9 mmol/L ± 1.2 for GL, p=0.64. Mean hypoglycaemia episodes/30 days were 2.7 ± 2.3 (total) and 0.5 ± 0.8 (nocturnal) for LY and 3.0 ± 2.4 (total) and 0.7 ± 1.1 (nocturnal) for GL, p=0.11 (total) and 0.43 (nocturnal). Mean GIR normalized to unit of insulin was less for LY than GL and persistent over 24 hours. The GIR profile for LY (shown in figure) is consistent with the peak to trough fluctuation ratio of <1.5 that was previously reported. Inter-subject GIR variability (SD) was 0.67 for LY and 0.53 for GL. Eight LY and 1 GL subjects had minimal GIRs over 24 hours (defined as <800 mg/kg), indicating optimal glucose control. Mean total glucose infused ($G_{TOT(024)}$) was 1.22 g/kg ± 0.82 for LY and 1.90 g/kg ± 1.01 for GL, p<0.001. Mean $G_{TOT(0-6)}$ was 0.21 g/kg ± 0.22 for LY and 0.41 g/kg ± 0.22 for GL, p<0.01. Mean $G_{TOT(18-24)}$ was 0.28 g/kg ± 0.18 for LY and 0.35 g/kg ± 0.23 for GL, p=0.20.
Conclusion: LY has a flatter profile than GL, with potentially more stable and predictable metabolic control. LY may have a novel mechanism of action as $G_{TOT(0-24)}$ for LY is less than for GL although LY and GL provide similar glycaemic control.

Mean LOESS-Fitted GIRs at Steady State

Clinical Trial Registration Number: NCT01049412
Supported by: Eli Lilly and Company

1031

Single dose of new insulin glargine Gla-300 formulation has a flatter and prolonged PK/PD profile than Gla-100 in Japanese subjects with type 1 diabetes

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Background and aims: Insulin glargine in its commercially available formulation, Gla-100, offers 24-hour basal insulin supply after single-dose subcutaneous (SC) injection. A new formulation containing 300 U/mL insulin glargine (Gla-300) is being developed to improve its activity profile.
Materials and methods: This is a double blind, randomized, cross-over study using the automated euglycemic clamp technique over 36 h. The pharmacodynamic (PD) and pharmacokinetic (PK) properties of Gla-300 versus Gla-100 formulation were compared in 18 Japanese subjects with type 1 diabetes (T1DM). Single SC doses of 0.4 and 0.6 U/kg Gla-300 and 0.4 U/kg Gla-100 were tested.
Results: The profiles of smoothed (locally weighted scatterplot smoothing factor = 0.12) bodyweight-adjusted glucose infusion rate (GIR), blood glucose (BG) and serum insulin concentrations (INS) were different for the two formulations. After administration, Gla-300 GIR gradually increased until approximately 12 h, and thereafter slightly declined up to 24 h and subsequently remained stable until the end of clamp at 36 h. In contrast, the Gla-100 profile was characterized by a smooth increase in GIR over the first hour, with a maximum GIR at around 12 h, and declining thereafter. BG was more tightly controlled with Gla-300 than Gla-100 beyond 24 h, consistent with more sustained action of Gla-300. In line with GIR profiles, INS profiles of Gla-300 were even flatter than Gla-100. All treatments were well tolerated with no differences in safety-related parameters between treatments.
Conclusion: Gla-300 formulation provides even flatter and prolonged PK/PD profiles as compared with Gla-100 in Japanese subjects with T1DM.
Clinical Trial Registration Number: NCT01493115
Supported by: Sanofi

1032

Metabolism of insulin glargine in humans is the same regardless of formulation, Gla-100 or Gla-300

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Background and aims: Gla-300, a new insulin glargine formulation comprising 300 U/mL with an improved pharmacokinetic (PK) and pharmacodymanic (PD) profile compared with Gla-100 has been developed. Insulin glargine (M0) is a 21^{A}-Gly modified mimic of the final intermediate of natural human insulin. Similar to maturation of human insulin in beta-cells, after subcutaneous (SC) injection of insulin glargine, enzymatic removal of the two C-terminal arginines upon redissolution from the subcutaneous depot yields 21^{A}-Gly-human insulin (M1), the predominant metabolite found in circulation responsible for metabolic effects, as demonstrated for Gla-100 (100 U/mL). Subsequent loss of threonine at position 30^{B} yields Des-29^{B}-Thre-M1 (M2). Both M1 and M2 have lower receptor affinity for Insulin-Like Growth Factor 1 (IGF-1), as well as lower mitogenic properties compared with the parent compound and human insulin. The present sub-investigation was to demonstrate that the metabolism of insulin glargine is the same regardless of formulation.
Materials and methods: Blood samples were obtained from 30 subjects with type 1 diabetes (T1DM) treated for 8 days in a single-center, double-blind, 2-treatment, 2-period, 2-sequence cross-over study in two cohorts. A 36 h euglycemic clamp was conducted on Day 8. Subjects received either 0.4 (cohort 1; n=18) or 0.6 U/kg/day Gla-300 (cohort 2; n=12) and 0.4 U/kg/day Gla-100 once daily in randomized period order. Trough values of M0, M1, and M2 were determined for 7 days, and a full PK profile was determined on Day 8. Liquid Chromatography Tandem Mass Spectrometry with prior immunoaffinity enrichment was applied with a Lower Limit of Quantification [LLOQ] ≤ 0.2 ng/mL to separately determine M0, M1 and M2. Conventional unspecific radioimmunoassay (RIA) determination (LLOQ ≤ 5.02 μU/mL) was used for total active insulin content (M0, M1, M2 combined) and comparison of Day 8 samples.
Results: At trough during the first 7 days, M1 was quantifiable after the 2nd to 3rd injection regardless of treatment and dose, pointing to some accumulation. On the profiling Day 8, M1 concentrations for Gla-300 were dose dependent, yet displayed similar flat PK profiles of lower peak-to-trough ratio compared with M1 profiles of Gla-100. This was in line with comparative RIA data. M0 and M2 were below LLOQ in all but a few trough samples of one subject of each cohort, and while M2 was detected in more than one sample of only these two subjects on the profiling Day 8, M0 was detected in few samples of seven further subjects. The findings match observations on insulin glargine metabolism obtained with Gla-100 in subjects with T1DM.
Conclusion: Insulin glargine metabolism in humans is the same regardless of the formulation (Gla-100 or Gla-300) and 21^{A}-Gly-human insulin (M1) is the main circulating active component.
Clinical Trial Registration Number: NCT01349855
Supported by: Sanofi

1033

Euglycaemic single dose clamp profile of new insulin glargine formulation in subjects with type 1 diabetes is flat and prolonged

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Background and aims: Insulin glargine 100 U/mL (Gla-100) has become a standard of care in diabetes as it provides a 24 h basal insulin supply after single-dose subcutaneous injection. A novel formulation containing 300 U/mL insulin glargine (Gla-300) with improved pharmacokinetics (PK) and pharmacodynamics (PD) is being developed. The aim of this study was to assess the PD and PK properties of this novel formulation in subjects with type 1 diabetes (T1DM).
Materials and methods: This study compared single subcutaneous doses of 0.4, 0.6, and 0.9 U/kg Gla-300 and 0.4 U/kg Gla-100 administered to 24 subjects with T1DM in a double-blind, randomized, 4-sequence, crossover study. The automated (Biostator) euglycemic clamp technique was used over 36 hours.
Results: The profiles of serum insulin concentrations (INS), blood glucose (BG), and smoothed body-weight-standardized glucose infusion rate (GIR) were different for the two formulations. Gla-100 GIR reached its maximum at 12 h and thereafter declined towards 36 h in line with the characteristic end-of-action phenomenon. This was consistent with BG values remaining at a euglycemic level (duration until last smoothed BG value ≤ 105 mg/dL) beyond 24 h and then slowly increasing until the end of the clamp period. GIR AUCs increased dose dependently for 0.4, 0.6, and 0.9 U/kg Gla-300 with a similar shape to the PD profiles over 36 hours, showing an increase from 2 h until around 12 h, followed by a slight decline thereafter, with ongoing activity up to 36 h. Gla-300 showed flatter profiles (i.e., lower maximum GIR), with less fluctuation in individual GIRs compared with Gla-100, and lasting BG control at the end of the clamp. All treatments were well tolerated with no differences in safety-related parameters between treatments. In line with GIR profiles, INS profiles of Gla-300 increased dose dependently and were flatter than with Gla-100.
Conclusion: The new insulin glargine formulation provides prolonged activity and a flatter PK/PD profile compared with Gla-100 in subjects with T1DM.
Clinical Trial Registration Number: NCT01195454
Supported by: Sanofi

1034

Insulin degludec multi-hexamers retain the native protein fold of human insulin

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Background and aims: Insulin degludec (IDeg), an ultra-long-acting basal insulin, forms a soluble and stable depot of multi-hexamers after subcutaneous injection with a subsequent slow release of monomers into circulation, resulting in a duration of action of greater than 42 hours. In a formulation that includes both zinc and phenol, IDeg is organised as finite di-hexamers, but upon injection, dissociation of phenol allows the soluble depot of IDeg multi-hexamers to form. The depot is composed of long strands of multi-hexamers with a width of 6.3 ± 0.9 nm as observed by transmission electron microscopy.
Materials and methods: We have conducted various types of spectroscopy to provide insight into the structure of the IDeg molecule and its organisation and self-assembly in the multi-hexamer.
Results: Circular dichroism, Infrared-, and Raman- spectroscopy showed that the fold of the protein backbone in the multi-hexamer is very similar to that of native insulin. The alpha-helical band is predominant and no signs of increased beta-sheet structure relative to that of human insulin were observed. Small angle x-ray scattering demonstrated the presence of strongly elongated structures with a repeated structural unit distanced by 34.8 Å, as indicated by a Bragg peak. The repeated distance originates from hexameric stacking and is close to the inter-hexameric distance also found in classical crystals of human insulin. In contrast, amyloid fibril structures are characterised by a Bragg peak of 4.7 Å and a predominant beta-sheet structure.
Conclusion: In conclusion, the IDeg multi-hexamers are composed of micrometer long linear arrays of hundreds of insulin hexamers. The ultra-long duration of action for IDeg relies on the formation of soluble multi-hexamers in subcutaneous tissue, and the protein fold adopted by insulin degludec in these multi-hexamers is very similar to that of human insulin.
Supported by: Novo Nordisk A/S

1035

Pharmacokinetics and pharmacodynamics of insulin glargine, changes in glucose and lipid metabolism after either evening or morning injection in type 2 diabetes mellitus

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Background and aims: We have already shown the differential pharmacokinetics and pharmacodynamics (PK/PD) of insulin glargine (GLA), when administered in T2DM at different time of the day.
Materials and methods: Here we report GLA effects on glucose (G) fluxes (deuterated G), lipid metabolism (plasma FFA, β-OH-butyrate) in 9 persons with T2DM (mean±SD: age 62±3.5 yrs; BMI 28±3.6 kg/m^2; A1C 7.2±1.1%, known T2DM duration 19±9.9 yrs), during a 24h euglycemic G clamp (cross-over study), with GLA given s.c. (0.4 U/kg), either at 10 AM or at 10 PM.

Results: Total G metabolism (GIR_AUC$_{0\text{-}24h}$) was similar (1012±731, 1058±606 mg/kg, p=0.654), but AUC$_{0\text{-}12h}$ was greater in AM vs PM (589±397 vs 358±259 mg/kg, p=0.010), whereas the opposite was observed for AUC$_{12\text{-}24h}$ (424±357 vs 700±420 mg/kg, AM and PM, p=0.005). PK of GLA and C-peptide levels did not differ in the two studies. The differential insulin activity in AM vs PM on G metabolism was explained by changes in endogenous G production (EGP) with greater suppression in initial 12 h and lower in second 12 h, when GLA was given at 10 AM; the opposite occurred whit GLA given at 10 PM (AUC$_{0\text{-}12h}$ 510±284 vs 744±384 mg/Kg, AM and PM, p=0.066). G utilization was not stimulated. Lipolysis was more suppressed with PM vs AM administration (FFA AUC$_{0\text{-}24h}$ 7.5±1.7 vs 9.2±1.7 mmol.h/L, p<0.001; β-OH AUC$_{0\text{-}24h}$ 6.8±5.0 vs 18±12 mmol.h/L, PM and AM, p<0.003).
Conclusion: PD of insulin GLA in T2DM is dependent on the time of daily injection. Evening administration results in greater GIR requirements in the second 12 h of day (afternoon), in contrast, to morning administration which displays a sensible reduction in metabolic activity from 12 h to 24 h after injection. GLA also suppressed lipolysis to a greater extend in PM vs AM administration. Since PK and C-peptide were similar in the 2 studies, the differential PD might reflect the contribution of diurnal fluctuation in insulin sensitivity (higher in afternoon vs early morning) inducing EGP changes

1036

Adipose tissue levels of human insulin and insulin detemir measured with open flow microperfusion (OFM) during constant intravenous infusion

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Background and aims: Human insulin and insulin detemir are easily measured in plasma, whereas sampling larger molecules (>50 kDa) directly in the tissue is difficult with conventional membrane-based techniques. In contrast to human insulin, the majority of insulin detemir is bound to albumin, resulting in a large molecular weight of 67 kDa. By using open flow microperfusion (OFM), a novel membrane-free sampling technique, we were able to compare the pharmacokinetics and pharmacodynamics of human insulin and insulin detemir in subcutaneous adipose tissue and plasma.
Materials and methods: Anaesthetized, diet-induced-obese Sprague Dawley rats were clamped to euglycemia (8 mM) by adjusting the glucose infusion rate (GIR) during constant intravenous insulin infusions. Human insulin was infused at 21 and 42 pmol/min/kg and insulin detemir at 209 and 417 pmol/min/kg to achieve equipotency. 3 OFM probes per rat were implanted in subcutaneous adipose tissue. OFM samples and arterial plasma samples were collected continuously and assayed for human insulin and insulin detemir with specific immunoassays.
Results: Steady-state GIRs were reached at similar times for both insulins. Steady-state concentrations of human insulin and insulin detemir were reached after 100 min in plasma and 200 and 300 min in tissue. At steady-state, GIR was ca. 50 and 90% of maximal GIR for human insulin and insulin detemir. GIRs correlated more strongly with tissue-insulin concentrations than plasma-insulin concentrations.
Conclusion: Our data show that OFM can be used to sample insulin in adipose tissue irrespective of the molecular weight of the insulin. By using OFM, we were able to show that compared to human insulin, insulin detemir appears considerably later in adipose tissue - probably due to its greater molecular size on albumin binding.
Supported by: Novo Nordisk

1037

Determination of glargine and the metabolites M1 and M2 by using a combination of insulin assays of different specificity

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Background and aims: Glargine is a long acting insulin analog, with a substitution of glycine for asparagine at A21 and two arginines added to the carboxy terminal of the insulin B chain. After injection, glargine is enzymatically transformed to its metabolites M1 and M2. The cross-reactivity of both glargine and its metabolites in insulin assays makes specific measurement of glargine a complex issue. The aim of this study was to find a method for determination of glargine and its metabolites along with endogenous insulin in human samples.
Materials and methods: We used two well-characterized, commercially available ELISAs to develop a method for determination of insulin, glargine and its metabolites in human samples. An alternative protocol was developed for one of the assays, where the cross-reactivity to glargine and its metabolites would be reduced, but endogenous insulin still detected - an "endogenous insulin ELISA". In the second assay, the cross reaction of glargine and its metabolites was determined - a "total insulin ELISA". The recovery of endogenous insulin, glargine, M1 and M2 was determined in human samples, using these two assays.
Results: The "endogenous insulin ELISA" was highly selective, with 100% cross reactivity to insulin, and non-detectable cross-reactivity to glargine, M1 and M2 within physiological insulin range. The "total insulin ELISA" showed a cross reactivity of 44% to glargine, 41% to M1 and 28% to M2. The recovery of endogenous insulin was 80 - 102 % using the "endogenous Insulin ELISA" in samples spiked with glargine, M1 or M2. Using the "total insulin ELISA", the recovery of glargine in spiked samples was 100 - 106%, the recovery of M1 was 91 - 102%, and M2 was 90-102%.
Conclusion: We conclude that measurement of insulin, glargine and its metabolites in human samples is possible by using two different insulin ELISAs with different specificity. The method described may be a valuable tool to determine glargine or its metabolites in patient samples.

PS 084 Insulin therapies: basal and novel combination

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Postprandial glycaemic control following a fixed-ratio combination of insulin degludec and liraglutide compared to each component individually in patients with type 2 diabetes

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Background and aims: Fasting and postprandial plasma glucose levels are key determinants of overall glycaemic control (HbA_{1c}). Here we investigate the complementary modes of action of insulin degludec (IDeg), an ultra-long-acting basal insulin, and liraglutide (Lira), a GLP-1 analogue, as the fixed-ratio combination IDegLira on postprandial glycaemic control following the 3 main meals of the day.
Materials and methods: In this 26-week randomised, open-label trial in patients with type 2 diabetes (T2D) inadequately controlled on metformin ± pioglitazone (n=1663; mean age: 55 yrs; diabetes duration: 6.6 yrs; HbA_{1c}: 8.3%; BMI: 31.2 kg/m^2), IDegLira was given once daily and compared to IDeg or Lira (1.8 mg) given alone. Postprandial control was assessed via 9-point self-measured plasma glucose (9P-SMPG) profiles and 72-h continuous glucose monitoring (CGM; conducted in a subgroup of patients [n=260]). Postprandial PG (PPG) increment (derived from 9P-SMPG) was calculated as the change in pre-meal levels (breakfast, lunch or dinner) to 90 min post meal. Postprandial increment in interstitial glucose was calculated as the area under the curve (AUC) above the pre-meal value from t=0 to 4 h ($iAUC_{0\text{-}4h}$).
Results: After 26 weeks, overall mean SMPG level of the 9-point profile had decreased to 7.1, 7.4 and 8.0 mmol/l with IDegLira, IDeg and Lira, respectively. The reduction from baseline in mean SMPG levels was significantly greater for IDegLira vs IDeg (3.2 vs 3.0 mmol/l; estimated treatment difference (ETD): -0.30 mmol/l [-0.50; -0.09], p=0.004), and for IDegLira vs Lira (3.2 vs 2.1 mmol/l; ETD: -0.93 mmol/l [-1.13; -0.73], p<0.0001). Pre-breakfast SMPG levels were similar for IDegLira and IDeg (5.8 vs 5.7 mmol/l) but higher in the Lira group (7.2 mmol/l). IDegLira reduced mean PPG increment over all main meals significantly more than IDeg (by 0.4 vs 0.2 mmol/l; ETD: -0.45 mmol/l [-0.63; -0.28], p<0.0001). A similar reduction was observed for IDegLira and Lira (ETD: 0.06 mmol/l [-0.11; 0.23], p=0.48). Similar results were obtained with CGM where $iAUC_{0\text{-}4h}$ (over all main meals) was significantly smaller with IDegLira vs IDeg (ETD: -0.34 mmol/l [-0.57; -0.11], p=0.0047) but similar for IDegLira and Lira (ETD: 0.10 mmol/l [-0.14; 0.33], p=0.41). Reductions in individual meal PPG increments were comparable for IDegLira and Lira. However, for each individual meal, significantly greater reductions in PPG increment (of similar magnitude) were observed for IDegLira vs IDeg. For CGM, $iAUC_{0\text{-}4h}$ was numerically lower at lunch and significantly lower at breakfast and dinner with IDegLira vs IDeg.
Conclusion: Once-daily IDegLira provides improved postprandial glycaemic control following all 3 main meals of the day (breakfast, lunch and dinner). For all meals, reductions in PPG increments were significantly greater than IDeg, whereas the effects of IDegLira and Lira were similar. The combined effects of IDeg and Lira on fasting and postprandial glucose levels results in a substantial overall improvement in glycaemic control as compared to each component individually.
Clinical Trial Registration Number: NCT01336023
Supported by: Novo Nordisk A/S

1039

A novel concentrated recombinant human insulin formulation with improved ultra-rapid prandial and similar basal absorption as insulin lispro protomine mixes

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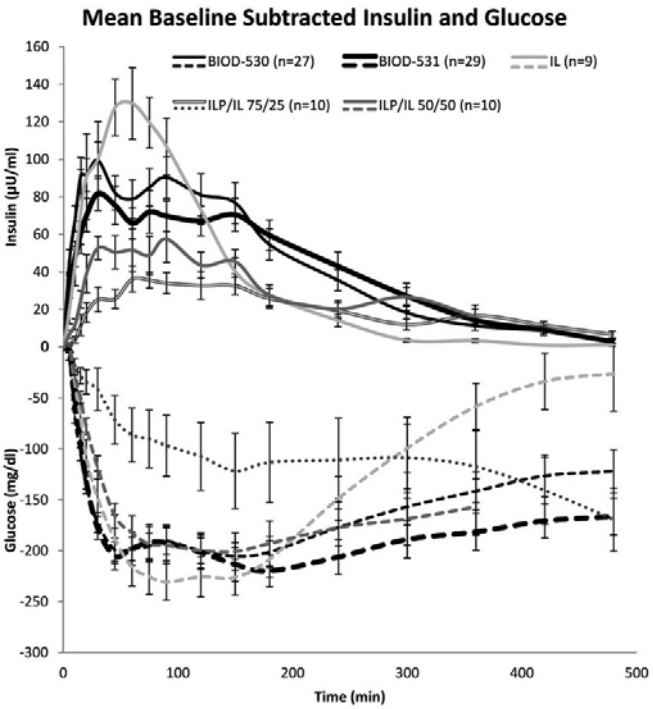
Background and aims: Formulations of U-100 recombinant human insulin (RHI) and insulin lispro (IL) containing EDTA and citrate show increased rates of absorption after subcutaneous (sc) injection in man compared to commercial formulations of RHI and IL. BIOD-530 and BIOD-531 are similar EDTA/citrate formulations with RHI concentrations of 400 U/ml. In previous studies in diabetic pigs, BIOD-530 had a significantly faster onset and similar duration of action to RHI U-500 which provides both prandial and basal coverage in type 2 diabetes patients requiring larger injection volumes. BIOD-531 was developed with $MgSO_4$ to mitigate EDTA related injection site discomfort. The aim of this study was to compare pharmacokinetic (PK) and pharmacodynamic (PD) profiles in diabetic miniature swine of BIOD-531 with U-100 formulations of mixtures of IL-Protamine (ILP) and IL 50/50 (ILP/IL 50/50) and 75/25 (ILP/IL 75/25) which also provide both prandial and basal coverage in patients with diabetes.
Materials and methods: Test formulations consisted of ILP/IL 75/25, ILP/IL 50/50 and BIOD-531. On the morning of each crossover study, miniature diabetic swine were given a sc dose (0.25 U/kg) of test formulation followed by a meal. Study 1 and Study 2 compared BIOD-531 with ILP/IL 75/25 and ILP/IL 50/50, respectively. Blood glucose and plasma insulin were sampled from -30 to 480 min post dose. Plasma insulin was measured by an ELISA method and glucose concentration determined by YSI. Time to half maximal concentration ($T_{50\%early}$) was calculated for each swine and averaged for each test article. Results of each study compared BIOD-531 to ILP/IL 75/25 or 50/50 mixtures using Students t-test.
Results: Concentration vs. time profiles are shown in the figure below; data for BIOD-530 and IL are included as a reference. Insulin concentration rose to higher peaks after BIOD-531 injection compared to either ILP/IL mixture. Despite rising to a higher peak, the absorption rates as reflected by $T_{50\%early}$ (min) and $AUC_{0\text{-}30min}$ (μU/ml*min) of BIOD-531 (Study 1: 25±5 and 1582±397; Study 2: 11±2 and 1475±134, respectively) were more rapid than ILP/IL 75/25 (35±6 and 351±82*, respectively) or ILP/IL 50/50 (23±3* and 777±128*, respectively) and resulted in a more rapid decline of glucose concentrations (*p<0.05 vs. BIOD-531). The duration of action as reflected by the percent total $AUC_{180\text{-}480min}$ was similar across the formulations: BIOD-531 (32-43%), ILP/IL 75/25 (45%) and ILP/IL 50/50 (35%). The glucose concentrations of all three formulations remained suppressed up to 480 min while insulin and glucose concentrations returned towards baseline levels around 240 min following IL administration.
Conclusion: BIOD-531 has a rapid onset of action comparable to IL and a similar basal control profile to ILP/IL pre-mixed insulins. BIOD-531 has the potential to deliver improved prandial and comparable basal coverage as pre-mixed insulins with lower injection volumes.



Clinical Trial Registration Number: NIH R43DK096604

1040

IDegAsp shows distinct prandial and basal glucose-lowering effects at steady state in subjects with type 1 diabetes

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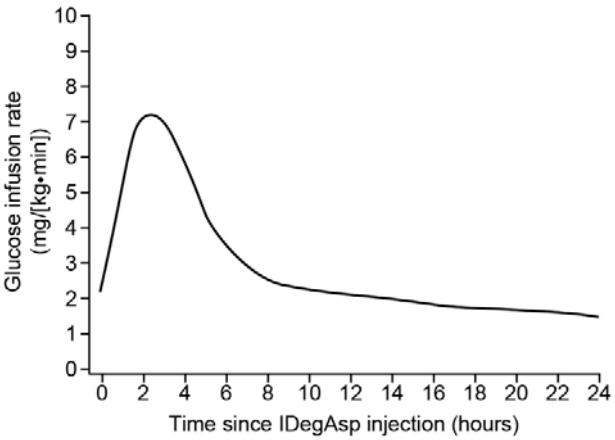
Background and aims: Insulin degludec/insulin aspart (IDegAsp) is a soluble co-formulation of 70% insulin degludec (IDeg) and 30% insulin aspart (IAsp) providing both ultra-long-acting basal coverage and a prandial insulin bolus in a single injection. The aim of this trial was to investigate the pharmacodynamic properties of IDegAsp at steady state in subjects with type 1 diabetes (T1DM).
Materials and methods: This was a single-centre, multiple-dose trial in subjects with T1DM. In order to achieve steady state of the basal component, subjects received IDeg once daily (0.42 U/kg) for 5 consecutive days. Separate bolus doses of IAsp were administered as needed for safety and glycaemic control. On Day 6 a 30-h euglycaemic clamp procedure was performed (Biostator, blood glucose target: 100 mg/dL) immediately following a single dose of 0.6 U/kg IDegAsp (comprising 0.42 U/kg IDeg and 0.18 U/kg IAsp).
Results: Twenty-two subjects (mean: age 40 years, duration of diabetes 23 years, BMI 24.6 kg/m^2, HbA_{1c} 7.9%) were exposed to treatment. The mean glucose infusion rate (GIR) profile showed a rapid onset of action and a distinct peak followed by a flat, prolonged basal action (Figure 1). The median time to maximum glucose-lowering effect of IDegAsp ($tGIR_{max}$) was 2.5 h. Duration of action (from dosing until blood glucose was consistently >150 mg/dL) extended beyond 30 h in all subjects.
Conclusion: In conclusion, at steady state the glucose-lowering effect of IDegAsp in subjects with T1DM was characterised by a distinct peak action due to IAsp, and a separate and stable basal action from IDeg sustained for >30 hours. This profile may constitute a clinical advance, allowing for the combination of specific meal coverage with full 24-h basal insulin coverage.

Figure 1: IDegAsp mean glucose infusion rate profile at steady state in subjects with type 1 diabetes



Clinical Trial Registration Number: NCT01590836
Supported by: Novo Nordisk A/S

1041

IDegAsp produces a dose-proportional glucose-lowering effect in subjects with type 1 diabetes

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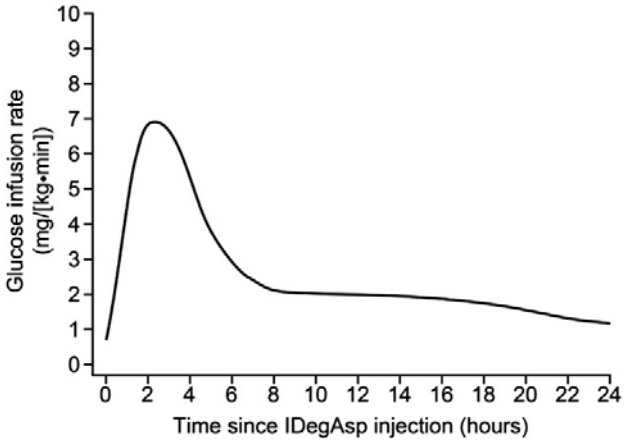
Background and aims: Insulin degludec/insulin aspart (IDegAsp) is a 70%/30% combination of insulin degludec (IDeg) and insulin aspart (IAsp), providing both an ultra-long-acting basal component and a rapid-acting bolus component in a single injection. The aim of this trial was to investigate the pharmacodynamic properties of three clinically relevant dose levels (0.4, 0.6 and 0.8 U/kg) following a single dose of IDegAsp in subjects with type 1 diabetes (T1DM).
Materials and methods: This was a double-blind, single-dose incomplete block crossover trial in subjects with T1DM. In order to measure glucose-lowering effect, a 26-h euglycaemic clamp procedure (Biostator, blood glucose target: 100 mg/dL) was performed immediately following each 0.4, 0.6 or 0.8 U/kg dose of IDegAsp.
Results: A total of 33 C-peptide negative subjects with T1DM (mean: age 39 years, duration of diabetes 17 years, BMI 25.1 kg/m^2, HbA_{1c} 8.0%) were included in this study. With increasing IDegAsp dose level, both the 24-hour area under the glucose infusion rate (GIR) curve (area under the curve $[AUC]_{GIR,0\text{-}24h,SD}$) and the maximum GIR ($GIR_{max,SD}$) increased significantly and proportionally. The estimated log dose-log $AUC_{GIR,0\text{-}24h,SD}$ (slope = 1.19, 95% CI: [0.99; 1.40] and log dose-log $GIR_{max,SD}$ (slope = 0.89 [0.66,1.13]) both supported dose proportionality as 1.0 was included in the 95% CI in each case. The 24-hour single-dose GIR profile of IDegAsp showed both a distinct peak action due to prandial IAsp and a separate and stable basal action from IDeg (Figure 1).
Conclusion: IDegAsp produces a proportional dose-response across three clinically relevant dose levels and a pharmacodynamic profile with separate prandial and basal components in subjects with T1DM.

Figure 1: Mean glucose infusion rate profile following a single dose of IDegAsp (0.8 U/kg) in subjects with type 1 diabetes



Clinical Trial Registration Number: NCT00993096
Supported: Novo Nordisk A/S

1042

Clinical efficacy and safety of insulin glargine as compared to other insulin preparations in the treatment of type 2 diabetes mellitus: a systematic review and meta-analysis

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Background and aims: Many patients with type 2 diabetes mellitus (T2DM) require insulin to achieve a proper glycemic control. Available systematic reviews have assessed only selected insulin preparations giving no definitive answer on whether insulin glargine (IGlar) provides additional clinical benefits to T2DM patients. To our knowledge this is the first systematic review combining all data for comparison of IGlar with other insulins enabling to make synthetic and reliable conclusions.
Materials and methods: The analysis was based on clinical trials, both randomized (RCTs) and nonrandomized (nRCTs), identified by a systematic literature search of medical databases (MEDLINE, EMBASE, CENTRAL and etc.) up to December 2012. Relevant studies compared IGlar, added to oral drugs (OAD) or in combination with bolus insulin, with human insulin (NPH) or insulin detemir (IDet) in the same regimen, as well as with premixed insulin (MIX). Results were presented as weighted mean difference (WMD), RR with a 95% CI.
Results: We found 29 relevant RCTs involving 7293 patients followed for 12-52 weeks. Pooled data from RCTs assessing insulin as add on to OAD showed that the probability of achieving target HbA1c level without any hypoglycemia was significantly higher with IGlar +OAD than with NPH + OAD (RR = 1.32 [1.09, 1.59]) or MIX without OAD (RR = 1.61 [1.22, 2.13]) and comparable to those observed for IDet+OAD (RR = 1.07 [0.87, 1,.33]) and MIX+OAD (RR = 1.09 [0.86, 1.38]). With respect to fasting glucose level IGlar+OAD showed significant advantage over NPH+OAD (WMD = -0.26 mmol/l [-0.32, -0.20]), MIX without OAD (WMD = 0.93 mmol/l [-1.39, -0.46]) and IDet+OAD (WMD = -0.30 mmol/l [-0.58, -0.02]). Symptomatic hypoglycemic episodes in patients on IGlar+OAD were observed with similar frequency as in those who were treated with IDet+OAD (WMD = -1.37 [-3.45, 0.71]), but were significantly lower in comparison with NPH+OAD (WMD = -2.32 [-4.14, -0.51]) and MIX with or without OAD (WMD = -5.80 [-8.72, -2.88]; WMD = -4.89 [-7.41, -2.36], respectively). Pooled data from RCTs assessing basal-bolus regimen revealed that the percentage of patients achieving target HbA1c was similar to NPH (RR = 1.14 [0.91, 1.44]) and significantly higher than MIX (RR = 1.26 [1.12, 1.42) or IDet (RR = 1.38 [1.11, 1.72]). The risk of severe hypoglycemia was lower in IGlar than in NPH (RR = 0.77 [0.63, 0.94]), with no statistically significant differences in comparison with MIX (RR = 0.74 [0.46, 1.20]) and IDet (RR = 1.10 [0.54, 2.25]). IGlar+OAD has comparable safety profile to NPH, with less frequent adverse events leading to treatment discontinuation than MIX+OAD (RR = 0.41 [0.22, 0.76]) and IDet+OAD (RR = 0.40 [0.24, 0.69]). Also severe adverse reaction were less common for IGlar+OAD when compared with MIX+OAD (RR = 0.71 [0.52; 0.98]). IGlar used together with bolus insulin revealed similar safety profile as other options. Supportive data from 21 nRCTs involving 26639 patients followed for up to 18 months confirmed that in a real-life setting IGlar is an effective option also for those patients in whom glycemic control remains inadequate despite human insulin treatment.
Conclusion: IGlar can provide better or comparable glycemic control with the same or even better safety profile as most of the alternative treatment options.
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Lower within-subject variability in mean blood glucose concentration with insulin degludec vs insulin glargine: a meta-analysis of patients with type 2 diabetes

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Background and aims: Insulin degludec (IDeg) is a new ultra-long-acting basal insulin shown to have low day-to-day variability in a euglycaemic clamp study. In this *post-hoc* meta-analysis of patients with type 2 diabetes (T2D), we compared day-to-day variability in mean self-measured blood glucose (derived from 9-point profiles; 9P-SMPG) between IDeg and insulin glargine (IGlar).
Materials and methods: This patient-level meta-analysis included all five phase 3a, randomised, open-label, treat-to-target trials (26 or 52 week) in which once-daily IDeg and IGlar have been compared. 9P-SMPG profiles comprised measurements made before and 90 min after the start of breakfast, lunch and main evening meal, before bedtime, at 4 AM, and before start of breakfast the next day. Within-subject variability (CV%) in the overall mean plasma glucose (PG) concentration of the 9P-SMPG profile (area under the profile) was estimated from profiles recorded at weeks 12, 16, and 26 (26-week trials) and weeks 12, 16, 26, 40, and 52 (52-week trials), using a linear mixed model.
Results: Estimated within-subject variability in mean 9P-SMPG was significantly lower by 7-10% for IDeg vs IGlar for patients on basal insulin plus OAD therapy, as well as the subset of previously insulin-naïve patients (Table).
Conclusion: In conclusion, IDeg is associated with significantly lower within-subject day-to-day variability in mean blood glucose concentration than IGlar in patients with T2D receiving basal insulin plus OAD therapy.

Population	Insulin degludec (IDeg)		Insulin glargine (IGlar)		IDeg/IGlar (ratio [95% CI])
	n	CV%	n	CV%	
T2D (BB)	692	16.0	236	16.3	0.98 [0.93; 1.04]
T2D (BOT)	1370	13.8	789	14.9	0.93 [0.89; 0.96]*
T2D (BOT-IN)	1162	13.8	581	15.2	0.90 [0.86; 0.94]*

n = number of subjects; CV% = coefficient of variation; T2D = Trials 3579, 3672, 3582, 3586 and 3668 (excluding IDeg flexible dosing group); BB = basal-bolus therapy (Trial 3582); BOT = basal insulin plus OAD therapy (Trials 3579, 3672, 3586, 3668 [excluding flexible dosing group]); BOT-IN = previously insulin-naive patients on BOT therapy (Trials 3579, 3762 and 3586); *p<0.05

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Superior FPG control and reduced hypoglycaemia with IDegAsp vs BIAsp 30 in adults with type 2 diabetes mellitus inadequately controlled on pre/self-mixed insulin: a randomised phase 3 trial

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Background and aims: Insulin degludec/insulin aspart (IDegAsp) is a soluble co-formulation of insulin degludec (70%) and insulin aspart (30%) that provides both an ultra-long-acting basal and a rapid acting prandial component in a single injection. This trial compared the efficacy and safety of

IDegAsp vs biphasic insulin aspart 30 (BIAsp 30) in adults with type 2 diabetes (TD2M) inadequately controlled with once daily or twice daily (BID) pre-mixed or self-mixed insulin regimens, with or without additional oral anti-diabetic drugs (OADs).

Materials and methods: In this 26-week randomised open-label multinational treat-to-target phase 3 trial, participants (mean: age 58.7 years, HbA_{1c} 8.4%, fasting plasma glucose [FPG] 8.7 mmol/L, BMI 29.3 kg/m^2) were randomised (1:1) to BID injections of IDegAsp (n=224) or BIAsp 30 (n=223) and ongoing OADs were permitted. Injections were administered with breakfast and the main evening meal and dose-titrated according to a pre-breakfast and pre-main evening meal self-measured plasma glucose target of 4-5 mmol/L.

Results: After 26 weeks, mean HbA_{1c} was 7.1% for both treatment groups and IDegAsp was within the prespecified non-inferiority margin for mean change in HbA_{1c} from baseline (primary endpoint; estimated treatment difference [ETD] -0.03%-points, 95% CI -0.18; 0.13). IDegAsp was superior in lowering FPG compared with BIAsp 30 (ETD -1.14 mmol/L, 95% CI -1.53; -0.76, p<0.001) by the end of the trial. Final mean daily insulin dose was 1.08 U/kg for IDegAsp and 1.20 U/kg for BIAsp 30 (estimated rate ratio [RR] 0.89, 95% CI 0.83; 0.96, p=0.002). Significantly fewer confirmed hypoglycaemia episodes (self-reported PG <3.1 mmol/L or severe episode requiring assistance) were reported for IDegAsp compared with BIAsp 30: 9.7 vs 14.0 events per year, respectively (estimated RR: 0.68, 95% CI 0.52; 0.89, p=0.0049). The rate of nocturnal confirmed hypoglycaemia episodes (onset 00.01-05.59, inclusive) was also significantly lower with IDegAsp compared to BIAsp 30 (0.7 vs 2.5 events per year, estimated RR 0.27, 95% CI 0.18; 0.41, p<0.0001); the rate of severe hypoglycaemia was numerically lower (0.09 vs 0.25 events per year, estimated RR 0.50, 95% CI 0.19; 1.30, p=ns). During the maintenance period (post-16 weeks' treatment) significantly lower overall rates of confirmed (estimated RR 0.61, 95% CI 0.45; 0.83, p=0.0015), nocturnal confirmed (estimated RR 0.23, 95% CI 0.13; 0.41, p<0.0001) and severe (estimated RR 0.11, 95% CI 0.01; 0.91, p=0.04) hypoglycaemia episodes were observed for IDegAsp vs BIAsp 30.

Conclusion: IDegAsp BID in T2DM effectively improves long-term glycaemic control measured by HbA_{1c} (non-inferior to BIAsp 30), and is superior to BIAsp 30 in lowering FPG. IDegAsp was also associated with significantly lower rates of overall- and nocturnal- confirmed hypoglycaemia episodes compared with BIAsp 30, especially during the maintenance period. These results indicate comparable overall glycaemic efficacy and a favourable safety profile compared with BIAsp 30 when switching from once- or twice-daily pre/self-mixed insulin to IDegAsp as part of an insulin intensification or dose optimisation regimen.

Clinical Trial Registration Number: NCT01009580

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Superior FPG control and less nocturnal hypoglycaemia with IDegAsp vs BIAsp 30 in Asian subjects poorly controlled on basal or pre/self-mixed insulin: randomised phase 3 trial

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Background and aims: Insulin degludec/insulin aspart (IDegAsp) is a soluble co-formulation of the novel basal insulin degludec (70%) and insulin aspart (30%) combining ultra-long-acting basal coverage and a rapid-acting bolus in a single injection. This trial investigated the efficacy and safety of IDegAsp and biphasic insulin aspart 30 (BIAsp 30) in Asian subjects with type 2 diabetes (T2DM) inadequately controlled on once or twice daily (BID) basal, premixed or self-mixed insulin, with or without metformin.

Materials and methods: In this pan-Asian (Hong Kong, Japan, Malaysia, South Korea, Taiwan) 26-week, randomised, open-label, treat-to-target phase 3 trial, participants (mean: age 59.8 y, HbA_{1c} 8.4%, fasting plasma glucose [FPG] 7.9 mmol/L, BMI 25.4 kg/m^2) were randomised (2:1) to BID injections of IDegAsp (n=282) or BIAsp 30 (n=142) plus continuing any previous metformin treatment. Both insulins were administered with breakfast and main evening meals and dose-titrated according to a pre-breakfast and pre-main evening meal self-measured plasma glucose target of 4-5 mmol/L.

Results: After 26 weeks, mean HbA_{1c} was 7.1% for IDegAsp and 7.0% for BIAsp 30; IDegAsp was non-inferior (predefined margin 0.4%) to BIAsp 30 for mean change in HbA_{1c} from baseline (primary endpoint) as expected in a treat-to-target trial (estimated treatment difference [ETD] IDegAsp-BIAsp 30: 0.05%-points, 95% CI -0.10; 0.20). IDegAsp was superior to BIAsp 30 in lowering FPG (ETD -1.06 mmol/L, 95% CI -1.43; -0.70, p<0.001), with levels reduced to 5.4 mmol/L and 6.5 mmol/L, respectively, by the end of the trial. The mean daily insulin dose after 26 weeks' treatment was 0.79 U/kg for IDegAsp and 0.99 U/kg for BIAsp 30 (estimated rate ratio [RR] 0.79, 95% CI 0.73; 0.85, p<0.0001). The rate of confirmed hypoglycaemia (self-reported PG <3.1 mmol/L or severe episode requiring assistance) was similar between IDegAsp and BIAsp 30 (9.6 episodes/y vs 9.5 episodes/y, estimated RR 1.00, 95% CI 0.76; 1.32, p=ns), while the rate of nocturnal confirmed hypoglycaemia (onset from 00.01 to 05.59) was numerically (33%) lower with IDegAsp (estimated RR 0.67, 95% CI 0.43; 1.06, p=ns). The rate of severe hypoglycaemia was not significantly different for IDegAsp and BIAsp (0.05 and 0.03 episodes/y, estimated RR 1.3, 95% CI 0.24; 7.03, p=ns). During the maintenance period (post-16 weeks' treatment) there was a trend towards lower overall confirmed (estimated RR 0.84, 95% CI 0.6; 1.19, p=ns), nocturnal confirmed (estimated RR 0.70, 95% CI 0.39; 1.26, p=ns) and severe (estimated RR 0.69, 95% CI 0.05; 9.8 p=ns) hypoglycaemia rates for IDegAsp vs BIAsp 30. There was no difference in observed adverse event rates between treatment groups.

Conclusion: IDegAsp dosed BID effectively improves long-term glycaemic control and provides superior FPG reductions with a trend towards fewer nocturnal confirmed hypoglycaemia episodes at approximately 20% lower final dose compared with BIAsp 30. These results indicate IDegAsp is a promising treatment option for Asian subjects with T2DM requiring both basal and prandial insulin coverage.

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PS 085 Hypoglycaemia in type 1 and type 2 diabetes

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Temporal variations in hypoglycaemia occurring in hospital in people with diabetes who are at risk of hypoglycaemia

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Background and aims: Hypoglycaemia is a major concern when tight glycaemic control is targeted amongst patients with diabetes in hospital. Our hospital is a medium sized acute district general hospital with approximately 550 inpatient beds. The aim of our study was to determine whether there were temporal patterns in hypoglycaemia episodes amongst patients with diabetes who are at risk of hypoglycaemia, whilst in hospital.
Materials and methods: All capillary blood glucose (CBG) readings in all hospitalised adult patients (≥16 years) measured on Precision Xceed Pro™ blood glucose meters, the only CBG measurement system across the entire hospital, were remotely captured over a two month period. Patients who did not have diabetes and those with diabetes on diet alone or single or combination of glucose lowering therapies not associated with hypoglycaemia, for e.g. metformin, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 analogues were excluded. Therefore, CBG measurements of only those at risk i.e. insulin and/or sulphonylurea treated patients were included in the analysis. We classified mild hypoglycaemia as CBG readings between 3.0 to 3.9mmol/l and severe as CBG readings <3mmol/l. CBG readings <4mmol/l within four hours of a previously documented hypoglycaemic episode were excluded since this could reflect re-testing following the previous hypoglycaemic episode.
Results: There were 15,844 CBG tests in the 'at risk' group, of which 8,549 tests were performed between 09:00 to 20:59 hours (daytime) and 7,295 tests between 21:00 and 08:59 hours (night time). There were 771 hypoglycaemic episodes, 4.9% of all tests. Nocturnal hypoglycaemia was significantly higher than daytime hypoglycaemia ($p<0.0001$); 542 nocturnal versus 229 during daytime. Both mild and severe hypoglycaemic episodes were significantly higher at night (338 and 204 respectively), 2.68 and 1.98 times more frequent than daytime hypoglycaemia (126 and 103 respectively), $p<0.0001$.
Conclusion: Hypoglycaemia occurred more frequently between 21:00 and 08:59 hours in patients with diabetes at risk of hypoglycaemia. We speculate that this could be due to inadequate carbohydrate intake during this period as the inter-meal timings between evening meal and next day breakfast in our hospital is between 12-14 hours and carbohydrate containing bedtime snacks are not always available.

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Frequency and characteristics of iatrogenic hypoglycaemia requiring medical assistance: a multicentre study of tertiary hospitals

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Background and aims: Hypoglycemia in diabetes usually results as a consequence of treatment, aiming at correction of hyperglycemia. Iatrogenic hypoglycemia is considered as a major factor contributing in morbidity and mortality of patients with diabetes. Aim of the present study was to examine the frequency, clinical characteristics and outcome of iatrogenic hypoglycemia requiring medical assistance.
Materials and methods: Eight hospitals (nine clinics) in five cities participated in this 22-month, prospective survey of documented iatrogenic hypoglycemia at the emergency departments (ED). The clinical characteristics of all patients were recorded at presentation and during hospitalization, if required. Patients were compared to a control group consisted of patients with diabetes, visiting the outpatient diabetes clinics of the same hospitals, during the same time period.
Results: During a median follow-up of 18 months, 295 episodes of iatrogenic hypoglycemia in 294 patients (146 men) were recorded. The frequency differed between centers and ranged between 0.25 and 0.78 cases per 100 patients. The majority (267 episodes, 90.5%) was patients with type 2 diabetes (T2D, mean age 76.8±10.0 years), while 24 (8.1%) episodes were recorded in 23 patients with type 1 diabetes (T1D, mean age 42.7±18.3 years). The mean plasma glucose value at presentation was 38.1±23.9 mg/dL. Hospitalization was required in 66.7% of patients (23.8% in T1D). Total in-hospital mortality was 4.4%, entirely regarding patients with T2D. Two deaths have been attributed to hypoglycemia itself and two others have been considered as its direct consequence (aspiration pneumonia). There were two documented fatal myocardial infarctions. Out of the 267 T2D patients, 113 (42.3%) had been on insulin therapy. Out of the 154 non-insulin treated T2D patients, 143 (94.1%) had been taking sulfonylureas (SU).60.5% of the patients had an e-GFR (aMDRD) < 60 ml/min. Compared to the control group, T2D patients with hypoglycemia were older, had longer disease duration, higher prevalence of serious comorbidities, lower eGFR and were more likely to receive treatment with SUs:
Conclusion: Hypoglycemia requiring medical assistance is a moderately frequent condition at the EDs of tertiary hospitals, with a mortality rate close to 5%. The majority of patients have T2D, they are elderly individuals suffering from serious medical conditions and in their great majority are treated with insulin or SUs.

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Diabetic autonomic neuropathy predicts severe hypoglycaemia in patients with type 2 diabetes mellitus: a ten-year follow up study

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Background and aims: We investigated factors that might influence the development of severe hypoglycaemia (SH) in patients with type 2 diabetes
Materials and methods: From 2000 to 2002, an autonomic function test (AFT) was performed on 955 patients with type 2 diabetes aged 25-75 years and these patients were followed-up in 2011 and 2012. SH was defined as hypoglycaemic episodes requiring hospitalization or medical care in an emergency department. The severity of AFT was classified using the AFT score. We used the Cox proportional hazard regression analysis to test associations between the SH episodes and potential explanatory variables
Results: The median follow-up time was 9.5 years. At baseline, the total study population consisted of 404 (60.4%) women, the mean age of the total study population was 56.0 ± 10.0 years and a mean duration of diabetes was 9.1 ± 6.4 years. The incidence of severe hypoglycaemia was 1.54 per 100 patient-years. The patients with severe hypoglycaemia were more frequently female, were older, had a longer duration of diabetes, and received more insulin and ACE inhibitor treatment. Poor glycaemic control, renal impairment, and diabetic microvascular complications also were more present in the group with severe hypoglycaemia at baseline. The Cox hazard regression analysis revealed that the development of SH was associated with an abnormal AFT score (normal autonomic function vs. mild autonomic dysfunction, hazard ratio 2.41, $P = 0.005$; normal autonomic function vs. moderate to severe autonomic dysfunction, HR 4.33, $P < 0.001$) in a univariate analysis. After adjusting for gender, age, duration of diabetes, estimated glomerular filtration rate, HbA_{1c}, treatment of insulin and ACE inhibitor/ARB, severe autonomic failure predicts the development of severe hypoglycaemia (normal autonomic function vs. moderate to severe autonomic dysfunction, HR 2.43, $P = 0.004$), and a higher AFT score tends to have a higher risk of the development of severe hypoglycaemia (P for trend = 0.015).
Conclusion: The development of SH was independently associated with autonomic dysfunction in patients with type 2 diabetes.

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Behaviours and impact of hypoglycaemia in type 2 diabetes mellitus

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Background and aims: To examine the behaviors and impact of hypoglycemia on health-related quality of life (HRQoL) in diabetes patients.
Materials and methods: Patients were adults with type 2 diabetes mellitus (T2DM) on antidiabetic treatment from a cross-sectional, epidemiological study conducted in Cyprus (HYPO-Cyprus). Demographic and clinical data were collected. Patients completed the Hypoglycemic Perspectives Questionnaire (HPQ), Audit of Diabetes Dependent Quality of Life (ADDQoL-19), treatment satisfaction questionnaire, and EuroQol-5 Dimensions (EQ-5D). HPQ assessed symptoms, behaviors, and impact of hypoglycemia on T2DM patients in 3 domains (Symptoms, Compensatory Behaviors [CB], Worry) and a single-item of global symptom awareness. Analyses examined the relationship between HPQ and other patient-reported and clinical outcomes.
Results: The sample was 500 T2DM patients with a mean age of 61±10 years; 32.6% were women. Nearly half were obese (45.6% BMI≥30). Average duration of diabetes was 11 years (±7.8); half of patients had uncontrolled diabetes (50.6% HbA1c≥7%). Better diabetes HRQoL was associated with needing fewer compensatory behaviors (r=-0.30) and having less worry (r=-0.34; p<0.001). Fewer CB (r=-0.12) and less worry (r=-0.16) also corresponded to higher overall health ratings (p<0.01). More symptoms, CB, and worry were related to lower patient satisfaction with their current medication (p<0.001) and more desire to change medications (p<0.05). A small association was identified between higher symptom awareness (r=0.21) with higher HbA1c level (p<0.01). Patients with one or more low blood sugar events in the past 7 days and those with at least one severe event in the past year engaged in more CB and had higher symptom awareness than those with no events (all p<0.001).
Conclusion: Results suggest that hypoglycemia behaviors and impact are related to HRQoL, treatment satisfaction, and recent hypoglycemia events for T2DM patients. The impact of hypoglycemic events should not be underestimated.
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Physical health status and nocturnal hypoglycaemia with insulin degludec vs insulin glargine: a 2-year trial in insulin-naïve patients with type 2 diabetes

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Background and aims: Insulin degludec is a new basal insulin with an ultra-long and stable glucose-lowering effect. We compared once-daily insulin degludec and insulin glargine (randomised 3:1) both in combination with metformin ± DPP-4 inhibitors in an open-label, treat-to-target trial of 2 years duration in patients with type 2 diabetes (T2D).
Materials and methods: Health status was assessed at baseline and 105 weeks using the Short Form 36 (SF-36 v2) questionnaire. SF-36 scores were analysed (intention-to-treat population) using ANOVA, with adjustments for relevant covariates. After an initial 1-year study period, insulin degludec improved overall SF-36 physical health and functioning compared with insulin glargine. This was followed by a 1-year extension in which subjects maintained their randomised treatment assignment.
Results: Of 1,030 subjects, 725 entered the extension and 659 (insulin degludec: 505; insulin glargine: 154) completed 2 years of treatment. At 105 weeks, insulin degludec was similar to insulin glargine with respect to change in HbA_{1c} from baseline, but was associated with a significantly greater reduction in fasting plasma glucose (FPG) (-0.38 mmol/L [95% CI: -0.70; -0.06], p=0.02). Rates of overall confirmed hypoglycaemia (PG <3.1 mmol/L, or severe) were similar between groups, whereas the rate of nocturnal confirmed hypoglycaemia (any episode between midnight and 6:00AM) was significantly lower (by 43%, p<0.01) for insulin degludec. Consistent with previously reported findings for the initial 1-year trial period, the overall physical component score was significantly better with insulin degludec vs insulin glargine after 2-years (treatment contrast (TC): 1.1 [0.1; 2.1], p<0.05). This was largely due to significantly better physical functioning (TC: 1.1 [0.0; 2.3], p<0.05) and bodily pain sub-domain scores (TC: 1.5 [0.2; 2.9], p<0.05). Consistent with 1-year data, other SF-36 domain scores showed no significant differences between groups.
Conclusion: In conclusion, a significantly better physical health status and lower risk of nocturnal hypoglycaemia is maintained for insulin degludec vs insulin glargine following the second year of treatment of insulin-naïve patients with T2D.
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Achieving fasting plasma glucose target without nocturnal hypoglycaemia: a pooled analysis of studies in type 2 diabetes comparing insulin degludec vs insulin glargine

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Background and aims: Insulin degludec (IDeg), a new basal insulin that forms soluble multi-hexamers after subcutaneous injection, has an ultra-long and stable glucose-lowering effect. These properties may lead to less nocturnal hypoglycaemia compared with other basal insulin analogues, and should allow more patients to reach fasting plasma glucose (FPG) target safely.
Materials and methods: In this pooled analysis, we investigated the proportion of patients with type 2 diabetes (T2D) achieving laboratory-measured target FPG (<5 mmol/L) without nocturnal confirmed hypoglycaemia in four open-label, randomised, treat-to-target trials where patients (n=2380) received either IDeg or insulin glargine (IGlar), both once-daily in combination with OADs, for 26 or 52 weeks. Confirmed hypoglycaemia was defined as PG <3.1 mmol/L or severe episodes requiring assistance, and nocturnal confirmed hypoglycaemia defined as any episode between midnight and 6:00AM.
Results: A greater proportion of patients achieved the FPG target, and fewer patients experienced nocturnal confirmed hypoglycaemia with IDeg than with IGlar (table). The chance of achieving FPG target without confirmed nocturnal hypoglycaemia was 82% higher with IDeg: estimated odds ratio IDeg/IGlar = 1.82 $[1.49; 2.22]_{95\%CI}$.
Conclusion: In conclusion, T2D patients are more likely to reach FPG target without nocturnal confirmed hypoglycaemia with IDeg than with IGlar. These findings may have important implications for achieving target levels of glycaemic control in clinical practice.

Trial ID; N FAS IDeg/IGlar	Number (%) meeting FPG target (<5 mmol/L)			Number (%) without nocturnal confirmed hypoglycaemia		Number (%) meeting FPG target without nocturnal confirmed hypoglycaemia		*Statistical Analysis Est. odds ratio*
		IDeg	IGlar	IDeg	IGlar	IDeg	IGlar	*IDeg/IGlar*
3579; 773/257	302 (39.1)	62 (24.1)	667 (86.3)	218 (84.8)	254 (32.9)	50 (19.5)		
3672; 228/229	86 (37.7)	66 (28.8)	214 (93.9)	209 (91.3)	79 (34.6)	56 (24.5)		*1.82*
3586; 289/146	124 (42.9)	53 (36.3)	231 (79.9)	111 (76.0)	100 (34.6)	40 (27.4)		*[1.49;2.22]95%CI*
3668; 228/230	109 (47.8)	72 (31.3)	204 (89.5)	181 (78.7)	97 (42.5)	59 (25.7)		
Total;1518/862	621 (40.9)	253 (29.4)	1316 (86.7)	719 (83.4)	530 (34.9)	205 (23.8)		

The endpoint was analysed using a logistic regression model with logit link. The model included treatment, sex, trial, antidiabetic treatment at screening, and region as fixed factors, and age and baseline FPG as covariates. Subjects were insulin-naïve in trials 3579, 3672, 3586. Trial 3668 included both insulin-naïve and non-naïve subjects. In trial 3668, subjects in the IDeg flexible-dosing group were excluded. N FAS = number of subjects in the full analysis set. In all trials, subjects were treated with basal insulin once daily + OADs.

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Higher rates of confirmed hypoglycaemia are associated with greater within-subject variability in fasting blood glucose in type 1 and type 2 diabetes: a meta-analysis

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Background and aims: In this *post-hoc* meta-analysis of insulin-treated patients with type 1 (T1D) and type 2 diabetes (T2D), we investigated whether there is an association between hypoglycaemia rate and the extent to which pre-breakfast blood glucose varies from day to day.
Materials and methods: This patient-level meta-analysis included all open-label, randomised, treat-to-target phase 3a trials (26 or 52 weeks) in which once-daily insulin degludec (IDeg) was compared to insulin glargine (IGlar) in T1D (2 trials) and T2D (5 trials). Within-subject variability (CV%) in self-measured pre-breakfast plasma glucose (PG) was determined from three consecutive daily measurements made in the final week (week 26 or 52) of each trial. CV% was compared between patients with a rate of confirmed hypoglycaemia (PG <3.1 mmol/L, or severe) within the upper quartile (top 25%) and those outside this range (bottom 75%) using a linear mixed model, which allows for heterogeneity in the residual variance for each group.
Results: For both IDeg and IGlar, estimated day-to-day variability in pre-breakfast PG was significantly higher for patients with the highest rates of confirmed hypoglycaemia (top 25%) than those with lower rates (bottom 75%), regardless of diabetes type or T2D subpopulation (Table).
Conclusion: In conclusion, for both IDeg and IGlar, higher rates of confirmed hypoglycaemia are associated with greater within-subject variability in fasting blood glucose in patients with T1D and T2D.

	Insulin degludec (IDeg)			Insulin glargine (IGlar)		
Population	Top 25% (n [CV%])	Bottom 75% (n [CV%])	Top 25%/Bottom 75% (ratio [95% CI])	Top 25% (n [CV%])	Bottom 75% (n [CV%])	Top 25%/Bottom 75% (ratio [95% CI])
T1D	158 [47.9]	478 [36.1]	1.33 [1.20; 1.46]*	80 [48.0]	239 [38.5]	1.25 [1.07; 1.42]*
T2D	565 [24.2]	1692 [16.1]	1.50 [1.43; 1.58]*	277 [24.3]	830 [16.6]	1.46 [1.36; 1.56]*
T2D (BOT)	379 [20.5]	1136 [15.4]	1.33 [1.25; 1.41]*	215 [22.5]	645 [15.4]	1.46 [1.34; 1.57]*
T2D (BB)	186 [29.6]	556 [18.0]	1.65 [1.50; 1.79]*	62 [31.8]	185 [19.2]	1.65 [1.40; 1.91]*
T2D (BOT-IN)	322 [20.1]	965 [15.1]	1.33 [1.24; 1.42]*	158 [22.9]	472 [15.3]	1.49 [1.35; 1.63]*

n = number of patients; CV%=coefficient of variation; T1D = Trials 3583 and 3770 (excluding flexible dosing group); T2D = Trials 3579, 3672, 3582, 3586 and 3668 (excluding IDeg flexible dosing group); BOT = basal insulin plus OAD therapy (Trials 3579, 3672, 3586, 3668 [excluding flexible dosing group]); BB = basal-bolus therapy (Trial 3582); BOT-IN = previously insulin-naive patients on BOT therapy (Trials 3579, 3762 and 3586); *p<0.05

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Recurrent severe hypoglycaemia in type 1 diabetes: potential for prevention?

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Background and aim: Recurrent severe hypoglycaemia (SH) in type 1 diabetes is a major clinical problem affecting up to 20% of patients in unselected cohorts. While solitary episodes of severe hypoglycaemia are hardly preventable, recurrent episodes are potentially subject to prevention. This study was aimed at characterising recurrent episodes of severe hypoglycaemia in order to explore the potential for prevention of such events in a well-defined cohort of patients with type 1 diabetes.
Material and methods: A cohort of 230 consecutive patients with type 1 diabetes was followed prospectively for one year and reported characteristics of SH within 24 hours. Characteristics of recurrent episodes were explored and compared to those of solitary episodes.
Results: A total of 239 episodes of SH were reported by 86 patients during the period, corresponding to 1.0 episode per patient per year; 82% of the episodes were recurrent (corresponding to 2 or more episodes per patient). The incidence of coma and seizure did not differ between solitary (21%) and recurrent (23%) SH. Recurrent SH occurred more often in subjects with reduced hypoglycaemia awareness (solitary: 65% vs. recurrent: 88%; p<0.005) and were characterised by absence of warning symptoms (solitary: 42% vs. recurrent: 66%; p<0.005) and occurrence during daytime (07-18, solitary: 26% vs. recurrent: 42%; p=0.042). The majority of episodes of SH took place at home (solitary: 65%; recurrent: 71%) and only few at work (solitary: 2%; recurrent: 8%) or elsewhere (solitary: 30%; recurrent: 19%) with no differences between solitary and recurrent episodes. Recurrent episodes tended to be less well explained (33% vs. 46%; p=0.11).
Conclusion: A large proportion of SH in patients with type 1 diabetes is recurrent and thereby potentially preventable. Focus should be directed towards patients with reduced hypoglycaemia awareness and measures include efforts to restore hypoglycaemia awareness, improvement of self-care including frequent scheduled carbohydrate ingestion and intensified blood glucose self-monitoring.

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Gender differences in personality traits and resilience concerning glycaemic control and hypoglycaemia in patients with type 1 and type 2 diabetes

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Aim: Structured therapy and education programs for patients with Type 1 (T1D) and Type 2 diabetes (T2D) have been shown to improve HbA1c and to reduce rates of hypoglycaemia. But not all patients succeed to reach their treatment goals after participating in such a program. We assessed personality traits and resilience to explain differences in therapeutic outcome with regard to gender differences.
Methods: We conducted a cross-sectional study of patients with T1D and T2D in a tertiary care centre in Germany during a period of three months (n=661, 161 T1D: 94 men / 67 women, 50.32 / 54.05 years, duration of diabetes (DD) 20.53 / 19.39 years, social economic status (SES) 13.22 / 11.91, HbA1c 7.4 / 7.64%, 500 T2D: 298 men / 202 women, 67.2 / 67.8 years, DD 15.4 / 15.5 years, SES 12.26 / 10.75, HbA1c 7.3 / 7.5%). We assessed the short version of the Big Five Inventory (BFI-S) with extraversion (EX), agreeableness (AG), conscientiousness (CO), neuroticism (NE) and openness (OP), resilience scale (RS-13), treatment satisfaction (DTSQ standard) and SES (score 3-15). Quality of diabetes care was measured by HbA1c, BP, BMI and rates of serious and severe hypoglycaemia (glucose or glucagon injection).
Results: In men with T1D OP explains 4.7% of the variance in severe hypoglycaemia/year. Men with more hypoglycaemic events have lower scores in OP and RES (R=-.262), p<.05. HbA1c is explained in 17.2% by a model of DTSQ (R=-.219), age (R=-.253) and EX in men and in 8.7% by OP (R=-.308) in women, p<.05. In women higher HbA1c is related to lower SES (R=-.269, p<.05) and lower mean HbA1c is accompanied by higher scores in OP (R=-.339, p<.01). Men with higher RES have lower scores in NE (R=-.35), less severe hypoglycaemia (R=-.262), higher scores in OP (R=.247), CO (R=.354) and SES (R=.25). NE and OP explain 22% of the variance in RES, p<.01. Women show higher RES having higher scores in CO (R=.30) explaining 9.6% of occurring differences and show lower scores in NE (R=-.326), p<.01. In men with T2D higher scores in EX mean less serious hypoglycaemic events compared to first visit, R=-.163 vs. .185, p<.01. In regression analysis EX explains 4.5% of the rates of serious hypoglycaemic events and 9.8% of the rates at first visit in a model with insulin dose adjustment (R=.273), p<.01. NE explains 7.6% of the variance in HbA1c in women, p<.01. Higher scores in HbA1c are associated with lower scores in CO, RES and DTSQ and higher scores in NE (R=.149), p<.05. In men variance in RES is explained in 19.9% by a model of NE (R=-.289), CO (R=.277) and OP (R=.28), in women in 23.3% by NE (R=-.335), SES (R=.293) and CO (R=.277), p<.01. In both T1D and T2D women show no significant findings concerning hypoglycaemia.
Conclusion: Out of five personality traits openness and extraversion can explain differences in metabolic control and the risk of serious and severe hypoglycaemia in men with diabetes mellitus.

PS 086 Insulin adjustment and self-management

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Evaluation of a real-time carbohydrate counting course during a clinic setting in the management of diabetes type 1

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Background and aims: DAFNE is a carbohydrate counting course used in patients with DM1. It is delivered over a week and effective provision of the program is difficult with staff curtailments in our current economy, and patients unable to commit a full week . Published results from Dafne are also disappointing. There was no evidence from the Irish Dafne group to show durable effect, although rates of hypoglycemia were lower. Recent Danish studies have suggested that one can deliver a modified Dafne program, within the remit of a clinic setting.
Materials and methods: Our study aimed to (1) examine the feasibilility of delivering a carbohydrate counting' (CC) program in the setting of a routine diabetes clinic , (2) evaluate the use of CC in managing DM1 and (3) compare outcomes with a control group not using CC . 20 patients with DM1 were randomly selected and assigned to one of 2 groups (CC or a dietary lecture). All had baseline HbA1c, lipids and BMI, recorded. All completed a Berger QOL questionnaire, and were specifically asked about hypogylcemia . They were all re-assessed after 24 weeks.The CC group attended a 3 hour workshop and met with the physician so that their insulin sensitivity index and carbohydrate ratios could be individually calculated. They had been asked to bring their own 'usual foods'. They also brought their own delphware (for more precise portion estimation), and were taught CC by the diabetes nurse. The control group attended a 2 hour lecture delivered by a dietician.The CC course was easily delivered in a clinic setting. It became clear however, that while patients were motivated, they found it laborious measuring food portions, and making calculations . Continuous engagement was problematic.
Results: At 24 weeks, HbA1c in the CC group increased, $p < 0.01$. It remained unchanged in the control group ($p = 0.944$). Independent T-Test showed that the CC intervention was not more effective ($p = 0.834$).There was a non- significant trend towards a reduction in hypoglycaemic events in the CC group ($p = 0.51$) . There was no diference between both groups.There was a non- significant difference in QOL between both groups, with the CC group reporting less anxiety about hypoglycemia. Interestingly, the CC group had poorer self reported QOL, (24.79 Vs 13.78). This may have hampered their ability to engage with interventions. Paired samples T test showed no significant difference in BMI in the CC patients, p value = 0.863, nor in the control group , p value = 0.536. Independent T-test confirmed this. 80% of the patients in CC group had a reduction in LDL, that was not statistically significant, p=0.253. 50% of patients in the control group had an increase in LDL. Independent T test confirmed no significant change in LDL (p=0.508). In summary, It is feasible to deliver this program. Patient selection is important. Enthusiasm does not equal success, and ongoing motivation is a limitation. Our results did not show improvement in A1c and the numbers were to small to show significant difference in hypoglycemia rates but the trend matches published data. CC success may not lie in primarily reducing HbA1c which hitherto has been the 'holy grail', but in reducing hypoglycemia (and improving safety) and improving QOL.
Conclusion: These qualitative results are additionally concordant with the recent position statements for management of DM.

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Which principles patients with diabetes type 1 use for self-adjustment of insulin dose?

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Background and aims: Self-adjustment of the insulin dose to different blood glucose levels, carbohydrate intake, exercise or illness is a core part of intensified insulin therapy an a prerequisite for the prevention acute and chronic complications. Structured treatment and education programmes for patients with type 1 diabetes provide rules for self-adjustment of insulin, which are extensively trained. The aim of this study was to register the patients' current principles for self-adjustment of the insulin dose and to check the ability for correct adjustment.
Materials and methods: In a tertiary care centre 117 patients with diabetes type 1 were included (mean HbA1c 7.9%, age 53 years, diabetes duration 23.6 years, BMI 27.7kg/m2) from 01.08 to 31.10.2012. The number of daily insulin dose adjustments was drawn from the last 28 days of the patients' diary. The type of insulin adjustment, insulin-to-carbohydrate ratio and the factor of correction were assessed by a structured interview as well as patients ability to find the correct dose in five structured case studies. All patients had participated in a structured education programme of 20 hours, 46% of them within the last 3 years.
Results: 103 patients (89%) are documenting their therapy in a diary regularly. The average self adjustment of the insulin dose was 2.6±1.3 per day. About half of all patients (51%) claimed to adjust their insulin dose using a rule (correction factor or correction scheme). The rest adapt their insulin dose based upon personal experience or feeling. There were no differences concerning HbA1c (7.8±0.9 vs. 7.9±1.0 p=0.56) or mild hypoglycaemia/ week (1.4±2.0 vs. 1.5±1.4 p=0.91) or severe hypoglycaemia/last 12 months (0.04±0.2 vs. 0.14±0.4 p=0.56) comparing patients using rules or not. 89 patients (76.7%) were able to give their meal-related insulin-to-carbohydrate ratio and 76 (65.5%) knew the associated correction factor/scheme. In case of high premeal blood glucose 26 patients (22%) reported to perform an injection-meal interval "always" or "often", 36 patients (31%) "sometimes" or "rarely" and 53 patients (46%) "never". Good ability to solve the case examples (4 or 5 of 5) showed 59 patients (49%) and 14 patients (12%) had moderate ability (1 to 3 of 5) and 44 (38%) poor ability (0 of 5 correct examples).
Conclusion: Although all patients participated in a structured treatment and education programme at least once, only about 49% were able to answer 4 or 5 of 5 case examples correctly. Patients, who make their daily adjustments of the insulin dose based upon feeling or personal experience did not show worse metabolic control. Teaching sophisticated rules for self-adjustment of insulin dose could be exaggerating for a considerable number of patients.

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Carbohydrate counting competency index (C31) is a clinically meaningful measure of carbohydrate counting skills in patients with diabetes: results from ABACUS

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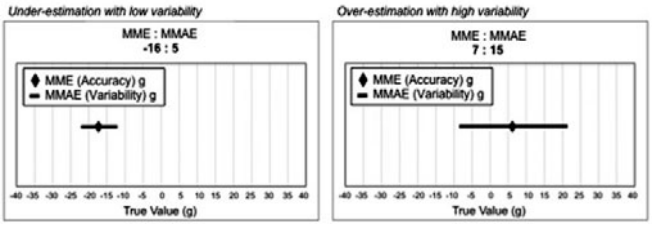
Background and aims: Carbohydrate (CHO) counting is a core skill for patients with diabetes who wish to practice effective meal-bolus insulin therapy. While the principles are straightforward, applying this skill reliably over diverse meals is challenging, and clinicians' ability to identify patients who have difficulties with CHO counting is often suboptimal. We developed a metric to assess and compare CHO skills in patients enrolled in the Automated Bolus Advisor Control and Usability Study (ABACUS).
Materials and methods: The ABACUS study was a large, 26-week, prospective, randomized, controlled, multi-national study of suboptimally controlled patients treated with multiple daily insulin injection (MDI) therapy, with mean (SD) baseline HbA1c 8.9 (1.2)%. At entry and study end, all patients were asked to provide CHO estimates for 10 photographic standard meal plates (DAFNE) of known CHO content, from which two scores were calculated.
Results: We assessed study patients' CHO counting competency based on two scores: mean meal error (MME) and mean meal absolute error (MMAE). The MME score is an indicator of accuracy in estimating the CHO content of a given meal, with "0" being a perfect score. The MMAE score was an indicator of variability, indicating the average range (number of CHO grams) above and below patients' MME; the lower the number, the less variability. The scores were combined into a single metric: CHO Counting Competency index (C3i). The C3i can be expressed numerically (MME:MMAE) or visually plotted on a scale as MME ± MMAE. Figure 1 presents examples of plotted C3i assessments.
Conclusion: The C3i metric provides information that allows clinicians to identify and address CHO counting deficits and then evaluate changes in

competency over time. The metric can also be applied in clinical research for comparison purposes, providing a standardized measure of average CHO competency within study groups at baseline and study end.

Figure 1. Examples of C3i assessments



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Use of an automated bolus advisor may improve carbohydrate counting competence in patients treated with multiple daily insulin injection therapy: results from ABACUS

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Background and aims: Use of multiple daily insulin injection (MDI) therapy is efficacious in achieving and maintaining optimal glycemic control. MDI therapy requires patients to appropriately utilize a number of parameters, including insulin-to-carbohydrate ratios (I:CHO), insulin sensitivity factors (ISF), target blood glucose (bG) range and current bG values, to determine their appropriate insulin dosages. Although correct use of these factors is essential for accurate bolus insulin calculations, patients' inability to accurately and consistently estimate planned CHO intake can adversely impact glycemic control in patients treated with MDI. We assessed the impact of using an automated bolus advisor on patients' CHO counting competence.
Materials and methods: We assessed the CHO counting competency of 218 diabetes patients who participated in the Automated Bolus Advisor Control and Usability Study (ABACUS), a large, 26-week, multi-national, prospective, randomized, controlled trial. The trial evaluated the use of an automated bolus advisor in suboptimally controlled MDI-treated type 1 diabetes and type 2 diabetes patients with baseline HbA1c 8.9 (1.2)%. Patients randomized to the control group (CNL) used a standard bG meter and manual bolus calculation; whereas, patients randomized to the intervention group (EXP) used the Accu-Chek® Aviva Expert meter, which provides an integrated automated bolus advisor to calculate insulin dosages. At entry and study end, patients were asked to assess the CHO content (grams) of 10 standardized meals (using life-size photographs), from which two scores were calculated. The mean meal error (MME) score was an indicator of accuracy, with "0" being a perfect score. The mean meal absolute error (MMAE) score was an indicator of variability, indicating the average range (number of CHO grams) above and below patients' MME. All patients then received refresher training and CHO reference materials prior to randomization. Patients were followed for 6 months, with regular reviews by their healthcare team. The CHO counting assessment was repeated at the end of the study.
Results: At study end, there was a significant improvement in MMAE: from 15.2 (9.0) grams to 12.4 (7.3) grams, $p < 0.01$; and a trend towards improvement in MME: 1.0 (10.1) grams to 0.3 (7.1) grams, p = NS in the EXP group but no change in MME or MMAE among CNL patients.
Conclusion: Our findings suggest that use of a bolus advisor may improve competency in CHO counting by providing frequent feedback on patient accuracy in estimating the CHO intake. Thus, automated bolus advisors may serve as educational tools that can improve the effectiveness of diabetes self-management in patients treated with MDI therapy.
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Use of an automated bolus advisor improves multiple outcomes in patients treated with multiple daily insulin injections: results from ABACUS

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Background and aims: Calculating bolus insulin dosages is challenging for many people with diabetes who use multiple daily injection (MDI) therapy. These calculations must take into account carbohydrate (CHO) intake, current blood glucose level, insulin sensitivity and insulin-to-carbohydrate ratios. This requires complex mathematics and, as a result, many patients rely on empirical estimates, which may increase the risk of hypoglycemia or hyperglycemia.
Materials and methods: The Automated Bolus Advisor Control and Utility Study (ABACUS) was a large, prospective, 26-week, randomized, multi-national study of patients with type 1 and type 2 diabetes who were treated with MDI therapy but maintained suboptimal diabetes control (HbA1c ≥ 7.5% / 58 mmol/l). The study enrolled 218 diabetes patients (202 type 1, 16 type 2), with mean (SD) baseline HbA1c 8.9 (1.2)%, age 42.4 (14.0) years, BMI 26.5 (4.2) kg/m^2 and 17.7 (11.1) years duration of diabetes. Patients randomized to the control group (CNL) used a standard blood glucose meter and manual bolus calculation; whereas, patients randomized to the intervention group (EXP) used the Accu-Chek® Aviva Expert meter, which provides an integrated automated bolus advisor to calculate bolus insulin dosages. Blood glucose measurements in both groups were downloaded during study visits and used to amend bolus parameters where appropriate and to record the frequency of hypoglycemic values. Frequency of bolus advice sought was recorded from the Expert meters; bolus calculations by CNL patients were reviewed from three 3-day intensive monitoring periods during the study. HbA1c, CHO counting accuracy and scores from the Diabetes Treatment Satisfaction Questionnaire (DTSQ) were recorded at the beginning and end of the study. The goal of the ABACUS trial was to achieve HbA1c reductions of >0.5% in all patients.
Results: Significantly more EXP than CNL patients achieved HbA1c reduction of >0.5% (56.0% vs. 34.4%, $p<0.01$); this difference was greater in patients aged <30 years (52.6% vs. 15.0%, $p<0.05$). The mean (SD) HbA1c reduction among all patients who achieved the study goal was 1.2 (0.5)%. Frequency of reported hypoglycemia and of severe hypoglycemia from self-monitored glucose measurements was similar in both groups during the study. EXP patients significantly improved their CHO-counting ability and experienced a significantly better improvement in DTSQ score by study end. EXP patients sought bolus advice for 73.5% of their average 3.8 daily boluses; whereas, CNL patients performed an average of 4.0 bolus calculations per day, of which up to 75% were incorrect. EXP and CNL patients performed similar frequency of blood glucose measurements per day at baseline and over the course of the study.
Conclusion: Use of the Accu-Check Aviva Expert meter supports insulin-treated patients in improving glycemic control, while improving CHO-counting ability, with no increase in hypoglycemia, insulin usage or blood glucose testing frequency.
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Evaluating the patient experience in the Asian Treat to Target Lantus Study (ATLAS): a 24 week randomised, multinational study

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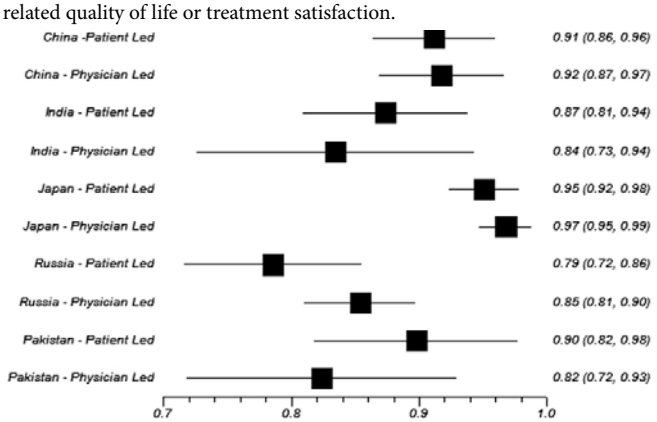
Background and aims: Approach to dose titration in patients initiating basal insulin, whether led by the physician or the patient themselves, may impact

diabetes management overall, particularly in terms of treatment satisfaction and quality of life. The ATLAS study was designed to evaluate titration of insulin glargine in insulin-naïve, Asian patients who were uncontrolled on 2 oral antidiabetic drugs. This analysis aimed to examine treatment satisfaction and quality of life in these patients, based on physician- or patient-led insulin titration.

Materials and methods: A total of 552 patients were randomised into patient-led (n=275) and physician-led (n=277) insulin titration. Insulin dose was adjusted using identical algorithms to achieve a target FBG of 110mg/dL.

Results: The ATLAS study demonstrated superiority of patient-led compared with physician-led insulin titration. The overall scores for the Diabetes Treatment Satisfaction Questionnaire status (DTSQs) and change (DTSQc) were similar in the 2 groups. For the patient-led titration group, the LS mean change in DTSQs score from baseline to 24 weeks was significant (mean Δ: 5.34; 95% CI: 4.48-6.20, p<0.001); the same was shown for the physician-led titration group (mean Δ: 5.05, 95% CI: 4.21-5.90, p<0.001). LS mean change in DTSQc score from baseline to 24 weeks was also significant for the the patient-led (mean Δ: 12.92; 95% CI: 12.32-13.52, p<0.001) and physician-led (mean Δ: 13.19; 95% CI: 12.60-13.78, p<0.001) titration groups. Between-group differences were not significant, and there were no differences in any DTSQc item. Health utility elicited with the EuroQol EQ-5D was high at baseline, despite the need for insulin initiation. Baseline mean utility was 0.86 (95% CI: 0.83-0.88) and 0.88 (95% CI: 0.85-0.90) for the patient-led and physician-led groups, respectively. At 24 weeks, the LS mean change from baseline in the EuroQoL-5D was not significant for either the patient-led (mean Δ: 0.021, 95% CI: -0.006-0.047, p=NS) or the physician-led (mean Δ: 0.018; 95% CI: -0-008-0.045, p=NS) titration group; between-group differences were not significant. Baseline EuroQoL-5D scores were similar between country-randomised groups, but different between countries. Russian patients had the lowest health utility (mean: 0.73, 95% CI: 0.68-0.78), while Japanese patients had the highest (mean: 0.94, 95% CI: 0.91-0.96). No systematic differences between country-randomised groups existed in the end of study utility.

Conclusion: The ATLAS results confirm that Asian patients initiating basal insulin with a self-titration plan may do so without compromising health-related quality of life or treatment satisfaction.



Clinical Trial Registration Number: 01169818
Supported by: Sanofi

1061

A Smartphone for adjustment of basal insulin dose and for coaching: benefits in terms of glycaemic control for type 2 diabetes patients

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Background and aims: The introduction of basal insulin is recommended in type 2 diabetes (T2D) when HbA1c exceeds 7-7.5%, despite well-titrated oral agents. Nevertheless, the insulin dose is often too low, chiefly due to fear of hypoglycemia.

Materials and methods: We have developed a smartphone modeled on the DIABEO system specifically for use by T2D patients. This system was tested in telediab 2 study, a large multicentre National Study that compared the use of the smartphone vs the use of the interactive voice server (IVS) and vs standard care. The smartphone incorporates algorithms that should help patients achieve their optimal insulin dose. Similar algorithms were used for the interactive voice server (IVS) utilized as a control. In addition, the smartphone includes educational coaching functions that allow patients to act on their blood glucose values where daytime BG values fall outside the target range. It also transmits data to the physician to enable remote telemonitoring. Patients were randomized to 3 groups (G1: control group: 1 face-to-face consultation every 3 months; G2: interactive voice server (IVS) group: IVS with phone consultations until M4 and then 1 face-to-face consultation every 3 months until M13; G3: smartphone group with 1 phone consultation every 2 weeks until M4, followed by 1 phone consultation per month until M13. The main evaluation criterion comprised HbA1c values at M4 in G2 and G3 vs. G1.

Results: This 13-month, open-label, parallel-group, multicenter study included 190 adult patients (64% male) with T2D (>1 year), BMI 29.5±4.9 Kg/m^2, age 58.7±9.6 years, diabetes duration 13.1±7.6 years, and HbA1c 8.9±1.1% despite oral agents (>6 months). The 4-month reduction in HbA1c was greater in G2 (-1.44%) and G3 (-1.48%) than in G1 (-0.92%) (p=0.0017 for G2 vs. G1 and G3 vs. G1 comparisons), but did not differ between the intervention groups. Basal insulin detemir was initiated at a mean dosage of 11.7±6.2 U/d and subsequently titrated. The insulin dosage was 35±24 (G1), 44±35 (G2) and 50±35 (G3) U/d. The percentage number of patients with normal fasting blood glucose levels was much higher in G2 and G3 than in G1 (82.5% and 82.7% vs. 41.8%, p<0.001 for both comparisons). No severe hypoglycemia occurred and the incidence of mild hypoglycemia was similar in all groups during the initial 4-month period.

Conclusion: At 4 months, the reduction in A1c was greater both with the IVS and the PDA than with standard care in T2D patients with poor glycemic control. This result appears to be due to more active titration of basal insulin without any increase in hypoglycemia. The potential benefits of the smartphone (i.e. coaching functions, telemonitoring allowing teleconsultations) will be assessed during the study extension phase (smartphone vs. standard care from M4 to M13) and presented during the session.

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Determinants of insulin dosing decisions made by diabetes care providers: a vignette study

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Background and aims: Many patients with type 2 diabetes require insulin to maintain adequate glycaemic control. Many options exist for implementing insulin therapy and little is known about strategies that are followed by care providers in practice. The objective of this study was to explore the decision making behavior of care providers regarding insulin dose adjustments.

Materials and methods: In the light of current guidelines and research, we postulated that certain patient characteristics (level of FPG and HbA1c, occurrence of hypoglycaemic events, demographics, medical history and insulin dose) influence the decision making behavior of care providers with regard to insulin dose adjustments. We constructed nine narrative vignettes presenting a fixed set of characteristics of patients using basal insulin. To test the effect of each characteristic, each vignette described one additional characteristic, which varied among two versions of an otherwise identical vignette. Each respondent received one set of nine vignettes. For each vignette respondents were asked to indicate whether they would advise to change the basal insulin dose, and if so, by how many units. 520 paper questionnaires were distributed among general practitioners, diabetes nurses in secondary care, nurse practitioners in primary care and internists. Multivariate linear and logistic regression analyses were performed.

Results: Of all distributed questionnaires, 190 (37%) were returned. Each additional 1 mmol/L FPG and each additional 1% HbA1c resulted in an average increment in insulin dose of respectively 1.28 IU and 1.25 IU. The occurrence of a hypoglycaemic event led to a decrement of 1.19 IU. In case of severe compared to mild hypoglycaemic events, care providers were nearly five times (odds ratio [OR] 4.77; 95% confidence interval [CI] 1.65 to 13.75) more likely to decrease the insulin dose. Also, care providers were almost eight times (OR 7.88; 95% CI 2.96 to 20.99) more likely to increase the insulin dose if a patient's FPG was between 5.5 and 7.0 mmol/L, than if a patient's FPG was

below 5.5 mmol/L. When the current insulin dose was low (30 IU) compared to a high insulin dose (90 IU), care providers were more than six times (OR 6.38; 95% CI 3.04 to 13.37) more likely to increase the insulin dose. There were no differences in insulin dose (OR 1.76; 95% CI 0.73 to 4.29) if a patient experienced symptoms of hypoglycaemia and measured plasma glucose (PG) > 3.9 mmol/l compared to PG ≤ 3.9 mmol/l. A history of cardiovascular disease did not trigger care providers to strive for less intensive glucose lowering (OR 1.17; 95% CI 0.56 to 2.43).

Conclusion: A substantial number of care providers strive for FPG target of 5.5 mmol/L, probably reflecting implementation of treat-to-target algorithms. Relative hypoglycaemic events and high insulin doses were barriers for intensifying insulin dose, although this has no scientific foundation. In contrast, a patient history of myocardial infarction did not restrain care providers from increasing the dose, although the ACCORD study suggests less intensive treatment among patients with known vascular disease. We recommend that titration guidelines should mention cut-off values for hypoglycaemia and give recommendations on coping with high insulin doses. Furthermore, awareness for the risk of intensive treatment in heart disease patients should be increased.

PS 087 Diabetes self care and adherence

1063

Asian Treat to Target Lantus Study (ATLAS): a 24 week randomised, multinational study

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Background and aims: Self-adjustment of insulin dose and patient empowerment have been effective in achieving better glucose control. The ATLAS study evaluated titration of insulin glargine in insulin-naïve Asian patients uncontrolled on 2 oral antidiabetic drugs.

Materials and methods: A total of 552 patients were randomised to 2 titration arms: 275 patient-led, 277 physician-led. Insulin dose was adjusted using identical algorithms to achieve a target FBG of 110mg/dl in both arms. The primary objective was non-inferiority of change in HbA1c at 24 weeks from baseline.

Results: Baseline demographics and HbA1c were similar in both groups. At 12 weeks, LS mean change in HbA1c was significantly decreased in both the patient-led (-1.27%) and physician-led (-1.16%) titration groups compared with baseline (both $p < 0.001$). At 24 weeks, HbA1c decreased further in both groups (-1.40% and -1.25%, respectively) from baseline (both $p < 0.001$). The between-group difference of the LS mean change from baseline (patient-led minus physician-led) was -0.15 (95% CI: -0.29 to -0.00; p=0.04), showing superiority. Proportions of patients with HbA1c < 7.0% without severe hypoglycaemia were not significantly different between the patient-led and physician-led titration groups (32.0% versus 26.0%, respectively; $p = 0.11$). Hypoglycaemia rates were low. Compared to physician-led titration, severe hypoglycaemia was similar, but nocturnal (p=0.002) and symptomatic hypoglycaemia (p=0.02) were higher in the patient-led titration group. Mean ± SD insulin daily dose was 8.2 ± 2.7 U at baseline and 24.4 ± 16.5 U at week 24, with a greater increase in the patient-led titration group ($p < 0.001$). Few unrelated serious adverse events (2.6%) were reported.

Conclusion: We conclude that insulin glargine titration, whether patient or physician-led, is safe and effective in achieving near-target glucose control in Asian patients uncontrolled on oral antidiabetic drugs. Contrary to common belief, Asian patients titrated up their insulin dose effectively when guided, similar to patients in the West. ´

	Patient-led Titration N = 275	Physician-led Titration N = 277	Overall N = 552
Demographics and baseline characteristics			
Age (years)	57.0 (8.67)	57.4 (8.45)	57.2 (8.55)
Gender - Male (%)	52.0	51.3	51.6
Diabetes duration (years)	10.3 (6.93)	9.1 (5.29)	9.7 (6.19
Duration of OAD treatment (years)	8.2 (6.18)	7.0 (4.91)*	7.6 (5.60)
BMI (kg/m^2)	27.5 (4.69)	27.3 (4.69)	27.4 (4.69)
Waist circumference (cm)	94.3 (11.99)	94.0 (11.12)	94.1 (11.55)
HbA1c (%)	8.70 (1.04)	8.76 (1.05)	8.73 (1.04)
FBG (mg/dL)	162.5 (39.0)	162.2 (36.7)	162.4 (37.8)
PPG (mg/dL)	220.9 (52.0)	221.0 (51.7)	221.0 (51.8)
	* p=0.01 for between-groups comparison		
Efficacy			
HbA1c at study end (Week 24)			
Mean (SD)	7.32 (0.87)	7.49 (0.98)	7.41 (0.93)
Adjusted LS mean change from baseline (95% CI)	-1.40 (-1.55, -1.25)	-1.25 (-1.40, -1.10)	
p-value	< 0.001	< 0.001	
Proportion (%) of patient achieving HbA1c < 7.0% without severe hypoglycaemia at study end (Week 24)	32.0	26.0	29.0
	p=0.12		
Hypoglycaemia			
Severe hypoglycaemia (%)	0.7	0.7	0.7
	p=0.98		
Nocturnal hypoglycaemia (%)	16.4	6.5	11.4
	p=0.002		
Symptomatic hypoglycaemia (%)	36.0	25.6	30.8
	p=0.02		
Insulin dose (units/day)			
Baseline insulin dose	8.2 (2.75)	8.1 (2.70)	8.2 (2.72)
Study end insulin dose - adjusted LS mean at study end (95% CI)	28.9 (27.14, 30.70)	22.2 (20.47, 24.02)	
p-value compared with baseline	< 0.001	< 0.001	
Between-group difference - adjusted LS mean insulin dose at study end (95% CI)	6.67 (5.03, 8.31)		
p-value	< 0.001		
Above results are presented as mean (SD) unless otherwise indicated			

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1064

The SimpleMix study with biphasic insulin aspart 30: a comparison between subject-driven titration vs investigator-driven titration

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Background and aims: Facilitating self-management of diabetes can help patients improve glycaemic control. SimpleMix was a 20-week, randomised, open-labelled, two-arm, parallel-group comparison of twice-daily subject- vs. investigator-driven (SD vs. ID) titration of biphasic insulin aspart (BIAsp) 30 in subjects with type 2 diabetes inadequately controlled with basal insulin analogues. The primary objective was demonstration of noninferiority of SD vs. ID titration of twice-daily BIAsp 30 assessed by change in HbA_{1c}.
Materials and methods: Subjects (n=348) were recruited from five countries (Argentina, China, India, Poland and the UK) and randomised 1:1 to treatment groups. BIAsp 30 was administered as two equal daily doses, before breakfast and dinner, (+metformin). BIAsp 30 starting dose was subject's previous basal insulin analogue dose split into two equal doses. The SD arm had five clinic visits and the ID arm had eight clinic visits. Phone contact could be made following changes to insulin dose and at any other time deemed necessary. No inferiority of SD vs. ID titration, assessed by HbA_{1c} change, was determined using a 0.4% noninferiority limit.
Results: Baseline data were comparable between titration groups (Table). During the first 12 weeks of treatment, HbA_{1c} reduction was similar between groups. By week 20, mean HbA_{1c} had decreased to 7.57 % in the SD titration group and 7.32 % in the ID group (difference 0.25%, 95% CI [0.04;0.46]). At the end of the trial (EOT) the proportion of patients achieving HbA_{1c} <7 % was 28.7% (SD) vs. 38.5% (ID), (OR 0.59, 95% CI [0.37;0.95]; p=0.032). For patients achieving HbA_{1c} ≤6.5 % at EOT the proportion was 12.1% vs. 20.7%, respectively (OR 0.50 [0.27;0.91]; p=0.022). Mean fasting plasma glucose (FPG) was similar in both groups (Table). Rate of hypoglycaemic episodes was slightly higher in the ID than the SD group (Table), but this was not statistically significant. Significantly higher weight gain was observed in the SD vs. ID group after 20 weeks (difference 0.68 kg, 95% CI [0.03;1.32]; p=0.041). Few serious adverse events were reported.

Table. Baseline characteristics and clinical outcomes at 20 weeks.

	Subject-driven	Investigator-driven
Demographic data, Mean (SD)		
Age, years	58.9 (9.8)	58.0 (9.5)
Female/Male, %	47.7/52.3	50.0 (50.0)
Duration of diabetes, years	9.3 (5.8)	10.6 (6.8)
Baseline, Mean (SD)		
HbA_{1c}, %	8.3 (0.9)	8.3 (0.9)
FPG, mmol/L	9.1 (2.7)	8.8 (2.8)
Weight, kg	81.0 (16.2)	77.9 (14.9)
Study outcome data*		
HbA_{1c}, % *		
Change at Week 12	−0.71 (0.07)	−0.82 (0.07)
Change at Week 20	−0.70 (0.08)	−0.97 (0.08)
Week 20 (LOCF)	7.57 (0.08)	7.32 (0.08)
FPG, mmol/L (LOCF) *		
Week 20	8.04 (0.21)	7.91 (0.22)
Change from baseline	−0.94 (0.21)	−1.07 (0.22)
Weight, kg (LOCF) *		
Week 20	82.6 (16.7)	78.6 (15.4)
Change from baseline	1.57 (0.24)	0.9 (0.25)
Mean dose, U/kg/day		
Week 1	0.37	0.37
Week 20	0.69	0.69
Hypoglycaemia, episodes/subject-exposure/year		
All episodes	9.59	12.08
Minor or severe episodes	3.90	4.47
Nocturnal episodes	0.80	1.09
*LS-Means (SE)		

Conclusion: Noninferiority of SD vs. ID titration with regards to change from baseline to EOT HbA_{1c} could not be confirmed. SD titration was as effective as ID titration in reducing FPG. There was no statistically significant difference between the groups in occurrence of overall, minor + severe or nocturnal hypoglycaemic episodes. 12-week HbA_{1c} results suggest a clinic visit ~12 weeks after BIAsp 30 initiation or intensification may benefit subjects' SD titration results. Overall, treatment with twice-daily BIAsp 30 was effective, safe and well tolerated.
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1065

Glycaemic control and quality of life: Is there any relationship in young patients with type 1 diabetes?

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Background and aims: To analyse the relationship between quality of life (QoL), emotional state and glycemic control in young patients with type 1 diabetes (T1D).
Materials and methods: We enrolled 140 T1D patients aged of 18-28 years (m/f - 46/94, mean age 22,6±3,2 years, duration of diabetes 12,3±5,6 years, HbA1c level 9,4±2,2%). Patients were evaluated by The Audit of Diabetes Dependent Quality of Life (ADDQoL), The Short Form (36) Health Survey (SF-36), The Well-Being Questionnaire, 12- Item (WB-Q12), The State-Trait Anxiety Inventory (STAI) (the Spielberger's scale), The Centre for Epidemiologic Studies - Depression scale (CES-D), The Hospital anxiety and depression scale (HADS). Statistical analysis: Spearman correlation tests.
Results: Patients with better glycemic control had a higher QoL. HbA1c level had a negative correlation with 3 scales of SF-36: bodily pain (rs=-0.341, p<0.001), social role functioning (rs=-0.211, p<0.05), physical role functioning (rs=-0.207, p<0.05) and with 6 scales of ADDQoL: generic QoL (rs=-0.184, p<0.05), impact of diabetes on QoL (rs=-0.296, p<0.001), physical appearance (rs=-0.198, p<0.05), motivation (rs=-0.175, p<0.05), finances (rs=-0.206, p<0.05), living conditions (rs=-0.191, p<0.05). Better glycemic control was also associated with better emotional state. There was a correlation between HbA1c level and anxiety scale of HADS (rs=0.209, p<0.05), state and trait anxiety scales of STAI (rs=0.184 and 0.208 respectively, p<0.05), level depression according to CES-D (rs=0.183, p<0.05) and negative well-being scale of WB-Q12 (rs=0.230, p<0.01).
Conclusion: There is a relationship of glycemic control with QoL and emotional state in young T1D patients. Better glycemic control is associated with less bodily pain, better social role functioning and physical role functioning, according to SF-36, better satisfaction of physical appearance, motivation, finances, living conditions, according to ADDQoL, and lower level of anxiety and depression, according to HADS, STAI and CES-D.

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Factors affecting non-adherence to drug, diet and physical activity advices among type 1 diabetic subjects

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Background and aims: Nonadherence to drug, diet and physical activity advices has been identified as a major problem in the management of type 1 diabetes mellitus (T1DM). Factors for nonadherence among diabetic subjects vary from population to population due to difference in lifestyle, culture, knowledge and beliefs. No study has yet been conducted regarding nonadherence to drug, diet and physical activity advices among T1DM patients in Pakistan. The current study was designed to determine the proportion as well as factors affecting nonadherence to these advices among T1DM subjects attending a tertiary care hospital in Karachi.
Materials and methods: An observational analytic study was conducted among 194 T1DM subjects [Age in yrs mean (±SD), 17.9± (6.4)]. Data were collected through pre-tested semi-structured questionnaire and face to face interview with respondents. Information on sociodemographic and clinical

characteristics, health care delivery system, and proportion of nonadherence were collected. Drug, diet and physical activity history were collected by recall method and a standardized scoring system, based on adherence to respective advices by the educators, were developed. For drug advices a subject was marked as 'Nonadherent' if s/he did not attain 100% of the adherence score. For dietary and physical activity advices, the cut-off value for 'Nonadherence' was 60%. Data were analyzed by univariate as well as multivariate statistics.
Results: The proportion of nonadherence was found to be high for drug (88.1%) and dietary (58.5%) advices; on the other hand it was relatively low (42.3%) for physical activity advices. On logistic regression analysis, factors which were found to have significant effect on drug nonadherence were: briefing of prescription other than respondent's own language by prescriber (p=0.04), negative family history for type 2 diabetes mellitus (p=0.04) and low maternal education (p=0.04). Factors significantly associated with dietary nonadherence were less attendance to diabetic clinic (p=0.05) and diabetic education programs (p=0.01), and large family size (p=0.01). Fear of hypoglycemia (p=0.05), respondent's low level of knowledge regarding their diabetes (p=0.03), maternal occupation status as housewife (p=0.02) and high monthly family expenditures (p=0.03) were the determinants which significantly contributed to nonadherence with their physical activity advices.
Conclusion: An alarmingly high proportion of Pakistani T1DM subjects are nonadherent to drug and dietary advices and a substantial proportion of them are also nonadherent to physical activity advices. The major factors for these nonadherences are communication barrier between patients and physicians, negative family history of T2DM, less apprehension for their disease, behavioral characteristics regarding their treatment, lack of parental support, poor maternal education status, large family size, high monthly expenses, and fear of hypoglycemia. These factors need to be specifically addressed in the management plan of T1DM subjects.
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Empowerment and motivation affects HbA_{1c} in subjects with low medical adherence and poor diabetes control

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Background and aims: Studies have shown that only around 50% of medications prescribed for the long-term treatment of chronic diseases are used as prescribed. Poor medical adherence (MA) contributes to suboptimal control and poor clinical outcomes. Moreover, very little knowledge of appropriate interventions exists. The aim of our pilot-study is to use new methods for individual empowerment to treat patients with type 2 diabetes and poor MA including poor glycaemic control. The end-points are change in HbA_{1c} at the end of the intervention in addition to six months later and medical possession rate during these 6 months.
Materials and methods: For all patients with HbA_{1c} of 63.9 mmol/mol or above from a single outpatient clinic, data from the Danish drug prescription database (Medicine Profile), were used to assess MA for oral hypoglycaemic agents, acetylsalicylic acid, lipid lowering drugs and liraglutide during the year 2010. A consultation program was developed and offered to 2/3 of patients with MA below 80%, the remaining 1/3 was used as a control group. The intervention consisted of three consultations and a phone call, and focused on facilitating empowerment, enhancing motivation and providing systematic, goal-oriented support, using tools to enhance dialogue, participation and informed patient decisions.
Results: We calculated MA for 172 patients, 105 (61%) had MA below 80% for at least one of the aforementioned drugs. Patients participating in other studies or not self-sufficient were excluded, thus 89 eligible patients remained. Altogether, 34 patients did not accept to participate and 11 patients died or migrated before or during the study and were excluded, resulting in 22 participants and 22 controls. At baseline the participants median HbA_{1c} was 69.5 (63-77) compared to 71.5 (62-80) mmol/mol in the control group (p=0.60). The participants showed a median decline in HbA_{1c} of 2 (IQ range -1 to 3) during the study compared to an increase of 2.5 (-2 to 4.5) mmol/mol (p=0.041) in the control group. The median decline from start to end of follow-up was 4 (-6 to 8) versus 0 (-8 to 7) mmol/mol for participants and controls respectively (p=0.45). Improvement in HbA_{1c} from start to end of intervention was seen in 64% of participants and 35% of controls (p=0.12), whereas from start to end of follow-up HbA_{1c} improved for 64% of participants compared to 48% of controls (p=0.36). At baseline participants were noncompliant for a mean of 1.3 (0.8) prescriptions whereas the same number was 1.4 (1.0) at follow up. The corresponding numbers for the control group were 1.6 (0.7) and 1.5 (1.0). There were no significant differences in mean change when the groups were compared (p=0.70). As were there no significant changes in total MA (p=0.95), or in mean MA for hypoglycaemic drugs (p=0.51).
Conclusion: In our pilot study, the intervention resulted in a decrease in HbA_{1c} of approximately 3 mmol/mol during the study period, with sustained effect 6 months after the intervention. When compared with the control group the differences were significant only at the former point in time. The intervention did not show improvement in medical possession rates for selected peroral drugs and liraglutide. This is in accordance with the patient's own goals that were more focused on improving glycaemia than MA. However, many participants improved their insulin routine which is not reflected in the estimated MA.

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Early utilisation of daily SMBG in insulin treated type 2 diabetes patients is associated with greater improvement in glycaemic control: results from the COMPASS study

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Background and aims: While self-monitoring of blood glucose (SMBG) is generally deemed essential in the management of insulin-treated type 2 diabetes (IT T2DM), not many IT T2DM patients in China perform SMBG on a daily basis. In the COMPASS study, we evaluated the effect of introducing a recommended SMBG regimen on glycemic control and SMBG behaviour in IT T2DM patients. The introduction of the SMBG regimen resulted in a substantial increase in daily SMBG utilization among the patients observed. In this sub-analysis, we examined the association between timing of daily SMBG utilization and glycemic control in poorly controlled IT T2DM patients in China.
Materials and methods: The COMPASS study is a multi-centre, observational study. Daily SMBG was introduced according to a recommended SMBG regimen. 820 patients who had been on insulin for at least 3 months and had poor glycemic control (HbA1c>8%) were followed up for 6 months. Patients were trained on how to individually respond to their SMBG readings by lifestyle changes and self-adjustment of insulin doses. Glycemic control and therapy regimen were assessed during quarterly physician visits. Retrospectively, we grouped the 820 patients into 4 quartiles, first according to duration of T2DM (<4.12 yrs; 4.12-9.19 yrs; 9.20-13.81 yrs; >13.81 yrs) and then according to duration of insulin therapy (<8.87 mths; 8.87-30.25 mths; 30.26-66.60 mths; >66.60 mths). We then compared glycemic control between the groups according to duration of T2DM and duration of insulin therapy respectively.
Results: Mean (SD) baseline HbA1c according to duration of T2DM (<4.12 yrs; 4.12-9.19 yrs; 9.20-13.81 yrs; >13.81 yrs) were 10.38(2.00)%, 9.51(1.30)%, 9.56(1.41)% and 9.38(1.18)%, respectively. Mean (SD) baseline HbA1c according to duration of insulin therapy (<8.87 mths; 8.87-30.25 mths; 30.26-66.60 mths; >66.60 mths) were 10.33(1.86)%, 9.53(1.43)%, 9.62(1.60)% and 9.37(1.06)%, respectively. According to the duration of T2DM, the mean (SD) HbA1c changes from baseline were -2.92(2.47)% (p<0.0001), -1.50(1.49) % (p<0.0001), -1.57(1.53)% (p<0.0001) and -1.28(1.42)% (p<0.0001) respectively at month 3 and -2.82(2.59)% (p<0.0001), -1.41(1.50)% (p<0.0001), -1.56(1.45)% (p<0.0001) and -1.24(1.54)% (p<0.0001) respectively at month 6. According to the duration of insulin therapy, the mean (SD) HbA1c changes from baseline were -2.93(2.38)% (p<0.0001), -1.70(1.60)% (p<0.0001), -1.55(1.57)% (p<0.0001) and -1.03(1.23)% (p<0.0001) respectively at month 3 and -2.88(2.39)% (p<0.0001), -1.54(1.68)% (p<0.0001), -1.47(1.58)% (p<0.0001) and -1.10(1.36)% (p<0.0001) respectively at month 6.
Conclusion: In this cohort of poorly controlled IT T2DM patients who were introduced to a recommended SMBG regimen and trained on individual therapy adaptation, which resulted in daily SMBG utilization, HbA1c improvements tended to be greater in patients with shorter duration of T2DM and with shorter duration of insulin therapy. This suggests that early adoption of daily SMBG in IT T2DM is associated with greater improvement in glycemic control.
Supported by: Roche Diagnostics Ltd.

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Correlates of patient barriers to medication taking in type 2 diabetes: the BENCH-D study

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Background and aims: Compliance to medication is a key point for the achievement of a good metabolic control and, consequently, for a better quality of life. In the context of the BENCH-D study, an integral part of the DAWN-2 initiatives in Italy, we evaluated socio-demographic, clinical and humanistic characteristics associated with patients' perceived barriers to taking their medication as scheduled.
Materials and methods: The study was conducted in 28 diabetes outpatient clinics in Italy. In each center, a random sample of patients filled in the Barriers to Medication (BM) questionnaire. Additional instruments were: WHO-5 well-being index, Problem Areas in Diabetes questionnaire (PAID-5), Patients Assessment of Chronic Illness Care (PACIC) questionnaire, Health Care Climate Questionnaire (HCCQ), Patient Support (PS) questionnaire, Diabetes Self-care Activities (DSCA) questionnaire, Global Satisfaction for Diabetes Treatment questionnaire (GSDT) and Diabetes Empowerment Scale (DES). For all the instruments higher values indicate a higher level of the dimension measured. Clinical data were extracted from computerized medical records.
Results: Overall, 2434 patients with T2DM were evaluated (mean age 65.0±10.2 years, diabetes duration 13.8±15.2 years, 59.9% males, 48.6% treated with oral agents, 25.3% treated with insulin+oral agents, 24.3% treated with insulin). When compared with patients in the lower quartile of the BM score, patients in the upper quartile (i.e. highest level of perception of barriers) had higher HbA1c levels (8.0±1.0% vs. 7.6±1.5%, p<0.001), showed significantly lower scores for WHO-5, PACIC, HCCQ, PS, GSDT (p<0.001 for all the scales), and DES (p=0.015), and higher diabetes distress (PAID-5; p<0.001). A higher perception of barriers to medication was associated with poorer adherence to diet and medication taking and higher adherence to blood glucose self-monitoring (DSCA; p<0.0001 for all the items). No association with type of diabetes treatment emerged. At multivariate analysis, adjusted for socio-demographic and clinical characteristics, the risk of presenting a BM score in the highest quartile increased for increasing values (by 5 score points) of PAID-5 (OR 1.07; 95%CI 1.04-1.10).
Conclusion: Higher perceived patient barriers to medication taking is associated with poorer metabolic control, higher levels of diabetes related distress, poorer quality of life and lower levels of satisfaction with care. Addressing perceived barriers should represent a key component of any educational activity in the context of patient-centered care.
Supported by: NovoNordisk SpA

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Correlates of patients' attitudes to check their feet as a diabetes self-care activity: the BENCH-D study

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Background and aims: Compliance with proper foot care reduces the incidence of foot ulcers and other serious complications. Optimum foot self-care practices include daily inspection of feet. In the context of the BENCH-D study, an integral part of the DAWN-2 initiatives in Italy, we evaluated socio-demographic, clinical and humanistic characteristics associated with patients' attitudes to inspect their feet.
Materials and methods: The study was conducted in 28 diabetes outpatient clinics in Italy. In each center, a random sample of patients filled in the Diabetes Self-care Activities (DSCA) questionnaire. Additional instruments were: WHO-5 well-being index, Problem Areas in Diabetes questionnaire (PAID-5), Patients Assessment of Chronic Illness Care (PACIC) questionnaire, the Health Care Climate Questionnaire (HCCQ), the Patient Support (PS) questionnaire, Barriers to Medication (BM) questionnaire, Global Satisfaction for Diabetes Treatment questionnaire (GSDT) and Diabetes Empowerment Scale (DES). For all the instruments higher values indicate a higher level of the dimension measured. Clinical data were extracted from computerized medical records.
Results: Overall, 2434 patients with T2DM were evaluated (mean age 65.0±10.2 years, diabetes duration 13.8±15.2 years, 59.9% males, 48.6% treated with oral agents, 25.3% treated with insulin+oral agents, 24.3% treated with insulin). When compared with patients in the upper quartile of the DSCA-Feet score, patients in the lower quartile (i.e. poorest compliance with foot monitoring) were more often male, had less diabetic complications, shorter duration of disease, showed significantly lower scores for PACIC, HCCQ, DES, PS, DSCA-subscales, and GSDT (p<0.001 for all the scales), and higher scores for BM (p=0.003). At multivariate analysis, adjusted for socio-demographic and clinical characteristics, the risk of presenting a DSCA-Feet score in the lowest quartile was higher in men (OR=2.4; 95%CI 1.7-3.6), in individuals taking oral agents as compared to those treated with insulin (OR=2.3; 95%CI 1.5-3.5), and decreased for increasing values of WHO-5 (OR 0.96; 95%CI 0.92-0.99; by 5 score points) and PACIC (OR 0.87; 95%CI 0.80-0.95; by 5 score points).
Conclusion: Poor adherence to foot monitoring is associated with lower perceived accessibility to chronic illness care-based services and lower levels of psychological well-being. Promoting patient-centered care and ensuring adequate psychological support are key elements to improve self-care activities in individuals with type 2 diabetes.
Supported by: Novo Nordisk SpA

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My Diabetes My Way: an electronic personal health record for people with diabetes

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Background and aims: Diabetes prevalence in Scotland is increasing at ~4.6% annually, with figures currently reaching 247,278 (4.7%). My Diabetes My Way (MDMW - www.mydiabetesmyway.scot.nhs.uk) is the official NHS Scotland information portal, containing validated educational materials for people with diabetes and their carers. Internet-based interventions have the potential to enhance self-management and shift the balance of power towards the patient, with electronic personal health records (ePHRs) identified as an ideal method of delivery. In December 2010, a new service was launched within MDMW, allowing patients from across Scotland access to their shared electronic medical record. We analysed the first two years of usage and uptake.
Materials and methods: We developed a diabetes-focused, population-based ePHR for NHS Scotland based on data sourced from primary, secondary and tertiary care via the national shared-electronic record, Scottish Care Information - Diabetes Collaboration (SCI-DC). The system includes key diagnostic information; demography; laboratory tests; lifestyle, foot and eye screening results; prescribed medication and clinical correspondence. Changes can be tracked over time using 'history' graphs and tables, data items link to detailed descriptions, explaining why they are collected, what they are used for and what 'normal' values are, and tailored information links refer individuals to further facts related to their condition.
Results: At the end of the second year of live use, 2601 individuals had registered to access their data (61% male; 30.4% with type 1 diabetes); 1297 had completed the enrolment process and 625 had accessed the system (most logins=346; total logins=5167; average=8.3/patient; median=3). Audit trails show 59599 page views (95/patient), with laboratory 'test results' proving the most popular (11818 accesses; 19/patient). The most utilised history graph was, unsurprisingly, HbA1c (2866 accesses; 4.6/patient). Feedback: "It is great to be able to view all of my results so that I can be more in charge of my diabetes".
Conclusion: The system is now a useful additional component for the self-management of diabetes in Scotland. Although there are other patient access systems available worldwide, the MDMW system is unique as it offers access to an entire national population and provides access to information collected from all available diabetes-related data sources. Although the system has been developed for, and by, stakeholders from across Scotland, it has the potential to connect to any electronic medical record. The current project is expected to reach over 5000 patients by the end of 2013.
Supported by: Scottish Diabetes Group

PS 088 Self blood glucose monitoring: utility and metrics

1072

Introduction of a recommended SMBG regimen improved glycaemic control in poorly controlled Chinese patients on insulin therapy: results from the COMPASS study

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Background and aims: Self-monitoring of blood glucose (SMBG) is deemed integral in the management of insulin-treated type 2 diabetes (IT T2DM). In China, utilization of SMBG among IT T2DM patients is low. Even among those who perform SMBG regularly, the daily testing frequency falls short of those recommended in clinical guidelines. We examined the effect of introducing a recommended SMBG regimen on testing frequency and glycemic control in IT T2DM patients in China.
Materials and methods: The COMPASS study is a 2-phase, multi-centre, observational study. The first phase evaluated the current state of SMBG practice, types of insulin therapy used and glycemic control in IT T2DM patients. The second phase evaluated the effect of introducing a SMBG regimen on glycemic control in poorly controlled IT T2DM patients. Inclusion criteria for the second phase included patients who were enrolled in the first phase, had poorly controlled diabetes (HbA1c>8%) and had been on insulin therapy for at least 3 months. At the start of this second phase, patients received a blood glucose meter and a paper tool to record SMBG readings. They were introduced to and advised to follow a SMBG regimen. They were also trained on how to respond to SMBG readings via lifestyle changes and insulin dose self-adjustment. During the 6-month follow-up period, physician visits were scheduled at the end of month 3 and 6. Physicians assessed glycemic control based on HbA1c and SMBG records, and gave further advice on lifestyle changes, medication adjustment and SMBG regimen, if applicable. Endpoints included (1) daily SMBG frequency; (2) HbA1c baseline change at month 3 and 6; (3) proportion of patients who achieved glycemic control (HbA1c<7%) at month 3 and 6; and (4) proportion of patients who self-adjusted insulin dosage based on SMBG reading.
Results: Of the 3,006 patients participated at 120 centres in China in the first phase, 820 patients (age 55±9.8 yrs, 50% females, BMI 24.9±3.6) met the inclusion criteria of the second phase and were enrolled (baseline HbA1c 9.7±1.6%, T2DM duration 9.8±7.1 yrs, insulin therapy duration 45.4±46.8 mths). At baseline, 30% of the patients reported performing SMBG daily (12% once, 12% twice, 3% thrice, 3% four times). At month 3 and 6, 99.8%/99.1% of the patients reported performing SMBG daily (20%/20% once, 64%/65% twice, 11%/10% thrice, 4%/4% four times), and 70%/74% of the patients reported having self-adjusted their insulin dose based on SMBG readings. HbA1c decreased substantially from baseline at month 3 [mean ΔHbA1c -1.88%, p<0.0001] and month 6 [mean ΔHbA1c -1.85%, p<0.0001]. 36% and 40% of the patients achieved good glycemic control (HbA1c<7%) at month 3 and 6, respectively.
Conclusion: In this Chinese cohort of poorly controlled IT T2DM patients, introducing a recommended SMBG regimen substantially contributed to an improved HbA1c. Based on the results, we hypothesize that training on how to interpret and respond to SMBG readings together with daily SMBG enable patients and physicians to better manage diabetes.
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Use of structured 3-day glycaemic profiles impacts glucose control in diabetes patients treated with multiple daily insulin injection therapy: results from ABACUS

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Background and aims: Recent studies have shown that use of structured self-monitored blood glucose (SMBG) regimens positively influences health behaviors in non-insulin treated diabetes, leading to improvements in glycemic control and other measures. We examined the effect of using 3-day, 7-point glycemic profiles in patients treated with multiple daily insulin injection (MDI) therapy.
Materials and methods: Data were drawn from 194 patients enrolled in the Automated Bolus Advisor Control and Usability Study (ABACUS) trial, a large, 26-week, prospective, randomized multi-national trial. The trial evaluated the use of an automated bolus advisor in suboptimally controlled MDI-treated type 1 diabetes and type 2 diabetes patients with baseline HbA1c 8.9 (1.2)%. Patients randomized to the control group (CNL) used a standard blood glucose (bG) meter and manual bolus calculation; whereas, patients randomized to the intervention group (EXP) used the Accu-Chek® Aviva Expert meter, which provides an integrated automated bolus advisor to calculate insulin dosages. All patients were asked to perform 7-point bG testing (pre-/postprandial, at bedtime) for 3 consecutive days prior to randomization and prior to 3 additional clinic visits. We compared bG levels obtained on profile days with values from non-profile days.
Results: All patients had significantly (p <0.01) lower mean bG values (Table) and a higher percentage of bG values within target range (70-180 mg/dL / 3.9-10 mmol/L) on more profile days than non-profile days. Glycemic variability (MAGE) was significantly (p <0.01) lower with significantly fewer bG values in the hypoglycemic range (<50 mg/dL / 2.8 mmol/L) and hyperglycemic range (>300 mg/dL / 16.7 mmol/L) on most profile days compared with non-profile days. No significant between-group differences were seen in these parameters.
Conclusion: Recent studies of non-insulin treated diabetes have demonstrated that use of structured SMBG as a basis for close collaboration between patients and their healthcare team facilitates therapy optimization. Our study suggests that performing periodic 3-day, 7-point bG profiles may be an effective stand-alone intervention to optimize glycemic control for patients treated with MDI therapy.

Table. Comparison of bG values on profile days vs. non-profile days (Intent-to-Treat cohort, n=194)

Profile Days	Mean bG on Profile Days (mg/dL / mmol/L)	Mean bG on Non-Profile Days (mg/dL / mmol/L)	P Value
Baseline	173.7 (39.6) / 9.7 (2.2)	181.9 (42.4) / 10.1 (2.4)	< 0.01
Visit 5	175.1 (39.1) / 9.7 (2.2)	183.5 (40.6) / 10.2 (2.3)	< 0.01
Visit 7	173.9 (38.7) / 9.7 (2.2)	184.8 (40.7) / 10.3 (2.3)	< 0.01
Visit 9	176.0 (37.1) / 9.8 (2.1)	179.8 (39.6) / 10.0 (2.2)	NS

Clinical Trial Registration Number: NCT01460446
Supported by: Roche Diagnostics GmbH

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Colour record in self-monitoring of blood glucose improves glycaemic control for insulin-treated type 2 diabetes patients

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Background and aims: We conducted a clinical research study to evaluate the effect of two color-indication methods used in SMBG, color display (CD)

and color record (CR), on glycemic control in insulin-treated type 2 diabetes patients.

Materials and methods: This was a prospective, 24-week, single center, comparison study. One hundred twenty outpatients of our University hospital were randomly allocated to four groups by two-by-two factorial design (Table). Group A used One Touch® Ultra Vue™ (Vue) with black and white indicator and recorded their blood glucose levels manually in black pencil. Group B observed a red or blue indicator light on the color Vue when their glucose levels were 160 mg/dl or greater, or less than 70 mg/dl, respectively, and recorded them manually in black pencil. Group C used the Vue with black and white indicator and recorded their blood glucose levels in red or blue pencil under the same glycemic conditions. Group D used both color display and color record. The primary endpoint was difference in HbA1c reduction in 24-week between CD (B+D) and non-CD (A+C) groups and between CR (C+D) and non-CR (A+B) groups. Secondary endpoints were differences in self-management performance change evaluated by the Summary of Diabetes Self-Care Activities Measure (SDSCA) and the Profile of Mood States (POMS) change.

Results: Demographics of subjects were almost the same among the 4 groups. Nine subjects (15.0%) in non-CR, 10 (16.7%) in CR, 9 (15.5%) in non-CD, and 10 (16.1%) in CD groups were dropped because of hospitalization for diabetes and comorbidities. Change in HbA1c in 24-week was -0.11% in non-CD group and -0.13% in CD group with no significant difference. However, change in the score on the exercise subscale of the SDSCA in 24-week was significantly improved by 0.50 points in CD group compared to that in non-CD group, suggesting that color display is favorable for an increase in physical activity. On the other hand, HbA1c levels at 24-week were significantly decreased in CR group by -0.28%, but were increased by 0.03% in non-CR group. Difference in HbA1c reduction in 24-week between the 2 groups was -0.31%, which was a significant difference. In addition, scores on diet and exercise subscales of SDSCA in 24-week were significantly increased by 0.44 points and 0.50 points, respectively, in CR group compared to those in non-CR group, implying that color record is helpful for improvement both in physical activity and in diet. There were no significant different in changes in scores on the POMS among the groups.

Conclusion: Color record has a favorable effect on self-management performance without any influence on psychological stress, resulting in improved glycemic control in insulin-treated type 2 diabetes patient.

Table. Two-by-two factorial design and subject number

n = 31 No color display No color record (A)	n = 29 Color display No color record (B)	Subtotal n = 60 No Color Record group (non-CR)
n = 27 No color display Color record (C)	n = 33 Color display Color record (D)	Subtotal n = 60 Color Record group (CR)
Subtotal n = 58 No Color Display group (non-CD)	Subtotal n = 62 Color Display group (CD)	Total n = 120

Clinical Trial Registration Number: UMIN00003589

1075

ExAct study: Integrated strip-free SMBG technology (Accu-Chek® Mobile) improves patient adherence to recommended testing frequency and patterns and glycaemic control

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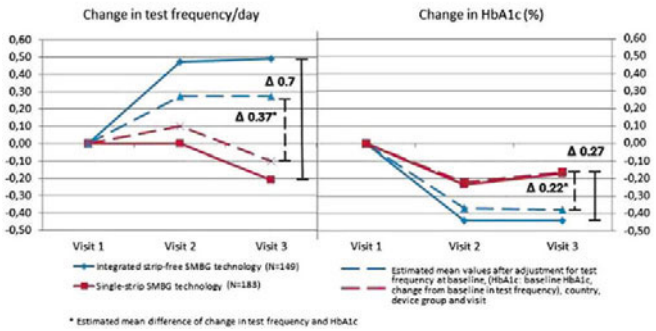
Background and aims: Current guidelines recommend a self-monitoring of blood glucose (SMBG) at least 3-4 times daily for diabetes patients on prandial insulin regimen. Patient adherence to this recommendation is often suboptimal, non-adherence is often device-related. Integrated strip-free SMBG technology has been developed to reduce testing barriers. This study investigates the impact of integrated strip-free SMBG technology on testing frequency and glycemic control of non-adherent patients.

Materials and methods: 478 non-adherent patients (<3.25 tests/day at baseline) were enrolled into this multicenter, prospective, cluster-randomized study with sites randomized to either "integrated strip-free" or "any single-strip" SMBG technology. Testing frequency and HbA1c were measured at baseline, after 3, and after 6 months. Changes from baseline were investigated using generalized linear mixed models.

Results: 332 patients were eligible for efficacy analysis. Three months after baseline, testing frequency increased by 0.48 measurements/day in patients using integrated strip-free technology. The average testing frequency was 0.37 measurements/day higher in "integrated strip-free" compared to "any single-strip" patients (p=0.007). Glycemic control improved with the 2 technologies, with a greater HbA1c reduction in patients using strip-free technology (mean difference 0.22%, p=0.04). Changes in test frequencies and HbA1c concentrations are represented below. A detailed analysis of daily measurement patterns revealed: Testing frequency at lunch (11am-2pm) and dinner time (5pm-8pm) in "integrated strip-free" patients increased from 12.3 to 16.6 and from 12.6 to 16.9 measurements/hour/100 patients, respectively, after 6 months. No change in testing frequency was found in "any single-strip" patients. (Figure)

Conclusion: Integrated strip-free SMBG technology resulted in a significant increase in testing frequency towards guideline recommendations. The change was associated with a clinically relevant HbA1c decrease in previously non-adherent patients. Examination of daily patterns revealed an increase in testing frequency in patients using the integrated strip-free technology before lunch and dinner time, resulting in a relatively large HbA1c impact given the small increase in daily testing frequencies.

Changes in test frequency and HbA1c in the two study groups over the 6 month observational period



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System accuracy evaluation of blood glucose monitoring systems for point of care testing and self monitoring

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Background and aims: Blood glucose (BG) monitoring systems for professional use only and systems designated for self-monitoring of BG (SMBG) are used for point of care testing (POCT) by health care professionals. These systems are often used interchangeably for capillary glucose monitoring, thus raising the question for comparability of measurement quality.

Materials and methods: In this study, we evaluated the accuracy of a BG system for self-monitoring based on dynamic electrochemistry (BGStar, Sanofi) and six different, commonly used systems for professional use (HemoCue Glucose 201+, HEMOCUE AB; StatStrip, nova biomedical; ACCU-CHEK Inform II, Roche Diagnostics; BIOSEN C_line, EKF-diagnostic; HITADO SUPER GL compact, Dr. Müller Gerätebau; GLUKOMETER PRO, BST Bio Sensor Technology). Investigational procedures were performed following the international standard ISO 15197:2003. Capillary blood samples of 100 subjects were measured and the results were analyzed according to the ISO/DIS 15197 by calculating the percentage of results within ±10%/±15% or within ±10 mg/dL/±15 mg/dL of the reference measurement results for BG concentrations above or below

100 mg/dL, respectively. Glucose oxidase was used as comparison method (YSI 2300 STAT Plus, YSI Life Sciences, USA). Two devices and one lot of reagents or test strips were used for each POCT system, except for the SMBG system BGStar, for which three reagent lots were evaluated separately.
Results: For all evaluated systems 99% to 100% of the measurement results were within ±15 mg/dL or ±15% of the reference method results. When applying more restrictive limits of ±10 mg/dL or ±10% larger differences between the evaluated systems were observed (82% to 98% of the results within these limits). For three POCT systems for professional use and for the three lots of the system for self-monitoring more than 95% of the measurement results were within these more restrictive limits.
Conclusion: In this study, the BG system for self-monitoring based on dynamic electrochemistry and the POCT systems for professional use fulfilled the accuracy requirements of the new ISO/DIS 15197 standard measuring capillary glucose values. Furthermore this study demonstrated that BG systems for self-monitoring which have to fulfil strict requirements prior to market launch can achieve similar system accuracy as POCT systems for professional use.
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Measurement error in estimated average glucose: a new approach
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Background and aims: Estimated average glucose (eAG) has been suggested as a way to link daily self monitoring blood glucose (SMBG) directly to long term glycaemia. However variation in hemoglobin A1c (HbA1c) and mean plasma glucose (MPG) determinations due to measurement error (ME) may mean that people with the same HbA1c have different eAG values. The aim of this study was to explore the impact of measurement error on the determination of an HbA1c derived eAG.
Materials and methods: SMBG data from the 'Efficacy of self-monitoring of blood glucose in patients with newly diagnosed type 2 diabetes' (ESMON) study were used to explore the relationship between HbA1c and MPG, in order to determine an eAG regression equation (with and without correction for ME) for this population. Monitoring data from 1, 6 and 12 weeks before HbA1c measurement were available for 73 individuals, with an average 18.5 SMBG values for each HbA1c measurement. Linear regression and structural equation modelling (SEM) were used to explore the impact of ME on the eAG regression equation.
Results: The correlation between weekly SMBG values and HbA1c ranged from 0.51-0.62, p<0.001, improving to 0.71, p<0.001 when all three weeks' SMBG data were combined. This is the same principle behind multiple sampling, the usual method to control for ME in MPG. The regression equation produced, eAG=1.5xHbA1c - 2.28 was close to that from the A1c Derived Average Glucose study (eAG=1.59xHbA1c -2.59) When structural equation modeling was used to correct both HbA1c and MPG for ME the correlation improved to 0.87, p<0.001, with eAG (mmol/L) = 2.06 x HbA1c.
Conclusion: Ignoring ME in the estimation of MPG leads to an attenuated correlation and a regression equation which gives an artificially low eAG for any given HbA1c value. When combined with other sources of variation in the HbA1c/MPG relationship the clinical usefulness of eAG is drawn into question.
Clinical Trial Registration Number: ISRCTN 49814766
Supported by: N. Ireland Department of Health R&D Office

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An innovative metric objective evaluation of glucose profiles
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Background and aims: Glucose monitoring (CGM or SMBG) has a high potential to improve significantly diabetes care and management. However, an easy, objective and practicable tool to evaluate the quality of measured glucose profiles is missing. It was the aim of our study to develop and to verify an easily to handle evaluation score (Q-score) that considers all important aspects of measured glucose profiles.
Materials and methods: 1495 registered glucose profiles provided the database for this study. First, a factor analysis addressing all parameters affecting glucose profiles (mean sensor glucose, intra- and inter daily variability, time and area above or below target range) was performed to identify factors with major impact on the measured profiles. For each factor one parameter was selected and used for the development of the Q-score.
Results: This study resulted in a Q-score for objective evaluation of glucose profiles. To verify the Q-score two diabetes specialists (DS) diagnosed independently 729 and 194 glucose profiles of type 2 diabetic patients, respectively. The results were analysed for the inter-individual variation as well as correlated with parameters to describe glucose profiles such as mean sensor glucose, ±SD, MAGE, -MODD. There was a high correlation between the Q-score and the results of both DS (Kendalls-Tau= 0.766 and 0.719; p<0.001). Both DS showed a high correspondence with the Q-score (Kendalls-Tau=0.763; p<0.001), although one DS seems to give a better diagnosis (McNemar-Bowker-Test p<0.001). To establish an easily to handle and practicable diagnostic tool, the Q-score was tested for categorisation of glucose profiles. 729 profiles were categorised by one DS in very good (Q-score: 3.4±0.8), good (4.9±1.2), satisfactory (7.1±1.6), borderline (10.0±1.9) and not satisfactory (13.8±2.6) and compared with the result obtained from the evaluation of the same glucose profiles by applying the Q-score. The Q-score was significantly correlated with the results obtained by the evaluation of the DS.
Conclusions: The Q-score combines all essential quality parameters to describe glucose profiles in only one parameter. The Q-score is independent of subjective opinions and can be used therefore for automated evaluation of glucose profiles. The Q-score has the potential to become a practical tool in diagnosis and therapy of type 2 diabetic patients.

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Robustness of dynamical real-time estimation of HbA_{1c} using routine self-monitoring of blood glucose (SMBG) data
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Background and aims: While A1c is the gold-standard marker for average glycaemia in diabetes, routine laboratory A1c results are only available to patients every few months. In studies, real-time estimation of A1c using self-monitoring of blood glucose (SMBG) data has shown improved glycaemic control and increased patient motivation. In a companion presentation, we introduce a new dynamical method for real-time *estimation of A1c* (*eA1c*) that tracks changes in average glycaemia in type 2 diabetes (T2D) patients. *eA1c* is shown to produce highly accurate estimates of A1c from SMBG. In this analysis, we study the robustness of the *eA1c* methodology.
Materials and methods: The data originated from a phase 3b trial of insulin-naïve subjects with T2D. The dynamical method was developed using a training data set (N=379 patients with T2D, who collected 17,925 fasting SMBG readings, 7-point profiles, and reference A1c values); then all model parameters and formulas were fixed. Robustness of the method was studied on an independent test data set (N=375 subjects; 17,925 fasting SMBG readings; 2314 7-point profiles; and 1628 reference A1c values; mean of 48 days of data/individual). We analysed: (1) robustness to reference A1c stratification; (2) robustness to initialisation comparing the first reference/eA1c pair for each subject to the rest of the data; (3) robustness to missing fasting SMBG, and (4) robustness to mislabelled profile SMBG (e.g. pre-post meal mislabelling). Analyses (3-4) were performed by randomly removing or mislabelling an increasing percentage of SMBG, assessing performance 10 times at each level.
Results: Stratifying the data by reference A1c, the dynamical method was most accurate in the 7% to 8% A1c range, with no bias and 4.48% MARD. Within the reportable A1c range of 6% to 10%, the bias of *eA1c* was always < 1% A1c; MARD < 10% (see Table). Stratifying the data by *eA1c*, the method was stable between 6% and 10% (bias:-0.23% to 0.19%; MARD: 6.74% to 7.24%). Performance at the initial *eA1c* for each subject was similar to overall performance (MARD 7.0% vs. 6.8%). Neither missing fasting SMBG (MARD 6.9% with 75% missing fasting SMBGs vs. 6.8% overall), nor mislabelling (MARD 7.0% with 75% mislabelled profile SMBGs vs. 6.8% overall) significantly altered *eA1c* performance when the method entry criteria were met (with increasing missing values the entry criteria are met less often).
Conclusion: The dynamical estimation procedure provides robust and accurate real-time estimation of A1c from self-monitoring data. Consistently achieving MARD < 10% in any strata defined by reference A1c, indicates that the model is capable of providing accurate tracking of changes in average glycaemia over time. The limited effect of missing or mislabelled SMBG values

supports that the algorithm is appropriate for field use. In addition, the computational requirements of the method are minimal; thus it is readily applicable into devices with limited processing power, such as home SMBG meters.

	HbA1c < 6	6= HbA1c < 7	7= HbA1c < 8	8= HbA1c < 9	9= HbA1c = 10	HbA1c > 10
Bias stratified by laboratory HbA1c , %	1.02 n =40	0.49 n = 516	-0.03 n = 608	-0.37 n = 265	-0.83 n = 113	-1.46 n = 19
MARD stratified by laboratory HbA1c, %	17.89 n = 40	8.01 n = 516	4.48 n = 608	6.51 n = 265	9.49 n = 113	14.16 n = 19
Bias stratified by eA1c, %	NA n = 0	0.11 n = 397	0.07 n = 870	-0.23 n = 243	0.19 n = 51	NA n = 7
MARD stratified by eA1c, %	NA n = 0	6.74 n = 397	6.80 n = 870	6.9 n = 243	7.24 n = 51	NA n = 7

Supported by: Sanofi-Aventis Deutschland GmbH

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Accuracy of dynamical real-time estimation of HbA_{1c} using routine self-monitoring of blood glucose (SMBG) data

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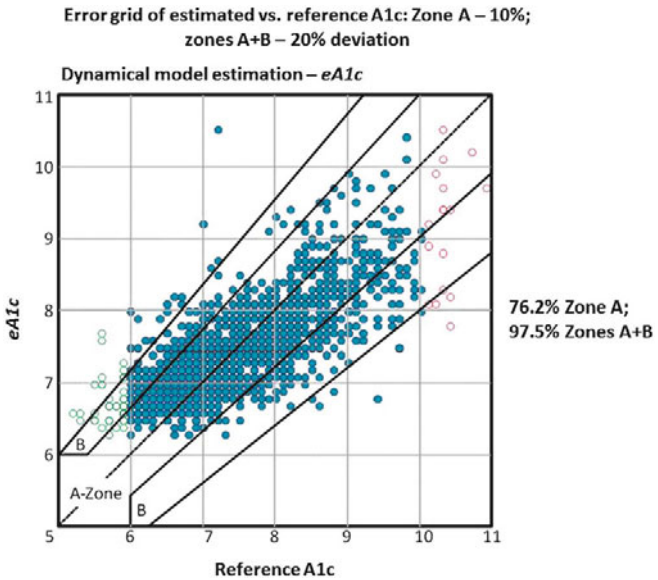
Background and aims: Although A1c is the gold-standard marker for average glycaemia in type 1 and type 2 diabetes (T2D), laboratory A1c assays are typically done only every few months. Self-monitoring of blood glucose (SMBG) offers the possibility for real-time estimation of A1c, which increases patients' motivation to improve diabetes control. We present a new dynamical method tracking changes in average glycaemia to provide real-time *estimation of A1c* (*eA1c*). The major advantage of this method is that it is designed to work with infrequent SMBG data typical in T2D - a setting in which previously introduced techniques would fail.

Materials and methods: Using compartmental modelling, a new 2-step algorithm was constructed that includes: 1) tracking average fasting glycaemia to compute *base eA1c* updated with every fasting SMBG data point; 2) calibration of the *base eA1c* trace with 7-point SMBG daily profiles taken approximately monthly, which uses a new factorial model to capture the principal components of blood glucose variability. Data originated from a multicentre phase 3b study of insulin-naïve subjects, 44.3% women (mean age 54 years) and 55.7% men (mean age 56 years); mean HbA1c 7.6%. A training data set (N=379 subjects; 17,863 fasting SMBG readings; monthly 7-point SMBG profiles; frequent reference A1c (n=1599 values); mean of 47 days of data/individual) was used to estimate all model parameters. The model was then fixed and applied without further modification to an independent test data set (N=375 subjects; 17,925 fasting SMBG readings; monthly 7-point profiles; frequent reference A1c (n=1628 values); mean of 48 days of data/individual). Accuracy was evaluated in the test data set by computing mean absolute and relative deviations (MAD, MARD) of *eA1c* from reference A1c; to visualise the results we also present HbA1c error-grid analysis (Figure 1).



Results: Test data set: MAD=0.51, MARD=6.8%; correlation between *eA1c* and reference A1c r=0.76. In comparison, the established ADA formula yielded MAD=0.98, MARD=13.1%, and r=0.73. In the HbA1c error-grid plot (Figure), 76.2% of all *eA1c* fell within 10% from reference A1c (Zone A) and 97.5% fell within 20% from reference (Zones A+B). If limited to a reportable A1c range (6%-10%), the accuracy of *eA1c* was 78.3% (Zone A) and 98.6% (Zones A+B).

Conclusion: A dynamical estimation model was developed using training data, then fixed and applied to independent test data, achieving accurate tracking of changes in average glycaemia over time (MARD <7%). This model is tailored to accommodate infrequent SMBG data typical for T2D, providing a new tool for A1c estimation on the patient level. Model accuracy is comparable to the accuracy of the original SMBG readings; thus, the model does not introduce bias in the estimate, beyond errors inherent with SMBG.

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PS 089 New devices for glucose monitoring and insulin delivery

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Development of a novel microprobe array continuous glucose monitor: pre-clinical validation

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Background and aim: Despite clinical benefits, continuous glucose monitoring (CGM) devices are invasive and can be uncomfortable, negatively affecting concordance and effectiveness. In addition, CGM sensor accuracy is suboptimal in the critical hypoglycaemic range. To overcome these challenges, a novel continuous glucose sensor has been developed based on microprobe technology. It consists of a small, wearable patch containing several microscopic projections (microprobes) that penetrate the stratum corneum to access interstitial fluid. Aim: Mechanical and functional validation of the sensor prior to in vivo clinical studies. Mechanical validation assesses the ability to penetrate the stratum corneum without mechanical failure, while functional validation assesses the current produced in response to changing glucose concentration and the effects of sterilisation and insertion.
Materials and methods: Each array had 64 microprobes arranged 8x8. Microprobe height is 1000 μm and tip diameter is 15 μm. Insertion tests were performed ex vivo in full thickness human skin. An Instron compression system was used to press the device into skin using forces of 7, 10, 15, 20 and 25 Newton. Skin penetration was confirmed by applying methylene blue dye to visualize created micropores and by histological examination. Fracture tests were performed in vitro using the Instron system to exert axial pressure against a metal probe using forces of 50, 100, 200, 300 and 400 Newton. Microprobes were examined using scanning electron microscopy before and after testing to detect failure and measure height reduction. To assess transverse fracture force, transverse pressure was exerted against one row of 8 microprobes till they fractured. Functional evaluation tests were performed in vitro by measuring the current generated in response to changing glucose concentration before and after gamma ray radiation and *ex vivo* skin insertion.
Results: Insertion tests (n=10) demonstrated successful penetration using forces as low as 7 N (equivalent to mild thumb pressure) without fracture. Axial fracture tests (n=10) have demonstrated the ability of microprobes to tolerate large forces without fracture of microprobes or base plate. There was reduction of microprobes' height between 4.1% for 50 N and 18 % for 400 N axial pressures. Transverse fracture tests (n=11) showed that the force required to fracture microprobes were 27 times higher than force required to penetrate the skin, implying a high safety margin of the device. Functional validation tests demonstrated the ability of the device to respond reproducibly to changing glucose concentrations in a linear fashion in the physiological range. This was not affected by either sterilisation or skin penetration. For glucose level ranging from 0 - 20 mM, currents measured (350 $x10^{-8}$ - 410 $x10^{-8}$ Amp) were approximately 250 times higher than those measured using a sensor licensed for clinical practice (1.0 $x10^{-8}$ - 16 $x10^{-8}$ Amp).
Conclusion: In vitro and ex vivo validation studies demonstrate a functional device, able to access the interstitial fluid compartment without mechanical failure, and the device can now be assessed in vivo. Glucose dependent responses are of high magnitude with a superior signal: noise to commercially available sensors in vitro.
Supported by: NIHR Imperial Biomedical Research Centre

1082

Performance of a novel sensor during induced glucose swings

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Background and aims: Continuous Glucose Monitoring (CGM) is expected to reduce the frequency and duration of hypoglycemic events. However, inaccurate CGM readings and associated high rates of false positive or missed warnings of hypoglycemic events diminish users' compliance of CGM sensor use. Sensor inaccuracy can be caused by a combination of erroneous or noisy sensor signals, mis-calibration of the sensor, physiological lag time between interstitial fluid (ISF) and blood glucose, physical lag time introduced by the CGM system and physiological effects at the sensor to tissue interface. Since the physiological lag time is an inherent element, efforts are being made to reduce the impact of the other factors.
Materials and methods: An early stage development CGM sensor (Roche Diagnostics, Germany) was investigated in an in-clinic setting. Two CGM sensors were worn in parallel for 7 days by 30 people with type 1 diabetes. The study design reflected some recommendations of the CLSI guideline POCT05-A Performance Metrics for Continuous Glucose Monitoring, especially regarding induced glucose swings. To obtain a wide range of glucose readings and rates of change, breakfast with a high glycemic index was given on two study days and the corresponding insulin bolus was slightly delayed and overdosed leading to a fast glucose rise followed by a glucose decline. Sensor accuracy was assessed by calculating MARD (Mean Absolute Relative Difference) between CGM value and self monitoring blood glucose (SMBG) measurements taken with a commercially available SMBG meter fulfilling the requirements of ISO 15197 (Accu-Chek Aviva, Roche Diagnostics, Germany). Precision between two sensors was assessed by calculating PARD (Precision Absolute Relative Difference). MARD and PARD were calculated over the complete data sets (7 days) as well as for the phases with induced glucose swings. Simulation of hypoglycemic warning thresholds was performed to estimate hypoglycemia detection rates.
Results: Rates of glucose change of up to ±6 mg/dL/minute were achieved. Overall MARD was 8.6% for the complete data sets (N=7039 paired values) and 10.6% for phases with induced glucose excursions (N=2250 paired values). Overall PARD was 7.6% for the complete data sets and 8.0% for phases with induced glucose swings. With a simulated hypoglycemic warning threshold set at 70 mg/dL, 97% of all hypoglycemic bG values ≤ 55 mg/dL would have been detected, with a threshold set at 65 mg/dL the detection rate would have been 92.7%.
Conclusion: The newly developed sensor showed high accuracy and precision in patients tested. During rapidly changing glucose levels the sensor followed the blood glucose concentrations very closely. Sensor precision during glucose excursions was comparable to the overall sensor precision.

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Presenting a truly non-invasive glucose monitor for home use

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Background and aims: GlucoTrack® is a truly Non-Invasive device for self-monitoring of blood glucose at home and home-alike environment, combining three technologies: Ultrasonic, Electromagnetic and Thermal. The device comprises user friendly Main Unit (MU), as well as sensors per each technology, located at a Personal Ear Clip (PEC). The PEC is clipped externally to the earlobe for less than a minute, to conduct a real-time spot measurement, after individual calibration. Glucose readings are heard and displayed on the smartphone sized MU with a color touch screen, designed for diabetics' needs. The PEC must be replaced every 6 months, to be followed by a calibration.
Materials and methods: GlucoTrack performances were evaluated in various clinical trials, simulating home-alike environment, for subjects of diverse genders, BMI, diabetes types and ages (currently over 18 years). At the beginning of the trial, each subject was calibrated. The measurements taken throughout the trial were based on this calibration. GlucoTrack eligibility for home use was assessed according to calibration validity period, device learning curve of use and feedback analysis regarding device usability and user satisfaction. Evaluation was conducted based on two groups: Clinic group, where measurements were performed by a skilled medical team; Home Simulated (HS) group, where measurements were conducted by the subjects, after a brief training. Subjects participated in the trial for up to 6 months, in order to verify the calibration validity period (6 months).
Results: Clarke Error Grid pulled data analysis of 135 subjects (7912 points) over 6 months of operation shows 96.5% of the points in the clinically accepted A+B zones. Mean Absolute Relative Difference (MARD) of 30.2% was observed. No degradation in performance was noticed as a function of time elapsed from calibration. Furthermore, GlucoTrack performances were maintained across Clinic and HS groups as 96.6% and 96.1 % of the points in A+B zones, and MARD values of 30.1% and 30.9%, respectively. Feedback

analysis shows that 99% of the subjects found the device comfortable; 78% found the device easy to operate, 90% expressed willingness to use the device regularly and 84% declared they will use the device more frequently than the invasive one.
Conclusion: GlucoTrack is technically available to use. Its key benefits include 6 months of operation without re-calibration and ability to perform numerous spot measurements with acceptable accuracy. Furthermore, it provides pain-free and inexpensive use, without the need to be continuously worn; it is user friendly and demonstrates a short learning curve for use. These advantages emphasize GlucoTrack suitability for home use and can lead to better blood glucose monitoring adherence and tighter glucose control.
Clinical Trial Registration Number: NCT00889668

1084

Six-month data from a clinical study of an implantable fluorescence-based glucose sensor
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Background and aims: A long-term implantable fluorescence-based glucose sensor has been developed. The small cylindrical sensor is designed to be implanted subcutaneously for at least 6 months. The outer shell of the sensor includes a polymer hydrogel containing a proprietary indicator molecule that becomes fluorescent when it binds glucose. The sensor includes a miniature fluorimeter for detection of the fluoresced signal. Prior clinical studies have demonstrated sensor functionality at one month post-implant. This study was designed to demonstrate functionality of the implantable glucose sensor while implanted over a 6-month period.
Materials and methods: In a pilot clinical study, 4 subjects were inserted with a single sensor in the upper arm. The sensors remained inserted for up to 6 months, and subjects came to the clinic every 14 to 28 days to have the sensors read for several hours. Blood samples were also taken every 15 minutes and processed using a YSI Blood Glucose Analyzer. A single blood glucose value from the beginning of each clinic day was used to calibrate each sensor for each session, and the sensor glucose values were calculated prospectively.
Results: The combined MARD for the 4 subjects is 13%, using an SMBG value twice daily (i.e., approximately once every 12 hours) for calibration and prospective calculation of the sensor glucose values. The percentage of remaining sensor fluorescence (relative to the fluorescence baseline at the time of implant) remains high after 180 days, suggesting that the sensor may last for more than 6 months after implant.
Conclusion: Clinical data from a 6-month pilot study of an implantable fluorescence-based glucose sensor has demonstrated that the sensor is able to maintain its fluorescence response and accuracy up to 6 months after implant.

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Catalytic protection from ROS using platinum nanoparticles: enabling a long-term, implantable glucose sensor
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Background and aims: Reactive oxygen species (ROS) that are generated as a consequence of the acute and chronic immune response to an implanted medical device have been shown to be a primary cause of loss of device functionality and reduced *in vivo* longevity. The Senseonics implantable continuous glucose monitoring (CGM) system utilizes an abiotic, fluorescent glucose indicator containing glucose binding elements that may be oxidized. A 10 nm thick layer of platinum, sputtered onto the sensor glucose indicating polymer layer, has been shown to catalytically degrade ROS, prevent indicator oxidation and enable sensors implanted subcutaneously in human subjects to remain functional for six months in clinical trials. We now establish protection against ROS (i.e., hydrogen peroxide) oxidation, through *in vitro* experiments, using a surrogate catalytic protection strategy in which platinum nanoparticles embedded into the glucose indicating polymer layer replace the sputtered platinum layer. A combination of both a sputtered platinum layer and embedded nanoparticles is shown to provide increased protection against oxidation by hydrogen peroxide.
Materials and methods: Sensor blanks (units prepared without electronic and optical components) were used for all tests. Samples were prepared with and without embedded platinum nanoparticles and sputtered platinum layers. Catalytic rate of degradation of hydrogen peroxide in PBS was measured using a commercial spectrophotometric hydrogen peroxide assay kit. Activity was measured at t = 0, 1 and 2 months storage in PBS at 37°C. Glucose indicator protection against oxidation was determined by immersing samples into hydrogen peroxide solutions in PBS at 37°C and monitoring loss of fluorescence using spectrofluorophotometers.
Results: Platinum nanoparticle embedded indicator hydrogels catalytically decompose hydrogen peroxide at rates comparable to platinum sputter coated samples. No loss in catalytic activity was observed after 2 months storage in PSB at 37°C. In oxidation challenge tests using 20 mM hydrogen peroxide, samples prepared without platinum nanoparticles were completely oxidized in under 15 minutes, whereas at one hour, samples containing either sputtered platinum or nanoparticles lost ~ 50% fluorescence and samples prepared with both sputtered and nanoparticle platinum lost < 10% fluorescence.
Conclusion: Platinum nanoparticles embedded into the glucose indicating polymer layer catalytically degrade hydrogen peroxide and provide protection against oxidation. A combination of both a sputtered layer of platinum and embedded platinum nanoparticles displayed the greatest level of protection against a high concentration of hydrogen peroxide. This combined catalytic approach offers the possibility of increased protection against *in vivo* oxidation and thus increasing device longevity from six months to one year.

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Evaluation of drug efficacy using glucose area under the curve monitoring system without blood sampling
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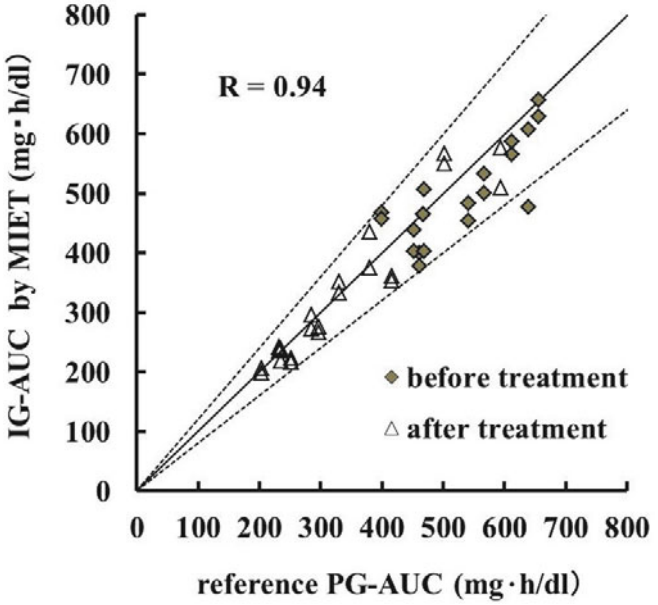
Background and aims: HbA1c is widely used as an index of average blood glucose over medium- to-long-term periods; however, it is not suitable for short-term evaluation because of its slow dynamics. OGTT is used to evaluate drug efficacy over the short term, but the need for multiple blood samples is burdensome on the patients and medical staff. We have developed a glucose area under the curve (AUC) measurement system using minimally invasive interstitial fluid extraction technology (MIET) that allows convenient monitoring of postprandial glucose levels without blood sampling. In this study, we have applied this system to patients receiving incretin-related drugs to evaluate the efficacy of these drugs.
Materials and methods: Eleven type 2 diabetic inpatients aged 30-70 years old with a mean HbA1c of 9.9% ± 1.2% underwent OGTTs (75g) before and 4-7 days after receiving alogliptin, exenatide, or liragluitide. Plasma glucose (PG) levels were measured every 30 min for 2 h after glucose load to calculate the reference AUC (PG-AUC). Interstitial fluid glucose AUC (IG-AUC) was measured using MIET simultaneously. MIET consists of two steps. First, a plastic microneedle array is applied to the skin of the forearm as a pretreatment to enhance transdermal interstitial fluid (ISF) extraction. Second, hydrogel patches are placed on the pretreated area to collect ISF over a 2 h period. Accumulated glucose and sodium ion levels in the hydrogels are subsequently measured for calculating the IG-AUC at 2 h after glucose load.
Results: Before drug administration, fasting plasma glucose (FPG), 2-h PG, and PG-AUC were 108 ± 18 mg/dl, 310 ± 68 mg/dl, and 526 ± 85 mg•h/dl, respectively. After drug treatment, FPG was unchanged (113 ± 22 mg/dl), but 2-h PG and PG-AUC significantly reduced to 143 ± 74 mg/dl ($P < 0.01$) and 339 ± 123 mg•h/dl ($P < 0.01$), respectively. A good correlation between PG-AUC and IG-AUC ($R = 0.94$) was confirmed before and after the treatment. The reduction levels of IG-AUC by the treatment highly correlated with those of PG-AUC ($R = 0.92$), indicating that drug efficacy was precisely monitored by IG-AUC. Furthermore, the majority of patients reported neither pain nor discomfort from the MIET procedure.
Conclusion: The glucose AUC measured by MIET provided a good index for short-term monitoring of incretin-related drug efficacies without the need for blood sampling. The MIET system could be used for evaluating the efficacy of other drugs used to control postprandial hyperglycemia, such as other dipeptidyl peptidase-4 inhibitors, sodium-glucose co-transporter-2 inhibitors, alpha-glucosidase inhibitors, and glinides.



Supported by: Sysmex

1087

Very well controlled patients using InsuPad experience reduced insulin requirements and less hypoglycaemic events: results from the real world BARMER study

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Background and aims: The InsuPad device has been developed to enhance insulin absorption by standardized warming of the injection site after insulin administration. The primary objective of this prospective controlled trial was to investigate the impact of InsuPad use on prandial rapid acting insulin dose and glycemic control when studied under real world conditions.
Material and methods: This study was performed with 145 patients (51 female, 94 male, 13 type 1 and 132 type 2 patients, age: 61.6±8.4 yrs., disease duration: 16.6±7.2 yrs., HbA1c: 7.19±0.50 %, body weight: 105.7±18.6 kg). All patients were treated with multiple daily injections with insulin glargine and any of the existing short acting insulin analogs (aspart, glulisine, lispro). After a run-in treatment optimization and basal insulin stabilization period of up to 4 weeks, the patients were randomized to continue therapy for three months without (Control, n = 72) or with InsuPad (n = 73 patients). Only 3 visits at the site (screening, baseline, endpoint) were performed to ensure real-world conditions. Observation parameters included HbA1c, insulin dose, frequency of hypoglycemia, and body weight.
Results: During the run-in period, HbA1c decreased in the whole group from 7.2±0.5 % to 6.8±0.5 % ($p<0.001$), and further improved in both arms until study end (Control group: 6.3±0.5 % InsuPad: 6.2±0.5 %; both $p<0.001$ vs. baseline, n.s. between the groups). To achieve this glycemic control, patients in the control group needed an increase in the daily prandial insulin dose from baseline by 8 % (from 66±32 U to 71±38 U, $p<0.05$) with stable basal insulin requirements (47±20 U vs. 47±20 U, n.s.). Patients in the InsuPad group required significantly less prandial insulin to reach these HbA1c results (70±43 U to 55±34 U; -19 %, $p<0.001$) and a slight increase in the basal insulin dose (from 50±32 U to 52±36 U, $p<0.05$). In consequence, total daily insulin dose increased in the control group (+3.7 %) and decreased with InsuPad (-8.6 %, $p<0.001$ between the groups). The number of hypoglycemic events (blood glucose readings <63 mg/dL) during the observation period was significantly higher in the control group (6.2±9.9/patient) than in the InsuPad group (3.4±4.9/patient, $p<0.05$).
Conclusion: When treating patients to target with intensified insulin therapy, use of the InsuPad device for three months resulted in a significant lower frequency of hypoglycemic events and a significant reduction in insulin analog requirements as compared to a control group not using the device under real-world conditions. InsuPad may be useful to achieve treatment targets with a safer and more efficient basal bolus therapy in insulin-treated patients with type 1 and type 2 diabetes.
Clinical Trial Registration Number: NCT01594801
Supported by: Insuline Medical

1088

Proof of reliability of intradermal devices under extended wear basal/bolus infusion conditions

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Background and aims: Intradermal (ID) insulin administration provides faster onset of insulin action, increased early phase bioavailability, and a less invasive delivery interface than subcutaneous (SC) infusion. The primary objectives of this study were 1) to characterise an investigational ID insulin infusion catheter's ability to infuse placebo solution to ambulatory human subjects under extended wear basal/bolus conditions using commercial insulin pumps and 2) to characterise variations in pressure during infusion.
Materials and methods: This was a single-center, 2-arm, randomised, open-label study in 50 healthy, non-diabetic subjects in an inpatient setting. Diluent was infused at a basal rate of 1U/hr with three 10U boluses at meals and one before bed (1U = 10 μL). Each subject participated in one study arm consisting of four infusion sets of a single type (ID or SC) worn simultaneously. The ID arm consisted of two ID catheter sets (34G, 1.5 mm, stainless steel microneedles with different proprietary geometries) delivering via two commercial pump types (OneTouch®Ping® and MiniMed Paradigm®723). The SC arm included two commercial catheter sets (28G, 6 mm, stainless steel (SC-SS) & 24G, 6 mm, polytetrafluoroethylene (SC-PTFE)) with a single pump type (OneTouch® Ping®). Study endpoints addressed pump occlusion alarm occurrence, leakage, adhesion, tolerability, pain, and infusion pressure. A proprietary algorithm was used to characterise infusion pressure, measured by a commercial blood pressure transducer in-line between the pump reservoir and the infusion set. Effects of delivery route (ID vs. SC), ID set configuration, SC set type, and pump type (ID only) were tested with a generalised linear statistical model.
Results: Overall, ID delivery was successfully maintained over the 24 hour infusion period. The number of pump occlusion alarms was not significantly different based on delivery route or set type, but there was a significant difference between pump types ($p<.001$). For all SC and ID conditions, 85-95% of devices had no observable leakage or statistical differences among conditions. ID devices had less bleeding ($p<.05$), similar erythema, and less edema compared to SC ($p=.04$). Mean pain scores did not exceed 1 (on a 0-10 visual analogue scale) for any device, delivery route, or time point. Based on the proprietary infusion pressure algorithm, one ID catheter design had no significant difference in pressure endpoints compared to the SC-PTFE catheter. The percentages of total infusion time for which there was a flow interruption detected were 8.6% (SC-PTFE), 1.5% (SC-SS), 2.6% (ID-catheter A) and 11% (ID-catheter B).
Conclusion: This was the first study demonstrating that extended duration ID basal/bolus infusion is feasible in ambulatory human subjects. One investigational ID catheter showed flow performance similar to a commercial SC set, with equivalent tolerability. Commercially available SC infusion sets exhibited flow problems undetected by the pump, particularly in the first few hours after insertion. These flow anomalies merit additional investigation and study. Results suggest that ID infusion is biomechanically feasible for extended duration basal/bolus use. Further study on extended ID pharmacokinetics is underway.

1089

Insulin therapy with a 4mm x 32G pen needle vs. larger needles in obese subjects, including those taking high doses and/or glargine

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Background and aims: Pen needles (PN) as short as 4mm for insulin therapy have been shown to provide equal glycemic control, less pain, and are usually preferred by patients using pens. However, clinician hesitancy to use short needles remains, especially in obese patients and those injecting high doses and/or glargine.
Materials and methods: We performed a prospective, randomized, multicenter, crossover non-inferiority trial to compare glycemic control in obese

subjects with a 4mm x 32G PN vs two larger PNs (8mm x 31G and 12.7mm x 29G). Subjects with BMI ≥ 30 kg/m^2, HbA1c 5.5-9.5% and who injected insulin ≥ 2 months using only pens entered a 3-week wash-in period, using each study PN for one week. They were then randomized to either 4mm vs 8mm (N=127) or 4mm vs 12.7mm (N=147) PN study arms - order of PN use was also randomized. The 1° outcome was HbA1c after each 12-week study period; 2° outcomes included: pain by Visual Analog Scale (VAS), preference, ease of use, ease of insertion, injection anxiety, insulin leakage from skin, and safety. We also report on pre-defined subgroups - subjects taking high insulin doses (≥ one daily injection ≥ 40 units) or glargine, pooled across both study arms.

Results: Of 274 subjects randomized, 52% were male; 75% Caucasian, 18% Black; 92% T2DM. Mean age ± SD = 56.7 ± 11.0 yrs; BMI 37.0 ± 6.1, range 29.1-59.9 kg/m^2; insulin use duration 6.9 ± 7.7 years; total daily dose 78.4 ± 52.9, range 6-350 units; HbA1c 7.5 ± 0.9%; 226 subjects are included in the 1° A1c analysis. HbA1c with 4mm PNs was 0.08% lower (95% CI -0.21, 0.06) vs 8mm PNs, and 0.10% lower (-0.19, 0.00) vs 12.7mm PNs, both within the ± 0.4% equivalence margin. Median insulin dose changes were -2 to +5 units within study periods. Differences in perceived relative pain by 150 mm VAS were 12.4mm and 30.1mm lower for the 4mm vs the 8mm and 12.7mm PNs, respectively (both $p < 0.05$). Subjects preferred the 4mm vs the 12.7mm PN ($p < 0.05$), but NS vs the 8mm PN. The 4mm PN was rated superior for ease of use, ease of injection, and anxiety vs both larger PNs ($p<0.05$). Of ~131,000 injections, subjects reported skin leakage in 4.2%, 4.1%, and 4.3% of injections, respectively, with the 4mm, 8mm, and 12.7mm PNs (NS). Hyperglycemic (BG > 400 mg/dL) and hypoglycemic (BG < 50 mg/dL) event rates per 1000 patient days (1.9-3.0, and 8.0-9.9, respectively) did not differ between PNs. In the subgroup analyses, 118 subjects (43%) took high dose injections (mean total daily dose 113.5 ± 54.9, range 40 to 350 units), 174 (64%) used glargine (mean daily dose 77.5 ± 53.3, range 7 to 350 units), with 107 and 154 subjects completing the study, respectively. In high-dose subjects, HbA1c levels with the 4mm PN were -0.09% (95% CL -0.23, 0.05) compared to pooled 8mm and 12.7mm PNs and -0.08% (-0.16, 0.01) vs the longer PNs in the glargine group, both within preset HbA1c equivalence criteria (± 0.4%). The 4mm PN was rated significantly less painful than 8mm PNs in high-dose subjects, and vs 12.7mm PNs in the glargine subjects, both $p < 0.05$. Pain ratings trended similarly in the other subgroups but were NS. Reports of leakage for the three PN lengths were infrequent and NS, ranging from 3.4 to 5.1% in high-dose subjects, and 3.7 to 4.7% in glargine users.

Conclusion: For obese patients, including those injecting high insulin doses and/or glargine, the 4mm x 32G PN is safe, provides equivalent glycemic control, is less painful, better tolerated, and does not increase skin leakage, compared to larger pen needles.

Clinical Trial Registration Number: NCT1231984

Supported by: BD

1090

Extra thin-wall pen needles: impact on thumb force, insulin flow, time-to-deliver, patient preference and confidence in dosing

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Background and aims: Pen needles (PNs) are an essential component of pen devices used to inject insulin and can significantly affect patients' injection experience. A new extra thin-wall needle (XTW) technology was evaluated in a laboratory study of thumb force, flow rate and time-to-dispense and in a clinical study that evaluated patients' injection experience in persons with diabetes injecting insulin with Lilly KwikPen™, sanofi-aventis SoloSTAR®, and Novo Nordisk FlexPen® insulin pens.

Materials and methods: Laboratory studies measured differences in thumb force, flow rate, and time-to-deliver medication between similar size, thin-wall PNs and XTW PNs using an Instron® Universal Testing Machine (Instron, Norwood, MA, USA). The clinical study was a prospective, randomized, 2-period open label crossover trial in subjects 35 to 80 years with T1 or T2 DM who injected insulin by pen ≥2 months. Subjects using 4 to 8 mm length PNs with 31-32G diameter were randomly assigned to use their current PN or the same/similar size XTW PN at home for ~1 week, followed by the other PN in the second week. Subjects completed several comparative 150 mm Visual Analog Scales (VAS) and direct questions at the end of Period 2 for overall patient preference, ease of injection, perceived time to complete the full dose, thumb button force to deliver the injection, dose delivery confidence and leakage/dripping. Both the lab and clinical studies included appropriate sample sizes to provide sufficient power to detect the impact of XTW PNs with each of the three insulin pens.

Results: Laboratory studies - XTW PNs showed thumb force reduced by 47% - 62%, increased flow rate by 108% - 149% and reduced time-to-deliver by 52% - 60% (all $p\leq0.05$). Clinical study - 216 subjects were randomized (80 SoloSTAR, 77 FlexPen, 59 KwikPen), 209 completed both periods; 198 were evaluable. Subjects were (mean (± SD)) 60.8 ± 9.3 years old; 50.5% female; 89.8% T2 DM; used insulin pens 4.3 ± 4.1 years; used 75.1 ± 52.3 U/day and 2.9 ±1.5 injections/day. Subjects rated the XTW PNs (mean (95% CI)) as more preferable than their current PN (68.2 vs 11.6%), requiring less thumb force (60.6 vs 7.1%), and giving more confidence of full dose delivery (51.5 vs 9.1%). XTW PNs were associated with less injection time (48.5 vs 4.5%), less pain (64.1 vs 9.6%), greater insertion ease (63.6 vs 6.1%), and more convenience (36.4 vs 4%) - all $p< 0.001$. Skin leakage and insulin dripping from the needle tip were rated as less frequent with the XTW PNs ($p<0.05$). There were no significant differences in reported AE's.

Conclusion: Extra thin-wall pen needles improve fluid flow, require less force and less time-to-deliver medication. Extra thin-wall pen needles were preferred overall, rated as requiring less time and less thumb force to inject, and provided greater confidence in completing a full dose in actual use, compared to usual needles.

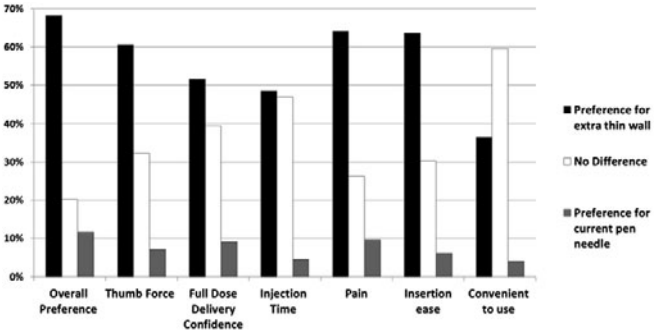

Figure - Distribution of preference responses for extra thin wall and current pen needle

Supported by: BD

PS 090 Closed loops and CGMS

1091

Inpatient evaluation of an automated closed-loop control-to-range system

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Background and aims: The Control-to-Range (CTR) study was a multinational artificial pancreas study designed to minimise the time spent in the hypo- and hyperglycaemic ranges using continuous glucose monitoring (CGM) and subcutaneous insulin infusion in individuals with type 1 diabetes. The controller was challenged by over- and missed-bolused meals and a period of exercise (50% VO2 max). The study was designed to demonstrate that the closed-loop system met a set of pre-defined metrics for efficacy and safety with an attempt to keep the glucose between 70-180 mg/dl. The range controller used an average model with patient-specific tuning and was accompanied with a safety algorithm to minimize hypoglycaemia.
Materials and methods: We studied 53 individuals: 27 ≥18 y.o. (mean 41±11), 26 <18 y.o. (mean 15±1); mean duration T1D 25±11 years and 8±3 years, respectively; mean A1c 7.7±0.6 % and 8.1±0.9%, respectively. Subjects were studied in-clinic for approximately 54 hours on 2 or 3 separate sessions. Glucose values from 1 of 2 CGM sensors (Dexcom) inserted two days prior to each session were the input of the system. The subjects arrived at 7 AM and a study CSII pump (Insulet) was inserted. Plasma glucose was measured every 15-30 min (YSI). Day 1, the subjects ate, and bolused for 3 mixed meals (1 gm carbohydrate (CHO)/kg body wt, 100 g max). On the morning after the first admission, subjects ate a meal that was not announced to the controller (1 gm CHO/kg body wt, 50 gm max). Subjects returned for a second session, similar to the first except that the afternoon included 1 hour of moderate exercise and the second breakfast was over-bolused by 30%.
Results: During the blinded CGM wear prior to the admission, the median percentage of values 71-180 mg/dl was 56% for adults and 46% for adolescents. Overnight time in target was 59% and 50% respectively, and daytime time in target was 61% and 45% respectively. During the first day of closed-loop control (large meal challenges, but no exercise or mis-bolusing challenges), adults' mean percentage of values 71-180 mg/dl was 66% overall (95% Lower Confidence Limit (LCL) = 62%), 82% overnight (95% LCL=77%), and 59% during daytime (95% LCL=52%). The median mean glucose was 161 mg/dl. YSI values ≤70 mg/dl occurred in 48% of admissions, and YSI values >300 mg/dl occurred in 22%. The controller requested CHO treatment for anticipated hypoglycaemia in 70% of admissions. For adolescents during the first day of closed-loop control, the mean percentage of values 71-180 mg/dl was 62% overall (95% LCL=55%), 82% during overnight (95% LCL=75%), and 53% during daytime (95% LCL=44%). The median mean glucose was 163 mg/dl. YSI values ≤70 mg/dl occurred in 20% of admissions, and YSI values >300 mg/dl occurred in 32%. The controller requested CHO treatment for anticipated hypoglycaemia on 60% of admissions. There were no cases of severe hypoglycaemia or diabetic ketoacidosis.
Conclusion: Inpatient glycaemic control with the CTR system was safe and effective. An updated CTR system is being tested in the outpatient setting.
Clinical Trial Registration Number: NCT01271023
Supported by: JDRF 22-2009-796 AP Consortium

1092

Tighter glucose control through a reduction of hyperglycaemia results from closed-loop intra-peritoneal vs subcutaneous insulin delivery in type 1 diabetes

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Background and aims: Closed loop insulin delivery aims at keeping blood glucose close to normal in patients with Type 1 diabetes (T1D) based on continuous glucose monitoring (CGM) and prediction of glucose levels according to a control algorithm. Faster insulin action from intra-peritoneal (IP) infusion is expected to improve control performance vs. subcutaneous (SC) delivery.
Materials and methods: Ten T1D patients (7M/3F, age: 49 +/- 11, BMI: 24 +/- 4, HbA1c: 7.7 +/- 1.0%, T1D duration: 29 +/- 14 years, insulin pump use: 8.5 +/- 7.8 years) underwent two 24-hour closed-loop insulin delivery trials in our Clinical Investigation Center: 1) with SC fast-acting insulin analogue infusion and 2) with IP regular insulin infusion after the implantation of a DiaPort system. For both trials, CGM was obtained from a Dexcom Seven Plus device and a zone-model predictive control-based algorithm was used. Control was evaluated based on ability to blunt glucose peak for large unannounced meals as well as the effect of pump suspensions to avoid hypoglycemia.
Results: Percentages of time (mean +/- SD) spent in 70-180 mg/dl and 80-140 mg/dl blood glucose ranges were both significantly increased with IP infusion: 65 +/- 9 vs. 42 +/- 14 (p=0.01) and 40 +/- 7 vs. 24 +/- 13 (p=0.02), respectively. Mean blood glucose levels (mg/dl) were also significantly lower with DiaPort use: 152+/- 11 vs. 192 +/- 31 (p=0.004). Tighter glucose control with IP infusion came from a significant reduction of time spent > 180 and 140 mg/dl: 32 +/- 9 vs. 54 +/- 17 (p=0.01) and 52 +/- 10 vs. 69 +/- 16 (p=0.02), respectively, while time spent < 70 mg/dl was similar: 2.8 +/- 3.3 (IP) vs. 4.4 +/- 5.4 (SC). Significantly higher daily units of insulin were infused with IP route: 46 +/- 17 vs. 35 +/- 11 (p=0.006).
Conclusion: This study comparing IP regular insulin vs. SC fast-acting insulin analogue infusions driven by the same CGM source and control algorithm design in the same patients supports the effectiveness of a faster insulin action thanks to DiaPort use in the reduction of post-meal excursions following unannounced meals. Besides, higher amounts of insulin can be delivered with IP route without impairing closed-loop safety through hypoglycemia.
Clinical Trial Registration Number: NCT01555788
Supported by: JDRF 17-2011-515

1093

Nocturnal glycaemic control with the MD-Logic artificial pancreas at type 1 diabetes mellitus patient's home

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Background and aims: Inevitable hypoglycemic events are still the reality and a true concern for all patients with diabetes, especially at night. Several multicenter, multinational, randomized studies have shown that MD-Logic Artificial Pancreas (MDLAP) can improve glucose control and reduce nocturnal hypoglycemia. This study assessed the feasibility, safety, and efficacy, of the MDLAP during the night at patients' home, under real-life conditions.
Materials and methods: Presented is an interim analysis of an ongoing randomized, multinational, crossover, single-blinded trail. It is a two-arm study, each consist of 4 consecutive nights, comparing the MD-Logic ("closed-loop" arm) versus sensor-augmented pump therapy ("control" arm). 15 patients [age 19±10.4y, A1c 7.5±0.5%, diabetes duration 9.9±8.2y] were randomly assigned either as "Group A" (first "closed-loop", then "control" arm) or as "Group B" (vice versa). All overnight sessions were monitored via the internet to remote sites, investigators were masked to treatment intervention. Primary endpoints were the time spent with glucose levels below 70mg/dl (3.9mmol/l), and the percentage of nights, in which the mean overnight glucose levels were within 90-140mg/dl (5-7.8mmol/l). Study is expected to end by 6.2013.
Results: Time of glucose levels spent below 70mg/dl was significantly shorter under closed-loop versus control night [3.8 (0, 11.6) and 48.7(0.6, 67.9) min, median (interquartile range, IQR) respectively, P=0.003]. The percentage of individual nights, in which mean overnight glucose level was within 90-140mg/dl was 67 (IQR, 33 to 88), and 50 (IQR, 25 to 75), under closed-loop and control nights respectively. Secondary endpoints' analyses demonstrate significant improvements in hypoglycaemia parameters. No serious adverse events were reported.
Conclusion: The feasibility, safety, and efficiency of a closed-loop system are shown in home use, with smooth integration within patients' lives. Results of the entire study will be presented.
Clinical Trial Registration Number: NCT01726829
Supported by: Sanofi

1094

Performance of a new continuous glucose monitoring (CGM) system in youth with diabetes

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Background and aims: Data from the TID exchange shows that CGM use in children and adolescents has been very limited and some past assessments of pediatric users suggest dissatisfaction with CGM performance. We studied the performance of the new Dexcom G4 PLATINUM (DG4P) CGM system in 176 youth (age 2 - 17, mean 11.5, at 6 centers. Most youth (72%) used insulin pumps; mean A1C was 8.2±1.3%; zBMI was 0.5 (range -4.7 to 2.6).
Materials and methods: Youth wore 2 systems (1 blinded, 1 displayed) on either the abdomen and/or upper buttocks for 7 days of home use. Youth/parents performed CGM calibrations using SMBG twice daily. Each youth ≥ 6 years old (n=147) had a single in-clinic session lasting up to 6 hours on sensor days 1, 4, or 7 in which "arterialized" (via a heating pad over an arm IV) venous YSI reference samples were collected up to q15 minutes for comparison with SMBG and CGM data. The in-clinic session for the 29 youth < 6 years old only included a comparison of fingerstick SMBG with CGM results q30 minutes for 4 hours.
Results: Using capillary glucose obtained with SMBG as reference, the DG4P MARD was 15% in 16318 paired samples with similar results from the abdomen (14%) and buttocks (16%). There were no meaningful differences when supplemental topical adhesives were used to secure the sensor. The mean absolute realtive difference (MARD) was 17% in the 2-5 age group, 16% in ages 6-12, and 15% in ages 13-17. The MARD of the sensor decreased from 19% on day 1 to 12% on day 7. In a comparison of fingerstick SMBG to YSI "arterialized" glucose in 1296 paired samples, the MARD was 13%, higher than in previous adult studies. Consequently, since capillary glucose is used to calibrate the system, the DG4P MARD using YSI reference was 17% (N=2922), discrepant from the performance observed in comparison to capillary glucose. After adjustment for the bias of YSI and capillary glucoses values in simulated post-hoc analyses, the MARD of CGM to YSI reduced to 15%, similar to accuracy using capillary glucose as reference.
Conclusion: This is largest pediatric CGM performance study to date, and included young children 2-5. DG4P performance compared favorably to the CGM system currently approved for pediatric use. There were minor differences at wear sites and across age groups. Performance of SMBG and CGM in comparison to YSI was likely impacted by arterialization challenges in pediatric subjects. After adjustment, the DG4P performance to YSI was similar to SMBG.
Clinical Trial Registration Number: NCT01667185

1095

Effect of repeated continuous glucose monitoring (CGM) profiles on glycaemic control of dialysis patients with diabetes: DIALYDIAB study

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Background and aims: Diabetes mellitus (DM) is a frequent condition in dialysis patients and increases morbi-mortality. Metabolic control is particularly difficult in this population with low accuracy of HbA1c and increased glycemic variability due to dialysis. CGM reliability has been recently validated in this population. The aim of our study is to explore the impact of repeated CGM on glycemic control of dialysis patients with DM (any type), versus usual care with self monitoring blood glucose (SMBG).
Materials and methods: Dialydiab is a before-after monocentric 12 weeks pilot study in a dialysis unit. There are two periods of 6 weeks during this study : first period = SMBG period (6 weeks): 3-6 SMBG/day; second period = CGM period (6 weeks): 5 days CGM (covering days with or without dialysis) every 2 weeks. SMBG (first period) and CGM (second period) profiles are evaluated by a single diabetes expert (centralized procedure) who suggests diabetic treatment adaptations to the dialysis center. CGM data, HbA1c and treatment adaptations are evaluated before (T0), between (T1) and at the end (T2) of the two 6 weeks periods.
Results: Population: 15 DM patients (type 1 (n=2), type 2 (n=9) secondary (n=4)) ; age 60.9±14.8 years ; diabetes duration 19.2±8.5 years ; dialysis duration 6.5±6.9 years ; treatment (lifestyle (n=3), insulin (n=12)). At T0, T1 and T2 (respectively) : mean CGM glucose was 151.64±44.60, 148.70±29.54 and 140.12±28.66 mg/dl (p=0.048 T2 vs T0) ; area under curve (AUC) hyperglycemia was 16.18±25.24, 10.27±14.83 and 7.23±8.32 (p=0.035 T2 vs T0) ; MAGE was 109.62±40.76, 101.70±54.83 and 90.85±31.96 mg/dl (ns) ; AUC hypoglycemia was 0.4±0.84, 0.01±0.03 and 0.09±0.16 (ns T2 vs T0) ; HbA1c was 6.85±1.48, 6.61±1.24 and 6.46±1.49% (p=0.039 T2 vs T0). There were more treatment adaptations during the CGM period, compared to the SMBG period : 2.1±0.9 vs 1.4±1.0 adaptations/patient (p=0.022). Quality of life was not different between the two periods. CGM profiles were significantly different for days with vs without dialysis : all day mean CGM glucose was 138.09±18.43 vs 142.52±11.86mg/dl (p=0.0197) and dialysis period mean CGM glucose was 123.28±10.94 vs 136.44±14.53 mg/dl (p=0.0147), respectively. We found no difference in the CGM profiles during the 12 hours period following dialysis.
Conclusion: We show that repeated CGM profiles, compared to SMBG, significantly improve glycemic control of dialysis patients with diabetes : decrease of mean CGM glucose, decrease of hyperglycemia exposure, decrease of HbA1c, without increase of hypoglycemia. This improvement is the result of more frequent treatment adaptations during the CGM period, reflecting the unknown glycemic drifts with SMBG. The process of remote centralized interpretation of CGM profiles by a diabetes expert opens the field of telemedicine. The results of this pilot study encourage further studies : first, it would be important to define the CGM glucose targets for these patients. Then, larger and longer studies should explore the effect of repeated CGM on glycemic control and morbi-mortality in this population. Medico-economic evaluations would also be important in regard of the cost of CGM technology.

1096

A comparison of utilising SMBG vs CGM data to optimise glycaemic profiles and glucose control in patients with type 2 diabetes

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Background and aims: Glycemic control in T2D is often judged by A1c and the rate of hypoglycemia. CGM better defines hypoglycemia and provides time in range and glucose variability. We report the first randomized, controlled, prospective study using CGM and A1c to compare the ability of structured SMBG testing (stSMBG) and real time CGM (rtCMG) to optimize glucose control.
Materials and methods: Subjects with T2D (N=114), age 59±0.9 yrs, duration 12±0.6 yrs, BMI 34±0.6 kg/m^2 used 2 wks of blinded CGM then were randomized to stSMBG [SMBG 4x /day and 3 x 7 point BG profiles (360 view) q2-4 wks] or rtCGM [CGM daily and CGM ambulatory glucose profile(AGP) report q2-4 wks] for 16 weeks. Therapy adjusted q2-4 wks. We evaluated if stSMBG or rtCMG led to improved glucose control and if one approach was safer or more effective based on a change in A1c and 2 wks of CGM in both groups (blinded CGM for stSMBG vs. rtCMG).
Results: See TABLE**:** Both stSMBG and rtCGM resulted in significant improvements in A1c, time in range and glucose variability (with no significant difference between groups). rtCGM resulted in a significant reduction in hypoglycemia compared to stSMBG, particularly in SU and insulin pts (not shown).
Conclusions: Collecting, analyzing and discussing structured SMBG or rtCGM (with AGP report) results in a significant improvement in glucose control and the glucose profile. CGM compared to SMBG also resulted in a reduction in hypoglycemia while improving glucose control particularly in SU and insulin treated pts.

Variables A1c & CGM data (14 days)	Baseline (Time 0)	Study End (16 weeks)	Change Baseline to Study End	Change within group p value	Change between groups p value
A1c (%)					
stSMBG	7.85±0.79	7.0±0.5	-0.82±0.12	<.0001	0.11
rtCGM	8.19±1.2	7.07±0.85	-1.12±	<.0001	
% in target (70-180 mg/dL)					
stSMBG	55±20	67±2.8	12±3.2	0.0006	0.1323
rtCGM	52±3.1	71±2.7	18.2±3.0	<.0001	
Variability (IQR)					
stSMBG	60±2.2	54±2.3	-5.91±2.2	0.0113	0.4264
rtCGM	62±3.1	53±2.8	-8.4±2.1	0.0002	
% > 180 mg/dL					
stSMBG	44±2.7	31±2.9	-12.8±3.3	0.0002	0.3151
rtCGM	46±3.2	29±2.8	-17.4±3.2	<.0001	
% <70 mg/dL					
stSMBG	1.04±0.20	2.26±0.44	1.22±0.38	0.0021	0.0044
rtCGM	1.68±0.62	0.88±0.23	-0.8±0.57	0.1671	
% <60 mg/dL					
stSMBG	0.51±0.11	1.21±0.28	0.7±0.25	0.0065	0.0041
rtCGM	1.05±0.44	0.38±0.12	-0.67±0.39	0.0906	
% <50 mg/dL					
stSMBG	0.21±0.05	0.62±0.16	0.41±0.16	0.0163	0.0064
rtCGM	0.58±0.28	0.16±0.06	-0.42±0.24	0.0893	

Clinical Trial Registration Number: NCT01237301
Supported by: Roche Diagnostics GmbH

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Improvement of metabolic control after three months use of RT-CGM in type 1 diabetics treated with insulin pump: the multicentre Greek DIAMOND study

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Background and aims: To evaluate the efficacy of adding continuous glucose monitoring (CGM) for three months to insulin pump therapy (CSII) in patients with siabetes mellitus type 1 (DMT1) in a multicenter Greek study (THE DIAMOND STUDY).
Materials and methods: In total, 11 Diabetes Centers in Greece participated to the study. Forty three patients (24 female, 55.8%), with mean age 33.6±8.1, mean duration of T1DM 14.5±9.8 years and treated with continuous subcutaneous insulin infusion were enrolled prospectively. All patients were on CSII (MiniMed Paradigm) for three months at least before the use of CGM. Then all participants were instructed to wear the MiniMed Paradigm REAL-Time System (Medtronic Inc.), which integrates both CSII and RT-CGM functionalities, at all times throughout the study for the next three months. At the end of the study we evaluated the following parameters: HbA_{1c}, BMI, hypoglycaemic episodes (HYPO), total daily insulin requirement (TDI), total daily insulin for boluses (TDIBOL), number of daily boluses (NOBOL), total daily insulin basal (TDIBASAL) and percentage of total time use of sensor (PTTU).
Results: The mean PTTU was 74±17.0%. The results of the other examined variables were as follows [mean±1SD] in patients before and after the use of CGM: HbA_{1c} 8.3±1.2% vs 7.5±1.0% (p< 0.001), TDI 45.7±15.3 vs 50.8±23.9 (p= 0.018), TDIBOL 24.1±10.4 vs 28.3±19.0 (p= 0.033) and NOBOL 4.7±1.5 vs 6.3±2.4 (p< 0.001). No significant change observed in BMI, HYPO and TDIBASAL before and after the use of CGM. No ketoacidosis observed throughout the study. 44, 2% of the patients achieved HbA_{1c} targets (< 7%).
Conclusion: In the present study continuous glucose monitoring was associated significantly with improvement of glycaemic control without BMI and hypoglycaemic episodes change, in patients with type 1 diabetes using CSII. Better self-management and increase of the doses and units of insulin may have contributed to these beneficial effects.
Clinical Trial Registration Number: 01369823
Supported by: Medtronic

1098

Poor agreement of computerised calculators to calculate the mean amplitude of glycaemic excursions

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Background and aims: Glucose variability has been identified as a predictor of hypoglycaemia and is associated with intensive care unit mortality. A popular metric to quantify glucose variability is the mean amplitude of glucose excursions (MAGE). The 'ruler and pencil' approach to calculate MAGE is operator dependent and time-consuming for analysis of continuous glucose monitoring (CGM) data. Therefore, several computer software programs have been developed for the automated calculation of MAGE. The aim of our study was to evaluate the agreement of currently available MAGE calculators when applied to the same set of CGM traces.
Materials and methods: Four software programs for calculation of MAGE from CGM data were identified. The software programs were used to calculate MAGE of forty-two CGM traces from seven patients with type 1 diabetes. Correlation between the MAGE values obtained by the different MAGE calculators was evaluated by Spearman's correlation analysis. A correlation-coefficient (r) of at least 0.95 was considered a sufficient correlation, given the fact that the calculators aim at assessing the same metric. Lastly, between-group comparison of the median MAGE per calculator was performed using analysis of variance.
Results: Only one calculator was able to calculate a MAGE for all the provided CGM traces. The other calculators showed 1, 2 and 15 missing MAGE values. The correlation between the calculators ranged from 0.3899 to 0.8765 ($p < 0.05$ for all). Mean differences ranged from -0.68 to 1.76. Between-group comparison showed that overall median MAGE was statistically different (P=0.002).
Conclusion: Available computer programs developed to calculate MAGE agree poorly. Although software programs for the calculation of MAGE would seem attractive to assess glycaemic variability, their use is limited by widely different outcomes, in the absence of a gold standard.

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Continuous glucose monitoring via telemetry in rats

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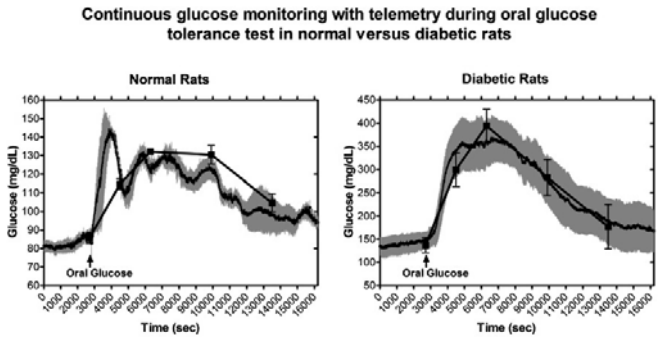
Background and aims: The current standard for routine glucose measurements in preclinical research is often glucometers and test strips. These pose significant limitations in terms of accuracy, animal stress, and frequency of sampling. Until now, continuous monitoring options for preclinical research have been very limited. The present study evaluates the use of a novel prototype device (Data Sciences International) for acute and chronic glucose measurements in rats.
Materials and methods: The device is 1.4cc and provides temperature, activity, and direct continuous blood glucose readings for 4 weeks or longer. The devices were evaluated in 4 diabetic and 4 normal Zucker fa/fa (ZDF) rats and in 10 Zucker diabetic/Sprague Dawley (ZDSD) rats. Each animal was surgically instrumented with glucose sensors in the abdominal aorta and the telemetry device placed in the intraperitoneal (ip) cavity. Continuous glucose readings were recorded for 5-7 weeks with periodic fasting GTTs (glucose, 2-3 g/kg, po or ip). Daily and GTT reference values were recorded with a StatStrip Xpress glucometer (Nova Biomedical).
Results: The glucose sensors provided high resolution data and demonstrated the ability to accurately assess chronic diurnal patterns matching with the feeding pattern of rats from 3 days up to 7 weeks after surgery.
Conclusion: These devices hold great potential for comparing physiologic processes associated with glucose regulation in normal and disease condition rats; monitoring diabetes progression and developing preventive treatments for type II diabetes.



PS 091 Insulin pumps in clinical practice

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Intermittent glucose monitoring in type 1 diabetics on insulin pump: is there difference in glycaemic control between real-time and retrospective analysis?

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Background and aims: The aim of the study is to describe glycemic outcomes in type 1 diabetics using an insulin pump with intermittent Real-Time and Intermittent Retrospective continuous glucose monitoring (CGM).
Materials and methods: A total number of 57 type 1 diabetics using insulin pump therapy (Medtronic 7xx, Medtronic, Northridge, CA) for at least one year, used CGM in 6 sessions (each session was 7 days every month) in a period of 6 months. The patients were randomized in two groups: Intermittent Real-Time (IRT) CGM group, 29 patients using real-time CGM (Minilink with En-lite sensor, Medtronic, Northridge, CA), where patients could see the glucose value and respond adequately (Real Time CGM) and Intermittent Retrospective (IRS) CGM group, 28 patients using retrospective CGM (Ipro2 with En-lite sensor, Medtronic, Northridge, CA), where patients could not see the glucose value (blinded CGM). After each session (both Real-time and retrospective CGM), data was downloaded using specific software (Carelink Personal and Carelink Ipro, Medtronic, Northridge, CA) and specific instructions for optimization the pump therapy (basal and bolus insulin, education on food, physical activity and hypoglycemia/hyperglycemia) were given to the patients. HbA1c was obtained before, three and six months after the study.
Results: Both groups significantly improved glucose control (HbA1c) from 7.5±0.9% to 6.6±0.7% in IRT group and from 7.4±1.1% to 6.5±0.8% in IRS group, but there was no significant difference between both groups at the end of the study. There was no severe hypoglycemia in both groups. There was no significant difference in TDD of insulin and number of boluses.
Conclusion: Both intermittent real-time and retrospective CGM can improve glucose control in type 1 diabetics on insulin pump where our study showed no significant difference in decreasing of HbA1c using both methods. Further investigation on larger groups should be performed to confirm our findings.

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Quantitation of the roles played by the main determinants of meal glucose tolerance in patients with type 1 diabetes on insulin pump therapy

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Background and aims: The relative roles of each component of the glucose (G)/insulin (I) system in determining post-meal hyperglycemia in type 1 diabetes (T1DM) are still under debate. The use of Metabolic Control Analysis allows to compute the coefficients of control of G (CC_G), which quantify the control exerted by each component of the G/I system on G concentration. Aim of this research was to quantify the CC_G of the main components of the G/I system during a mixed meal test (MMT).
Materials and methods: 7 T1DM patients (age: 41±5 yrs; BMI: 24.3±0.6 $kg \cdot m^{-2}$; HbA1c: 7.9±0.2%) on I pump and continuous G monitoring (CGM) participated in 2 studies:1. Euglycemic I (240 $pmol \cdot min^{-1} \cdot m^{-2}$ BSA) clamp (duration: 120 min, M value: 1209±169 $\mu mol \cdot min^{-1} \cdot m^{-2}$ BSA) including CGM; 2. MMT (292 Kcal; 38.9 g complex CHO, 8.9 g lipids, 14 g proteins) with plasma I/G assessment and CGM for 300'. With our modeling strategy, data from both clamp and MMT are used to build an in silico replica ("virtual patient") of the G/I system of each patient (CGM included), which behaves as the real patient during the MMT. Virtual patients were used to compute the CC_G of both plasma G and CGM measured G.
Results: During the MMT, plasma G and I started from 9.5±1.8 mM and 75±8 pM and peaked at 12.2±1.4 mM (time: +90') and at 136±18 pM (time: +60') respectively. The CC_G of key components of the G/I system are in the Table. The apparent size of s.c. I depot had the highest CC_G ($p<0.01$ or less vs other CC_G). The CC_G of meal I bolus peaked at 300' ($p<0.01$ vs CCs at 30'-

60'). The CC_G of CHO transit time across the gut, including also absorption, was relevant in the first half and became negligible in the second half of the meal (p<0.01 CC_G 180-300' vs CC_G 30'-120'). The CC_G of CGM measured G were parallel, but not superimposable to CC_G of plasma G, with significant differences (p<0.03-0.01) in CC_G of gut CHO transit time.
Conclusion: In patients with T1DM on I pump therapy, after a MMT: 1. a primary determinant of plasma G may be the size of subcutaneous I depot; 2. pre-meal I bolus may be much more influential in the last 3 than in the initial 2 hours. These findings may have important implications for the development and the refinement of closed loop control of s.c. I delivery systems. Table. CC_G of meal carbohydrate (CHO) content, gut mean transit time (MTT) of meal CHO, rate of splanchnic CHO extraction (SGE), pre-meal I bolus, apparent size of s.c. I depot, meal I sensitivity (S_I) and G effectiveness (S_G) after the meal. The signs +/- indicate that an increase in the parameter causes an increase/decrease in plasma G.

		Control coefficients of plasma glucose after MMT (mean±SEM)					
		Time after MMT ingestion					
	Parameter value (mean±SEM)	+30'	+60'	+120'	+180'	+240'	+300'
Meal CHO content (g)	38.9	+0.36 ±0.02	+0.57 ±0.02	+0.72 ±0.02	+0.72 ±0.02	+0.66 ±0.03	+0.61 ±0.03
Gut CHO MTT (min)	85.3 ±8.6	-0.36 ±0.02	-0.50 ±0.02	-0.56 ±0.04	-0.05 ±0.03	+0.05 ±0.03	+0.08 ±0.03
Rate of SGE (min^{-1})	0.13 ±0.06	-0.08 ±0.02	-0.12 ±0.02	-0.18 ±0.04	-0.15 ±0.03	-0.14 ±0.03	-0.14 ±0.03
Meal insulin bolus (IU)	4.0 ±0.5	-0.14 ±0.04	-0.29 ±0.06	-0.50 ±0.05	-0.53 ±0.05	-0.58 ±0.05	-0.60 ±0.05
Apparent size of s.c. insulin depot (µl)	109 ±64	-0.54 ±0.14	-1.1 ±0.21	-1.8 ±0.33	-1.75 ±0.33	-1.81 ±0.34	-1.82 ±0.34
Meal S_I [$(ml\,min^{-1})/(pmol\,L^{-1})$]	0.72 ±0.15	-0.11 ±0.01	-0.19 ±0.02	-0.34 ±0.02	-0.37 ±0.02	-0.44 ±0.02	-0.48 ±0.03
Meal S_G ($ml\,min^{-1}$)	18.4 ±10	-0.05 ±0.01	-0.07 ±0.01	-0.13 ±0.02	-0.10 ±0.02	-0.11 ±0.02	-0.10 ±0.02

Clinical Trial Registration Number: NCT01800734

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HbA_{1c} and weight development one year after initiation of continuous subcutaneous insulin injection therapy in adult subjects
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Background and aims: The success of improvement in HbA1c after initiating or intensifying multiple daily injection (MDI) insulin therapy in subjects with diabetes is typically prevented by weight gain or hypoglycemia. Here we performed a retrospective observational analysis in adults with diabetes initiating continuous subcutaneous insulin injection (CSII) therapy in order to find out whether the effect of CSII is similar in clinical practice.
Materials and methods: All subjects initiating CSII at our Diabetes Center after year 2002 were analyzed for age at CSII initiation, HbA1c, weight and total insulin dose prior to and one year after pump initiation by non-parametric paired analysis and multiple regression analysis to find independent variables explaining the change in HbA1c or weight and adjust for relevant variables. Data are presented as mean ± SEM.
Results: 505 subjects had sufficient data to enter into the analysis, 184 males and 321 females; age at CSII initiation 40 ± 0.6 years (ns between males and females), weight before CSII 82.9 ± 1.1 and 70.0 ± 0.8 kg for males and females, respectively (p<0.0001), HbA1c before CSII 68.1 ± 0.6 mmol/mol (ns between males and females). At follow-up (after 312 ± 2.5 days) HbA1c had decreased significantly to 62.6 ± 0.9 mmol/mol in males and 60.6 ± 0.6 mmol/mol in females (p<0.05) with a significantly greater reduction in female patients (-7.6 ± 0.6 vs. -5.3 ± 0.7, p<0.05). While there was no significant change in weight for male subjects (-0.8 ± 0.6 kg), there was a slight but significant increase in weight for female subjects (+1.2 ± 0.4 kg, p=0.01 vs male subjects). The required total daily insulin dose decreased significantly in both males and females from 64 ± 6 to 46 ± 1 (p<0.0001) and 49 ± 1 to 38 ± 1 (p<0.0001) units, respectively, with a significant greater decrease in males compared to females in absolute (p<0.0001) but not in relative terms (ns). By univariate Spearman analysis a decrease in HbA1c was positively correlated with an increase in weight with no relation to the change in insulin dose whereas an increase in weight was negatively correlated to the decrease in insulin dose. Adjusting for relevant covariates by multiple regression analysis gender (p<0.05) and HbA1c prior to CSII (p<0.0001) were independent predictors of decrease in HbA1c (greater reduction for female sex and higher HbA1c) explaining 29% in the variability of change in HbA1c. Independent predictors for an increase in weight were HbA1c prior to CSII (p<0.05), weight prior to CSII (p<0.0001), and relative decrease in insulin dose (p<0.01) with no effect of gender (greater increase at lower HbA1c, lower weight, and lesser of a decrease in dose) explaining 17% of the variation in weight change.
Conclusion: Switching from MDI to CSII in adult patients with type 1 diabetes may lead to successful lowering of HbA1c in a clinical setting after one year. The HbA1c decrease is relatively greater in females with a higher starting HbA1c which is mirrored by an increase in weight in females compared to no change in males, despite the same relative reduction in total insulin dose. Meanwhile, the increase in weight in most cases was very marginal, particularly with regard to the observed substantial decrease in HbA1c (eq. to a 1.6 kg weight gain for every 10 mmol decrease in HbA1c). Gender differences may be important to consider when initiating CSII but neither this nor weight change appear to be significant barriers to a switch to CSII.

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Factors affecting glycaemic control in adult type 1 diabetic patients treated with personal insulin pumps
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Background and aims: Continuous subcutaneous insulin infusion (CSII) improves glycemic control and quality of life as compared to conventional insulin therapies. However, it is not clear which factors play the most significant role in optimizing therapy in CSII treated patients. The purpose of this analysis was to investigate retrospectively variables affecting metabolic control in T1DM (type 1 diabetes) patients treated with personal insulin pump.
Materials and methods: Data from 145 patients treated with CSII were analyzed. Sources of information about the patients included: available medical records, memory read-outs from insulin pumps, blood glucose meter data, biochemical analysis results and questionnaires. Data from the last three years were used for this analysis. The Shapiro_Wilk test was performed as a test for normality. Correlation analysis was used to study the relationships between various variables. The difference between the groups was analyzed with the Student t test. Nonparametric tests were used when necessary. Additionally, a multivariate regression analysis was performed for mean HbA1c. We indicate a significant result when p<0.05. Statistica 10.0 software was used for statistical analysis.
Results: The mean age of the patients was 28.6, mean BMI 23.1 kg/m^2 with an average duration of diabetes of 13.4 years. Mean HbA1c level for the whole studied group was 7.3% (mean glycemia from glucose meter readings - 151 mg/dl). Negative correlations between HbA1c and age (r = -0.31, p = 0.0003), number of blood glucose measurements per day (r = -0.31, p = 0.0010) and number of hypoglycemic episodes per day (r = -0.48, p = 0,0000) were found. Mean glucose levels from glucose meter memory (r = 0.71, p = 0.0000), blood glucose variability measured with standard deviation from glucose meter data (r = 0.54), daily insulin requirement (r = 0.23, p = 0.0100), insulin requirement per kilogram of body weight (r = 0,25, p = 0,0050) and CSII duration (r = 0,24, p = 0,0065) correlated positively with HbA1c level. Patients with higher education were characterized by lower HbA1c (p = 0.0384). The use of an extended/dual wave bolus had no effect on metabolic control (p = 0.30). Both the Bolus Calculator (BC) (p = 0.0303) and Continuous Glucose Monitoring System (CGMS) (p = 0.0119) option was associated with lower HbA1c. In multivariate regression analysis, HbA1c significantly correlated with mean blood glucose, number of daily blood glucose measurements and insulin dose per kilogram of body weight. The adjusted coefficient of determination was 71%.
Conclusion: This study demonstrates that both patient-related and technology-related variables have a strong influence on glycemic control in CSII-treated individuals. Self monitoring of blood glucose and usage of some advanced pump options like BC and CGMS are associated with a better chance of achieving treatment goals, while extended/dual wave bolus functions have no significant impact. CSII-treated patients with higher insulin doses are characterized with poorer metabolic control.

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Carbohydrate-to-insulin ratio in type 1 diabetic patients treated with continuous subcutaneous insulin infusion therapy (CSII)

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Background and aims: Patients with type 1 diabetes (T1DM) under CSII therapy usually calculate insulin boluses with the carbohydrate-to-insulin ratio (CH/IR), with the addition of a correction if pre-prandial blood glucose levels are above the objective. The initial calculation of the CH/IR is often done using the 500 formula (500/total daily doses of insulin-TDD). Some authors have suggested that the formula tend to underestimate this ratio and on the other hand the ratio is usually different along the day. The aims were to know the CH/IR used by T1DM patients under CSII therapy, the differences observed with the CH/IR obtained from the 500 formula, and also the differences of CH/IR along the day.
Patients and Methods: A total of 170 patients with T1DM under CSII therapy for at least 6 months and relatively good control were evaluated (132 women, age 45.5 ±10 years, diabetes duration 26.65 ± 10 years, CSII duration 9.15 ± 6 years, HbA1c 7.6 ± 0.8%). CSII indications were: non optimal glucose control with multiple daily injections (45%), optimization before pregnancy (29.8%), high glucose variability (8.2%) among others.
Results: The proportion of TDD as basal rate was 57.7 ± 13% and for boluses was 42.3 ± 12%. The table shows the CH/RI used in the main 3 meals, the CH/IR obtained with the 500 formula and the formula that should be used according to the real results obtained.

All patients	**Breakfast**	**Lunch**	**Dinner**
CH/IR	1.20±0.7 ui/10 g.	1.09±0.6 ui/10 g.	1.07±0.7 ui/10 g.
Calculation by 500 formula	1.55 ui/10 g.	1.55 ui/ 10 g.	1.55 ui/10 g.
Real calculation	507/TDD	460/TDD	455/TDD
Patients with HbA_{1c}<7%	**Breakfast**	**Lunch**	**Dinner**
CH/IR	1.2 ui±0.7 ui/ 10 g.	1.1±0.6 ui/10 g.	1.1±0.6 ui/10 g.
Calculation by 500 formula	1.6 ui/10 g.	1.6ui/10 g.	1.6 ui/10 g.
Real calculation	478/TDD	445/TDD	459/TDD

The CH/IR was significantly different between breakfast and lunch and between breakfast and dinner (p<0.05). Real CH/IR was also different that that obtained with the 500 formula in the 3 meals (p<0.05). CH/IR was higher at breakfast (p<0.05) that in the other meals.
Conclusions: The 500 formula for the calculation of CH/IR overestimate the real CH/IR used by T1DM patients under CSII therapy, especially for lunch and dinner. The CH/IR is higher at breakfast. A more approximate calculation can be obtained using the formula 480/TDD for breakfast and 450/TDD for lunch and dinner.

1105

Outcomes in people with type 1 diabetes transferring to insulin pump therapy after structured education

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Background and aims: In interpreting the results of studies of the use of Continuous Subcutaneous Infusion of Insulin (CSII) in people with Type 1 diabetes (T1DM), it can be difficult to disentangle the effects of education and of CSII per se on outcomes. Firstly, patients in such studies may not have attained optimal diabetes management through contemporary structured education before CSII is initiated. Secondly, those starting on CSII are necessarily provided with additional education and professional input, even in studies with a comparator group. Also, in many studies of the use of CSII, there is a paucity of data on psycho-social outcomes. We have therefore studied biomedical and psycho-social outcomes in people transferring to CSII compared to those remaining on Multiple Dose Insulin injections (MDI) after a one week UK Dose Adjustment For Normal Eating (DAFNE) intensive education course.
Materials and methods: The DAFNE research database is active in 10 UK diabetes centres and has ethical approval with patients required to give informed consent. Information on biomedical measures and from psycho-social questionnaires is collected at baseline and at 12 months after structured education. 1450 people with T1DM were registered on the database between January 2009 and June 2011.
Results: Of the above cohort, 938 people had paired baseline and follow-up data. Mean age was 44 (SD 14.0) years with duration of diabetes 20 (13.7) years. During 12 months follow-up, 57 (6.1%) patients transferred to CSII. At baseline, the Problem Area In Diabetes (PAID) score was higher in those that later transferred to CSII at 36.4 (19.9) compared with 28.3 (19.3) in those that remained on MDI (p=0.006). PAID scores of 21.7 (16.5) and 20.1 (17.0) respectively were similar at 12 months after structured education. At baseline, the frequency of severe hypoglycaemia was 1.31 events per patient year in those that transferred to MDI and 0.96 in those that continued on MDI (p=0.03). After DAFNE rates of SHE fell by approximately 75% overall and did not differ between groups at 12 months. In those that continued on MDI, HbA1c fell from 8.74 (1.53) % (72 mmol/mol) at baseline to 8.54 (1.49) % (70) at 12 months, and in those that transferred to CSII, was reduced from 8.79 (1.96) % (73 mmol/mol) to 8.20 (1.58) % (66 mmol/mol), with a greater fall in the latter (p=0.03).
Conclusion: In a group of people with T1DM undergoing structured education, those that subsequently transferred to CSII had normalisation of the higher levels of psychological stress (PAID score) and the increased rate of severe hypoglycaemia apparent at baseline and also had better overall glycaemic control at 12 months follow-up. Even after intensive structured education there remains a group of people with T1DM that gain additional benefits from CSII.
Supported by: NIHR UK

1106

Dose Adjustment for Normal Eating (DAFNE) structured education is associated with reduced progression to insulin pump among patients considered for pump before enrolment

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Background and aims: Current UK national guidelines recommend insulin pumps for people with type 1 diabetes suffering from 'disabling hypoglycaemia' or whose HbA_{1c} remains >8.5% on optimised multiple dose injection (MDI) regimens, but the meaning of 'optimised MDI therapy' is not clearly defined. Our aim was to determine if further training provided through the DAFNE (Dose Adjustment for Normal Eating) structured education programme could reduce the need for insulin pump therapy among patients already established on MDI.
Materials and methods: Retrospective study using a national database of participants attending DAFNE courses at 10 UK centres in 2009-10 being considered for insulin pump at time of enrolment. Baseline and one year data including treatment modality, HbA_{1c} , hypoglycaemia awareness status and severe hypoglycaemia prevalence (SH) were collected.
Results: 108 participants were considered for pump therapy at baseline. 34 proceeded to pump initiation after 202 ± 105 days. 69 remained on MDI after 384 ± 43 days follow-up; outcomes were unavailable for 5. Participants had mean and standard deviation age of 39 ± 13, diabetes duration of 20 ± 12 years and baseline HbA_{1c} 8.7 ± 1.8 %. 48% reported impaired hypoglycaemia awareness (IA) and 33% reported SH in the preceding year. 75% of patients enrolled for DAFNE who were considered for pump at enrolment fulfilled at least one criterion for pump, which fell to 60% at one year follow-up (p<0.05). Mean HbA_{1c} had fallen to 8.4 ± 1.5 % (p=0.04 vs. baseline) and there was a significant reduction in HbA_{1c} in participants who met insulin pump criteria based on HbA_{1c} (mean HbA_{1c} 10.2% at baseline vs. 9.4% at one year) (p<0.01). IA and SH prevalence reduced by 16% (p<0.001) and 20% (p<0.01) respectively.
Conclusion: Provision of DAFNE structured education was associated with reduction in proportion of participants meeting insulin pump criteria and prevalence of IA and SH, enabling 69% of patients being considered for insulin pump to remain on MDI. These findings strongly support optimisation of MDI through effective structured education prior to considering insulin pump as part of the treatment pathway for all patients with type 1 diabetes.
Supported by: National Institute for Health Research funded database

1107

Comparison between individually and group-based insulin pump initiation by time-driven activity-based costing

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Background and aims: Controlling cost and increasing the precision of decision making is important for efficient and cost-effective delivery of health care. Time-driven activity based costing (TDABC) offers the possibility of absorbing costs in terms of activities and may be particularly suited for service businesses, not least where alternatives for the same service are available. Here we used TDABC to compare initiating insulin pump therapy in groups (GT) to individual treatment (IT) in adult subjects with type 1 diabetes.
Materials and methods: Activities and cost drivers were identified and routines timed or estimated at location. Medical quality and patient satisfaction were assumed to be equal or non-inferior and were not measured. Other quantitative data were obtained from departmental sources. Overhead costs were allocated differentially based on profession and manager(s). The alternatives were compared under different scenarios and conclusions adjusted for the net effect of alternate cost, cost of opportunity, and differential reimbursement.
Results: GT was about 30% less time-consuming and 17% less cost driving per patient per activity compared to IT. When analysed as a batch driver GT produced an upward jig-saw cost accumulating curve compared to the incremental increase incurred by IT. *Variable batch*, i.e. also taking into account the alternate cost that taking care of patients who did not attend group sessions, was less cost driving than IT already when only five out of 16 patients attended the sessions. Estimated cost accumulation curves for variable batch treatment and the cost of opportunity that was generated in comparison to IT converged already before filling the first session with 16 individuals suggesting that another group could be booked at no additional cost.
Conclusion: TDABC is an effective method for comparing treatment alternatives yielding estimates of the opportunity costs and the possibility to increase cost control and improve decision making. In this particular case, pump therapy initiation in groups was clearly favoured over an individual approach from a financial point of view.
Supported by: Swedish Research Council, NN Foundation, ALF

1108

Experience with V-GO insulin delivery device in clinical practice

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Background and aims: V-GO is a disposable insulin delivery system providing set basal rates and bolus insulin on demand for 24 hours. We describe our early clinical experience with V-GO for the treatment of adults with suboptimally controlled type 2 diabetes (T2D).
Materials and methods: Adult T2D patients with suboptimal glycaemia control followed at a diabetes center were selected for insulin management using the V-GO device. Subjects were eligible for V-GO treatment if they demonstrated one of three difficult-to manage problems: 1) needle phobia limiting multiple dose insulin (MDI), 2) unwilling or unable to use conventional insulin pumps because of pump complexity, literacy/numeracy limitations, inability to carbohydrate count, or difficulty comprehending corrective insulin algorithms. 3) refractory hyperglycaemia despite self-reporting large daily insulin doses. All patients initiating insulin treatment with V-GO or transitioned from MDI to V-GO received either fixed basal doses of twenty (20) units, thirty (30) units or forty (40) units per day, with additional ability to receive 36 units of bolus insulin per day.
Results: Thirty six (36) patients with difficult-to-manage glycaemia issues received V-GO-based insulin treatment, twenty-one (21) continue to use the V-GO. Overall, fifteen (15) of the 36 have discontinued V-GO use; five (5) due to hypoglycaemia despite using the lowest basal V-GO device, three (3) due to skin irritation from the V-GO adhesive, four (4) withdrew for behavioral health reasons, one (1) withdrew as a result of resolved hyperglycaemia following diet/exercise, one (1) withdrew for discomfort, and one (1) for emergency surgery. Twenty one (21) are presently receiving insulin therapy as early V-GO adopters. Two (2) patients with severe needle phobia used the V-GO as their initial experience with basal/bolus insulin. Nineteen (19) patients with refractory hyperglycaemia despite self- reported high daily insulin requirements have all demonstrated a 40-60% decrease in their daily insulin need. Percentage of V-GO insulin users presently using V-GO 20 (which delivers 20 units basal insulin over 24 hours), V-GO 30, and V-GO 40 devices are (4/21) 19%, (5/21) 23% and (12/21) 57%, respectively. Our preliminary analysis of available A1C data from the 6 patients using the V-GO the longest (at least 8 weeks) finds a 17% improvement in A1C ; pre-V-GO A1c mean 9.8% (range 8.3 - 14.3%) with the post-V-GO A1c mean being 8.1% (range 7.1- 9.1%).

Patient	A1C Change (%)	Pre V-GO	Post V-GO
1	-43.90%	Lantus 140 units + Novolog 30 units, Januvia 100 mg, Metformin 2000 mg	V-GO 20, Metformin 2000mg
2	2.40%	Lantus 40 units + Humalog 24 units , 10 Glyburide mg, Metformin 2000 mg	V-GO 30, Metformin 2000 mg
3	-14.50%	Lantus 40 units + Novolog 25 units	V-GO 30
4	-19.10%	Lantus 60 units, Metformin 1500 mg	V-GO 40, Metformin 1500 mg
5	-11.70%	Lantus 70 units + Novolog 83 units, Victoza 1.8 mg	V-GO 40
6	-5.20%	Lantus 180 units + Humalog 125 units, Victoza 1.8 mg, Metformin 2000 mg	V-GO 40, Metformin 2000 mg

Conclusion: Our preliminary experience shows patients with suboptimally controlled T2D despite taking high daily dose MDI can be successfully transitioned to V-GO insulin delivery devices in clinical practice. Patients using the V-GO insulin delivery system experienced improved glycaemia control within the initial three month time framework, and required fewer medications and less daily insulin.

1109

A feasibility assessment of PaQ®, a simple 3-day basal/bolus insulin delivery device, in patients with type 2 diabetes

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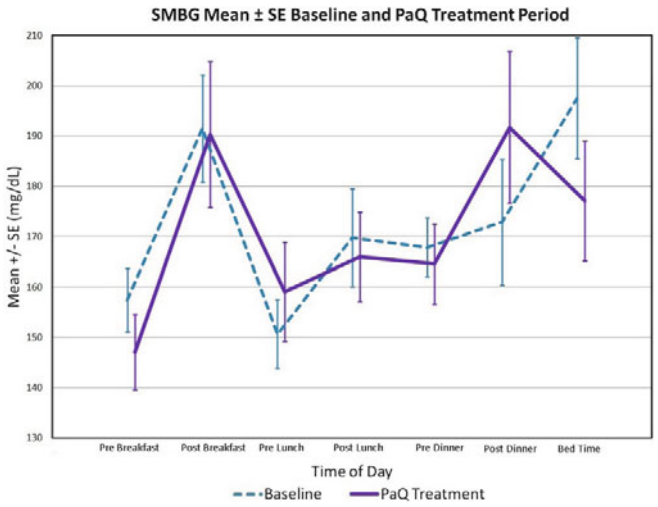
Background and aims: PaQ (CeQur, SA) is a simple patch-on device that provides preset basal rates and bolus insulin on demand. In this 6-week open-label single-arm study we assessed feasibility of use, transition from multiple daily injections (MDI), ability to maintain glycemic control and safety. The study was done in patients with type 2 diabetes (T2D) treated with PaQ.
Materials and methods: Twenty patients on a stable MDI regimen were enrolled (age 59 ±5 y, T2D duration 15±7 y, A1C 7.7 ±0.7 %). The study was comprised of three 2-week periods: baseline (MDI), transition to PaQ, and PaQ therapy. The first PaQ basal rate (dose/day) selected was ≤ the patient's basal MDI dose. There was no attempt to treat to target.
Results: Eighteen patients completed the study. All patients were able to assemble and use PaQ. No cannula site infections occurred and PaQ was well tolerated. At the end of PaQ therapy the mean total daily dose (TDD) was similar to baseline (57±15 U vs. 60.4 ± 19 U). Self-monitored blood glucose values (mean ± SD mg/dL) showed a trend towards better glycemic control pre and post breakfast and at bedtime compared to baseline values; pre- and post-breakfast; -10.7±28 (P=0.12), -13.0±34 (P =0.13), and bedtime -17.9±45 (P =0.12). Blinded continuous glucose monitoring (CGM) data revealed a trend towards improved glycemic control, with a mean change in average 24 hour glucose exposure of -190.3 mg/dL (P = 0.18) compared to baseline. The reduction in glucose exposure occurred overnight and during the day. CGM also revealed no episodes of severe hypoglycemia. The improved glucose exposure was consistent with a mean change in A1C of -0.3±0.4%.
Conclusion: MDI treated patients with T2D were easily and safely transitioned from MDI to PaQ. Despite similar TDD during MDI and PaQ study periods, there was a trend toward improved glycemic control with PaQ therapy. Future studies will assess long-term PaQ efficacy and safety.



Clinical Trial Registration Number: NCT01535612
Supported by: CeQur Corp.

PS 092 Delivering diabetes care

1110

Marked improvement of glycaemic control and insulin therapy associated problems of patients with diabetes mellitus after intervention in a diabetes day clinic

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Background and aims: During the last decade diabetes care in Germany migrated from inpatient to now almost exclusively outpatient care. Health care associations´ more and more stringent stipulations after introducing the DRG system makes it nearly impossible to treat even complicated diabetes cases in hospitals. We evaluated, if therapeutic problems which could not be solved after repeated outpatient consultations on tertiary care level (university out-patient clinic) improved after intervention in a diabetes day clinic.
Population and methods: From 10/2010 to 09/2011 117 patients with diabetes mellitus (DM) had a problem orientated intervention in a diabetes day clinic (DDC) in a university out-patient clinic for Endocrinology and Diabetes. The intervention was performed by an experienced, specially trained diabetes nurse ("Diabetesberater") hand in hand with a diabetologist. All patients received problem orientated, structured education and training. After 11.9 months 93 (80%) of the patients had a follow-up examination, another 3 patients had died. Mean time of the intervention was 4.1 days. 26 patients had DM1 (age 58.3y.(SD15.2), BMI 27,8kg/m2(SD4.8), 54% male) and 67 DM2 (age 65.0y.(SD10.8), BMI 33.4kg/m2(SD5.9), 60% male). The HbA1c was measured locally with an HPLC-method (TOSOH G8) and DCCT adjusted (mean HbA1c 5.05%).
Results: Most frequent reasons for the DDC admission were for DM1 high Hba1c (19/26; 73%), extreme plasma glucose fluctuations (11/26; 42%) and frequent hypoglycemic events (5/26; 19%), for DM2, respectively, high HbA1c (47/67; 70%), optimization of insulin therapy (10/67; 15%) and initiation of insulin therapy 7/67; 10%). In DM1 patients HbA1c improved 0.6% (69.4±1.1 to 63.9±1.0 mmol/mol; p=0.001), the daily insulin dose decreased from 52.9±34.8 to 48.9±32.5 IE/d and the weight reduced 0.55 kg. Frequency of mild hypoglycemic episodes per week decreased from 1.75±1.8 to 1.23±1.6. In patients with DM2 the HbA1c improved 1.7% (83.6±8.3 to 65.0±1.3 mmol/mol; p=0.013), daily insulin dose increased slightly from 58.8±50.9 to 59.5±47.6 IE/d whereas weight decreased by 1.36 kg.
Conclusion: One year after a problem orientated intervention in a diabetes day clinic performed by a specially trained diabetes nurse and a diabetologist in patients with diabetes mellitus type 1 and 2 with therapeutic problems which could not be solved after repeated presentations in an outpatient setting beforehand, resulted in a marked and sustained improvement of glycemic control and hypoglycemic events with a reduced (DM1) or stable daily insulin dose (DM2) and reduced weight.

1111

How quickly do HbA_{1c} levels respond to a change in oral glucose lowering therapy? Results from a cohort study

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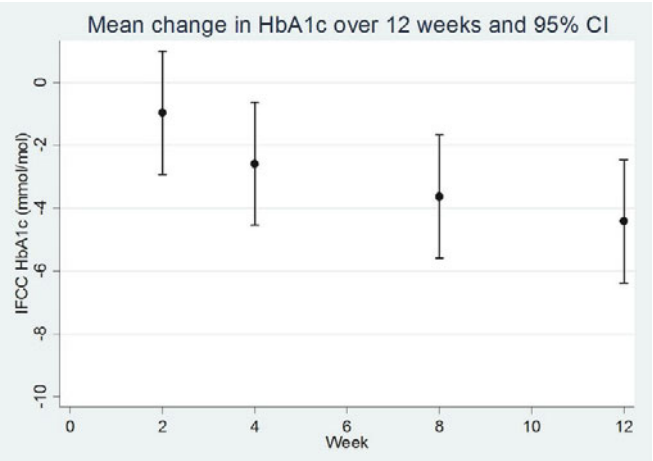
Background and aims: A routine monitoring interval of 3-6 months for glycated haemoglobin (HbA1c) is recommended for people with type 2 diabetes. However, this recommendation is based on expert consensus with limited supporting empirical evidence. Using a prospective clinical research design, we measured how quickly HbA1c responds after a change in oral glucose lowering therapy (OGLT).
Materials and methods: In a 12-week cohort study, adults with non-insulin treated type 2 diabetes were recruited from 18 GP practices in the United Kingdom. Eligible participants had a diagnosis of diabetes for ≥ 3 months and were initiating or increasing dose of their OGLT. HbA1c was measured at baseline and 2, 4, 8 and 12 weeks after the medication change. Adherence to medication was recorded using the 8-point Morisky scale. High adherence was defined as a score of 0 at the final consultation. Results for change in HbA1c are presented for participants who have both a baseline and 12-week HbA1c result.
Results: A total of 94 eligible patients entered the study. Fifteen were excluded from the analysis because of changes to their diabetes medication during follow-up (n=7); the 12-week HbA1c was measured more than 3 days late or

early (n=7) and death from non-study related causes (n=1). Eighty-one patients have completed the study to date. The mean (SD) age of 56 per-protocol participants was 61.9 (11.0) years, BMI was 39.6 (8.1) kg/m^2, diabetes duration 5.9 (4.3) years and 36% were female. After 12 weeks 21 patients (38%) had achieved glycaemic control (defined as an HbA1c≤59mmol/mol), and 35 (63%) had not. Mean±SE change in HbA1c from baseline at 2, 4, 8 and 12 weeks was 1.0±0.4 (n=53) mmol/mol, 2.6±0.6 (n=53) mmol/mol, 3.6±0.9 (n=52) mmol/mol and 4.4±1.0 (n=56) mmol/mol (Figure). Twelve weeks after the medication change the change in HbA1c ranged from a reduction of 22mmol/mol to an increase of 13mmol/mol. In 26 patients with a Morisky score of 0, mean ±SE change after 2, 4, 8 and 12 weeks was 0.1±0.5, 1.6±0.6, 2.7±1.0 and 2.3±1.4 mmol/mol. Twenty-nine patients (52%) had a greater change in HbA1c at 12 weeks than at 8 weeks. However, in 27 patients the change in HbA1c by 8 weeks was as great as, or greater than, the change in HbA1c by 12 weeks; of these patients, two-thirds had not achieved optimal glycaemic control by the end of the study.

Conclusion: This preliminary analysis suggests that 8 weeks after a medication change the mean change in HbA1c was 82% of the 12 week change in all patients, and >100% of the 12 week change in the most adherent patients. Forty-eight percent of patients had achieved their maximum change in HbA1c within 8 weeks of their medication change but the majority still had sub-optimal glycaemic control. This data suggests that many patients would benefit from returning to their GP earlier than 12 weeks following a change in their medication to have their HbA1c checked and to review their medication adherence.



Supported by: NIHR National School for Primary Care Research

1112

Use of sulfonylureas in patients with multiple risk factors for CV disease or those with existing CV disease - CREST Study: are we following current ADA/EASD guidelines?

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Background and aims: Current literature suggests increased risk of cardiovascular disease (CVD) and all-cause mortality with sulfonylureas (SU) use. The new ADA/EASD guidelines also recommend that SUs be avoided in patients with CVD risk. This study examines the use of SUs in a high risk population.

Materials and methods: A retrospective cohort study design was employed for this project. Records were obtained for 529,468 patients with at least 2 records of T2DM diagnosis. Data was obtained from a large administrative claims database from 01/01/2004 to 01/01/2011. High risk for a CV event was defined as: GROUP 1 (N= 97,762): having established CVD; and GROUP 2 (N= 151,700): having multiple risk factors for CVD but without established CV disease. Group 3 (N=280,006) included patients who neither had established CVD nor any risk factors. Frequency of SU use was reported as rate per 1,000 patient-years and as an aggregate use frequency (%). Chi-square tests were used to test the differences in the use frequency of SUs across the CV risk cohorts. As exploratory analyses, logistic regression models were used to examine the associations between baseline characteristics and use of SUs within each of the study cohorts.

Results: In all the three groups, approximately one-quarter of patients reported the use of SUs in the study period (Group 1: 26.9%, Group 2: 28.6%, Group 3: 26.0%). For SUs, the overall use frequency over the study period was 486.3/1,000 person-years for Group 1; 521.4/1,000 person-years for Group 2; and 396.5/1000 person-years for Group 3. Compared with Group 3, the frequency of use of SUs for Group 1 or 2 were significantly higher ($p<0.0001$) for overall (all ages) and within age groups: 55-64 years, 65-74 years, and ≥75 years. From 2004-2011, the overall number of patients on SUs declined 24.4%. There was a greater decline in SU use observed in the CV risk groups compared to the low risk group over this time period [Group 1 (-28.6%), Group 2 (-29.3%), Group 3 (-18.5%), $p<0.001$ for Group 1 vs 3 and Group 2 vs 3, respectively]. Using exploratory multivariate logistic regression, several baseline clinical factors were associated with the current use of SUs, compared to other anti-diabetic medications. Within each of the CV risk groups, the following factors were statistically significant: (OR [95%CI]) Group 1: nephropathy (1.55 [1.30-1.84]), congestive heart failure (CHF) (1.06 [1.03-1.11]), chronic kidney disease (CKD) (0.67 [0.62-0.72]), and obesity (0.88 [0.84-0.92]); Group 2: nephropathy (1.68 [1.39-2.02]), CHF (1.07 [1.01-1.14]), CKD (0.77 [0.70-0.84]), and obesity (0.80 [0.76-0.84]); Group 3: CKD (0.61 [0.55-0.68]), nephropathy (1.56 [1.33-1.84]), obesity (0.84 [0.81-0.86]) and hypertension (1.13 [1.11-1.15]).

Conclusion: This study highlights that in clinical practice the use of SUs has declined over time in all CVD risk groups, but the current use of SUs is still quite high and similar to patients who do not have established CVD or risk factors for CVD, despite recommendations against such use in the ADA/EASD guidelines. Future research examining the association between patient's co-morbidities at baseline and new SU prescriptions is needed as well as the impact of SU use on outcomes in high risk patients.

1113

National Diabetes Action Programme: a monitoring study regarding the Dutch care standard for diabetes among health care professionals and patients

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Background and aims: The National Diabetes Action Program (2009-2013) was carried out with funding of the ministry of Health, Welfare and Sport. The main objective of the action program is the systematic implementation of the Care Standard (CS), an instrument for the content, organisation, quality and funding of diabetes prevention and care. The Netherlands can be regarded as unique in the use of the CS for prevention and care for diabetes. The CS is also translated for patients into the so called 'Zorgwijzer', describing what care should be provided. The aim of the present study was to suggest ways to optimise the implementation of the CS by examining the perceptions of Dutch health care professionals and diabetes patients regarding the CS and the delivery of care in accordance with the CS and the barriers to using it. In addition, we aimed to evaluate the efforts as part of the NAD until now.

Materials and methods: A cross-sectional questionnaire survey was conducted among health care professionals (N=1370) and diabetes patients (N=5650) between October 2012 and February 2013. In 2010 a similar study was conducted to monitor the action program. Two sample z-tests were conducted to assess the differences between both studies.

Results: In 2013, 43.7% of the professionals were in possession of the CS and 17.9% was unfamiliar with the CS. Only 18.1% of the professionals that were somehow familiar with the CS in 2013 perceived themselves as working completely in accordance with it. Professionals' familiarity with the CS has significantly improved compared to the monitor in 2010, when 37.6% was in possession of the CS. The majority perceived the CS as the norm for high quality diabetes care (92.6%), thought the CS made a major contribution to ensuring the quality of care (80.7%) and 78.7% judged the feasibility of working in accordance with the CS to be high. These scores were significantly higher compared to 2010. Logistic regression analyses showed significant differences in possession of the CS, working in accordance with the CS and appreciation of the CS between professional groups. The most important barrier professionals perceived was the care for groups that are difficult to reach, such as migrants or low SES patients. The majority of the professionals (80.4%) thought Dutch diabetes care has (strongly) improved since the past 2.5 years. Of the patients, 15.3% were in possession of the 'Zorgwijzer', while 40.4% was

unfamiliar with it. Patients were satisfied with their caregivers and perceived a high degree of autonomy support (M=4.1 (SD 0.8)) and self-management skills (M=4.1 (SD 0.7)) on a 5-point scale, but scored lower on perceived mastery (M=3.7 (SD 0.7)).
Conclusion: Overall, professionals were largely positive about the CS, although only a minority indicated that they were working completely in accordance with it. Professionals' perceptions regarding the CS have significantly improved compared to 2010 and the majority reported that diabetes care in general has improved in the past 2.5 years. Patients were generally satisfied with their care. Attention for the most important perceived barriers is however needed to further optimise the delivery of care and maintain the positive trend.
Supported by: Ministry of Health, Welfare and Sport

1114

Differences in healthcare professionals' perceptions of diabetes healthcare provision in the DAWN2 study
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Background and aims: The 2nd Diabetes Attitudes, Wishes and Needs (DAWN2) study sought cross-country comparisons of healthcare provision for benchmarking of clinical practices. The study aimed to identify any differences in attitudes between different healthcare professional (HCP) disciplines towards diabetes healthcare.
Methods: 4785 HCPs (2066 primary care physicians, 1350 diabetes specialists, 827 nurses, 542 dieticians) from 17 countries across 4 continents completed an online questionnaire to assess healthcare provision, self-management and training between March and August 2012.
Results: There were significant differences between professional disciplines in most components of the survey. There was agreement, however, that people with diabetes needed to take more responsibility for their diabetes by becoming more active, maintaining a healthy weight and taking their medications as recommended. The greatest discrepancies between professions were in the areas of attendance for professional training in medical management of diabetes and healthcare organisation. The table shows the results to the survey questions by discipline where >5% of the variance in responses was explained by professional discipline - see Table: HCPs indicating this as an area of concern is shown as clustered mean, then % (95% CI). Nurses and dieticians expressed a greater need than GPs and specialists for better access to communication training and psychological care. Overall the rate of HCPs attending professional training for a better management of psychological aspects of diabetes was low, particularly for GPs. It was surprising that less than half of HCPs had attended training in the provision of diabetes self-management education. Members of all professional disciplines agreed that there was a need for better communication skills and better access to psychological treatments.
Conclusion: This survey has highlighted the similarities and differences in concerns of diabetes HCPs regarding diabetes healthcare provision, self-management and training. Understanding these differences in several areas will allow for better interdisciplinary working.

Table.

Question	Clustered mean	Specialists	GPs	Nurses	Dieticians	Variance explained by discipline
Healthcare organization: better access to psychologists	62.6	63.5 (53.5-72.4)	48.9 (38.8-59.0)	71.1 (61.8-78.9)	65.9 (55.9-74.7)	5%
Healthcare organization: formal training in effective communication	67.5	65.5 (55.2-74.6)	54.2 (43.4-64.5)	73.6 (64.2-81.2)	74.7 (65.4-82.2)	5%
% Attending professional training in provision of diabetes self-management education	35.4	41.1 (30.9-52.1)	23.8 (16.7-32.8)	45.8 (35.0-57.0)	32.9 (23.7-43.7)	6%
% Attending professional training in management of psychological aspects of diabetes	19.7	26.3 (18.2-36.4)	13.3 (8.7-19.8)	27.1 (18.8-37.4)	15.2 (9.9-22.7)	6%
% Attending no professional training	19.5	11.5 (7.2-18.0)	23.5 (15.5-34.0)	29.3 (19.7-41.0)	16.7 (10.6-25.5)	7%
% Attending professional training in medical management of diabetes	64.5	78.4 (59.7-89.9)	67.4 (45.7-83.5)	47.1 (26.6-68.6)	31.8 (15.9-53.5)	20%
% Attending professional training in dietary/ nutritional management of diabetes	57.1	58.1 (42.8-72.1)	42.6 (28.5-57.9)	49.4 (34.4-64.6)	75.6 (62.3-85.4)	11%

1115

Emergency medical identification use in adults with diabetes
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Background and aims: Emergency medical identification (ID) is recommended for use by patients with diabetes. Prior studies show awareness of emergency medical ID and its importance among emergency providers. The purpose of this study is to characterize adherence of emergency medical ID use, types used, and reasons for non-adherence among adult patients with diabetes.
Materials and methods: Questionnaires were given to patients at a diabetes comprehensive care center in a university hospital. The survey was comprised of questions regarding the use of emergency medical ID, type used, if not used reasons why, and demographic information.
Results: The study population (N=185) were 57% female, 75% in the age range of 36-65 years old, 65% had type 2 diabetes, and 73% were on insulin therapy (Table). Most patients (87%) agreed with the importance of the use of emergency medical ID. Only 47% of patients carried or wore one, and only 25% were the jewelry type which would be more readily visible. Over half reported the barrier to use as lack of awareness with only 7% reporting concern for privacy. Cost was a factor in 19% of those patients who did not use emergency medical ID (Table). Patients with type 1 diabetes and patients on insulin therapy were more aware of the use of emergency medical ID and more likely to use it. In patients with type 1 diabetes, 64% reported using emergency medical ID compared to 42% of patients with type 2 diabetes, $p<0.05$. Visible emergency medical ID (jewelry) was reportedly used by 28% of patients with type 1 diabetes and only in 14% of patients with type 2 diabetes, $p<0.05$. In patients with type 1 diabetes, 29% reported no provider recommended the use of emergency medical ID as the reason for not using it, compared to 56% in patients with type 2 diabetes, $p=0.05$.
Conclusion: Medical providers and patients with diabetes agree as to the importance of the use of emergency medical ID. More patients with type 1 diabetes are aware of the recommendation to use emergency medical ID and are more adherent to the use of it, especially visible emergency medical ID (jewelry) compared to patients with type 2 diabetes. As unawareness of emergency medical ID use was the main reason for not using it, discussion of this with patients with diabetes may improve awareness and adherence to emergency medical ID use.

Table. Characteristics and medical ID use and reasons for non adherence in percentage (N=185)

	Characteristic	Any ID	Jewelry	No ID	Nobody Recommended it	Cost to much	Don't know where to buy	Don't want everyone to know medical problem	ID is important
All		47	25	53	53	19	21	7	87
Male	43	41	45	59	41	28	31	25	37**
Female	57	59	55	41	59	72	69	75	63**
18-35 years old	11	13	21*	87	10	11	23	25	79**
36-65 years old	75	72	52*	28	82	72	62	75	9**
66 years and older	14	15	27*	85	8	17	15	0	12**
Type 1 diabetes	26	38*	45*	62*	12**	24	27	50	18
Type 2 diabetes	65	62*	55*	38*	88**	76	73	50	82
Insulin use	73	74	90	26	60**	78	85	50	75*
No insulin	27	26	10	74	40**	22	15	50	25*

* p<0.05, ** p=0.05

1116

Optimal target range for blood glucose in hyperglycaemic patients in a neurocritical care unit

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Background and aims: Hyperglycemia is common among patients with critical neurological injury, even if they have no history of diabetes. The optimal target range for normalizing their blood glucose is unknown.
Materials and methods: Two groups were divided according to retrospective data, which were extracted from 890 hyperglycemic patients admitted to our neuroscience critical care unit (NCCU) with targeted blood glucose levels of 180 mg/dL: intensive control to ≤ 140 mg/dL, moderate control to 140-180 mg/dL. The groups were stratified according to the hyperglycemia type (pre-existing diabetes or stress-related). We defined the primary endpoint as death from any cause during NCCU admission.
Results: In the NCCU, tighter control of blood glucose at ≤ 140 mg/dL increased the mortality of the preexisting diabetic patients compared with moderate control (29 of 310 patients [9.4%] vs. 15 of 304 patients [4.9%], P =0.034). Patient age (adjusted odds ratio [OR] = 1.12; 95% confidence interval [CI] = 1.05-1.19; P < 0.001), level of glycated hemoglobin (adjusted OR = 1.24; 95% CI = 1.04-1.48; P = 0.017) and hypoglycemia (adjusted OR = 10.3; 95% CI = 2.92-36.6; P < 0.001) were positively associated with higher mortality. Death decreased among the stress-related hyperglycemic patients with tighter glucose controlled at ≤ 140 mg/dL (6 of 140 patients [4.3%] vs. 15 of 136 patients [11.0%], P = 0.035).
Conclusion: The blood glucose levels of hyperglycemic NCCU patients with pre-existing diabetes or stress-related hyperglycemia should be normalized at 140-180 or ≤ 140 mg/dL, respectively.

Table 1. Baseline Characteristics of the Study Patients

	Diabetic mellitus		Stress hyperglycemia	
	Intensive Glucose Control (n=310)	Conventional Glucose Control (n=304)	Intensive Glucose Control (n=140)	Conventional Glucose Control (n=136)
Age (year)	68.9±14.4	67.5±16.5	68.1±16.8	69.8±16.1
Hb_{A1c} (%)	10.8±2.7	10.7±3.0	5.4±0.7	5.3±0.6
NIHSS score (n)	13.5± 4.6 (278)	13.4±3.3 (273)	13.4±3.5 (126)	13.4±3.1 (122)
Treated with insulin (n, %)	276, 89.0**	189, 62.2	113, 80.7**	78, 57.4
Fasting blood glucose (During admission)	131±18**	164±21	124±19**	163±26
Infection with detectable septic focus (n, %)	94, 30.3*	117, 38.5	45, 32.1	57, 41.9
Severe hypoglycemia (n, %)	18, 5.8**	0	8, 5.7*	0
Death (n, %) During NICU admission	29, 9.4*	15, 4.9	6, 4.3*	15, 11.0
Death (n, %) During in-hospital admission	33, 10.6	20, 6.6	9, 6.4	18, 13.2

Data were presented as mean ± SD or frequencies. *P <0.05, ** P <0.001 compared with conventional glucose control of diabetic mellitus or stress hyperglycemia. NIHSS represents National Institutes of Health Stroke Scale score on admission.

1117

Predictors of medication usage for incident diabetes patients: a SUrveillance PREvention and ManagEment of Diabetes Mellitus (SUPREME-DM) study

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Background and aims: Patients with diabetes use numerous medications to control blood glucose and treat comorbid conditions. However, no studies have documented the specific level and correlates of medication usage in the U.S. diabetic population, or trends in medication usage over time. By leveraging a large national sample of incident diabetic patients (the SUPREME-DM DataLink), this study aims to examine prescription drug usage pre- and post-diabetes diagnosis; determine patient-level correlates of drug usage; and examine if drug usage is changing over time.
Materials and methods: We analyzed data from a longitudinal, observational cohort study of 196,654 incident diabetes patients from 11 U.S. integrated health care delivery systems. Patients were included if they were ages 20 and above diagnosed with incident diabetes from January 1, 2005-December 31, 2010. We calculated overall medication usage using therapeutic classes defined by the American Hospital Formulary Services (AHFS) classification system for each patient in the 12 months prior to, and the 12 months following, their incident diabetes diagnosis date. We defined overall drug usage as the total number of therapeutic classes a patient had taken in each period. We also calculated the usage of 4 specific therapeutic classes: antihyperglycemic, antihypertensive, antihyperlipidemic, and mental health medications. A multivariate linear regression model was used to examine the relationship between the total number of therapeutic classes in the 12 months post-diabetes diagnosis and year of diagnosis; gender; age; race/ethnicity; baseline A1c value; and body mass index (BMI); and diagnosis of depression, hypertension, and/or hyperlipidemia within 2 years prior to diabetes diagnosis.
Results: Mean patient age was 58.6 years; 48% were female; 47% were white; and mean baseline A1c value was 58 mmol/mol. In the 12 months prior to diabetes diagnosis, patients used a mean of 5.72 drug classes, 0.35 mental health, 1.24 antihypertensive, and 0.39 antihyperlipidemic therapeutic drug classes. In the 12 months post-diabetes diagnosis, these averages increased to 7.97 total (including 0.60 antihyperglycemics), 0.39 mental health, 1.54 antihypertensives, and 0.74 antihyperlipidemic drug classes (p<.0001 for all pre vs. post comparisons). In the multivariate model, overall drug usage after diabetes diagnosis decreased by almost one therapeutic class (coefficient =-0.98, p<.001) between 2005 and 2009. Drug usage increased significantly with greater patient age, baseline A1c level, and BMI; and was lower for Hispanics, African-Americans, Asians, and Pacific Islanders compared to Whites.
Conclusion: Data from a large U.S. sample of incident diabetes patients suggests medication usage for these patients is high prior to diagnosis and increases substantially afterwards, primarily due to increases in antihyperglycemics and other cardiovascular disease risk factor medications. However, total drug usage appears to be decreasing over time.
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1118

Process- and intermediate outcome indicator results in the 2011 LOK survey (DUDE-2)

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Background and aims: In the Netherlands, professional health care organisations, patient organisations, insurance companies and regulatory agencies aim to assess the quality of diabetes care. The Landelijke Organisatie Ketenzorg (LOK (Dutch initiative for shared care organisations)) has initiated data collection over larger groups of subjects in the years 2010 and 2011, concentrating on subjects with type 2 diabetes (T2DM), treated in shared care

groups within a primary care setting. Information is collected (partly) based on the "kernset DM" ("core data set" (CDS)), which is supported by the relevant organisations mentioned above.
Materials and methods: All shared care groups were invited to voluntarily submit relevant information to the LOK; 66 out of a total of more than 100 did so with sufficient information to allow at least partial analysis of the known information. These 66 groups reported regarding 380,688 subjects with DM, as treated within the shared care environment, out of a grand total of 439,956 reported with DM. 54 groups also provided sufficient information regarding their total population (7,266,316). Data were analyzed as part of the DUDE-initiative (DUtch Diabetes Estimates).
Results: Results are observational and self-reported, which might lead to bias. Selection of main findings in 2011: in 380,688 subjects in 66 groups, HbA1c was measured in 91.7±6.7% (mean±SD, comparing groups), HbA1c was <7% in 68.5±7.6%; LDL < 2.5 mmol/l was reached in 52.7±6.3%, and lipid-lowering drugs prescribed in 63.6±14. Urine analysis was performed in 78.7±14.4%. BP could be traced in 92.5±5.8%, a BP<140 mmHg reported in 54.8±5.7%; fundus control in 74±14.8%, foot control in 68±15.9%. Part of the low score and high standard deviation in some items proved to be due to insufficient reporting.
Conclusion: In general, diabetes care within shared care groups is well organized, with overall good to excellent results. Still, improvement can be made both in actual care and noting down and properly reporting activities. LOK initiatives are ongoing to support lower-than-average performing groups in learning from better-than average performing groups.

1119

European vs non-European HCP attitudes and practices: results from DAWN2

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Background and aims: The 2nd DAWN (Diabetes Attitudes, Wishes, and Needs) study assessed healthcare provider (HCP) attitudes and practices regarding psychosocial issues faced by adults with diabetes across 10 European and 7 other (Algeria, Canada, China, India, Japan, Mexico, USA) countries.
Materials and methods: ≥4785 HCPs caring for people with diabetes - comprising general practitioners, specialist physicians (endocrinologists and diabetologists), diabetes nurses and dietitians - participated in an online survey; each country generated a parallel sample. Data were ranked across the nations to compare highest (rank = 1) and lowest (17) responses to each question and identify between-country variances.
Results: Highest between-country variance was for opinions related to healthcare organisation (HCO) (13-23%); least variance was for need for more training (2-7%). All numbers reported are relative ranking among the 17 nations. HCPs from European countries fared better than other countries in discussing emotional issues (France-1, Germany-3) and involvement and empowerment of people with diabetes (PWD; Poland-2, Netherlands-3). Healthcare organization was rated better in Europe (Netherlands-1, Denmark-3), as assessed by a lower perceived need for: more qualified nurses (Denmark-1, Netherlands-2); better communication between HCPs (Denmark-1, Netherlands-2, Germany-3); improved access to psychologists (Netherlands-2, Denmark-3). Involvement of PWD (Russia-1, Spain-2, Poland-3) and family members (FM; Russia-2, Turkey-4) by HCPs is rated higher in Europe. Societal discrimination against PWD was less in Europe (UK-2, Denmark-3); only Poland (15) reported high levels. European HCPs do not fare as well in postgraduate training (PT): Danish and German HCPs score 2 and 3 in attending PT, but Spain (17), Russia (14), France (12) and Italy (11) score lowest. Fewer European HCPs attend PT in the medical management of diabetes (only Denmark-1 figures in the top 3), nutritional management (only Poland-2 is in top 3), or provision of diabetes self-management education (SME; Denmark-2). PT for psychological management was more common in Europe (Poland-2, Netherlands-3), as was 'effective communication and motivation strategies to support long-term behavior change' (Denmark-1, Netherlands-2, Germany-3). Non-European countries may exemplify better attitudes and practices towards psychological support (India-1, Mexico-3). These countries, along with Turkey (2), also report more optimal SME; HCP collaboration and formal training; and involvement of FM. Availability of reimbursement is better in Canada (1) and China (3) than most European countries except Denmark (2).
Conclusion: These data may facilitate best practice sharing and help improve global diabetes care delivery. Other countries can learn from PWD and FM involvement in European HCO. European countries should work to improve support in providing psychological care, SME, HCP collaboration/formal training, and FM involvement.

PS 093 Type 1 diabetes education

1120

A randomised controlled trial of an internet-based mentoring programme for type 1 diabetes patients with inadequate glycaemic control

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Background and aims: This study is to determine whether an internet based mentoring program can improve glycemic control in subjects with type 1 diabetes mellitus (T1DM).
Materials and methods: Subjects with T1DM on intensive insulin therapy and with HbA1c ≥ 8.0% were randomized to mentored (glucometer transmission with feedback from mentors) or control (glucometer transmission without feedback) groups for 12 weeks. Five mentors were interviewed and selected. Two were T1DM patients themselves and three were parents of at least one child diagnosed with T1DM for more than 5 years ago.
Results: A total of 57 T1DM adult subjects with a mean diabetes duration of 7.4 years were recruited. The mentored group failed to show significant improvements in HbA1c levels or other outcomes, including quality of life, after completion of the study. However, the frequencies of blood glucose monitoring (1.41 vs. 0.30) and website logins (20.59 times vs. 5.07 times) were higher than in the control group.
Conclusion: A 12-week internet-based mentoring program for T1DM patients with inadequate glycemic control did not prove superior to the usual follow-up. However, the noted increase in the subjects' frequency of blood glucose monitoring may lead to clinical benefits.
Clinical Trial Registration Number: NCT01157611
Supported by: i-SENS Inc.

1121

The efficiency of telemedicine to optimise metabolic control in patients with type 1 diabetes mellitus

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Background and aims: The application of interactive telematic systems between the patient-healthcare team may improve the cost-effectiveness of healthcare programmes for the optimization of metabolic control in persons with diabetes. To evaluate the impact of the Medical Guard Diabetes® telematic system on the economic and clinical management of human resources and material used in a programme of optimization of metabolic control in patients with type 1 diabetes mellitus (DM1).
Materials and methods: This prospective, randomized, comparative, open, multicentre 6-month study included patients with DM1 >18 years of age receiving treatment with multiple insulin doses (MID) and HbA1c >8%. An intervention group (IG) (two face-to-face and 5 telematic appointments) was compared with a control group (CG) (7 face-to-face appointments). The variables studied were: 1) patient and healthcare team costs, 2) metabolic control 3) knowledge of diabetes 4) quality of life and 5) treatment self-care adherence.
Results: 118 (76.6 %) of the 174 patients included completed the study (IG: 54, CG: 64). The time (minutes) used by the patients to follow the programme in the CG was 823.7 ± 645.81 vs. 353.6 ± 222.2 in the IG (p <0.0001). Compared with the CG, the IG required less healthcare time from the professional (288 ± 105 vs. 232 ± 89, p=0.0001).

The following table shows the remaining variables studied in both groups:

Study Group	CG		p	IG		p
Months	0	6		0	6	
HbA1c (%)	9.1 ± 0.9	8.5±0.9	.000	9.1±1.4	8.6±1.5	.000
Knowledge (DKQ2)	24.7 ± 4.3	26.7±4.0	.000	24.7±4.4	26.3±4.5	.006
Quality of life (EuroQol)	67.1± 17.7	66.8± 17.3	.922	64.5± 17.9	69.9± 18.7	.904
Adherence (SCI-R) (%)	64.1 ± 10.7	69.8 ± 9.6	.000	61.2± 11.9	66.1± 11.0	.003

Conclusion: The use of interactive telematic appointments in subjects with DM1 treated with MID and inadequate metabolic control is an efficient strategy, providing comparable results to those of face-to-face appointments in relation to improvement in glycaemic control, acquisition of knowledge and treatment self-care adherence, with a significant reduction in the time used, especially by the patient, without a change in quality of life perception.
Clinical Trial Registration Number: NCT01337141
Supported by: CIBERDEM

1122

Evaluation of a 5 days education programme by regards to individualised patient target for patients with type 1 diabetes

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Background and aims: The aim of our study was to see if the DAFNE model was adapted to the French clinical practice in routine health care delivery, and to evaluate if the same in-patient education training program in flexible intensive therapy (FIT) could achieve individualized therapeutic targets according to different typologies of patients.
Materials and methods: Prospective, observational monocentric study (Dec 2009-Dec 2010). All patients with type 1 diabetes participating in our educational FIT program were consecutively included.Patients have attended the same educational program (5 days) but were sub- divided in 3 groups according to their main goal: decrease HbA1c for patients with baseline HbA1c ≥ 7.5 % (58 mmol/mol) and no recurrent hypoglycaemia Group 1 (G1;n=74), improve diet flexibility and quality of life without worsening glycaemic control in patients with baseline HbA1c <7.5% (58 mmol/mol) and no recurrent hypoglycaemia Group 2 (G2;n=12) and decrease frequency of hypoglycaemia in patients who had recurrent hypoglycaemic episodes (≥ 2 severe hypoglycaemia in the preceding year or more than 5 episodes of minor hypoglycaemia per week) Group 3 (G3;n=35).Clinical data were collected at baseline and 12 months and quality of life and treatment satisfaction were evaluated with ADDQoL,DTSQs and DSTQc questionnaires at baseline and after 6 months.
Results: Baseline characteristics: 121 patients, mean duration of diabetes 19.7 ± 12.5 years,mean HbA1c 8.3± 1.5%(68 ± 16 mmol/mol).113 patients out of 121 had already a basal bolus insulin therapy with long acting analogue insulin or SCII therapy (n=17). In G1, mean HbA1c decreased significantly from 9.0 ±1,4 % (75 ± 15mmol/mol) to 8,4 ±1,4 % (68 ±15mmol/mol)(p=0.0004) and 53% of patients experienced a fall by 0.5% or more in HbA1c level. This decrease occurred without weight gain or more frequent hypoglycaemia. In the sub group of patients with at least a 0.5% decrease in HbA1c, global perception of quality of life assessed by overview I item (ADDQol) statistically improved between baseline and M6 (mean OvI Baseline= 0.46; mean OvI M6= 0.94; Δ=0.43; p=0.01) so did satisfactions treatment assessed with DSTQc score:2.04. Baseline HbA1c was the only predictable factor of success in G1 (OR=1.7 [1.08 ; 2.66] for an 1% increase). In G2, patients' satisfaction with treatment improved significantly after the program,DTSQc score: 2.17 [1.57;2.77] p<0.0001.The programme did not modify perception of dietary freedom. In G3, minor hypoglycaemia significantly decreased from 6.6 ± 4.7 to 3.2 ± 3 hypos/week p<0.001 and incidence of severe hypoglycaemia dropped significantly from 2.31 to 0.86 Ev/Patients/Year (E/P/Y) p<0,001. Among patients who had at least 2 severe hypoglycaemia in the past year, the rate of severe hypoglycaemic attacks significantly decreased from 4.6 ± 3.0 to 1.30 ±3.3 E/P/Y; p=0.0019.Patients felt more comfortable with their treatment and changes in perception of treatment were positive:DTSQc=1.91[1.5;2.3] p<0.0001.
Conclusion: Our study shows that the outcomes seen in DAFNE and Dusseldorf studies can be achieved in the clinical French routine hospital care, and that a patient-centered approach can solve different patient's problem and is appropriate even when patients with different primary outcome criteria attend the same FIT program.

1123

Gender differences in the effect of motivational interviewing and cognitive behavioural therapy in Austrian adolescents with type 1 diabetes

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Background and aims: Living with diabetes is a challenge especially for chronically ill adolescents who are known to show decreased dedication to their diseases´ therapy during the period of adolescence. Deterioration of metabolic control is common and cannot only be explained by physiological changes in puberty, also psychological factors are contributing. The aim of our study was to assess the changes in metabolic control after a psychological intervention with elements of motivational interviewing (MI) and cognitive behavioral therapy (CBT) in adolescents with poorly controlled type 1 diabetes (T1DM) after 6 months of intervention in a randomized controlled trial
Materials and methods: 151 consecutive T1DM patients, age 13-20 years, diabetes duration >1 year and Hba1c >8% in the in the past year from 5 diabetes outpatient clinics in Austria were eligible to participate in this study. 39 refused participation, 37 could not be reached or did not attend their scheduled outpatient controls. 75 patients (male n=32) were included in the study. Participants were matched for age, sex and contributing clinic and randomly assigned to an intervention group (n=40) and a control group (n=35). In the intervention group (IG) 12 sessions of 45-60 minutes (4 sessions of motivational enhancement therapy, 8 sessions of cognitive behavioral therapy) and 10 supportive email exchanges performed by specially trained clinical psychologists were accomplished during a time span of 6 months. In the control group (CG) treatment as usual was performed with an additional offer of a fortnightly email contact. Hba1c was assessed before participation and within 4 weeks after completing the intervention. Groups were compared by using chi2-test, t-test and Wilcoxon test for statistical analysis.
Results: There was no significant improvement in metabolic control in either of the groups after intervention (IG 9.95% vs. 9.74%, p=.412; CG 9.25% vs. 9.18%, p=.978). When separated for gender, boys in the IG showed improvement in HbA1c after the intervention (9.74% vs. 9.14%, p=.017), girls did even slightly increase their HbA1c (10.09% vs. 10.14%, p=.475). The gender difference of the change of HbA1c in the IG was significant (p=.023) with -0.60% in boys vs. +0.05% in girls. In the CG and in refusing patients no significant change of HbA1c was found in either gender.
Conclusion: A six month intervention with MI and CBT showed different changes in metabolic control according to gender. While a moderate improvement was observed in boys, girls did not benefit directly after intervention. Long term effects will be observed.
Supported by: ONB Fund

1124

Do young adults with poorly controlled type 1 diabetes benefit from a flexible nurse-led guided self-determination intervention? Results of a randomised controlled trial

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Background and aims: Psychosocial distress combined with impaired glycaemic control is common among young adults with type 1 diabetes (T1D). As these patients have poor prognosis due to early appearance of late complications and poor quality of life, health promoting and preventive interventions tailored young adults are needed. However, such interventions are difficult to implement as an integral part of clinical practice. As a solution we offered an autonomy supportive intervention, Guided Self-Determination (GSD), in a flexible nurse-led program for young adults with poorly controlled T1D. Eighteen months results available primo September will be presented.
Materials and methods: January 2010 - February 2012 we invited young adults with T1D (18-35 years, diabetes duration > 1 year and HbA1c ≥ 64 mmol/mol) at two diabetes clinics in the capital region of Denmark to participate in a waitlist trial randomized 2:1 to GSD immediately (intervention) or 18 months delayed (control). GSD advocates person-centred care using reflection sheets filled in by patients before appointments with GSD-trained nurses who use advanced communication skills in mutual problem solving of person-specific difficulties. Evidence of risks entailed to high and low blood glucose is translated to patients in a meaningful way enabling them to choose self-concordant goals in glucose-management. HbA1c was measured at baseline and every three months (primary outcome). Questionnaires with psychometric scales were answered at baseline before randomisation and again after 9 and 18 months (secondary outcomes). Analyses include repeated measurement analysis.
Results: 201 young adults (101 male) mean age 25.7(5.1), diabetes duration 13.7(6.8) were randomized to intervention (n=135) or control group (n=66). Overall at baseline participants had a mean HbA1c 81.3(15.1) mmol/mol/9.6(1.3) % and 30.3 % had complications. The patients' psychosocial functioning showed a mean of 24.8(6.5) in perceived competence in managing diabetes (PCD), 35.9(21.5) in burden experienced from diabetes-related problems (PAID), 28.4(5.8) in autonomy supportive climate with health professionals (HCCQ), 20.9(6.0) in self-esteem (RSES), 55.2(19.4) in well-being (WHO-5) and concerning amount and kind of motivation (TSRQ) mean 44.2(7.1) in autonomous, 37.1(10.8) in controlled and 9.9(5.1) in amotivation. Continuous Subcutaneous Insulin Infusion (CSII) was used by 15.9% and self-monitored glucoses (SMBG) were measured 23.4(21.7) times weekly in average. No baseline differences were seen between intervention and control groups concerning primary and secondary outcome measures. Among all participants significant differences were seen between men and women at baseline. Compared to men women perceived higher degree of burden from diabetes-related problems (PAID mean 43.2(22.2) vs. 28.7(18.2)), lower competence in managing diabetes, (PCD mean 22.9(6.7) vs. 26.6(5.8)) and lower self-esteem (RSES mean 19.1(6.1) vs. 22.7(5.4)) all p<0.001. Women more frequently used CSII and measured more SMBG weekly, 29.7(25.3) vs.16.6(14.2) both p<0.001.
Conclusion: Male and female young adults with poorly controlled type 1 diabetes are equally willing to participate in a nurse-led GSD program. Results will determine the effectiveness of such an intervention both concerning glycemic control and psychosocial functioning.
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1125

A cross country qualitative investigation of structured education programmes for adults with type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) is associated with huge human and economic costs. T1D, its treatment and the skills necessary to self-manage are similar across the world but interventions and related outcomes are varied. Since the introduction of structured education programmes (SEPs) in Germany in the 1970s such programmes have been widely adopted. The aim is to explore differences and similarities in outcomes in relation to the self-management behaviours of people with T1D in 3 different cultures and healthcare systems to identify the "active ingredients" that lead to positive outcomes.
Materials and methods: Diabetes centres in Ireland (n = 7), the UK (n = 10) and Germany (n = 5) participated in this qualitative study which is embedded in a larger quantitative study, incorporating the Dose Adjustment for Normal Eating (DAFNE) programme in Ireland and the UK and the Insulin Training and Teaching Programme (ITTP) in Germany. Prior to completing a SEP, in-depth interviews were conducted with 16 patients and 4 educators in both Ireland and the UK and 13 patients and 2 educators in Germany. Repeat interviews were conducted 6 months after completing a SEP. NVivo, a qualitative software package was used to analyse the data.
Results: Patients' experiences around the time of their diagnosis appears to have an impact on their subsequent level of engagement with healthcare professionals and the health services. In some instances, patients described being initially misdiagnosed with type 2 diabetes or not being satisfied with the care

received from their diagnosing doctor, especially those who were diagnosed as children, particularly amongst UK participants. This led to many participants expressing feelings of "frustration", "bad first impressions" and "guilt". A pre-SEP knowledge difference is emerging among participants, with German participants expressing more in-depth understanding of their disease at baseline. Participants report dramatically increasing blood glucose (BG) diary usage, weighing foods and using BG corrections immediately following SEP. Many Irish and UK participants express a degree of frustration with this flexible intensive insulin approach, saying that while they enjoy more "freedom" they express a sense of loss to the more conventional fixed, "routine" approach. German patients by comparison were happier with the flexible approach.

Conclusion: Identifying differences across countries and understanding why these differences occur will help identify what are the "active ingredients" of a SEP to inform the development of more effective programmes and to help inform decision-makers of the most effective strategies to managing T1D.

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1126

Evaluation of a one day a week re-education course (EDWARD) for type 1 diabetes: 6-year follow up

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Background and aims: To evaluate the EDWARD (Education for Diabetes without a Restrictive Diet) course. This structured education course was established in 2005 for people with Type 1 diabetes and who were already using a basal-bolus insulin regimen. One day group sessions were held weekly for four weeks, to allow consolidation of new knowledge between sessions. Topics focused on carbohydrate counting, with general information about diabetes and complications.

Materials and methods: Outcomes were examined in all those attending between 2005 and 2011, with follow-up to July 2012. Multiple measures were determined at baseline, 3, 6 and 12 months from completion of the course. HbA_{1c} was also determined annually for up to 6 years.

Results: (a) Follow-up over 12 months: Of 489 (52% male) who attended at least one session, median (range) age was 39 (17-77) years, and diabetes duration was 14 (<1-58) years. Median HbA_{1c} was 74 mmol/mol (8.9%) at baseline and 69 (8.5%) (2-tailed paired t-test, 70 (8.6%) and 72 (8.7%) at 3, 6 and 12 months (ANOVA p<0.00625). Between baseline and 12 months there was a fall in short acting insulin dose from mean 34 to 29 units/day in men, and from 23 to 19 units/day in women (both p<0.01), but none in the dose of basal insulin. Estimated mean daily blood tests rose by almost 50% (p<0.001). There was no change in BMI. The incidence of episodes of hypoglycaemia was not documented. There were significant falls in HADS scores for both anxiety and depression (p<0.001), although baseline results were within the normal range (anxiety baseline 5.52 men, 6.97 women; depression 3.41 and 4.07). There were similar falls in all domains of PAIDS, but also from a low baseline. (b) Follow-up over 6 years: Median HbA_{1c} values at 2 (n=290), 3 (n=206), 4 (n=165), 5 (n=135), and 6 (n=81) years after completion of the course were 68 (p=<0.0083, 2-tailed paired t with Bonferroni correction), 69 (p<0.0083), 69 (p=0.170), 68 (p=0.028) and 69 mmol/mol (p<0.0083).

Conclusion: These data indicate that this model of weekly structured education is associated with significant changes in both metabolic control and well-being at 12 months, and that the fall in HbA_{1c} is maintained for up to 6 years. Consideration may be given to introducing refresher courses at 3 years.

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Midterm impact of a specially designed therapeutic education programme in patients transferred from a paediatric to an adult diabetes unit

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Background and aims: Type 1 Diabetes (T1D) management in young patients is a challenge and troublesome in adolescence and emerging adults. In this period, T1D patients are usually transferred to adult Diabetes Units. We aimed to evaluate the mid-term impact of a specially transition therapeutic education program (TEP) on metabolic control, self-management and quality of life (QoL) 1-year after the transfer T1D patients from a pediatric to an adult diabetes Unit.

Methods: We included 189 subjects with T1D (aged 18.0±1.1 years, 92 women, disease duration 7.9±3.8 years) consecutively transferred (2005-2011). The TEP included a co-ordinated transfer between the Units, individual visits and group sessions. At baseline, we registered data on BMI, insulin dose, HbA_{1c} and the frequency of hypoglycaemia. For evaluation of the QoL and knowledge in T1D management, self-report questionnaires were assessed. After 12-months, all subjects were re-evaluated.

Results: Our TEP reduced HbA_{1c} from 8.6±1.4 to 8.0±1.4 (p=0.02) with a concomitant decrease in hypoglycemic episodes (severe events per subject year, from 0.31±0.46 to 0.07±0.25 (p<0.001) and frequency of > 5 mild episodes/week, 9,4% to 6.5 % (p=0.04). The TEP improved the knowledge in diabetes management (DKQ2 score from 25.0±4.1 to 27.7±4.0) and increased the frequency of SMBG (20.5±8.6 to 22.7±7.3 determinations7week, p<0.01). TEP was also associated with an improvement in some aspects of Qol (Diabetes-related issues worrying, 8.7±3.4 to 7.4±2.3). The significant increase in the proportion of subjects with $HbA_{1c} \leq 7\%$, (from 15.2 to 26.2 %, p=0.02) was related to a lower HbA_{1c} (7.2±1.1 vs. 8.6±1.5 %,p<0.001) at entry and less duration of the disease (5.9±1.6 vs 8.1±3.7 years,p=0.001).

Conclusion: The use of a specific transition TEP improves metabolic control, self-management abilities and some aspects of diabetes-related QoL of young T1D subjects transferred from pediatric care to an adult diabetes unit.

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Influence of basal insulin regimen on outcome from structured education in type 1 diabetes

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Background and aims: There is little evidence on whether the type or frequency of injection of basal insulin (BI) influences outcomes after structured education in flexible intensive insulin therapy in people with type 1 diabetes. Early successful programmes such as the German ITT course were designed with twice daily BI using NPH insulin, but more recently once daily basal regimens, often with analogues, have been introduced. In current UK programmes such as DAFNE, clinicians and patients choose a BI regimen which may include long-acting analogue insulin once daily. This study investigates the relationship between BI regimen and outcomes.

Materials and methods: We analysed baseline and 12 month follow-up data from 10 UK centres enrolled in the DAFNE Research Database Study. Outcomes were assessed in relation to BI type and frequency of injection; using a linear multiple regression model for HbA_{1c} and negative binomial models for hypoglycaemia and diabetic ketoacidosis (DKA) data. All models used population-averaged exchangeable correlation and robust standard errors to allow for clustering by centre.

Results: 558 pairs of complete baseline and 12 month data were identified. The cohort was 49.6% male, mean age 41.9 years (SD 14.2) and mean duration of diabetes 17.4 years (SD 14.0). At 12 months the proportions of people using NPH, detemir and glargine were 17.2%, 41.0% and 41.8%. The proportion using twice daily BI rose (40.3% to 61.1% CI: -24.6% to -17.0%, p<0.001). HbA_{1c} for the cohort fell from 8.68% (SD 1.50) to 8.55% (1.47) (-0.12, 95% CI: -0.22 to -0.03, p=0.012) but with significant fall only in those on twice daily BI (twice daily baseline HbA_{1c} 8.62% (SD 1.38), 12 month HbA_{1c} 8.42% (SD 1.37) difference -0.19, 95% CI: -0.31, -0.07, p=0.002 vs. once daily baseline HbA_{1c} 8.79% (SD 1.68), 12 month HbA_{1c} 8.77% (1.60) difference -0.02, 95% CI: -0.18, 0.14, p=0.802). In the multiple regression model at 12 months, use of twice daily BI was associated with significantly lower HbA_{1c} than once daily (-0.19, 95% CI: -0.34 to -0.04, p=0.012). There was no significant difference in HbA_{1c} at 12 months between analogue and NPH basal regimens (0.12, 95% CI: -0.14 to 0.39, p=0.369), nor between glargine and detemir (0.00, 95% CI: -0.20 to 0.20, p=0.997), and no significant interaction between type of basal insulin and frequency of injection. All associations were independent of age and duration of diabetes. Severe hypoglycaemia (SH) reduced from 0.74 to 0.21 events per patient per year, an estimated relative risk for SH of 0.28 (95% CI: 0.19 to 0.42, p<0.001). DKA episodes per patient per year reduced from 0.07 to 0.03, an estimated relative risk of DKA of 0.36 (95% CI: 0.18 to 0.71, p=0.003) with no significant difference in the relative risk of SH or DKA by type or frequency of BI.

Conclusion: Outcomes from structured education in flexible insulin therapy may be in part related to choice of BI regimen, with twice daily basal regimens offering lower HbA_{1c} and equal benefit in reduced risk of SH and DKA. There may be a potential for cost saving from the lack of apparent added benefit of basal analogue, although these data do not examine individual indications for either insulin. Optimising basal insulin regimen should be the subject of further investigation.
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Impact of ethnicity and social deprivation on uptake of structured education for type 1 diabetes in a multi-ethnic urban population
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Background and aims: Structured education on flexible insulin therapy for is recommended by national guidelines for the management of type 1 diabetes (T1D) in the UK and is provided at no direct cost to patients and with sufficient capacity to meet demand. Despite this, overall uptake of structured education remains poor and a large proportion of patients do not receive formal training in insulin self-management. The aim of this study was to determine the factors influencing uptake of structured education in a cohort of patients attending two inner city London teaching hospitals serving a socially and ethnically diverse local population.
Materials and methods: We have conducted a retrospective analysis of combined biomedical (HbA1c), demographic (age, sex, diabetes duration, ethnicity and socioeconomic status) and health resource utilisation data collected over a 10 year period for a cohort of T1D patients (n=1365) resident in a defined geographical catchment area of London, UK, who attended one of two specialist diabetes clinics offering the DAFNE (Dose Adjustment For Normal Eating) structured education programme.
Results: 405 (29.7%) of the cohort attended DAFNE between 2001 and 2012. There were no differences in age (37±11 vs 38±13 yrs, p=0.05), duration of diabetes (15±12 vs 14+11 yrs, p=0.2) or baseline HbA1c (8.7±1.8 vs 8.6±2.0, p=0.5)) between those who received training and those who did not. However, ethnic minorities were under-represented among those receiving DAFNE (21.2 vs 30.5%, p <0.001) as were individuals residing in more socially deprived districts (mean index of multiple deprivation score 27.2 ± 9.8 DAFNE vs 30.3 ±9.6 others, p <0.001). Those who attended DAFNE achieved significantly better long-term glycaemic control (mean HbA1c at study end 8.1±1.2 vs 8.4±1.8 %, p=0.001, mean follow-up interval post-DAFNE 4.8 years) and a lower prevalence of severe hypoglycaemia requiring hospitalisation (1.7 vs 4.8%, p=0.006).
Conclusion: There are significant inequalities in uptake of structured education which impact on outcomes. The reasons for poor uptake by patients from ethnic minority and poor socio-economic backgrounds merit further study, with the aim of informing the development of specific targeted interventions for these groups and improving equality of care for type 1 diabetes.
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PS 094 Education and motivation in type 2 diabetes

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NOTOS: an electronic questionnaire structures individualised diabetes management
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Background and aims: Successful individual management of patients with diabetes requires a multifactorial treatment to avoid and manage late complications. In addition, individualised goal setting - depending on disease progression and other individual risk factors - has become a standard of modern diabetes care. However, these approaches require careful data collection and consideration of appropriate therapeutic strategies and might be challenging for patient self-management. As active patient involvement is an important element to achieve outcome improvements, and as limited time budget and lack of patient engagement are major reasons for suboptimal achievement of therapy goals in diabetes, support for effective consultations can be useful. Electronic tools that include guidance on treatment have been shown to improve clinical outcomes. We developed a tablet based (iPad2, Apple), electronic questionnaire which is completed by the patient prior to a routine visit in the waiting room. The questionnaire covers quality of life, the patient's social environment, communication and disease knowledge issues, thereof generating a summary report on individualised patient needs and areas to be emphasized on. The report serves as basis for the subsequent consultation, may be used for documentation of goal achievement and therapy progress and serve as a self-management support by written documentation of the therapy goal.
Materials and methods: The evaluation quantitatively assessed handling and usefulness of NOTOS from a patient perspective. In brief, 63 patients (T1D and T2D) from two practices in Germany - one general practitioner and one diabetologist - who had previously used the NOTOS tool participated in an online survey on handling and usefulness of the tool. Questions were mainly answered as 5-point Likert-type scales and analyzed by descriptive statistics.
Results: 68% of the patients rated the tool as helpful for their diabetes therapy, with a high level of acceptance (average scores of 4,2 to 4,7). The majority of patients (83%) stated they would fill in such a questionnaire again in general, but only 35% would agree to fill in this type of questionnaire prior to every visit. A considerable share of the patients rated different aspects of the consultation to be better than usual consultations (20% mentioned a more target-oriented discussion, 24% felt motivated to put more effort into diabetes therapy, 25% rated the discussion to be more structured, 26% stated that it addressed additional important aspects). The share of patients who experienced a decrease in their motivation was below 2%. A share of 30% of patients recognized for themselves a beneficial behavioral change.
Conclusion: We demonstrated that application of the electronic, tablet based questionnaire on diabetes-related issues and the discussion of a subsequently generated individualized report with the physician was well accepted by patients with Diabetes, with a positive effect on a considerable share of patients in attitude towards the consultation and their awareness of the disease. Electronic consultation support tools as NOTOS might provide a way towards improved patient engagement in individualised diabetes management.
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Information seeking behaviour of patients with diabetes mellitus: a cross-sectional study in an outpatient clinic of a university-affiliated hospital
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Background and aims: Self-care in diabetes mellitus (DM) is of fundamental importance for the successful management of this chronic disease. Informa-

tion behavior in diabetic patients is a relatively unexplored field of DM care. The purpose of this study was to examine the information behavior of patients with type 1 and 2 DM, including their stated needs for information, resources used, obstacles encountered and their degree of satisfaction regarding available possibilities for DM related information.
Materials and methods: The study was undertaken in a cohort of diabetic patients being followed-up in the outpatient diabetes and special diabetic foot clinic of a University-affiliated major hospital. The participants' related issues were assessed using a validated questionnaire that inquired into patients' information needs, resources, obstacles to information seeking and degree of satisfaction of their current ability to seek information.
Results: A total of 203 patients (M/F:110/93, DM type 2/1:172/29) completed the questionnaire. Patients identified diet (61.4%) and diabetic complications (41.9%) as "of great importance" as their needs of information. The treating physician (94.6%) and ophthalmologist (31.5%) were considered as of "extreme importance" as information resources, while internet importance and frequency of use ranked low (91.1% reported access of once a month or less). Main reported obstacles to information seeking were "lack of time" and "cost". Most patients (71.4%) stated they were "quite" or "very satisfied" with the current possibilities of information seeking.
Conclusion: Diabetic patients seemed particularly interested in diet and diabetic complications. Their main stated source of information was the treating physician and main obstacles to obtaining information were lack of time and cost. The results of this study could potentially have important implications in designing an information campaign for diabetic patients.

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The art of creating dialogue and participation in patient education: health educator experiences using a person centred patient education tool kit

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Background and aims: Participation and dialogue are core values in diabetes education, leading to increased likelihood of improved empowerment and improved action competence in persons with diabetes. Creating participation and dialogue as well as more participant-educator interactivity is shown to increase the effectiveness of self-management education programmes in diabetes. We tested the ability of a dialogue-based education tool kit to enhance participation and dialogue from a health educator perspective. The tool kit is designed for group-based sessions in chronic disease education and is part of an innovative health education concept called The Balancing Person including two theoretical models. It contains 24 different health education tools. The choice of how many and which tools to use is optional. We studied the dialogue between participants as well as the dialogue between health educators and participants.
Materials and methods: We tested the use of the tool kit in 49 chronic illness patient education settings in all parts of Denmark. We applied a mixed method approach drawing on questionnaires, observations, and interviews. Data was collected March-September 2012. Questionnaires about the experiences using the tool kit resulted in 432 responses. 19 observations and 18 interviews with health educators were performed in 12 selected settings. During observations we did 10 sec. interval coding to estimate the distribution of participant talk, educator talk, and group work. This gave an indication of the ability of the tool kit to support the creation of participation and dialogue. During interviews health educators were asked to complete in writing a number of unfinished sentences regarding their experiences using the tool kit. Data was analysed using descriptive statistics and meaning condensation.
Results: In all, 82% of the educators reported that the tools supported them in creating participation to a high or a very high degree. Furthermore, 75% of the educators reported that the tools enhanced dialogue between the participants, and 80% reported that the tools enhanced dialogue between educators and participants to a high or a very high degree. The 10 sec. interval coding demonstrated a higher degree of participant talk and group work in sessions where the tool kit was used compared to more traditional lecture inspired sessions showing a higher degree of educator talk. Additionally, data from the unfinished sentences suggested that the educators especially valued the flexibility of the tool kit and the ability of the tools to support the opening dialogue which appeared as a consistent theme throughout the analysis.
Conclusion: The health education tool kit constitutes a valuable approach for the health educators in creating participation and dialogue between participants as well as between educators and participants. Results indicate that the tool kit is especially well suited for establishing the initial contact and dialogue which is crucial to further learning during the entire educational programme. Furthermore, the flexibility enhances the use of the tool kit.

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Motivation to change towards healthy diet and habitual physical activity in subjects with type 2 diabetes mellitus

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Background and aims: Motivation to change is a critical issue for education to healthy lifestyles in patients with type 2 diabetes (T2DM). We aimed to check the level of motivation for change towards healthier diet and increased habitual physical activity in a cohort of patients with T2DM.
Materials and methods: In 1743 patients with T2DM (males, 879) recruited in 14 diabetes Italian centers, motivation to change was tested by the validated EMME 3 questionnaire, which assesses the stage of change (Precontemplation, Contemplation, Determination, Action, Maintenance) according to the model of Prochaska & DiClemente. The questionnaire derives from a previously validated tool for individuals with alcohol problems. It consists of two parallel sets of instruments (for diet and physical activity, respectively): an 18-item questionnaire (MAC 2) on a Likert scale from 0 (totally false) to 6 (totally true) and a set of 6 visual analogue scales (VAS) from 0 to 100, which also provide scores on Discrepancy, Self-Efficacy, Importance, Temptation, Readiness-to-change and Stabilization-of-change. Discrepancy refers to the contradiction between what one is or behaves like and what one aims to be or to behave like, related to personal "image of self", values, goals and expectations. Discrepancy (or internal fracture) reflects concern and dissatisfaction with the present situation (need for change) and the perceived importance of change (desire for change). Self-efficacy is the perceived confidence in attaining and maintaining the predefined goals of change. Importance and Temptation are defined as the importance attributed to the new lifestyle and the attractive value of the old lifestyle; finally Readiness-to-change and Stabilization-of-change offer a summary assessment of the stage of change. A third part of the test, containing 9 brief descriptions (PORTRAITS) of imaginary people, confirmatory of the same motivational components of the MAC 2 questionnaire, was not used in this setting. Data are reported as means [SD] and significance of differences (paired T-test).
Results: The scores on stage of change are different between diet and physical activity, with a much higher motivation to diet (P<0.0001). Men have higher scores in Maintenance and Self-Efficacy for Diet, while women have higher levels of Temptation to unhealthy habits in both questionnaires, despite higher levels of Determination for Diet and Contemplation and Internal Fracture for Physical Activity. The levels of Internal Fracture (in Diet) and Stabilization (in Physical Activity) are significantly higher in men. Finally, for both behavioral changes, most scores are dependent on time since the diagnosis of diabetes, in keeping with a progressive loss of motivation with time and/or comorbidities.
Conclusion: Data may be used to address tailored interventions in individual patients

Mean scores of changes for Healthy Diet and Habitual Physical Activity

	Healthy Diet	Habitual Physical Activity	P Value
Precontemplation	40 [22]	28 [22]	< 0.0001
Contemplation	57 [23]	60 [23]	ns
Determination	64 [24]	56 [28]	< 0.0001
Action	58 [24]	39 [31]	< 0.0001
Maintenance	59 [24]	46 [31]	< 0.0001
Internal Fracture	44 [25]	49 [26]	< 0.0001
Importance	81 [16]	78 [19]	< 0.0001
Self Efficacy	66 [20]	61 [24]	< 0.0001
Temptation	54 [27]	37 [26]	< 0.0001
Readiness-to-Change	63 [33]	61 [28]	ns
Stabilization	62 [35]	54 [29]	< 0.0001

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Group-based diabetes self management education for refugees with type 2 diabetes in Sweden: an observational study on short term effects on metabolic control

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Background and aims: Refugees are faced with a number of difficulties in order to benefit from the opportunities provided by medical care. Very few published studies have investigated the special conditions that arriving to a new country and seeking asylum provokes in subjects with diabetes and the proper mode of health care delivery under these conditions. The purpose of this exploratory pilot study was to evaluate the effect of nurse-led group-based self-management education (SME) of refugees with diabetes compared to standard care based on individual visits (SC).
Materials and methods: We conducted a retrospective analysis of 134 refugees who were consecutively enrolled at a primary health care centre dedicated to refugee care in Malmö, Sweden, during 2007 and 2009. In addition to SC on an individual basis subjects were offered group-based structured nurse-led education sessions. Treatment effect and potential gender effects were investigated. HbA_{1c}, weight, type of treatment and other relevant clinical variables were recorded and compared by paired non-parametric analysis and multiple regression analysis was employed to identify independent predictors of treatment success. Data are mean ± standard error of the mean unless otherwise indicated. A p-value below 0.05 was considered statistically significant.
Results: A significant reduction in HbA_{1c} (from 7.63 ± 0.22 to 6.80 ± 0.16 %, $p = 0.0002$) was observed in the total patient population (mean duration of treatment 197 ± 13 days) resulting in subjects choosing SME no longer exhibiting an increased HbA_{1c} compared to those choosing SC alone. While improving metabolic control, a significant weight loss (BMI from 34.1 ± 1.9 to 32.5 ± 1.7 kg/m^2, p=0.005) was also seen in the group receiving SME which was not seen in patients receiving SC. When analyzed separately the decrease in weight was significant in female patients only ($p = 0.03$).
Conclusion: We conclude that SME may add significant value and benefits beyond what SC can offer to patients with diabetes in the particularly stressful circumstances of refugee/assylum seeking status. Alternatively, subjects that are willing to accept a group-based education setting under these circumstances are characterized by being able to improve metabolic control without the commonly associated weight gain. There may also be differences between the way males and females benefit from SME that need to be investigated further. Randomized studies are called for to address these important issues in a group of particularly vulnerable subjects.
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Physical activity and life quality in patients with type 2 diabetes mellitus after therapeutic education in "diabetes school"

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Background and aims: Tolerance to physical exercise may be reduced in patients with diabetes as result of concomitant diseases, complications and deconditioning. Therapeutic education of patients is aimed to increase level of physical activity and tolerance to exercise may be changed as result which may be important for life quality. Aim of this study was to evaluate tolerance to physical activity, usual level of activity and their relation to life quality in patients with T2DM before and after educational course in "diabetes school"
Materials and methods: 40 patients with T2DM there included in open prospective study with evaluation of performance in 6-minute walk test (6MWT) at baseline and 3 and 6 months after therapeutic education program. Habitual physical activity was self-assessed by patient as total duration of exercise in a week. HbA1c, body weight, life quality using SF-12 questionnaire were also evaluated. 24 patients so far have finished 6-months observation. Statistical analysis was performed using Wilcoxon matched pairs test, Spearman correlation test.
Results: At baseline distance in 6MWT had significant negative correlations with HbA1c (R = -0.56 p=0.0001), diabetes duration (R=-0.44 p=0.005), BMI (R=-0.34 p=0.03). It was associated with better life quality on physical functioning (PF) and role-physical (RP) scales of SF-12 (R=0.46 and 0.43 p=0.003 and 0.005). Usual level of activity correlated with BMI (R=-0.37 p=0.028). After education patients had trend to decrease in HbA1c (7.4% baseline, 6.9% at 3 and 6 months p=0.08 and 0.06) with no change in BMI (30 at all visits). At 3 and 6 months after education patients had significant increase in average 6MWT distance (421 and 424 meters vs. 404 meters at baseline, p = 0.003 and 0.03). Increase in 6MWT distance from baseline for both 3 and 6 months correlated with diabetes duration (R=-0.57 and -0.42 p=0.0037 and 0.043), NSS neuropathy score (R=-0.43 and -0.44 p=0.033 and 0.035). Also there was increase in usual physical activity from 149 to 331 min/week (p<0.0001).
Conclusion: Education can be an effective way in improvement of physical performance in patients in T2DM which is an important contributor in physical component of life quality. Physical performance is dependent on diabetes compensation, obesity, diabetes polyneuropathy which all can be potential targets for interventions.

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Exploring diabetes education and information: perspectives of people with diabetes (DAWN2 study)

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Background and aims: Diabetes education is essential for diabetes self-management and improved diabetes outcomes, quality of life and prevention of complications. The 2nd Diabetes Attitudes, Wishes and Needs (DAWN2) study assessed access to diabetes education/information among people with diabetes (PWD) in 17 countries.
Materials and methods: In each country, a sample of 500 PWD, stratified by diabetes type and treatment, completed a psychometrically and cross-culturally validated questionnaire in local languages. This incorporated access to diabetes education and information and validated scales, eg Problem Areas in Diabetes (PAID), Diabetes Empowerment Scale (DES), and Summary of Diabetes Self-Care Activities (SDSCA). Binary logistic regression models estimated factors explaining participation in education and use of diabetes information.
Results: 8596 patients completed the questionnaire: 16% with type 1 diabetes (T1DM), 84% with type 2 (T2DM). 68% of people with T1DM (N=934) and 58% with T2DM (N=4221) participated in education. Approx. 40% never participated in education, with higher nonparticipation for those with T2DM (OR 1.5 [1.4-1.7]). Participation rate between countries varied (range 27-86%) and was highest in Germany, Poland and Canada (80-86 %). Participation in education decreased with increasing age (p<0.0001), and was likely to be lower for people living in large cities (OR 0.5 [0.5-0.6]) whereas the participation rate was higher for people living alone (OR 1.5 [1.3-1.7]). Participation was associated with fewer psychological problems, better diabetes empowerment and better self-management (p=0.0001).The 79% of the patients who participated in education and found it helpful had fewer psychological problems, better diabetes empowerment and better self-management (p<0.01) compared to participants who did not find education helpful. Experiencing helpfulness decreased with increasing age (p=0.02). Preferences for future education included access to "printed information," "ongoing advice and support outside regular office visits" and "general health and diabetes websites." Preference for "printed information" decreased with increasing age (p<0.001). "Websites" were less preferred by males (OR 0.84 [0.76-0.93]), more preferred by people living in large cities (OR 1.31 [1.18-1.44]) and increased with increasing age (p<0.001).
Conclusion: PWD who access education find it valuable and have higher psychological well-being than those who have not accessed education. This multinational analysis has highlighted significant variations in access to education and identified groups of patients who may be less likely to participate. Best practices can guide improvements in access to diabetes education.

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Effect of individual patient education for people with type 2 diabetes mellitus in Bangladesh

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Background and aims: Diabetes patient education has long been recognized as a vital and integral component of successful diabetes care. Education that increases patients' understanding of diabetes can prevent or delay complications and reduce the number and duration of hospitalizations, which in turn can improve quality of life. To the best of our knowledge, there are no other studies addressing to conduct patient education to make its present health care services more comprehensive in Bangladesh. The aim of the study was designed to assess the effectiveness of individual patient education on metabolic control, diabetes knowledge and psychosocial outcomes through intervention.
Materials and methods: An Intervention study was conducted with one thousand newly diagnosed type 2 diabetic patients (aged ≥18 years) who were randomly selected from a tertiary care Diabetic hospital. Subjects were divided into a control group (500) and an intervene group (500). A structured questionnaire in simple understandable local language was used to collect socio-demographic history by face to face interview. Subjects in intervene group were visited every month; along with standard care and structured intervention especially self-care education was given. Control group were visit twice (0 and 6 month) and given only the standard care. Anthropometric measurement was done by standard techniques. Diabetic profile (HbA1c) and total cholesterol (TC) were examined by high-performance-liquid chromatography (HPLC) and enzymatic-endpoint method. Data were analyzed by univariate as well as multivariate analysis.
Results: Among the intervene group HbA1c was reduced significantly compared to control group. The weighted mean difference (WMD) of HbA1c was 0.8% with a trend to favour individual patient education (95% CI 0.3 to 1.3, P = 0.0007). There was no significant decrease in BMI at 0 to 6 months with a WMD of -0.2 BMI units (95% CI -1.0 to 0.6, P = 0.62). Intervene group with individual patient education had no significant effect on total cholesterol compared with control group with a WMD of -0.03mmol / L (95% CI -0.2 to 0.10, P = 0.66). There was a significant reduction in the number of participants who quit or reduced the amount of smoking in those receiving individual education versus control group (16.7% versus 5.7%, P = 0.031).
Conclusion: Individual education appears to be a significant effect among the intervene group compared to control groups. Repeated reinforcement of health education and strong motivation are needed in self-care practices with regard to diabetes control for the control groups as well as all diabetic subjects.
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PS 095 Psychosocial aspects of diabetes

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Socioeconomic differences in families of children with well and poorly controlled type 1 diabetes

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Background and aims: Numerous studies show that the achievement of near-normoglycaemia reduces the development of chronic complications of diabetes. The management of type 1 diabetes always poses a challenge, particularly in prepubertal children. The treatment completely depends on the parents' decisions and presents a unique set of problems. Difficulty in achieving glycemic targets in this age group stems both from children developmental stage and parents themselves; incorrect eating habits, family stress, fear of hypoglycaemia, parental depression, problems in insulin dosage, frequent infections and unpredictable physical activity affect glycaemia. Family socioeconomic status is also found to play a role in diabetes treatment. Our clinical experience shows that some parents manage to adhere to the treatment requirements while others constantly have problems with achieving good metabolic control in their children. The aim of the study was to identify the family factors affecting metabolic control of type 1 diabetic children under nine years of age.
Materials and methods: During the routine visit in the outpatient clinic 104 parents of type 1 diabetic children under nine years of age were asked to fill in Beck Depression Inventory, Quality of Life Questionnaire, a 58-item questionnaire, based on DCCT Quality of Life Measure, and a questionnaire specially prepared for this study on psychological, social, demographics and disease-related topics. 16 participants were rejected due to incomplete data. Out of the remaining 88 (84%) 45 were completed by mothers and 43 by fathers. At the same time other data was collected: sex of a child, age, diabetes duration, HbA1c, BMI, daily insulin dose. Families were divided into two groups depending on children metabolic control: HbA1c<7% and HbA1c≥7%.
Results: 36% (32/88) of children had HbA1c≥7%. There was no difference between groups with HbA1c<7% and HbA1c≥7% in age (6,8±1,8 vs. 6,5±2,0 yrs; p=0.51) and diabetes duration (2,6±1,6 vs. 2,9±1,9, p=0,448); respectively. The poorly controlled children had higher HbA1c 8,1±0,6% than the well controlled ones 6,6±6,5% (p<0.0001). In comparison with well controlled subjects, in families of children with HbA1c≥7% parents had lower education (p<0,05), more parents were employed as physical than office workers (p=0,004) and family income was lower (p=0,013). In the group with HbA1c≥7% there were more single parent families than in families of well controlled subjects (p=0,008). Parents of children with HbA1c≥7% had more problems with extra feeding by other family members (p=0.0008). Surprisingly we didn't find any difference in reported depressive symptoms (p=0.7824) and quality of life (p=0.6978). Children in both groups didn't differ in BMI (p=0.0545) or daily insulin dose (p=0.6456).
Conclusion: Our results show that families of prepubertal children with poorly controlled type 1 diabetes come from a disadvantage group. These families may require particularly careful screening for social and psychological problems. Additional help, including financial and psychological support, more re-education should be individually tailored according to each patient's needs.

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Association of personality traits with diabetes-related distress and psychosocial factors in subjects with long duration of type 1 diabetes

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Background and aims: Type 1 diabetes is a chronic irreversible disease. Proper diabetes management is multidimentional and comprises issues of insulin therapy, prevention of chronic complications, psychological support, motivation and patient empowerment and is performed by a therapeutic team. The aim of this study was to assess the impact of particular personality

traits on diabetes-related distress and psychosocial factors in subjects with long history of type 1 diabetes.
Materials and methods: 255 subjects (138 women), aged 42.8±10.7 years, type 1 diabetes duration 27.1±6.2, HbA1c 8.2±1.5% were included. Personality was assessed using NEO-FFI (Neurotism-Extraversion-Openness Five Factor Inventory). Additionally Problem Areas in Diabetes Questionnaire (PAID) assessing diabetes-related distress, Beck Depression Inventory (BDI) and Multidimensional Fatigue Symptom Inventory- Short Form (MFSI-SF) were completed. Data on social, economic and lifestyle factors were collected. Metabolic control expressed by HbA1c, lipid profile and chronic complications was assessed via medical chart review.
Results: The main results are shown in Table 1. There was no correlation of personality traits to HbA1c, lipid profile or microvascular complications.
Conclusion: The results of this study may be useful in patient-centered, individualized therapeutic approach. Although there was no relationship of personality traits with metabolic control of diabetes, we indicated the association of several personality traits with psychosocial status. This may enable therapeutic team to better delineate the treatment plan and adjust it to patient's needs and behaviours.

Table 1. Correlation of personality traits to psychosocial status and diabetes-related issues in studied group. *p<0.05, **p<0.001

	Conscientiousness [score]	Openness to Experience [score]	Extraversion [score]	Neuroticism [score]	Agreeableness [score]
PAID [score]	r=-0,1294	r=-0,0058	r=-0,2499**	r=0,4642**	r=-0,2467**
BDI [score]	r=-0,2095*	r=-0,0211	r=-0,3559**	r=0,6022*	r=-0,2354**
MFSI-SF, General [score]	r=0,0557	r=-0,0035	r=-0,2264*	r=0,3793**	r=-0,1363
MFSI-SF, Physical [score]	r=-0,0100	r=-0,2250*	r=-0,3004*	r=0,3177**	r=-0,0793
MFSI-SF, Emotional [score]	r=-0,0514	r=-0,0334	r=-0,1965*	r=0,4615**	r=-0,1740
MFSI-SF, Mental [score]	r=-0,1304	r=-0,1988*	r=-0,1943*	r=0,3671**	r=-0,0833
MFSI-SF, Vigor [score]	r=0,0161	r=-0,0553	r=0,3472**	r=-0,3437**	r=0,1453
MFSI-SF, Total [score]	r=-0,0083	r=-0,0740	r=-0,3247**	r=0,4790**	r=-0,1598
Education [degree]	r=0,0051	r=0,3575**	r=0,0619	r=-0,1466*	r=-0,0097
Financial status [income]	r=0,0986	r=0,0501	r=0,0884	r=-0,2173*	r=-0,0020
Fear of hypoglycaemia [y/n]	r=-0,0618	r=-0,0776	r=-0,1470*	r=0,2001*	r=-0,0376
Fear of "the needle" [y/n]	r=-0,0660	r=-0,0435	r=-0,1616*	r=0,1571*	r=-0,1073
Physical activity [y/n]	r=0,0860	r=-0,0352	r=0,1416*	r=-0,0769	r=-0,0311

PAID - Problem Areas in Diabetes Questionnaire; BDI - Beck Depression Inventory; MFSI-SF - Multidimensional Fatigue Symptom Inventory - Short Form

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Socio-economic status - combination of occupation, education and income level - and diabetes risk: the Hoorn study
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Background and aims: Low socio-economic status (SES) is associated with increased risk of type 2 diabetes. Most studies however only include 1 indicator of SES: education, income or occupation. The aim of our study is to evaluate the strength of the association between education, income, occupation or the combination of all 3 SES indicators and diabetes risk in the general population.
Materials and methods: Design, setting, patients**:** The Hoorn study; a population-based cohort study among older men and women, who had a physical examination at baseline in 1989 and after 6y follow-up. Main outcome measure**:** Incident diabetes based on WHO Consultation criteria for fasting plasma glucose levels, 2h plasma glucose levels and HbA1c levels.
Results: We included 1162 participants (46% male; 59.9 ± 6.8 years). At follow-up, 106 (9.1%) participants had developed type 2 diabetes. Low levels of education (7-level scale), occupation (5-level scale) and income (3-level scale) were all associated with increased diabetes risk; however, only the association with income was statistically significant. When we combined the 3 SES indicators into 1 score, we observed that compared to the highest tertile, the age and sex adjusted HR (with 95% CI) for diabetes incidence for the middle and lowest tertile were 1.65 (1.01-2.67) and 1.62 (0.98-2.68). Adjustment for BMI and waist-to-hip ratio explained part of this increased risk.
Conclusion: The combined SES score of income, occupation and education level better predicts the increased risk of type 2 diabetes in those with low SES status, compared to the separate scores of the SES indicators in an elderly population-based cohort.

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Low self-efficacy and insulin resistance in a Swedish population: a cross-sectional study
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Background and aims: The high prevalence of insulin resistance and depressive symptoms is well documented. The underlying psychological mechanisms and interactions are interesting both in prevention and treatment. The aim of the present study was to investigate the association between the self-efficacy and insulin resistance in men and women.
Materials and methods: A random sample of 2816 men and women from the municipalities of Vara and Skövde in South-western Sweden, were invited to a health survey in 2002-2005 (participation-rate 76%). After excluding participants with missing information on any of the study variables, 2564 subjects (1286 women and 1278 men) remained for the present study. Information on self-efficacy was assessed by the question "Do you believe you can do something yourself to maintain a good health", with the three answer alternatives "Yes, I believe that one's own effort is very important"; "Yes, I believe that one's own effort has some importance"; and "No, I do not believe that one's own effort has any importance". Information on leisure-time physical activity (LTPA), smoking, and education was also collected by questionnaires. Glucose metabolism (OGTT), insulin resistance (HOMA-ir), blood pressure, and BMI were measured using standard methods.
Results: Among men, 294 (22%) reported low self-efficacy, while corresponding findings in women were 280 (21%). In a linear regression model, there was a significant association between insulin resistance and low self-efficacy in both men (regression coefficient (ß): 0.042, 95% CI:0.005-0.079, p=0.025, Beta 0.042) and women (ß: 0.048, 95% CI: 0.010-0.086, p=0.013, Beta 0.067). When differences in BMI, LTPA, smoking and educational level were accounted for, these associations remained in women (ß: 0.033, 95% CI:0.001-0.065, p=0.043, Beta 0.047), but not in men (ß: 0.012 95% CI:-0.020-0.045, p=0.466, Beta 0.017).
Conclusion: Screening for self-efficacy related to the ability to sustain good health may be a way to target women who would benefit most from interventions to prevent impaired glucose metabolism, including type 2 diabetes.
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Societal discrimination and emotional well-being in people with diabetes: results from DAWN2
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Background and aims: Suffering from a chronic condition such as diabetes may still be a reason for societal discrimination. The second Diabetes Attitudes, Wishes and Needs (DAWN2) study assessed perceptions of being

discriminated against because of having diabetes in persons with diabetes (PWD) across 17 countries within 4 continents. Data from DAWN2 allow exploration of correlates between perceptions of being discriminated against and psychosocial distress.
Materials and methods: All country samples comprised ~500 PWD (~80 type 1 [T1DM] and ~420 type 2 [T2DM], of which ~150 were insulin treated). Survey questionnaires, developed using new items as well as items adapted/modified from existing validated measures, were designed to assess self-management, attitudes/beliefs, health-related quality of life (QOL), social support and priorities for improving future diabetes care. Questionnaires were administered online, by telephone or in person. Comparisons between PWD who reported being discriminated against (or not) were made for various scales including WHO-5 Wellbeing index, Problem Areas in Diabetes (PAID-5), WHO-QOL-BREF (% of PWD reporting their QOL as poor/very poor), Impact on Life Dimensions, a support scale and Diabetes Empowerment Scale (DES). Data were analyzed using multilevel analysis (patients clustered within countries).
Results: 1368 adults with T1DM and 7228 with T2DM completed questionnaires in local languages. Overall, 19.2% of PWD reported being discriminated against because of diabetes, with wide country variation [11.1- 30.1%]. After adjusting for clustering effect, discriminated against PWD had a significantly lower WHO-5 score (-5.0; p<0.0001), a higher PAID-5 score (+12.4; p<0.0001), a 67% higher likelihood of reporting poor/very poor QOL (OR 1.67; 95% CI 1.43-1.92), lower perceived support (-1.6; p<0.0001), and higher empowerment (+9.6; p<0.0001). PWD who reported being discriminated against reported a negative impact on several life dimensions: financial situation (OR 1.44; 95% CI 1.28-1.62); relationship with family, friends or peers (OR 1.99; 95% CI 1.75-2.26); leisure activities (OR 1.42; 95% CI 1.26-1.59), work or studies (OR 1.76; 95% CI 1.55-2.00); and psychological well-being (OR 1.19; 95% CI 1.06-1.33).
Conclusion: Perceptions of being discriminated against because of diabetes are common in PWD and should be taken seriously. These perceptions are associated with negative impacts on emotions and well-being, coping strategies and different aspects of living with diabetes during work as well as leisure activities and with friends or relatives. Almost half of PWD with perceptions of discrimination also reported a negative financial impact of diabetes. It is not clear why discrimination was associated with higher empowerment.

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Psychosocial stress is associated with higher mortality: the Hoorn study
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Background and aims: Psychosocial stress is associated with chronic disease. Experiencing stressful life events may lead to premature mortality, by inducing behavioural, endocrine and metabolic changes. The aim of our current study is to evaluate whether in the general population (the number of) stressful life events are associated with all-cause and cause-specific mortality, and whether this relationship is mediated by behavioural, endocrine and metabolic disturbances.
Materials and methods: *Design, setting, patients*: The Hoorn study; a population-based cohort study among older men and women. *Main outcome measure*: Stressful life events experienced during the previous 5 years were assessed by questionnaire. We calculated Cox proportional hazard ratios (HRs) for all-cause and cause-specific mortality during follow-up for those who experienced no stressful life events compared to those who did.
Results: We included 2385 participants (46% male; 61.6 ± 7.4 years). During 20 years of follow-up 834 (35%) participants died, of whom 239 (28.6%) died of cardiovascular disease and 235 (28.2%) of cancer. Compared to the group with no stressful life events, the age and sex adjusted HRs (with 95% confidence intervals) for all-cause mortality for those who had 1 event, 2 events, 3 events and ≥ 4 events were 0.92 (0.75-1.11), 1.02 (0.83-1.25), 1.29 (1.01-1.65) and 1.51 (1.15-1.99), respectively. Adjustment for prevalent obesity, diabetes and cardiovascular disease and deleterious behaviour partly explained the increased risk. Similar results were observed for cardiovascular mortality and cancer mortality.
Conclusion: Having 3 or more stressful life events is associated with a significantly increased risk for all-cause mortality, cardiovascular mortality and cancer mortality in an elderly population-based cohort. This association is presumably mediated by behavioural, endocrine, behavioural disturbances, resulting in obesity, cardiovascular disease and diabetes.

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Qualitative insights into diabetes psychosocial needs and strategies from the perspective of healthcare professionals in DAWN2
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Background and aims: To identify professionals' perceptions of the needs, challenges and wishes for people living with diabetes, the second Diabetes Attitudes, Wishes and Needs (DAWN2) study surveyed 4785 healthcare professionals (HCPs) who care for people with diabetes (PWD) in 17 countries across 4 continents. These included 2066 PCPs/GPs, 1350 diabetes specialists, 827 nurses and 542 dietitians.
Materials and methods: Qualitative data were drawn from responses to open-ended survey questions about challenges and successes in managing diabetes, and wishes for improvement in support for PWD. Emergent coding with input from multinational collaborators identified thematic content about psychosocial aspects of diabetes care, and yielded 30 macro-level (parent) codes, and 114 micro-level (child) codes.
Results: Two common challenges are not having time or support to help PWD manage the disease; that is, to "make time to think about things from the patient's perspective." PWD access to support groups and psychologists is limited. HCPs find a challenge in knowing how to listen to patients, understand causes of depression and find solutions. They state PWDs are not aware of complications, and "think that diabetes is nothing more than elevated blood sugar." Strategies considered successful in helping PWD achieve better outcomes include reducing the fear of taking insulin, and involving families and patients in goal-setting. Providing encouragement is helpful, such as giving hope that people can manage the disease and verbal reinforcement to raise motivation. One HCP encourages patients to "write down your thoughts, fears and experiences about diabetes." Across all countries, HCPs wish for psychological support for PWD "since there are personal and behavioral problems for which we don't have the ability to take action." This also includes helping PWD overcome depression. HCPs wished for more time with PWD and proper reimbursement, as well as learning motivational strategies to improve A1C. HCPs also wish for new advances in affordable oral medications with fewer side effects.
Conclusion: The narratives indicate that changes are needed to give HCPs enough time to assist PWD and to provide psychological assistance for the effective treatment of comorbid depression. The results highlight the psychosocial needs and strategies of HCPs globally to improve care and support for PWD.

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A psychological support programme for patients with nonalcoholic fatty liver disease (NAFLD)
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Background and aims: Lifestyle changes towards healthier diet and physical activity (PA] are mandatory in nonalcoholic steatohepatitis (NAFLD), but PA is more difficult to implement in the population. We aimed to determine the clinical effectiveness of an intense psychological support to PA in NAFLD.
Materials and methods: In a pilot study, 22 NAFLD cases were enrolled into an individualised motivational and psychological support to exercise (PA group], chaired by a psychologist and tailored to their motivational needs. Patients were helped to identify their preferable leisure activities (such as going for a walk or riding the bike) and then supported in developing a weekly schedule and implementing that plan. The sessions were then

focusing on how to deal with common psychological barriers to physical activity and on the importance of self-monitoring. Defined and reasonable goals for physical activity were set during the sessions. Only one hour was devoted to nutritional counselling. The effects on weight, body fat and physical fitness were compared with the data of 44 NAFLD subjects enrolled in a cognitive-behavioural program for weight loss (CBT group], consisting of 13 weekly group sessions, 120 min each (approx. 15-20 subjects), chaired by physicians, dieticians, psychologists, education experts. Education focuses on the alimentary pyramid, size of portions and regular eating, calorie counting, and marginally on physical activity. Physical fitness was measured by the 6-min walk test, VO2max and the PA rating questionnaire (PA-R]; body fat by the fatty liver index (FLI) and the visceral adiposity index. Measurements were performed at baseline, at 4 months and at 1-year follow-up. Data were adjusted for propensity score, based on baseline clinical characteristics.

Results: The two groups were well balanced at baseline, without significant differences in socio-economic, anthropometric and clinical data. The PA program produced a larger effect of physical fitness, the CBT program a larger effect on body weight. After adjustment for propensity, weight loss > 7% was associated with CBT (odds ratio (OR], 2.52 (95% confidence interval, 0.60-10.53] at 4 months and to 6.21 (1.23-31.30] at one year. By contrast, the probability of PA-R >3 was significantly associated with PA group (OR, 10.31; 2.02-52.63]. Liver enzymes and body fat similarly decreased in the groups, without significant differences at 4 months and 1-year follow-up.

Conclusion: An intensive psychological counselling for PA produces effects on the liver similar to those of a standard CBT, improving physical fitness independently of weight loss. Strategies aimed at promoting exercise are worth implementing in motivated patients.

Parameters at baseline and follow-up (mean or % [SD])

	PA Group			CBT Group			ANOVA (time x treatment)
Parameter	Baseline	4 months	12 months	Baseline	4 months	12 months	P Value
BMI (kg/m2)	30.4 [5.0]	29.3 [4.4]	29.7 [4.6]	32.4 [3.9]	30.3 [3.7]	30.4 [3.7]	=0.005
Waist circum. (cm)	99 [15]	97 [15]	98 [15]	105 [10]	100 [10]	99 [10]	< 0.001
ALT (U/L)	68.5 [28.5]	45.0 [20.1]	46.2 [21.0]	64.1 [32.2]	39.5 [15.9]	40.8 [20.7]	=0.983
Fatty Liver Index (%)	77.0 [23.7]	65.5 [26.8]	69.7 [25.1]	84.7 [14.8]	71.0 [20.9]	70.8 [20.6]	=0.083
Visceral Adiposity Ind. (%)	2.84 [1.83]	2.38 [1.66]	2.27 [1.32]	2.79 [1.54]	2.29 [1.16]	2.26 [1.34]	<0.001
6-Min Walk Test (m)	521 [92]	660 [112]	---	497 [82]	540 [76]	---	<0.001
PA-Rating > 3 (%)	14	91	---	36	46	---	<0.001
VO2max (ml/kg/min)	29.0 [7.4]	37.1 [7.7]	---	28.3 [3.8]	31.5 [3.6]	---	<0.001

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Eating disorders and food anomalous behaviour in type 2 diabetes adults

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Background and aims: It is well-known that Eating disorder (ED) and other anomalous eating behaviours (AEB) in young diabetics are associated with anomalies in their metabolic control. These anomalies occur more commonly in youth with type 1 diabetes than their non-diabetic peers. However, ED and AEB, have not been well studied in adults with type 2 diabetes. The aims of the present study were to determine the rate of ED and AEB in type 2 diabetes adults and to determine whether the presence of ED or AEB influences on patients metabolic control.

Materials and methods: Adult (over 40 years) patients with type 2 diabetes of both sexes from three Primary Care Centers in Southern Galicia, Spain, without previous selection and adult non-diabetic aged sex and BMI matched as controls from the same population were included. It is a prospective, case-control, multicenter and observational study of 1 year duration. This project was approved by the Research Ethics Committee of Galicia. All patients were submit to two self-administered, validated, psychological questionnaires to assess the presence of ED, the Bulimic Investigatory Test Edinburgh (BITE) to assess bulimic symptomatology, and Questionnaire of Eating and Weight Patterns-Revised (QEWP-R) to diagnose Binge Eating Disorder (BED) and Bulimia Nervosa (BN), followed for a structure interview on eating patterns. The study variables were ED and AEB rates, glycosylated hemoglobin (HbA1c), and body mass index.

Results: The rate of ED for diabetics and controls were similar, 4.8% and 3.9% respectively. Statistical significance differences were no found for the rates of BN (0% vs. 1.3%), BED (0.8% vs. 0%) and eating disorder not otherwise specified (3.9% vs. 3.8%) for patients and controls respectively. Picking at foods and dissatisfaction with body weight or shape were significantly more frequent rates of ED were observed. Table 1.

Table 1. Comparison of AEB rates between patients and controls

Rates (%)	DM2 (N=230)	CONTROLS (N=177)
Binge eating	4.8	5.2
Skipping meals	3.9	3.8
Picking at foods	28.7*	19.5
Dissatisfaction with body weight or shape	34*	15.6
Food addiction	16.1	15.6

*p<0.05 vs. controls

The mean values of HbA1c (%) were 7.0±1.3; 8.0±1.5** and 7.2±1.3** for the group of patients non ED and AEB, patients with ED and the group of patients with AEB. **p<0.05 vs. patients non ED and AEB.

Conclusion: These findings highlight the importance of evaluating type 2 diabetes over 40 years for the presence of AEB and ED.

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1147

Study on the support of compatibility between work and medical care and of return to work in diabetic patients in Japan

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Background and aims: Many previous reports showed that the increasing prevalence of lifestyle-related diseases (i.e. diabetes mellitus (DM) is one of the major factors leading to a decline in the working population. Therefore the prevention of DM is the most important issue for public and labor health in Japan. From 2000, the Ministry of Health, Labor and Welfare (MHLW) has been promoting measures against lifestyle-related diseases in Japan (Healthcare Japan 21th Century). But in a recent report of this plan in progress from MHLW, the population of DM and pre-DM patients is still increasing and is now more than 20 million in Japan. In another MHLW report in 2009, less than 25% of diabetic patients had received medical care. Patients without medical care and the condition of these workers are not clear.

Materials and methods: To clarify the problem for the compatibility between work and DM treatment, we conducted surveys in the form of a questionnaire. The surveys were conducted with 1184 employees with DM and with the health management staff in 800 companies in Japan. We used SPSS ver. 19.0 for statistical analyses, and set P<0.05 as significance level.

Results: Patient questionnaire results showed that the average of HbA1c adjusted by age and sex in employees working at companies that provide industrial physicians is significantly lower than for employees who do not have access to such physicians (7.5% vs. 7.2%, p=0.002). Similarly, the prevalence of diabetic retinopathy (P=0.046) and that of nephropathy (P=0.014) in employees at companies with industrial physicians (IPs) are significantly lower than those without IPs. In multiple regression analysis, existence of IPs is an independent factor for less prevalence of diabetic nephropathy after adjusting for age, gender, duration of diabetes, body mass index, HbA1c, existence of hypertension and dyslipidemia (odds ratio 0.51, 95% confidence interval 0.27-094, P=0.032). In questionnaire results from the companies, the age-adjusted prevalence rate of DM at small (employee number<50) companies is significantly higher than that at large (employee number<300) and medium-sized (50<employee number<300) companies (p=0.04). This tendency can be seen regardless of category of business. The questionnaire results of the survey conducted with health management staff in companies also showed that the prevalence of DM patients adjusted by age and sex in employees working at companies that provide full-time IPs tends to be lower than part-time or

nonscheduled IPs (38.2, 46.0, and 59.4 diabetic patients / 1000 employees, p=0.062).
Conclusion: Our results suggest that the IPs play an important role in DM management for employees and the quality of its management depends on the size of companies and type of employment of IPs. The goal of this study is to establish a system of cooperation among DM patients, medical doctors, and company through the adaptation of new guidelines prepared from our results.
Supported by: JLHWO

PS 096 Good news for depression in diabetes?

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Multiple analysis of risk factors for depression in Chinese type 2 diabetic patients
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Background and aims: Depression is common in patients with type 2 diabetes. Different studies have showed contradictory results on the risk factors for the depression in type 2 diabetic patients. A multiply factors analysis is needed for the a system study on the all-scaled factors affecting depression in diabetes patients. This study we first using multiply factors analysis to find the risk factors for depression in patients with type 2 diabetes in China.
Materials and methods: A total of 397 patients with type 2 diabetes were recruited from July 2012 to September 2012 in Nanjing. Depression was evaluated with the nine-item Patient Health Questionnaire (PHQ-9). Classification criteria for depression: <=4 points, normal group; 5-9 points, mild-depression group; 10-14 points, moderate-depression group; 15-19, moderate-to-major-depression group; 20 points or higher, major-depression group. While the demographic data (gender, age, education, marriage, income, way of payment, insulin injection and diabetic duration), metabolic data (fasting plasma glucose, postprandial blood glucose, HbA1c, triglyceride, total cholesterol, low density lipoprotein, high density lipoprotein, BP, BMI and obesity index) and chronic complications related to diabetes (diabetic angiocardiography, diabetic cerebrovascular disease, diabetic neuropathy, diabetic oculopathy, diabetic nephropathy and diabetic pedipathy) were collected, diabetic knowledge and self-management behaviors were also investigated by using Deborah scale and Diabetic Knowledge Test scale. Correlation tests were conducted between depression and all these potential factors. The risk factors associated with depression were screened by logistic regression.
Results: Of all the 397 patients with type 2 diabetes (137 males, 260 females, age 68.16 +/- 7.84 years, disease duration 9.43 +/- 6.53 years), a total of 94 (24.2%) patients were categorized as having depression, including 16.8% with mild, 5.8% with moderate, and 1% with moderate-to-major depression. Compared with the normal group, depression was correlated with gender (P=0.000), economic income (P<0.05), diabetes duration (P<0.05), economic income (P<0.05), tryglyceride (P<0.05), high density lipoprotein (P<0.05) and obesity index (P=0.000) in patients with type 2 diabetes. Although there was no significant relationship between depression and the number of chronic diabetic complications, the depression symptom was more severe in patients with diabetic pedipathy (P<0.05). Multiply logistic regression showed that obesity (OR=1.57), long diabetic duration (OR=1.37), diabetic pedipathy (OR=1.48), and female (OR=1.21), were risk factors for depression, while hight high density lipoprotein (OR=0.72) showed a protective effect on depression.
Conclusion: More attention should be paid on depression symptoms in patients with obesity and diabetic pedipathy especially the females with long diabetic duration. High density lipoprotein may act as a protective factor for depression in patients with diabetes and further research on the mechanism for this relationship is necessary.
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Depression and the metabolic control of type 2 diabetes mellitus
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Background and aims: Depression is a frequent co-morbidity in patients with diabetes mellitus (DM), especially in those with chronic diabetic complications. It was estimated that half of depression cases in DM are undiagnosed and untreated. The aims of this study were to estimate the prevalence of depression in persons with type 2 DM and to evaluate the impact of depression on the metabolic control of type 2 DM.
Materials and methods: The study included 364 patients with type 2 DM, 180 men and 184 women, with mean age 54.2 ± 3.8 years. For the screening

of depression, all patients filled the questionnaire PHQ-9 (The 9-item Patient Health Questionnaire) and those identified with depression were subsequently subjected to psychiatric evaluation. For each subject we determined the waist circumference (WCF), body mass index (BMI), systolic blood pressure (SBP) and diastolic (DBP). Laboratory analyzes included: fasting plasma glucose, insulin, HbA1c, total cholesterol (TC), triglycerides (TG), HDLc. Insulin resistance was estimated using HOMA-IR index.
Results: The prevalence of depression in persons with type 2 DM was 22.8%. Of subjects with type 2 DM affected by depression only 38.5% were previously diagnosed with depression and were under psychiatric antidepressant treatment. Patients with type 2 DM and depression had poor glycemic control. The goal of HbA1c < 7% was achieved only in 13.7% of type 2 diabetics with depression previously undiagnosed, in 34.3% of those with previously known depression (under antidepressant treatment), and in 45.1% of patients without depressive symptoms. Depressed diabetics had significantly higher values of BMI, WCF, TG, SBP, DBP and insulin resistance than those without depression (Table 1).

Table 1. Characteristics of patients with type 2 DM with and without depression

Parameter		type 2 DM + depression previously undiagnosed	type 2 DM + previously diagnosed depression	type 2 DM without depression	P (ANOVA)
N		51	32	281	-
Men / women		24/27	15/17	142/139	-
Age (years)		54.7 ± 3.2	54.5 ± 3.1	54.1 ± 3.6	0.475
Duration of diabetes (years)		6.2 ± 1.8	6.5 ± 1.4	6.1 ± 1.3	0.292
WCF (cm)	M	106.5 ± 5.1	101.3 ± 5.2	96.6 ± 4.7	<0.001
	W	94.7 ± 4.9	89.3 ± 4.6	85.4 ± 4.1	<0.001
BMI (kg/m^2)		30.4 ± 3.8	28.9 ± 3.5	27.2 ± 3.4	<0.001
TG (mg/dL)		211.4 ± 22.9	182.7 ± 20.5	164.3 ± 21.4	<0.001
TC (mg/dL)		207.2 ± 23.3	210.6 ± 21.5	204.8 ± 22.7	0.341
HDLc (mg/dL)	M	34.8 ± 4.7	37.2 ± 4.2	40.5 ± 5.1	<0.001
	W	41.9 ± 4.8	42.7 ± 4.5	48.5 ± 4.6	<0.001
SBP (mmHg)		141.6 ± 9.8	136.9 ± 10.5	132.6 ± 11.2	<0.001
DBP (mmHg)		92.3 ± 5.6	88.4 ± 6.1	85.5 ± 6.8	<0.001
HOMA-IR		7.4 ± 1.2	6.5 ± 1.1	4.6± 0.9	<0.001
HbA1c (%)		8.1± 0.7	7.5 ± 0.9	7.3 ± 0.8	<0.001
HbA1c < 7% n(%)		7 (13.7)	11 (34.3)	127 (45.1)	

Conclusions: Depression negatively influences the management and the prognosis of type 2 DM, being associated with poor glycemic control, with an adverse cardiovascular risk profile and, subsequently, with an increased risk of chronic diabetic complications. Recognition and treatment of depression in type 2 DM patients is imperative, in order to achieve better adherence to diabetes management plan, in particular to lifestyle optimization measures, and consecutively to improve their prognosis and quality of life.

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Diabetes and depression among pregnant women in Bangladesh: a hospital based study

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Background and aims: Diabetes (DM) is increasing worldwide along with gestational diabetes mellitus i.e.; GDM (1-4%)[1] and Depression is an alarming disease which ranks 4th among worldwide disease burden. In Bangladesh the prevalence rate of depression in adult population is only 4.6%[2]. Both depression and GDM are common in the antenatal period and result in serious consequences for mother and foetus. However there has been little research specifically examining the association between them in pregnancy, revealing that depression is more common in GDM cases with much complicacy [3,4,5]. To our knowledge data regarding this issue in South Asia are inadequate. Till date we could not find any single study in Bangladesh. This study was the 1st one in this aspect, designed to find out the prevalence of depression and associated factors, among GDM subjects and to compare with Non GDM.
Materials and methods: A total of 745 pregnant women participated in the case control study. They were followed up from their 1st visit (not included after 13th week) to 1st week after delivery for at least 4 checkups. Blood glucose was measured on every visit following WHO criteria; GDM was diagnosed within 24th to 28th weeks. Depressive symptoms was scored following MADRS scale (0-12=not, 13-19=mildly, 20-34 moderately, 35-60=severely - depressed). Semi structured questioner was used to assess their socio-demographic status and clinical history. BP, Height, weight was measured on each visit. Birth weight and APGAR score were assessed for the neonate.
Results: 382 subjects found to have GDM while 363 were without glucose abnormality. Prevalence of depression among pregnant women was 12.69%. The rate was higher in GDM subjects (21.73%) with mean age 28.34 years than NGDM subjects (7.73%) with mean age 27.17 years. Depression was more common in Middle aged group (26-35years, 22.53%). Surprisingly depression was found more in higher educated group. Occupation and habit of physical exercise do not seem to have any influence over depression but ove DM. Financial status may have an effect on depression, as the total income had a significant association with depressive symptoms (OR.18.5, 95%CI, 2.4, 9.6, P<0.001). Interestingly, though most of the participants were housewives, depression was more common in working women but with low income. Multipara women were more prone to both the diseases. FBS had some significant association with depression. Depression did not seem to have any association with neonatal status like APGAR and Birth weight but more analytical study is recommended to confirm it. Table 1: Prevalence of Depressive symptoms by age

Age	Non Depressive (MADRS<13)		Depressive (MADRS>13)		Prevalence of Depression (% and 95% CI)		
	NGDM	GDM	NGDM	GDM	NGDM	GDM	Total
≤15		3		1	na	25.00 (0.47, 0.37)	25.00 (0.48, 0.37)
16-25	131	81	10	21	7.09 (0.04,0.03)	20.59 (0.09, 0.07)	12.76 (0.04, 0.04)
26-35	196	196	16	57	7.55 (0.03, 0.02)	22.53 (0.06, 0.04)	15.70 (0.03, 0.03)
36-45	8	19	2	4	20.00 (0.13, 0.13)	17.39 (0.17, 0.14)	18.18 (0.14, 0.11)
Total	335	299	28	83	7.73 (0.03, 0.02)	21.73 (0.04, 0.03)	12.69 (0.02, 0.02)

Conclusion: The study confirmed again that depression boosts up in diabetes of all type and status. But the important finding of this study was the elevated prevalence rate of depression in pregnancy which was greater than assumed. Developing countries do not focus much on mental health but it is becoming ultimate necessity for future.
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Diabetologist perceived barriers to addressing psychological matters in diabetes consultations

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Background and aims: In approximately two out of three cases health professionals working with diabetes fail to identify psychological problems and disorders in their patients. This has potential serious clinical implications: 1) diabetes is associated with an increased risk of psychological disorders, such as depression and anxiety; 2) the co-existence of psychological disorders and problems and diabetes are associated with poor diabetes outcomes, for example treatment adherence, higher HbA1c, and higher mortality; 3) psychological problems, such as diabetes distress and low quality of life, have clinical relevance in their own right. Diabetologists report poor psychological health to be widespread among patients with diabetes. This indicates a considerable gap between clinician awareness and clinical practice. It seems important to explore why hospital-based diabetologists fail to identify their patients' psychological problems. This study aims at identifying barriers as perceived by diabetologists to addressing psychological matters in diabetes consultations.
Materials and methods: We conducted a qualitative study based on individual semi-structured interviews with 12 diabetologists from four diabetes specialist clinics. The interviews lasted 50-60 minutes and were audio taped. The interview guide consisted of themes such as the individual physician's

definition of psychological problems and perceived barriers to addressing psychological problems in their patients. All interviews were transcribed verbatim. Analysis was performed according to the method of systematic text condensation (STC) formulated by Kirsti Malterud. The procedure consisted of four steps: 1) identification of preliminary themes 2) identification of meaning units related to previously identified themes 3) condensation and 4) synthesizing.
Results: Some diabetologists defined psychological problems as exclusively psychological pathology, for example depression or eating disorders whereas others perceived psychological matters more broadly, including problematic challenges of everyday life with diabetes. The barriers reported fell in three categories: system-related barriers, patient-related barriers and physician-related barriers. System-related barriers included perceived limited time and demands of time consuming documentation in the consultations. Patient-related barriers included the seriousness of patients' psychological problems and patients' apparent indifference to their diabetes. Physician-related barriers included perceived lack of skills in the field of psychology, lack of skills in opening and closing dialogue about psychological problems and the feeling of being powerless against the psychological burden of patients.
Conclusion: We identified several barriers, potentially amendable, for addressing patients' psychological problems in diabetes consultations. Diabetologists emphasized lack of time as a major barrier. However, the individual diabetologist receive very little guidance, and the organizational structure of consultations appears to exclude psychological health and problems from the systematic agenda for diabetes consultations. There seems to be a huge challenge in the trade-off between time for including diabetes health, psychological health and written documentation in diabetes consultations.

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Depression in diabetes: the role of diabetes-related distress on incidence and recovery
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Background and aims: Prevalence of depression in diabetic patients is nearly twice as high as in the non-diabetic population. As recent meta-analytic findings suggest, diabetes seems to be a risk factor for the incidence of depression or at least for elevated depressive symptoms. Mechanisms linking the diagnosis of diabetes to the development of depression are unknown. This study prospectively investigates the role of diabetes-related distress on incident depression and recovery in diabetic patients.
Materials and methods: Participants completed the CES-D and the Problem Areas in Diabetes Scale (PAID) at baseline and at a 6-month follow-up. Elevated depressive symptoms were indicated by a CES-D score of ≥16. A PAID score of ≥30 indicated moderate diabetes-related distress. Logistic regression analyses were performed with recovery from and incidence of depressive symptoms as dependent variables. Independent variable was diabetes-related distress adjusted for possible demographic (age, gender) and medical confounders (diabetes type, diabetes duration, HbA1c, BMI, late complications and intensification of treatment).
Results: Data from 370 patients with insulin therapy were analysed (age 53.9 ±14.2 yrs.; 47% female; 46% type 2 diabetes; diabetes duration 16.6 ±11.1 yrs.; HbA1c 8.5 ±1.4%; BMI 29.9 ±6.3 kg/m²; 57% with late complications; 4.2 ±1.7 injections/day). At baseline, 145 patients (39.2%) reported elevated depressive symptoms and 37 of these patients (25.5%) recovered 6 months later. Out of the 225 patients without elevated depressive symptoms, 35 (15.6%) had elevated depressive symptoms 6 months later. If diabetes-related distress was not present at baseline the chance to recover from elevated depressive symptoms was 2.8 times higher than if diabetes-related distress was present (OR=2.8; p=.017; 95% CI 1.2-6.6). Furthermore, if diabetes-related distress was present at baseline but could be reduced (PAID-score<30) at follow-up, the chance to recover from elevated depressive symptoms was substantially increased (OR=8.8; p=.003; 95% CI 2.1-37.0). In addition, the chance for incident depressive symptoms was reduced by 73% if diabetes-related distress was not present at baseline in contrast to present diabetes-related distress (OR=0.27; p=.003; 95% CI 0.11-0.64). For all analyses, none of the confounders reached a significant Odds Ratio.
Conclusion: This prospective study demonstrates that elevated diabetes-related distress is a risk factor for the incidence of elevated depressive symptoms and influences the reduction of depressive symptoms. These effects are independent and not mediated by demographic or medical confounders. One limitation of this study is the fact that depressive symptoms were assessed via a self-report questionnaire and not via interviews according to ICD-10. Nevertheless, the diagnosis of diabetes does not seem to be a risk factor for depression per se but the perception of diabetes-related distress seems to be much more important. Future research should address intervention strategies to reduce diabetes-related distress and the effect on depression to corroborate the findings presented. The findings suggest that it seems to be a promising approach to reduce depressive symptoms by reducing diabetes-related distress at first.

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Impact of comorbid depression on glycaemic control and family functioning in treatment-resistant Japanese patients with type 2 diabetes: a 6-month follow-up study
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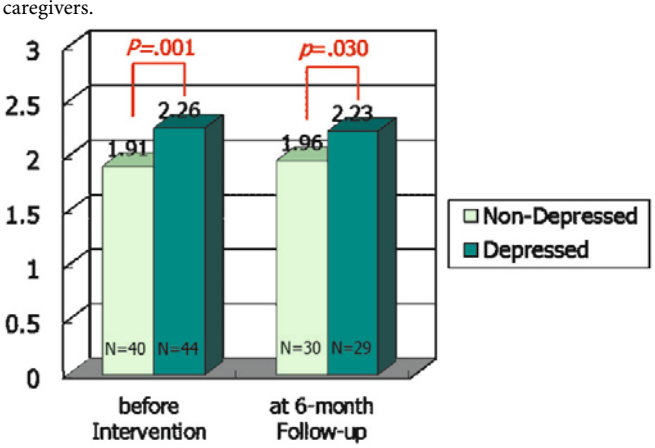
Background and aims: Depression in patients with diabetes is associated with poorer adherence and worse health outcomes. However, evidence for the efficacy of treating depression in improving glycemic control was mixed. The aim of the study was to investigate the impact of depression on glycemic control and quality of life including family functioning among treatment-resistant Japanese patients with type 2 diabetes and their caregivers.
Materials and methods: Ambulatory patients with type 2 diabetes were drawn consecutively from the treatment-resistant inpatient population participated in a two-week educational intervention program at two general hospitals affiliated with our university. Those who could have cognitive impairment were cautiously excluded. Among 123 out of 159 patients who gave written informed consent, we enrolled 84 patients living with his/her caregiver in the study to focus on family functioning. Before and after the intervention program, and also 6 months later, the subjects and their caregivers completed the Zung Self-rating Depression Scale(SDS), the Zung Self-rating Anxiety Scale, and the Problem Areas In Diabetes scale. Family functioning was assessed by the Family Assessment Device before the program and 6 months later. This study was approved by the Institutional Review Board and the Ethics Committee of those two hospitals and our university.
Results: "Depressed Patients" (N=44; SDS score at baseline >= 40) perceived significantly worse diabetes-related quality of life including family functioning than "Non-Depressed Patients" (N=40; SDS score at baseline < 40) before the intervention. At the 6-month follow-up after the intervention, Depressed Patients apparently improved in their mood, quality of life including family functioning, but still showed significantly worse general health, inappropriate familial communication and higher HbA1c values than Non-Depressed Patients.
Conclusion: The findings of the short-term longitudinal study suggested that, as for treatment-resistant patients with diabetes, depression at baseline might predict worse quality of life, less effective family functioning, and worse glycemic control afterward. Consequently, diabetes care professionals should devote attention to taking care of mood status of patients, and intervene to promote more effective communication among patients and their caregivers.



Gap between „Communication" scores by the Family Assessment Device (the higher score, the worse functioning): No improvement even at 6-month follow-up.
Supported by: Grant-in-Aid for Scientific Research, Japanese Ministry of Education

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Low prevalence of depression and burden by diabetes mellitus at secondary care level in Germany
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Background and aims: The importance of psychosocial factors for the treatment of diabetes mellitus is accentuated in the recommendation of all guidelines. A recent survey showed that every third patient with diabetes mellitus (DM) suffers from depressive disorders. Furthermore, it is suspected to be a trigger for diabetes complications. To detect diabetes-related burden and depression the PAID questionnaire ("Problem Area In Diabetes") provides a valid and reliable instrument.
Materials and methods: From 01.11.2012 to 31.01.2013 we assessed diabetes related burden and depression in 785 patients with DM (24.6% type 1, mean HbA1c 7.9%, age 54.3 years, diabetes duration 22.6 years, blood pressure 154/84 mmHg, BMI 27,0 kg/m2, 80/89 without retinopathy and 97/170 without neuropathy; 75.4% type 2, HbA1c 7.6%, age 66.6 years, diabetes duration 15.6 years, blood pressure 146/85 mmHg, BMI 32.8 kg/m2, 252/289 without retinopathy and 252/535 without neuropathy) with the PAID questionnaire in an university outpatient department for endocrinology. A PAID score <40 was considered as no increased risk of depressive disorders. As confounders of the "diabetes mellitus" effect on mental health we checked age, gender, type and duration of diabetes, method of treatment (with or without insulin), HbA1c and marital status. The department takes part in the disease management programme DM, therefore half of the patients are in permanent care. Patients attend the clinic usually every three months, or more often according to clinical needs. Participation in at least one of the following five structured patient education programmes, given by trained diabetes nurses and a diabetologist, is part of the standard treatment of all patients. If necessary patients got refresher courses. This is mainly the case in patients on insulin therapy.
Results: The main PAID score was 17.1 (DM1 17.8 vs. DM2 16.8, p=0.42). 91.1% of all responders had no diabetes-related signs of depression (score <40). There was a negative correlation with respect to the age of the patients (r=-0.205, p<0.001). The older the patients, the lower was the burden of diabetes. Metabolic control was associated to the PAID score, as we found a positive correlation with HbA1c (r=0.168, p<0.001). In contrast to age we did not found a correlation with duration of diabetes (r=-0.017; p=0.63). Furthermore, there were no significant differences regarding PAID score between patients with type-1 and type-2 (17.8±15.7 vs. 16.8±14.9; p=0.42), as well between patients with or without insulin (17.3±15.4 vs. 16.5±15.4; p=0.55). But there were significant differences between women and men (19.0±16.6 vs. 15.6±13.6; p=0.002). The marital status (living alone vs. married/cohabitee) had no effect (16.5±14.8 vs. 17.3.±15.2; p=0.51).
Conclusion: In contrast to a recent survey in a German inpatient diabetes centre with 30.8% of patients scored 40 or more in the PAID questionaire, less than 10% of our outpatients with DM showed a suspicion of depressive mood or high burden of diabetes. Beside the patients selection in an inpatients setting, possible reasons for the low prevalence of depression in our sample could be the participation in the disease management programme for outpatients and the high percentage of patients with permanent care in the department, as we could show recently social inequalities of diabetes care disappeared after care in our centre.

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Depression, anxiety, cognitive impairment and their correlations with clinical and demographic variables in people with type 2 diabetes: a 4-year prospective study
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Background and aims: Depression is at least twice as common among people with type 2 diabetes (T2D) as in the general population. The prevalence of depression, anxiety and cognitive impairment and their associations with clinical and socio-demographic variables were investigated in a 4-year follow up study in a population with T2D.
Patients and methods: 498 consecutive T2D patients were investigated, 249 Non Insulin-Treated (NIT) and 249 Insulin Treated (IT), aged 40-80 years. Demographic and clinical variables, depression, anxiety and cognitive impairment were measured at baseline and after 4 years by the Zung Self-Rating-Depression-Anxiety-Scale and Mini-Mental-State-Examination (MMSE).
Results: At baseline NIT patients were younger and had shorter disease duration (p<0.001, both). The IT did more blood glucose monitoring (BGM) and had higher blood glucose and HbA1c (p<0.001, both). The mean scores for depression were lower among the IT (p=0.006) with no differences for anxiety or MMSE. After 4 years, 72 patients were lost to follow-up, of whom 18 had died. The latter were older (p=0,0003), did more frequent BGM (p=0,019), smoked more (p=0.02) and had higher prevalence of microalbuminuria (MA) (p=0.03) than the survivors. The remaining 171 NIT had improved their fasting glucose (p=0.006), total cholesterol (p<0.0001), triglyceride and HbA1c (p=0.0006). Although the prevalence of MA and diabetic retinopathy (DR) had increased (p<0.0001, both), there was a small improvement in depression (p=0.04) and MMSE (p=0.0007). 41 NIT had been switched to IT and increased BMI (p=0.004), blood pressure (p<0.001), DR severity (p=0.03) and MA (p=0.0045), but did not change depression, anxiety or MMSE scores. The remaining original 214 IT patients increased the prevalence and severity of foot ulcers (p=0.03), DR (p<0001), MA (p=0.0047) and numbers treated for hypertension (p<0.0001). They had worse depression (p=0.0005) and anxiety (p<0.0001) but MMSE improved slightly (p=0.0002). Multivariate analysis showed that women with lower education had higher levels of anxiety but no difference in depression.
Conclusion: Depression and anxiety are associated with T2DM but not modified by time or switching to insulin therapy. Anxiety is associated with female gender and low schooling. Depression and anxiety are likely to worsen when complications become more severe.
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Simple self-reported anger and memory loss as risk factors for the development of type 2 diabetes in Japanese individuals
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Background and aims: Although behavioral symptoms or psychological stresses can be considered as novel markers for risk of future type 2 diabetes, whether responses to simple statements on the presence of one or more of such factors would be effective in screening individuals at high risk of type 2 diabetes remains uncertain. We tested the hypotheses that a simple self-report questionnaire on negative behavioral or psychological characteristics including perseverance, anger, memory loss or sleep disorders would be predictive of the development of type 2 diabetes, and that the association would be independent of metabolic factors including fasting plasma glucose (FPG) and HbA1c concentrations in Japanese individuals.
Materials and methods: We investigated 2869 non-diabetic Japanese individuals. At the baseline examination, behavioral or psychological factors were assessed using a questionnaire. Firstly, individuals were asked as to whether they gave a positive answer to items on lack of perseverance, anger (testy, or easily annoyed), memory loss or sleep disorder. Cumulative incidence rate and hazard ratios (HRs) for the development of T2DM over 7-13 y were evaluated according to the presence of anger, lack of perseverance, memory loss or sleep disorders.
Results: Results of Cox regression analysis showed that anger, (age-sex-adjusted HR 1.71 (95% CI: 1.20, 2.42) or memory loss (1.49 (1.13, 1.96)) was predictive of the development of type 2 diabetes. Even after adjustment for metabolic factors including fasting plasma glucose and HbA1c concentrations, the two factors were significantly associated with an increased risk of future type 2 diabetes. We found that individuals with both anger and memory loss had a 2.44 (95% CI: 1.50, 3.97) times increased risk of type 2 diabetes than those without those two symptoms.

Conclusion: Responses to a simple self-report questionnaire as to whether individuals were aware of anger or memory loss could predict new onset of type 2 diabetes independent of traditional predictors. Using this easy-to-implement instrument might be an adjunctive screening method for detection of individuals at high risk of future type 2 diabetes.
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Psychological, behavioural and biological changes following treatments of subsyndromal depression in people with type 2 diabetes
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Background and aims: Research on clinical benefits of treating subsyndromal depression is scarce. This study aimed to determine the effects of three behavioural treatments -psychoeducation, physical exercise and diabetes re-education- in type 2 diabetic patients with mild depressive symptoms.
Materials and methods: A sample of 209 type 2 patients with subsyndromal depression (aged 57±6 yrs, 54% female, with diabetes duration of 11±8 yrs, 32% insulin treated, with HbA1c of 7.3%±1.2 and BMI of 30±5 kg/m2) were randomly assigned to either psychoeducation, physical exercise, or the control arm. The interventions were aimed at enabling patients to alleviate depressive symptoms. Psychoeducation was focused on cognitive-behavioural skills, and physical exercise on physical activation. The interventions comprised 6 weekly group sessions. The control arm consisted of one patient-centred re-educational session. The assessments of depressive symptoms, diabetes distress, diabetes self-care and health-related quality of life (QoL) were made by using validated questionnaires. Metabolic status was assessed by HbA1c and lipid panel (triglycerides, total-, HDL- and LDL-cholesterol). Leukocytes (total, neutrophils, lymphocytes and mononuclears), myeloperoxidase index (MPXI) and CRP, and plasma uric acid were used as endogenous inflammatory and antioxidant markers. The same psychological and laboratory measures were repeated after the completion of the interventions, and after a 6-month follow-up period. Repeated measures ANOVA was used to determine within- and between-group differences at follow-ups.
Results: Psychological outcomes including depressive symptoms, diabetes distress, subjective health, emotional roles and mental health improved after the interventions and remained so after six months, regardless of the group assignment (all time effects' $p<0.001$). Diabetes diet improved after the treatments ($p<0.001$) but deteriorated at six months follow-up ($p=0.01$). Physical exercise, self-monitoring blood glucose and foot care demonstrated stable improvement (all time effects' $p<0.001$). Glycaemic control was slightly improved in all groups (HbA1c : 7.24%±1.13 vs 7.09%±0.97 vs 7.08%±0.89 $p=0.002$). A significant drop in total cholesterol was found after the treatment ($p=0.01$), but this was not maintained after 6 months in any of the groups. CRP was not changed, whereas leukocyte-, neutrophil- and lymphocyte-counts significantly decreased (all $p<0.001$) throughout the follow-up period. MPXI recovered to baseline levels after a significant post-treatment drop ($p<0.001$). Plasma uric acid remained unchanged in males, but increased significantly at follow-up ($p<0.001$) in females in all three groups.
Conclusion: The employed behavioural interventions had comparable positive effects on 6-month-outcomes including depressive symptoms, diabetes distress, health-related QoL, diabetes self-care, glycemic control, and markers of chronic inflammation. Treating subtreshold depressive symptoms in type 2 diabetic patients by even minimal behavioural intervention has favourable clinical implications.
Clinical Trial Registration Number: ISRCTN05673017
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PS 097 Health economics: Living better? At what cost?

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Defining the health economic value of avoiding weight gain and hypoglycaemia in type 2 diabetes mellitus
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Background and aims: Treatment algorithms for the medical management of people with type 2 diabetes mellitus (T2DM) are based on a combination of glucose lowering efficacy and other important clinical effects such as the avoidance of weight gain and hypoglycaemia. Based on glucose lowering potential only, the addition of a sulphonylurea or basal insulin in those uncontrolled on metformin mono-therapy is considered the most effective and cost effective treatment strategy. From the patient perspective, weight gain and hypoglycaemia can negatively impact quality of life, treatment satisfaction and the attainment of glycaemic goals. The objective of this study was to assess the economic value associated the three key components of T2DM: changes in HbA_{1c}, hypoglycaemia and body mass index (BMI).
Materials and methods: This study utilized the IMS CORE diabetes model (CDM) to model four profiles associated with managing type 2 diabetes; Treatment 1: -0.5% HbA_{1c}; Treatment 2: -0.5% HbA_{1c} and BMI -1 Kg/m^2; Treatment 3: -0.5% HbA_{1c}, BMI -1 Kg/m^2 and 2 non-severe hypoglycaemia (NSHE) avoided; Control: no effect. Lifetime analyses were conducted using patient level data (NHANES) to populate the modelling. Health dis-utilities of -0.0052 and -0.0038 were applied to each NSHE and 1 unit increase in BMI respectively. Discounting was applied at 3.5% and GBP 2011 costs were used.
Results: Compared to Control (no effect), Treatments 1, 2 and 3 were associated with discounted gains in lifetime quality adjusted life expectancy (QALE) of 0.05, 0.107 and 0.233 respectively. Each unit decrease in NSHE and BMI were associated with similar gains in QALE associated with a 0.5% HbA_{1c} reduction. The maximum annual therapy specific costs (to remain cost effective at a willingness to pay threshold of GBP 20,000) for treatments 1, 2 and 3 were GBP 109.4, GBP 205.4 and GBP 428.6 respectively.
Conclusion: The health utility gains associated with weight reduction and avoidance of NSHE impact patients in the short-term and are therefore subject to less discounting within health economic models; in contrast to changes in HbA_{1c} that impact the probability of a future cardiovascular or microvascular event. Consequently, treatment strategies that avoid weight gain and hypoglycaemia exhibit favourable cost effectiveness profiles compared to improvements in glycaemic control only.

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Health-related of quality of life using EQ-5D in patients with type 2 diabetes
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Background and aims: Diabetes is a lifelong disease that affects a patient's general health and well-being in various ways. Consequently, diabetes management requires a fundamental change in the patient's lifestyle and the one of the important outcome criteria in this view is quality of life. The aim of the study was to assess the health-related quality of life (HRQoL) and to examine the factors associated with HRQoL.
Materials and methods: An analytical cross-sectional study was conducted among 500 type 2 diabetics (age>25 years and diabetes duration >1 year), who were selected conveniently from the OPD of Bangladesh Institute of Health Sciences Hospital (BIHSH). Data were collected by a pre-tested, interviewer-administered questionnaire. HRQoL was assessed by an adapted and validated Bangla version of the EQ-5D (EuroQol Group, 2009) questionnaire which has five domains- mobility, self-care, usual activities, pain/discomfort

and anxiety/depression and three levels on each dimension. In addition, the Euroqol visual analogue scale (VAS) was used for assessing patients' overall actual health state where 0 represents the worst imaginable health state and 100 indicate perfect health.
Results: Mean age of the patient was 54.2 (±11.2) years; About 50.2% were females. Most of the patients (92.2%) belonged to middle to upper-middle age group; 41% had completed high school and 50.8% were from lower-middle income family. Mean BMI was 26.1(±6.7) kg/m2 and about 78.8% were overweight or obese according to Asian BMI cut-off value. About 49.6% patients had no problem in mobility, 71.8% in self-care, and 52.4% in usual activities though 57.8% had some problem in pain/discomfort and 59.4% in anxiety/depression. The mean ±SD EQ VAS score was 65.4(±18.3). The EQ VAS score of the female patients was significantly lower than the male patients (60.4±17.6 vs 70.5±17.5, p<0.0001). In binary logistic regression, age (OR 1.05; 95% CI 1.02 to 1.07), gender (OR 4.87; 95% CI 1.48 to 15.99), taking of OHA & insulin (OR 1.62; 95% CI 1.02 to 2.59) and lower-middle income group (OR 2.51; 95% CI 1.08 to 5.85) were significantly associated with mobility. Self-care was significantly related with age (OR 1.03; 95% CI 1.01 to 1.06), family history of DM (OR 0.43; 95% CI 0.26 to 0.73) and duration of DM (OR 1.05; 95% CI 1.01 to 1.08). Gender (OR 4.79; 95% CI 1.45 to 15.78), family history of DM (OR 0.46; 95% CI 0.28 to 0.74) and lower-middle income group (OR 2.67; 95% CI 1.13 to 6.29) had significant association with usual activities. Pain/discomfort was significantly associated with age (OR 1.05; 95% CI 1.02 to 1.07), taking of OHA & insulin (OR 1.75; 95% CI 1.04 to 2.93), lower-middle income (OR 2.89; 95% CI 1.25 to 6.64) and upper-middle income group (OR 2.54; 95% CI 1.12 to 5.79). Higher education (OR 0.24; 95% CI 0.08 to 0.68) was significantly related to anxiety/depression.
Conclusion: Around half of the patients have problem in mobility, usual activities,pain/discomfort,anxiety/depression and majority in self-care. Age, female gender, lower-middle & upper-middle income group, higher education, taking of medication, family history of DM and duration of DM are important factors associated with HRQoL in patient with type 2 diabetes.
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Flexible insulin dosing improves health-related quality of life (HRQoL): a time trade-off survey
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Background and aims: The rigidity of insulin regimens may negatively impact on health-related quality of life (HRQoL) and therapy adherence, with the requirement for basal insulin dosing at the same time every day being a significant contributing factor. We examined the HRQoL impact of both flexible timing of the basal insulin injection and the number of basal insulin injections, in basal and basal-bolus regimens using time trade-off (TTO) methods.
Materials and methods: HRQoL was examined via an online TTO survey in the UK, Canada and Sweden with separate analyses of 2,465 respondents from the general population, and 265 and 392 people with type 1 (T1D) and type 2 diabetes (T2D), respectively. HRQoL was measured on a utility scale: 0 (dead), 1 (perfect health). Respondents traded off length of life for improving HRQoL in described health states. Respondents evaluated health states with diabetes and basal injections that were once-daily time-flexible, once-daily time-fixed and twice-daily time-fixed injections as part of a basal or a basal-bolus regimen.
Results: A time-flexible basal injection was associated with 0.016 (95% CI 0.011; 0.022) and 0.013 (95% CI 0.007; 0.019) higher utility vs. a fixed time of injection for basal-only and basal-bolus regimens, respectively, as evaluated by the general population (Table 1). The diabetes respondents confirmed the basal-only results, but the difference in utility was small and insignificant for basal-bolus. Once-daily injections had significantly higher utility compared with twice-daily injections for basal (0.039 [95% CI 0.032; 0.046] and basal-bolus (0.022 [95% CI 0.016; 0.028]) regimens, as evaluated by the general population. These results were confirmed by the respondents with diabetes.
Conclusion: Flexible dosing and fewer injections have a positive HRQoL impact, which may enhance therapy adherence and potentially contribute to improved long-term outcomes. The impact of flexibility is greater in people treated with basal-only insulin regimens, and diminishes if bolus injections are part of the treatment regimen.

Table 1: Utility differences from the time trade-off survey

	Basal-only				Basal-bolus			
	N	Utility	CI95L	CI95H	Utility	CI95L	CI95H	CI95H
Population								
1 Flex vs. 1 Fixed	1,121	0.016	0.011	0.022	0.013	0.007	0.020	0.020
1 Fixed vs. 2 Fixed	1,121	0.039	0.032	0.046	0.022	0.016	0.028	0.028
1 Flex vs. 2 Fixed	1,121	0.055	0.048	0.063	0.036	0.029	0.043	0.043
Diabetes populations*								
1 Flex vs. 1 Fixed	192	0.015	0.004	0.027	0.004	-0.006	0.014	0.014
1 Fixed vs. 2 Fixed	192	0.042	0.025	0.061	0.021	0.013	0.031	0.031
1 Flex vs. 2 Fixed	192	0.057	0.040	0.076	0.025	0.015	0.035	0.035

CI95L, confidence interval 95% (low); CI95H, confidence interval 95% (high).
*Diabetes population with basal-only questions: 192 patients with type 2 diabetes; Diabetes population with basal-bolus questions: 265 patients with type 1 diabetes; 200 patients with type 2 diabetes.
Supported by: Novo Nordisk A/S

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Economic models of diabetes mellitus: Are we making decisions on the best available evidence?
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Background and aims: It is almost a decade since a number of important computer simulation models of diabetes mellitus were published. Several regulatory agencies have recently raised concerns that models based on historic clinical data may have limited relevance as care patterns and therapeutic choices change over time. This concern was echoed in a recent validation study, which reported limitations in the UKPDS Outcomes Model in predicting endpoints such as stroke, amputation, heart failure, and death from any cause. As the global economic crisis further restricts healthcare resources, accurate economic evaluation and prediction (and its influence on clinical decisions in diabetes care) will be increasingly important. To understand how models perform in predicting outcomes in the current diabetes care environment, a systematic literature review was undertaken to identify publications on diabetes models, clinical data relevant to complications, disease progression and mortality risk.
Materials and methods: In October 2012, literature searches including studies of both type 1 and type 2 diabetes were performed based on Medical Subject Heading terms in the PubMed and Cochrane Library databases. Hand searches of health technology assessment databases and landmark trial websites were also performed. Searches were limited to publications in 2004 or later. Abstracts were screened to exclude studies that did not provide longitudinal data, had follow up of less than 1 year, or study populations of fewer than 100 subjects. Full-text review of all remaining publications was performed to understand the capabilities of current diabetes models and identify opportunities for improvement.
Results: The literature searches identified a total of 12,405 hits for screening since 2004. Screening identified a total of 523 publications in type 2 diabetes and 53 papers in type 1 diabetes that met criteria for full-text review. Little has been published to validate the performance of diabetes models in recent years, or to describe how recent clinical observations have been integrated into models. In type 2 diabetes, data from meetings (e.g. Mount Hood) indicate that, although modeled estimates of relative risk between interventions may be acceptable, estimates of absolute risk represent an opportunity for improvement (comparison with CARDS, ASPEN, ADVANCE and ACCORD). The review identified many type 2 diabetes studies that may provide valuable information in terms of risk adjustment since publication of the models themselves. In type 1 diabetes, there are few data validating model performance in recent years. Long-term follow-up data from longitudinal studies (EDIC and Pittsburg EDC) may offer potential to update models of type 1 diabetes.
Conclusion: Literature review has demonstrated that there is a paucity of studies relating to the validation and ongoing development of existing models of diabetes. There may be an opportunity to incorporate data, where appropriate, from recent clinical approaches to management in both type 1 and type 2 diabetes to ensure that economic models reflect current practice and can best inform cost-benefit decisions for care.
Supported by: Eli Lilly and Company

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Acute coronary syndrome patients with type 2 diabetes had higher healthcare costs vs patients without diabetes in the US population from 2006 to 2011

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Background and aims: Acute Coronary Syndrome (ACS) contributes significantly to morbidity and mortality and represents an enormous economic burden to society. T2D has been recognized as one of the major risk factors for ACS; however, there are limited published data quantifying the impact of T2D on healthcare costs, especially the long-term costs following ACS. This study evaluated the total and cardiovascular (CV)-related healthcare costs for ACS with T2D versus patients without diabetes.
Materials and methods: Patients with ≥ 1 ICD-9 code for acute myocardial infarction (MI) (410.xx) or unstable angina (411.1x) during hospitalization were identified from the HealthCore Integrated Research Database (HIRD^{SM}) between 01/01/2006 and 09/30/2011. The first ACS hospitalization date was defined as the index event date. T2D patients were selected if ≥ 2 claims for T2D (250.x0, 250.x2) at least 30 days apart; or ≥ 1 claim for T2D and ≥ 1 claim for oral or injectable anti-diabetics; or ≥ 2 prescriptions of oral anti-diabetics or glucagon-like peptide 1 medications. Those with no evidence of a type 1 or 2 diabetes diagnosis claim (250.xx) in the study period were identified as ACS patients without diabetes. Patients with < 12 months' plan eligibility pre- and post-index ACS or age < 18 years were excluded. Total and CV-related healthcare costs following the index ACS event were evaluated in patients with T2D and without diabetes, for 1, 2 and 3 years, respectively, adjusting for baseline differences including demographic characteristics, co-morbidities, treatment utilization and index ACS characteristics.
Results: Of the 140,903 ACS patients identified, 38,553 (27%) and 81,845 (58%) patients have been selected into the ACS with T2D and without diabetes cohorts, respectively. Patients with T2D vs. without diabetes were older (mean age 68.2 vs. 65.6 years, respectively) and had higher mean baseline comorbidities (Deyo-Charlson Index score 3.15 vs. 1.11, respectively). Mean (median) length of stay of the index ACS hospitalization was longer in patients with T2D vs. without diabetes (7.62 [4.00] versus 5.20 [3.00] days, respectively). Adjusted mean (median) 1-, 2-, and 3-year post-index total and CV-related costs for T2D cohort were significantly higher than those without diabetes (Table). After adjusting for baseline differences, compared to non-diabetes, ACS patients with T2D had significant 14.4%, 16.2% and 14.8% increased total annual costs at 1, 2 and 3 years, respectively.
Conclusion: T2D patients have increased total and CV-related healthcare costs at 1, 2 and 3 years post-ACS event as compared with non-diabetes patients. These results provide further confirmation that new therapies directed specifically at T2D patients with ACS are needed to not only improve the quality of care, but also to help reduce the cost of care.

Table: Comparison of total and CV-related adjusted annual healthcare costs at 1, 2 and 3 years following an ACS event in patients with type 2 diabetes versus without diabetes

		With T2D			Without diabetes			P-value
		Mean	SD	Median	Mean	SD	Median	
Total costs, $	1-year	37,728	62,159.0	19,617	29,976	54,815.2	16,470	< 0.0001
	2-year	24,728	37,866.9	14,137	18,379	32,038.6	10,850	< 0.0001
	3-year	19,887	29,392.8	12,132	14,174	22,455.5	8,776	< 0.0001
CV-related costs, $	1-year	28,659	58,322.4	11,032	23,963	50,362.8	10,220	< 0.0001
	2-year	16,653	30,600.8	6,902	13,205	27,632.3	5,679	< 0.0001
	3-year	12,292	21,964.1	5,475	9,452	17,367.2	4,336	< 0.0001

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Cost predictors in type 2 diabetes mellitus: a retrospective claims database analysis

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Background and aims: This study aimed to identify cost predictors in type 2 diabetes mellitus (T2DM) patients using oral antidiabetic therapies (OADs).
Materials and methods: Adult patients with T2DM using OADs were identified in the Truven Health Analytics MarketScan® databases (2004-2010). Patients with 4 or more HbA_{1c} tests within 1 year were classified into cohorts based on quartiles (Q) of the distribution of total 12-month diabetes-related costs following a randomly selected OAD prescription date (i.e., index date). Diabetes-related costs were defined as the sum of costs for medical services with a diagnosis for diabetes and antidiabetic (AD) medications. Patient demographics, comorbidity profile, AD use, HbA_{1c} level, and resource use were measured over the 12-month pre-index period and compared between cohorts with the highest costs (i.e., Q4) and lower costs (i.e., Q1-Q3). Multivariate logistic regression was used to identify predictors of the highest diabetes-related costs.
Results: Among the 3,921 selected patients with HbA_{1c} tests (i.e., 5% of the eligible T2DM patients using OADs), the average 12-month diabetes-related cost was $12,623 for the highest cost cohort (N=981) and $1,871 for the lower cost cohort (N=2,940). Compared to the lower cost cohort, during the pre-index period, fewer patients in the highest cost cohort were female and achieved the glycemic goal (HbA_{1c} <7%), more patients used insulin, and patients had a higher comorbidity burden, in particular microvascular diabetic complications, hypertension, and lipid disorders (all $p<0.01$). The main predictors (by greatest ORs) of the highest diabetes-related costs include insulin use (OR=3.08), coronary artery disease (OR=2.27), endocrinologist visit (OR=1.69), number of AD classes used (OR=1.56), diabetic retinopathy (OR=1.53), neuropathy (OR=1.44), and lipid disorder (OR=1.43) (all $p<0.05$).
Conclusion: In a sample of T2DM patients receiving OADs, the main predictors for high diabetes-related costs included factors suggesting greater T2DM severity and diabetic complications.
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Trends in the management of type 2 diabetes and its prescription drug costs in Greece (2006 and 2012)

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Background and aims: We had previously reported that cardiovascular risk (CVR) management of patients with type 2 diabetes (T2D), treated by diabetes specialists improved significantly in 2006 as compared to 1998. This finding was associated with a considerable increase in the prescription cost. Shortly thereafter, incretin-based medications launched and acquired a substantial market share. At the same time a major economic crisis emerged in Greece, leading to health system reform and consecutive reductions in drug prices. Aim of the present study, was to compare CVR factor control and prescription drug cost in patients with T2DM between the years 2006 and 2012.
Materials and methods: Medical files of all patients with T2D treated in three diabetes centers, located in Athens and Piraeus, during 2006 and 2012, were examined. Only patients with at least six months of follow-up prior to the recorded visit, were included. The prescription cost was calculated in € per patient/year (PY) according to the official Greek market prices. All comparisons were performed after adjustment for age and duration of T2D.
Results: 938 medical files from 2006 and 1015 from 2012 were included. Patients in the 2012 group were older (67.4±9.6 vs. 64.5±10.5 years, p<0.001), and had longer duration of T2D (13.9±8.1 vs 10.4±8.3, p<0.001). In 2012 a significantly higher proportion of T2D patients used glucose-lowering, lipid-lowering and antihypertensive medications, while no significant difference was observed for antiplatelet agents. The use of sulfonylureas, glitazones, glinides and acarbose decreased significantly. There was no change in the use of insulin, while a significant increase in the use of metformin occurred. In 2012, 34.7% of patients were treated with DPP-IV inhibitors and 3.8% with GLP-1 agonists. There was no significant difference in HbA1c, systolic blood pressure and HDL-cholesterol between 2012 and 2006 (7.0% [7.0-7.1] vs 7.0% [6.9-7.1], 134.1 mmHg [133.0-135.4] vs. 135.2mmHg [134.0-136.9] and 47.7mg/dl [46.8-48.7] vs. 48.4mg/dl [47.4-49.5] respectively). Significant reductions were observed in LDL-cholesterol (97.0mg/dl [94.9-99.0] vs. 112.6mg/dl [110.4-114.9]; p<0.001), triglycerides (138.8mg/dl [134.1-143.5] vs. 150.6mg/dl [145.4-155.7]; p=0.001) and diastolic blood pressure (DBP) (75.8mmHg [75.5-76.8] vs. 77.6mmHg [76.6-77.9]; p=0.03). The cost of prescriptions against hypertension declined slightly in 2012, while no significant difference was observed regarding the cost of lipid-lowering and antiplate-

let agents. A highly significant cost increase was found for glucose-lowering (615.6€PY [593.2-638.1] vs 391.2€PY [364.8-417.6]; p<0.001) and for total prescriptions (1309.4€PY [1272.61353.0] vs 1122 [1080.0-1164.0]; p<0.001).
Conclusion: In 2012, the lipid profile of T2D patients improved significantly, glycemic control remained stable and DBP slightly decreased. Despite a government-derived, overall decrease in medication prices, the glucose-lowering and total prescription cost increased, mainly due to intensification of CVR management and the introduction of new glucose-lowering medications.

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Cost of diabetes care and its determinants: a hospital based study in Bangladesh

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Background and aims: Diabetes is creating a major socio-economic burden on individual, societal and state levels, and cost-effectiveness of interventions against this disorder is a high priority particularly in developing countries. Primary evidence on these issues are scarce even in developed world and these are almost absent in the developing countries. The present study was undertaken to assess the cost analysis of diabetes and its determinants in a Bangladeshi type 2 dietetic population.
Materials and methods: A cross-sectional study was conducted among 496 registered participants (aged ≥30 years) of Out-Patient Department of the central hospital of Diabetic Association with more than 1 year duration of diabetes. All treatment related records of the last 1 year were collected from patients' guide books. The degree and extent of complications like cardiopathy, retinopathy, nephropathy and vasculopathy were recorded and direct, indirect & incremental cost of the management of diabetes and complications were calculated from consumers' point of view. Direct cost included outpatient visits, drugs, laboratory testing and other medical services. Indirect cost included opportunity cost of participants and his/her attendance calculated by human capital approach.
Results: Among 496 patients 46% were males and 54% were females with mean±SD duration of diabetes 8.8±6.8 years. The average annual cost of care was US$ 313 (direct US$ 283 & indirect US$ 31) per patient. Drugs accounted for the largest share of direct cost US$ 193 (67%), followed by laboratory investigations US$ 27 (13%) and consultation fees US$ 23 (12%). Correlation analysis showed that average annual cost increases with increasing age (p=.018), duration of diabetes (p=.001) and increasing blood sugar level (p=.001). Participants with both cardiopathy-nephropathy had 1.8 times, with both cardiopathy-retinopathy had 2.3 times and with both nephropathy-retinopathy had 3.4 times higher cost of care compared to participants without complications. The annual medical costs of participants increased with increase in the number of complications/co-morbidities. Without complications/co-morbidities participants spent US$ 268, but the cost increased to US$ 303 with one/two and US$ 417 with more than two complications/co-morbidities (F=13.7, p=.001). Multiple regression analysis showed that blood sugar level (p=.001), number of complications/co-morbidities (p=0.01) and duration of diabetes were significant (p=0.01) explanatory variables of annual cost of care (R^2=0.21; F=32.5, P<0.001).
Conclusions: The average annual cost of diabetes care per patient in Bangladesh is US$ 313. With an estimated 9.5 million diabetic patients in the country the total annual burden will be US $ 2973.5 million which is huge for a developing country like Bangladesh. Better metabolic control should be in focus which can help in controlling complications and thus lead to substantial saving in the cost diabetes care.
Supported by: Bangladesh Diabetic Somiti

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The cost of type 2 diabetes: a population-based study in Catalonia

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Background and aims: To estimate the direct cost of care associated to type 2 diabetes (T2DM) in patients with the disease compared with non-diabetic subjects in a population-based primary care database during 2011.
Materials and methods: Retrospective analysis of resource consumption (ambulatory care and in-patient care, laboratory tests, pharmacy, strips and disability days) during 2011, from the SIDIAPQ database, including 1.878.816 persons assigned to the Institut Català de la Salut in Catalunya. For each patient with T2DM (age 30-90 years), one control matched for age, sex and managing physician was randomly selected. Data on the diagnostic and procedure codes for outpatient and in-patient care were obtained. All costs were based on the official prices provided by the local Health Care Authority.
Results: We compared the costs of 126,811 T2DM patients (53.5% male, mean age 67.7 years, mean disease duration 7.2 years and mean HbA1c: 7.14%) with 126,811 patients without diabetes. The annual average of visits was 16.3 for diabetic and 10.1 for non-diabetic patients. Mean days of hospitalization were 8.2 and 6.7, respectively and those of temporary incapacity for work of 5.8 and 4.3, respectively. The annual average cost per patient was € 3,362.8 and € 2,156.5 for diabetic and non-diabetic subjects, respectively (difference € 1,206.3, i.e. 55.9% increased cost). The costs of in-patient care were € 1,226.6 and € 886.4 (38.4% increase in cost), pharmacy costs € 925.0 and € 489.2 (89.1% increase), and other costs of € 634.3 and € 412.4 (54.5% increase), in diabetic and non-diabetic subjects, respectively. Patients with poor control (HbA1c> 7%) had an average cost of € 3,631.7 vs € 3,119.5 for patients with good control. In the absence of macrovascular complications, average cost was € 2,925.2 for diabetic and € 1,950.9 for non-diabetic subjects, while the presence of any complication increased the cost to € 4,717.2 and € 3,478.7, respectively.
Conclusion: The direct costs of care were 56% higher in diabetic compared with non-diabetic subjects. Higher costs were associated with poor glycaemic control and diabetic complications.
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Distribution of costs in patients with type 2 diabetes mellitus: a retrospective claims database analysis

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Background and aims: Patients with diabetes are known to have healthcare costs that are two to three times higher than the costs of the population without diabetes. This study aimed to describe the distribution of different cost components among adult patients with type 2 diabetes mellitus (T2DM), the most prevalent type of diabetes, receiving oral antidiabetic medications (OADs).
Materials and methods: Adult patients with T2DM using OADs and having records for HbA_{1c} tests were identified in the Truven Health Analytics MarketScan® databases (2004-2010), which cover a representative sample of employer-provided health insurance in the United States. Direct healthcare costs (2011 USD) were measured from a payer perspective over a 1-year period. Distribution of all-cause and diabetes-related costs were reported by cost components (Table, columns 1 and 2). Diabetes-related costs were defined as claims with a reported diagnosis for T2DM or an antidiabetic medication.
Results: Cost distribution of a total of 4,104 selected T2DM patients using OADs is detailed in the Table. Diabetes-related costs represented 34% of total costs (1-year means $13,548 versus $4,583, respectively). Medical costs accounted for the majority of all-cause and diabetes-related total costs (64%

and 60%, respectively), with outpatient services being the most costly component for all-cause medical costs (34%) and inpatient services being the most costly component of diabetes-related medical costs (30%). Less than 10% of the patients accounted for 50% of diabetes-related medical costs and antidiabetic medications accounted for 40% of diabetes-related total costs. Less than half of patients accounted for >80% of the total all-cause and diabetes-related costs (42% and 38%, respectively) and less than a quarter of patients accounted for >80% of the medical all-cause and diabetes-related costs (23% and 15%, respectively).

Conclusion: In a sample of T2DM patients receiving OADs, a small proportion of patients was found to incur most of healthcare costs, especially for the diabetes-related cost component. Identifying these costly patients is warranted to improve the health, enhance the understanding of cost drivers and reduce the economic burden of T2DM patients.

Cost type	Cost Component	Annual Cost Distribution (2011 USD)				
		Across Patients			Across Components	
		Mean±SD	Median (IQR)	P90	% of the Total	% of the Component
All-cause†	**Pharmacy**	**4,913 ± 5,042**	**3,822 (2,024 - 6,372)**	**9,617**	**36%**	**100%**
	Antidiabetic medication	1,855 ± 1,922	1,469 (223 - 2,751)	4,429	14%	38%
	Other pharmacy	3,058 ± 4,387	2,082 (937 - 3,767)	6,284	23%	62%
	Medical	**8,635 ± 21,670**	**2,431 (1,016 - 6,933)**	**19,511**	**64%**	**100%**
	Inpatient	3,132 ± 14,990	0 (0 - 0)	4,784	23%	36%
	Emergency room	195 ± 927	0 (0 - 0)	483	1%	2%
	Outpatient	4,610 ± 11,133	1,953 (890 - 4,599)	9,933	34%	53%
	Other medical service	698 ± 5,073	24 (0 - 338)	1,548	5%	8%
	Total	**13,548 ± 23,260**	**7,389 (4,188 - 13,714)**	**27,484**	**100%**	-
Diabetes-related‡	**Antidiabetic medication**	**1,855 ± 1,922**	**1,469 (223 - 2,751)**	**4,429**	**40%**	**100%**
	Medical	**2,728 ± 10,187**	**620 (384 - 1,149)**	**4,249**	**60%**	**100%**
	Inpatient	1,370 ± 8,873	0 (0 - 0)	0*	30%	50%
	Emergency room	76 ± 430	0 (0 - 0)	0*	2%	3%
	Outpatient	1,151 ± 3,404	530 (310 - 874)	1,833	25%	42%
	Other medical service	131 ± 658	0 (0 - 68)	201	3%	5%
	Total	**4,583 ± 10,449**	**2,546 (961 - 4,361)**	**8,048**	**100%**	-

*SD, standard deviation; IQR, interquartile range from Q1 to Q3; P90, 90th percentile showing the cost cut-off for the 10% most expensive patients; †All-cause costs were defined as the sum of all costs for medical services and pharmacy claims; ‡Diabetes-related costs were defined as the sum of costs for medical services associated with a diagnosis for diabetes or any claim for an antidiabetic medication. *P90 = 0$ implies that <10% of the patients incurred all diabetes-related inpatient and emergency room costs*

Supported by: Takeda Pharmaceuticals International

PS 098 Inflammation and immunity: links to clinical practice

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The effect of intensive insulin treatment on plasma level of LP-PLA2 and sPLA2 in patients with newly diagnosed type 2 diabetes

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Background and aims: Recent studies have shown that lipoprotein-associated phospholipase A2 (LP-PLA2) and secretory phospholipase A2 (sPLA2) are closely related to chronic inflammation and atherosclerosis. In this study, we aimed to evaluate plasma level of LP-PLA2 and sPLA2 in patients with newly diagnosed type 2 diabetes mellitus (T2DM), and investigate the effect of short-term intensive insulin treatment on it.

Materials and methods: We enrolled 90 patients with newly diagnosed T2DM and 58 people with normal blood glucose, 30~60 years old, from Oct.2010 to Mar. 2012. Plasma LP-PLA2 and sPLA2 were determined in all subjects with an empty stomach. All the patients received continuous subcutaneous insulin infusion with an insulin pump for 10~14 days. IVGTT and OGTT were carried out before and after treatment.

Results: As to gender, age, body mass index, waist-hip ratio, blood pressure and lipid profile, there were no statistically differences between these two groups ($P > 0.05$). Plasma levels of LP-PLA2 and sPLA2 were significantly higher in diabetic patients ($P < 0.05$), especially in those with macroangiopathy ($P < 0.05$). After treatment, level of sPLA2 was significantly reduced ($P < 0.05$), while there was no statistically difference of LP-PLA2 ($P > 0.05$). Regarding to β cell function, AUC_{Ins} of IVGTT and OGTT, acute insulin response (AIR), $\Delta Ins_{30}/\Delta G_{30}$, modified beta cell function index (MBCI) and HOMA-β were all increased after treatment even when corrected by the influence of insulin resistance ($P < 0.001$). With respect to insulin resistance, HOMA-IR was lower after treatment, while insulin sensitivity index of Cederholm (SIM), IAI (1/(FBG*FIns)) and quantitative insulin-sensitivity check index (QUICKI) were higher ($P < 0.05$). Correlation analysis showed that LP-PLA2 and sPLA2 were positively correlated with HOMA-IR in all the subjects ($P < 0.05$). In a multiple linear regression analysis, LP-PLA2 and sPLA2 were independent correlative factors of HOMA-IR ($P< 0.05$).

Conclusion: We conclude that plasma level of LP-PLA2 and sPLA2 is closely related to T2DM, insulin resistance and macroangiopathy of diabetic patients. And we inferred that early intensive insulin therapy in patients with newly diagnosed T2DM might be helpful for the improvement of insulin resistance as well as the protection of diabetic macroangiopathy.

Clinical Trial Registration Number: ChiCTR-TRC-10001618

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Intensification of antihyperglycaemic treatment with DPP-4 inhibitor: influence on body composition and adipokine level

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Background and aims: Obesity, insulin resistance and chronic inflammation are very serious problems in treatment of type 2 diabetes (DM 2). The aim of this study was to make a comparative analysis of the weight and body composition and adiponectin level in patients with DM 2 in a 6 months after the addition of inhibitor DPP-4 vildagliptin or sulfonylurea to basal metformin therapy.

Materials and methods: 50 patients with poor controlled DM 2 on basal metformin therapy (2000 mg/day) alone (mean age 55.1 y, mean duration of diabetes 2.5 years, mean HbA1c level 8.0 %) were divided into 2 groups. Group 1 (26 people): vildagliptin (100 mg/day) was added to metformin in stable dose for 6 months. Group 2 (24 people): sulfonylurea was added to metformin for 6 months with titration due to achieve fasting glycemia < 7.0 mmol/l. Group1 and 2 were comparable in basic glycemic control level, weight and adiponectin level (mean=5.04mcg/ml). HbA1c level, body weight, BMI, waist circumference, adiponectin level, body composition (2-energy X-ray absorptiometry DEXA Lunar Prodigy GE) were evaluated at baseline and after 6 months of combination therapy. Exclusion criteria: liver failure, GFR <60 ml/min/1,73m^2, BMI ≥ 40 kg/m$^{2/}$

Results: After 6 months of therapy improvement of glycemic control was comparable in both groups: HbA1c 6.7% [6.2, 7.0] vs 6.5% [6.1, 7.0] (p>0.05), but hypoglycemiae were significantly often in group 2. In group 1: adiponectin level was significantly improved (5.0[3.0; 8.8] vs 10.6 [7.1; 14.9] mcg/ml, p < 0.0001); weight decreased from 81.8 [81.3, 96.0] to 77.0 [73.4, 93.6] kg (p <0.0001); BMI decreased from 32.2 [29.2, 34.0] to 30.4 [28.1, 31.9] kg/m^2 (p <0.0001); waist circumference decreased from 105.0 [98.6, 118.0] to 98.9 [94.7, 108.6]cm, (p < 0.0001) and fat mass from 34128.0 [31850.5, 37805.1] to 29863.0 [29408.2, 34184.1] g, (p = 0.001), the percentage of tissues fat from 42.9 [33.8, 45.7] to 39.2 [33.9, 41.1]%, (p = 0.001) and the percentage of regional fat from 41.7 [32.6, 43.9] to 38.5 [32.7, 39.8]% (p = 0.001); lean body mass was not significantly changed (44910.2 [42738.0; 55189.5] vs 44798.2 [43056.2, 54933.5] g, p = 0.3). In group 2 adiponectin level was not significantly changed (5.1 [3.1; 7.5] vs 5.9 [3.2; 8.0]mcg /ml, p = 0.39); weight increased from 78.5 [73.0, 105.0] to 81.4 [74.1, 107.0] kg (p <0.0001); BMI increased from 31.4 [28.6, 36.7] to 32.2 [29.2, 34.0] kg/m^2, (p <0.0001) and waist circumference increased from 101.9 [105.0, 119.3] to 105.5 [100.1, 123.6] cm, (p <0.0001), and fat mass increased from 30648.0 [30081.5, 6572.0] to 33320.4 [31250.3, 35100.1] g, (p = 0.047). Changes in the percentage of tissues fat, lean body mass and the percentage of regional fat were NS.
Conclusion: Both combination therapies (metformin + vildagliptin and metformin + sulfonilurea) are effective in glycemic control. Well-known negative effect of addition of sulfonilurea as part of combination therapy is risk of hypoglycemia and increase of weight and body fat. However, combination of metformin and vildagliptin has additional positive effects, such as the reduction of total body weight, decrease of body fat (without reduction of lean body mass) and waist circumference (as surrogate marker of visceral obesity), and increase of adiponectin level (antiinflammation adipokine). These observations should be taken into account during intensification of diabetes therapy.

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Depression in newly diagnosed type 2 diabetes is associated with raised levels of systemic inflammatory markers

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Background and aims: There is an increased prevalence of depression in type 2 diabetes mellitus (T2DM) which is associated with more frequent diabetes complications and increased mortality. T2DM and macrovascular disease are independently associated with raised levels of circulating inflammatory markers and in non-diabetes participants there is evidence that depression is linked with inflammation. Since inflammation may be the common antecedent of both T2DM and depression and may play a pathogenic role, we tested the hypothesis that patients with new-onset T2DM and depression have higher levels of circulating inflammatory markers than T2DM patients without depression.
Materials and methods: Adults with newly diagnosed T2DM recruited from primary care were assessed for depression using the Patient Health Questionnaire-9 (PHQ-9). Markers of inflammation were measured from fasting blood samples and included: high sensitivity C-reactive protein (hs-CRP), white blood cell count (WBC), interleukin-1 receptor antagonist (IL-1RA). Covariates included socio-demographic factors, adiposity, smoking and HbA_{1C}. Univariate analyses were used to determine relationships between depression and inflammatory markers and multiple linear regressions were used to adjust for covariates.
Results: 1489 data sets were available for analysis; mean [SD] age = 55.4 [11.2] years, 44.9% female, prevalence of depression = 14.7%. Depressed participants were younger at diagnosis (mean [SD] age = 52 [9.9] vs. 56 [11.2] years, p <0.001) but had similar HbA_{1C} (mean [SD] HbA_{1C} = 7.0 [1.5] vs. 7.2 [1.5] %). Inflammatory markers were significantly increased in depressed compared to non-depressed participants; median [IQR] hs-CRP = 3.4 [1.5 - 8.9] vs. 2.6 [1.1 - 6.2] mg/L, p=0.002; median [IQR] WBC = 7.1 [5.7 - 8.6] vs. 6.5 [5.3 -7.9] x10^9/L, p < 0.001; median [IQR] IL-1RA = 501.6 [334.6 - 786.3] vs. 435.4 [292.0 - 678.2] ng/L, p = 0.004. Associations between levels of inflammatory markers and depressive symptoms remained after adjusting for covariates: hs-CRP (standardised b = 0.10, p = 0.002), WBC (standardised b = 0.11, p < 0.001, IL-1RA (standardised b = 0.13, p < 0.001). After one year inflammatory markers remained significantly increased in participants who were depressed at baseline compared participants who were not depressed at baseline: median [IQR] hs-CRP = 3.5 [1.3 - 7.9] vs. 2.2 [0.9 - 5.0] mg/L, p < 0.001. This association also remained after adjusting for covaraites: hs-CRP (standardised b = 0.11, p < 0.001). In those who were depressed at baseline after twelve months there was no difference in levels of inflammatory markers between those with improved depressive symptoms and those without.
Conclusion: Circulating markers of inflammation are significantly elevated in T2DM participants with depression compared to non-depressed T2DM participants, these levels were still raised 12 months later. These consistently increased levels of inflammation and activated innate immunity observed in T2DM with depression may help to explain the persistent increased risk of complications and mortality in this group.
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Relation of the obesity-related gene (FTO) polymorphism with depressive symptoms and quality of life in type 1 diabetic children treated with insulin pumps: a pilot study

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Background and aims: A single-nucleotide polymorphism rs9939609 of fat-mass and obesity-associated (FTO) gene is associated with weight gain and obesity. The A allele of the FTO gene predisposing to obesity occurs in approximately 40% of the European population. Type 1 diabetic children who are carriers of the AA genotype of the FTO gene polymorphism (rs9939609) are at risk of more weight gain in the course of insulin therapy when compared to carriers of the TA and TT genotypes of this polymorphism. There is confirmed that obesity-related disorders are more often observed among people with depression than in general population. Moreover, depressive disorders are more common in children with type 1 diabetes. Some authors confirmed that polymorphisms in the FTO gene are associated with increased BMI in the depressive patients. There is also suggested that FTO rs9939609 A variant may be associated with a lower risk of depression independently of its effect on BMI. The aim of this study is to evaluate the frequency of depressive symptoms in type 1 diabetic children with AA genotype of the FTO gene polymorphism (rs9939609) and compared the results of youth the TA and TT genotypes of this polymorphism.
Materials and methods: The analysis included 277 children (93 girls) with type 1 diabetes diagnosed at least 1 year priori, treated with insulin pumps. The mean age was 13.1±2.9 years, the mean diabetes duration was 5.6±3.1 years and the mean HbA1c 7.5±1.1%. Gene polymorphism analysis in the extracted DNA were made with the real-time PCR method using TaqMan 7900 HT by Applied Biosystems. During the routine visit in the outpatient clinic children were asked to fill in Polish version of Children's Depression Inventory (CDI) by Maria Kovac. This self-report questionnaire is often used in clinical studies due to high coefficient of reliability (α-Cronbach between 0.81 - 0.89). Patients from age 11 and above were additionally asked to answer questions in 58-item Quality of Life Questionnaire, based on the DCCT Diabetes Quality of Life Measure. At the same time, other data was collected, including: sex, age, diabetes duration, HbA1c, BMI, daily insulin dose/kg body mass/24h (TDD).
Results: There were carriers of the genotype of the FTO gene polymorphism (rs9939609): AA (n=62), AT (n=139), TT (n=76). No difference between groups AA vs. AT vs. TT was observed depending on the results of: age (p=0.897), diabetes duration (p=0.601), BMI (p=0.968), TDD (p=0.388) and HbA1c (p=0.878). Fifteen percent (14 girls, 28 boys) of all participants reported depressive symptoms based on CDI scores ≥13. The percentage of children with depressive symptoms was slightly higher in the group TT (20.6%) in comparison to the groups AA (16.9%) and AT (16.8%), however without statistical significance (p=0.857). The quality of life was similar in all examined groups (p=0.803).
Conclusion: FTO gene polymorphism (rs9939609) had no impact on BMI, daily insulin dose, glycaemic control and quality of life in type 1 diabetic children treated with insulin pumps. The frequency of depressive symptoms was a little lower in children who were carriers of the A allele of the FTO gene in comparison to control subjects, however without statistical significance.
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Is there a relationship between depression and inflammatory markers in people with type 1 and type 2 diabetes?

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Background and aims: Previous studies suggest a relationship between depression and increased inflammatory markers in the general population. In people with diabetes, depression is the most common mental illness, and the risk of depression twice that of the general population. The objective of this study is to analyse the relationship between depression and inflammatory markers in patients with type 1 and type 2 diabetes, and to analyze the influence of the main risk factors of both variables.
Materials and methods: The sample consisted of 190 patients with diabetes (type 1: 144 and type 2: 46). Sociodemographic and medical variables were collected by structured interview. Biological values: Glycosylated haemoglobin (HbA_{1c}), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) were collected through analytical blood. The assessment of depressive symptoms was performed with the Spanish version of the Beck Depression Inventory (BDI-II). To analyse the relationships between the variables was used Pearson correlation coefficient. To analyze differences between patients with depressive symptoms and without depressive symptoms an analysis of variance was performed (ANOVA). We used SPSS version 19.0.
Results: In the sample studied, 31.1% of patients had depression symptoms (type 1: 23.2%; type 2: 7.8%). After analysing the three inflammatory markers, only found significant relationships between depression symptoms and IL-6 marker. In this case, in type 1 diabetes the marker IL-6 was associated with: depression symptoms, age and complications of diabetes. Type 1 diabetes patients with depressive symptoms had higher values of IL-6 than those without depressive symptoms ($p = 0.037$). However, in type 2 diabetes were not differences. After controlling for age and complications of diabetes, there were still significant differences in the values of IL-6 in patients with type 1 diabetes between those with depressive symptoms and those without depressive symptoms ($p < 0.001$).
Conclusion: This study shows a relationship between depressive symptoms and IL-6 levels in type 1 diabetes. While in type 1 diabetes patients with depressive symptoms have higher values of IL-6 than patients without depressive symptoms, in type 2 diabetes does not occur. None of the variables associated in this study with elevated levels of IL-6 in type 1 diabetes patients (age and complications) explain this relation. Therefore, the results of this study highlight the role of inflammatory markers, specifically IL-6 marker as a biomedical indicator of depressive symptoms in patients with type 1 diabetes.
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Insulin antibodies are associated with use of protaminated insulin, and the uncommon TNF -857T allele in subjects with type 2 diabetes mellitus

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Background and aims: The clinical significance of insulin antibodies (IA) cannot be ignored, even after the introduction of either recombinant human or analog insulin. We recently reported IA to be associated with use of protaminated insulin, as well as showing an independent positive association with diabetic retinopathy in type 2 diabetic subjects receiving recombinant human or analogue insulin therapy. Herein, we tripled the number of subjects, and endeavored to further assess the clinical features of IA-positive type 2 diabetic subjects. The genetic background for the emergence of IA was also examined, focusing on functional tumour necrosis factor (TNF) promoter polymorphisms.
Materials and methods: The study group was comprised of inpatients at our University Hospital. There were 247 females and 342 males, with a mean age of 58± 16 years, diabetes duration of 12± 9.8 years, and HbA1c of 13± 2.1%. After obtaining informed consent from all subjects, blood samples were collected. IA levels were measured with RIA kits for IA (Yamasa, Tokyo, Japan). TNF promoter polymorphisms at -857 and -863 were determined by sequencing the PCR products of the 5'-flanking region of the TNF gene. Statistical significance was analysed by the Mann-Whitney U test, or multiple linear regression analyses.
Results: One-hundred-nineteen of the 259 subjects (46%) receiving current or previous therapy with recombinant human or analogue insulin were IA positive (percent binding 10± 18%), whereas 92% of those without such therapy (n=330) were IA negative. Among the subjects treated with insulin, those with IA had significantly longer durations of diabetes (16± 8.1 vs 13± 9.5 years, P=0.013), and lower fasting CPR levels (1.5± 0.13 vs 1.9± 0.65 ng/ml, P=0.005). Multiple regression analyses revealed treatments with protaminated insulin to be the only significant explanatory variable for the percent binding with IA ($\beta=0.352$, $R^2=0.124$, P=0.000). Among the subpopulation of subjects genotyped for the TNF polymorphisms (n=43), the mutant -857T allele was found to significantly predict percent binding with IA ($\beta=0.433$, $R^2=0.187$, P=0.019). Diabetic retinopathy was predicted by the percent binding with IA ($\beta=0.214$, P=0.009), as well as by diabetes duration ($\beta=0.216$, P=0.011).
Conclusion: Our recent findings, the positive association of IA emergence with current or previous use of protaminated insulin, and with diabetic retinopathy, were reconfirmed after tripling the number of subjects. Our finding on protaminated insulin could be interpreted, based on previous observations, as indicating that protaminated proteins had augmented antigenicity. To our knowledge, this is the first report showing the -857T allele of the TNF promoter polymorphism, which may lead to a high TNF producer phenotype, to be positively associated with IA generation. This finding is in line with previous observations indicating that some functional TNF polymorphisms confer humoral immune responsiveness in settings of infectious immunity or autoimmunity. In conclusion, we propose that the emergence of IA and its concomitant adverse effects should be taken into account when starting insulin therapy in subjects with type 2 diabetes, and that the -857T allele of the TNF polymorphism could be a good marker for the emergence of IA.

PS 099 Nephropathy: epidemiology and clinical correlates

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Prevalence of albuminuria and reduced renal function in type 2 diabetes and its association with vascular co-morbidities in a Swedish primary care setting

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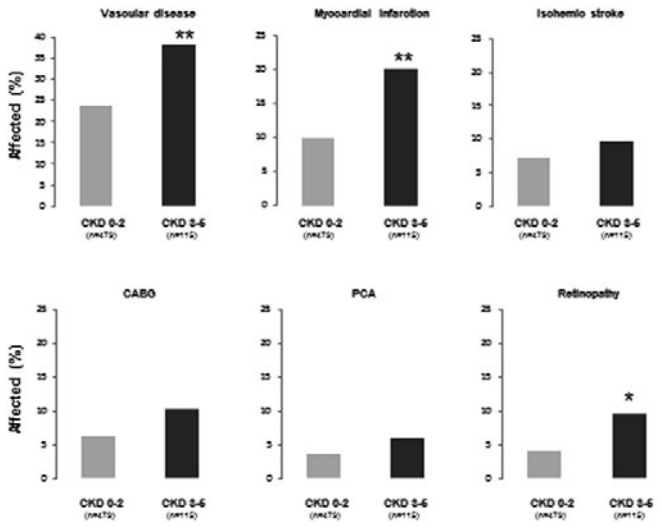
Background and aim: To estimate the prevalence of chronic kidney disease (CKD) and albuminuria and it associations to prevalent cardiovascular co-morbidities in patients with type 2 diabetes in a primary care setting in Sweden.
Patients and methods: Cross-sectional data on demography, laboratory measurements, current medication, previous vascular disease and major events of hypoglycaemia during the last 12 months was consecutively recorded from 53 primary care centres. Stage of CKD was defined based on the presence of albuminuria and estimated glomerular filtration rate (eGFR) according to KDOQI. Albuminuria was measured using the urinary albumin-creatinine ratio.
Results: We included 362 men and 226 women aged 68.5±9.3 years, mean diabetes duration 9.9±7.2 years. 220 patients (37%) were categorized as having CKD stage 1-5, 105 subjects (18%) CKD 1-2, and 115 subjects (20%) CKD 3-5. Albuminuria was present in 155 subjects (26%), microalbuminuria in 128 (22%) and macroalbuminuria in 27 (5%) subjects. The proportion of patients (%) with prevalent micro- and macrovascular disease according to CKD stage (CKD 0-2 vs. 3-5) is shown in figure 1. The OR, adjusted for age and gender, for subjects with CKD 3-5 for having retinopathy was 2.71 (95% CI 1.14 to 6.25, p=0.02) and for myocardial infarction 1.91 (95% CI 1.04 to 3.45, p=0.03) compared with CKD 0-2. Severe hypoglycaemia was more common in CKD 3-5 (n=3; 2.6%) and OR for subjects in CKD stages 3-5 for having hypoglycaemia was 8.71 (95% CI 1.19 to 80.11; p=0.03) compared with CKD 0-2 (n=2; 0.4%). Levels of haemoglobin were lower (133 ±14 g/L vs. 143 ±12 g/L) in CKD 3-5 compared to CKD 0-2 (p<0.01). In addition, the prevalence of vascular disease (p=0.03) and ischemic stroke (p=0.02) were higher in subjects with albuminuria compared with individuals without albuminuria.
Conclusion: Chronic kidney disease is common in type 2 diabetes in a primary care setting and associated with prevalent vascular co-morbidities.

Figure 1 Proportion of patients (%) with prevalent micro- and macrovascular disease according to CKD stage (CKD 0-2 vs. 3-5) in study population included in PRIDE: Prevalence of Renal Impairment in Diabetes. Vascular disease; composite of myocardial infarction, ischemic stroke, CABG and PCA, CABG; coronary artery bypass graft, PCA; percutaneous coronary angioplasty



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Albuminuria significantly predicts cardiovascular events in patients with type 2 diabetes independently from the baseline coronary artery state

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Background and aims: Albuminuria is an important indicator of cardiovascular risk. We have recently shown that it is also associated with angiographically determined coronary artery disease (CAD). Whether albuminuria predicts cardiovascular events independently of the baseline coronary artery state in patients with type 2 diabetes (T2DM) has not been investigated yet.
Materials and methods: We measured urinary albumin and creatinine concentrations in 211 consecutive patients with T2DM undergoing coronary angiography for the evaluation of suspected or established stable CAD. Albuminuria was defined as a urinary albumin to creatinine ratio (ACR) of 30 µg/mg or greater. Prospectively, we recorded vascular events over 3.2±1.4 years.
Results: During follow up, 24.6% of our patients suffered cardiovascular events. The cardiovascular event rate was significantly higher in patients with albuminuria (n=85) than in those with normoalbuminuria (35.3 vs. 17.5%; p = 0.003). Cox regression analysis adjusting for age, gender, BMI, smoking, systolic and diastolic blood pressure, LDL cholesterol, HDL cholesterol, eGFR, and use of ace inhibitors/angiotensin II antagonists confirmed that albuminuria significantly predicted cardiovascular events independently from conventional risk factors (adjusted HR 1.96 [1.11-3.46]; p=0.021). Further adjustment for the angiographically determined presence of CAD at baseline did not significantly attenuate the predictive power of the ACR (HR 1.84 [1.04-3.27]; p=0.037). Similar results were obtained when the ACR was entered into the final regression model as a continuous variable (standardized adjusted HR 1.30 [1.02-1.65]; p = 0.037).
Conclusion: Albuminuria significantly predicts cardiovascular events in patients with T2DM independently of established cardiovascular risk factors and of the baseline coronary artery state.

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Impact of morbid obesity on kidney function in type 2 diabetes

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Background and aims: Type 2 Diabetes (T2D) and obesity are risk factors for kidney dysfunction (Glomerular Filtration Rate (GFR) and Urinary Albumin Excretion (UAE)). People with T2D are often obese. We looked on the separate effects of T2D and of obesity on kidney function.
Materials and methods: We enrolled consecutively in our clinic for kidney function 822 participants with various levels of obesity (of them 784 T2D). Their GFRs were assessed using the slope-intercept method derived from ^{51}Cr-EDTA plasma clearance, corrected or not for 1.73m^2 body surface area, and their UAE status (normo-, micro-, macro-albuminuria) from 3 consecutive urine collections. Obesity stages were classified as: absent (BMI < 25kg/m2), overweight (25 - < 30), obesity (30 - < 40), and morbid obesity (≥ 40). GFR was analysed by 2-factor ANCOVA (T2D yes/no; obesity stages, adjusted for age and sex), and UAE status by Cochran-Mantel-Haenszel test. Logistic regression analyses were used to determine the risk for micro- and macro-albuminuria in patients with obesity and morbid obesity compared to subjects with normal weight.
Results: There was an interaction between obesity stages and T2D on body surface-adjusted GFR (p=0.01). In non-diabetic subjects, GFR increased with obesity stages up to 40 kg/m^2 and declined over 40. For patients with T2D, a progressive decline of GFR was observed when crossing the whole obesity range (table 1). Results on unadjusted GFR were similar. The effects on UAE status were similar: diabetes-obesity interaction (p<0.0001), diabetes effect (p=0.0005) and obesity effect (p=0.001). The prevalence of normo-, micro- and macro-albuminuria according to morbid obesity was 38.1%, 42.9% and 19.0%, respectively. Regressive model indicated an increased risk of microalbuminuria (OR 2.86, 95%IC 1.55 - 5.17, p=0.0005) and macro-albuminuria (OR 2.22, 95%IC 1.40 - 3.55, p=0.0007) in patients with morbid obesity, after adjustment for sex, age and T2D status.

Conclusion: We found a strong interaction between obesity status and T2D with a reduced GFR and an increased UAE in patients with morbid obesity.

Table 1. Body surface adjusted GFR according to obesity stages and T2D status

		Normal (N=155)	Overweight (N=301)	Obesity (N=296)	Morbid obesity (N=70)	p*
Diabetes	Yes	91 ± 3	89 ± 2	88 ± 2	73 ± 4	0.0004
	No	93 ± 7	99 ± 7	115 ± 6	105 ± 5	0.04

Results expressed as means ± SEM. *ANCOVA adjusted for sex and age. Diabetes x obesity stages interaction: p=0.01.

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Heterogeneity of chronic kidney disease (CKD) phenotypes in patients with type 1 diabetes mellitus

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Background and aims: According to traditional paradigms, increased albuminuria precedes GFR loss in the progression to CKD. However, mainly in type 2 diabetes, but in type 1 too, recent findings demonstrate that GFR decline may occur also in longstanding normoalbuminuria. Indeed, in the DCCT/EDIC over 19 years 24% of type 1 diabetic subjects (T1DM) develops sustained eGFR <60 ml/min/1.73 m2 with no previous albuminuria. Furthermore, different sets of risk factors are related to albuminuria and eGFR suggesting they represent parallel phenotypes. Prevalence and correlates of different CKD phenotypes have been explored in 777 adults with T1DM consecutively recruited at our Outpatients Clinic between 2001 and 2009.
Materials and methods: We studied 408 males and 369 females (52.5/47.5%). Mean age (±SD) was 40.2±11.7 (IQR 32-47), duration of diabetes 19.4±12.2 (9-28) years. BMI was 24.8±3.6 kg/m2; HbA1c 7.83±1.17% (IQR 7.1-8.4); 29.6% were current smokers, 35.2% had hypertension, 19.4% were treated with anti-hypertensives (17.5% with RAS blockers). Diabetic retinopathy (DR) was detectable in 41.3% of subjects (25.7% background, bDR; 15.6% proliferative, pDR); peripheral polyneuropathy in 8.1%, major cardiovascular (CV) events occurred in 8.5%.
Results: Normoalbuminuria (nA, albumin to creatinine ratio, ACR <30 mg/g), microalbuminuria (μA, ACR 30-299) or macroalbuminuria (MA, ACR ≥300) were present in 91.6, 6.4 and 1.9% of subjects, respectively. Stage 1 (≥90), 2 (60-89) and ≥3 (<60 ml/min/1.73 m2) eGFR were present in 57.3, 39.0 and 3.7% of patients. Combining eGFR and ACR strata, 89.4% (n. 695) had no-CKD, while stages 1-2 and stages ≥3 CKD were detected in 6.8% (n. 53) and 3.7% (n. 29) of subjects. The albuminuric (Alb+) and non-albuminuric (Alb-) phenotypes were present in 17 (58.6%) and 12 (41.4%) of stages ≥3 CKD. Alb+ and Alb- showed similar age, duration of diabetes and gender distribution. Patients with nA were splitted in subjects with "normal albuminuria" (ACR <10 mg/g; 77.2%) and those with "low-microalbuminuria" (ACR 10-29 mg/g; 14.4%); patients with stage 2 eGFR were stratifed in 2a (75-89) and 2b (60-74 ml/min/1.73 m2). Also in 2b eGFR, nA (88.7%) and normal albuminuria (70.4%) were highly frequent. Prevalence of normal albuminuria was lower but still significant (35.7%) in stages ≥3 CKD. Prevalence of DR (37.3, 70.6 and 85.2%; pDR 11.4, 47.0 and 63.0%), polyneuropathy (5.8, 18.8 and 48.0%) and CV events (7.3, 11.3 and 34.5%) increased from no CKD to stages ≥3 CKD (p<0.0001). In logistic backward regressions (model 1), CKD 1-2 and CKD 3-5 (versus no CKD) were both independently associated with age (inversely in CKD 1-2), HbA1c, gammaGT, fibrinogen, hypertension and (model 2) also with pDR, but not with gender, BMI, smoking, HDL-C and triglycerides. CKD ≥3 Alb+ correlated with male gender, diabetes duration, HbA1c, HDL-C (inversely), fibrinogen but not with hypertension, while CKD ≥3 Alb- was associated with age, gammaGT, hypertension, but not with HbA1c.
Conclusion: Alb- CKD phenotypes are detectable in a significant proportion of T1DM subjects. These findings hardly can be due to a misclassification artifact and support the hypothesis of two distinct pathways (Alb+ and Alb-) that progress to advanced kidney disease in T1DM as also suggested by the observation that different sets of covariates are related to different CKD phenotypes.

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Diabetes increases renovascular impedance in patients with liver cirrhosis

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Background and aims: The increase of renal Doppler indices (pulsatility index, resistance index, PI- RI) in cirrhosis, has usually been associated to a higher risk of deterioration in renal function. An increase of PI-RI has also been reported in type 2 diabetes (T2DM). It's still unknown if T2DM modifies renal hemodynamics in cirrhosis.To compare renal Doppler indices in cirrhotics with and without type 2 diabetes (T2DM) and in diabetics without cirrhosis.
Materials and methods: We evaluated 89 consecutive patients with normal renal function: 37 cirrhotics with T2DM (CD-Group), 41 cirrhotics without diabetes (C-Group) and 11 diabetics without cirrhosis (D-Group). The kidney pulsatility index (PI) and the resistance index (RI) were measured with Doppler-ultrasound. Renal function was expressed as the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal-Disease (MDRD) formula, the microalbuminuria (μAlb), was also evaluated.
Results: No significant differences were present regarding age, Child-Pugh's class and serum creatinine. The eGFR was weakly reduced in CD-Group compared to C-Group and D-Group, μAlb was present in 24.4% of the CD-Group and in 9% of the D-Group. The PI and RI were significantly increased in CD-Group and in D-Group compared to C-Group. Both PI and RI were significantly related to μAlb independently of age and Child-Pugh's class.
Conclusion: Diabetes is associated with an increase in reno-vascular resistance in cirrhotics that is related to μAlb values. The novel finding of this study was that DM impairs renal hemodynamics in cirrhosis.

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Poor glycaemic control is related with increased nitric oxide activity within the renal circulation of patients with type 2 diabetes mellitus

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Background and aims: Experimental studies have shown that glucose releases endothelial nitric oxide (NO), and that NO contributes to renal hyperperfusion in models of diabetes. To examine whether this translates into the *human* condition, we studied the relationship between glycemic control and renal NO activity in patients with type 2 diabetes.
Materials and methods: A total of 113 patients with type 2 diabetes mellitus and a wide range of glycated hemoglobin A1c (HbA1c) concentrations were included. Renal plasma flow (RPF) and glomerular filtration rate (GFR) were determined by constant infusion input clearance. Functional NO activity in the renal circulation was determined as change of RPF to infusion of the NO synthase (NOS) inhibitor N(G)-monomethyl-L-Arginine (L-NMMA, 4.25 mg/kg). As additional markers, we measured urinary excretion of NO (UNOx) and L-arginine/asymmetrical dimethylarginine (ADMA) ratio in plasma.
Results: Subjects within the highest tertile of HbA1c concentration had increased RPF (low, medium and high tertiles: 576±17 versus 585±22 versus 627±33 ml/min/m^2, P=0.05 by 1-way ANOVA), while GFR was similar across tertiles. The response of RPF to NOS-blockade was augmented in subjects with higher HbA1c levels (-55±7 versus -64±8 versus -86±8 ml/min, P=0.04 by 1-way ANOVA). Further, L-arginine/ADMA ratio and UNOx were increased in subjects with higher HbA1c levels.
Conclusion: In line with experimental evidence, we could demonstrate *in humans* that poor glycemic control is related with higher NO activity and hyperperfusion of the kidney. The renal NO system may thus be a novel therapeutic target for improving renal hemodynamics in patients with diabetes mellitus.
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Haemodialysis-associated hypoglycaemia and inter-day glycaemic variability assessed by continuous glucose monitoring system in patients with type 2 diabetes

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Background and aims: The proportion of diabetic patients undergoing hemodialysis is rapidly increasing. Glucose control among such patients is difficult to assess, because hemodialysis seems to alleviate uremia-induced insulin resistance and is therefore likely to induce significant glycemic variability. The aims of the study were to assess the glucose variability in type 2 diabetic patients undergoing hemodialysis, using continuous glucose monitoring system (CGMS), and to perform a comparative analysis of CGMS glycemic curves in days with and without hemodialysis.
Materials and methods: We investigated 12 hemodialysed type 2 diabetic patients (HD DM; 6M/6F, 56±7.3 years, diabetes duration 8.6±3.9 years) and 11 type 2 diabetic patients without renal impairment (nonHD DM; 5M/6F, 57.6±9.5 years, diabetes duration 8.1±5.7 years). Subjects were monitored 48-h using CGMS (Medtronic MiniMed). The glycemic variability was quantified by mean interstitial glucose, percent of time with interstitial glucose >180mg/dl and <70mg/dl, coefficient of variation (%CV), mean amplitude of glycemic excursion (MAGE), fractal dimension (FD), mean of daily differences (MODD). SPSS 17 software was used for statistical analysis. The comparisons between different groups were performed using parametric tests.
Results: HbA1c levels were lower in HD DM patients than in non-HD DM controls (6.0±0.5 vs. 6.9±1.6%), but the differences did not rich statistic significance. The percent of time spent with glucose <70mg/dl was significantly higher, during the 1st day with dialysis session, in HD DM patients comparing with non-HD DM subjects during the equivalent 1st day (6.6±3 vs 0.7±0.2%, p<0.001). MODD was significantly higher in HD DM patients, comparing with nonHD DM subjects (36.2±10.3 vs 19.8±5.7, p=0.01), indicating the influence of hemodialysis on inter-day glycemic variability. Although there were no differences for indices of glycemic variability between the day with and without hemodialysis, hypoglycemia occurred predominantly in day on hemodialysis (6.6±3% vs 2.1±0.4% time with glucose <70mg/dl, p=0.03). There were no significant differences for indices of glycemic variability or time in hyper/hypoglycemia between the 1st and 2nd day, in nonHD DM subjects.
Conclusion: Our findings suggest that HbA1c was not as good marker of glucose control in hemodialysis and, according to the CGMS readings, hemodialysis seems to increase inter-day glucose variability and the risk of hypoglycemia. This study provides evidence that CGMS can be a useful tool in glycemic variability assessment in hemodialysed diabetic patients.

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Glucose profile in patients with type 2 diabetes and ESRD: comparison between haemodialysis sessions and inter-haemodialysis periods

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Background and aims: The main difficulty to obtain adequate glycaemic control in diabetic patients with end stage renal disease (ESRD) treated by hemodialysis is due to the consecutive dialysis and inter-dialysis periods. In this population, glucose profile is not well documented. We aimed to analyze the glucose profile by continuous glucose monitoring (CGM) during consecutive dialysis and inter-dialysis periods in patients with type II diabetes and ESRD.
Materials and methods: CGM (Navigator, Abbott) was performed in 33 diabetic patients (14 female/19 male, age: 66±8yrs, diabetes duration: 23±11yrs, hemodialysis duration: 3.8±2.6yrs) during 54 hours, including 2 consecutive hemodialysis sessions at 0, 1 and 3 months with determination of the mean CGM glucose value, maximal glucose excursion, MAGE during consecutive dialysis and inter-dialysis periods. Comparisons were done using ANOVA for repeated measures. Each patient used a dialysate solution with a 100mg/dL glucose concentration.
Results: Mean glucose value of 78 CGM was 171±36 mg/dL with coefficient of variability: 34±9% and MAGE: 104±42 mg/dL. During dialysis sessions, mean glucose value was significantly lower: 136±46mg/dL vs. 171±35 mg/dL between two dialysis sessions (p< 0.001). Standard deviation, coefficient of variability and MAGE significantly decreased by 35±5mg/dL (p<0.001), 20±1% (p<0.001) and 58±7mg/dL (p<0.001) respectively. Percentage of glucose value < 60mg/dL increased significantly during dialysis: 4.4±9.6 % vs. 2.1±7.9% between two dialysis sessions (p<0.001) with a mean glucose value significantly higher: 49±10mg/dL vs. 37±24 mg/dL (p< 0.001).
Conclusion: During hemodialysis sessions, mean glucose CGM value and glucose variability are improved in patients with type II diabetes and ESRD. Hypoglycemic risk increases but low glucose values are less severe. The use of 100mg/dL glucose dialysate solution in all dialysis sessions may contribute to the decrease of glycaemic variability. The continuous glucose monitoring (CGM) allows documenting the glycaemic status in this group of patients with high cardiovascular risk.
Supported by: Novo Nordisk, Abbott

PS 100 Nephropathy: clinical risk factors, markers and outcomes

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Socioeconomic risk factors for developing end stage renal disease in patients with childhood onset type 1 diabetes: a population based register study

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Background and aims: Sweden has one of the highest incidences of childhood onset type 1 diabetes. The aetiology of ESRD in diabetes mellitus is multifactorial. Well known risk factors are genetic traits, disease duration, metabolic control and high blood pressure. The latter two may be associated with psychosocial stress. Therefore the aim of the present study is to investigate the influence of some early social determinants on ESRD development.
Materials and methods: The Swedish Childhood Diabetes Registry (SCDR) covers almost all patients in Sweden with onset of type 1diabetes before 15 years of age since 1 July 1977. The SCDR was linked to the Swedish Renal Registry, which includes virtually all cases of ESRD in Sweden since 1991. Information on the patients and their parent's socioeconomic status was retrieved anonymised from Statistics Sweden. Age at onset was grouped in five year groups; 0-4, 5-9 and 10-14 years. In Cox regression analyses we analysed the effect of parental education (up to college / higher than college) and the family's use of income support (any / none) on the risk for ESRD in the proband, using follow up time from diabetes onset to ESRD.
Results: Out of 7836 patients with diabetes duration longer than 15 years, 129 (68 men, 61 women) developed ESRD due to diabetes. Mean duration of diabetes was 28 years for those who developed ESRD, 24 for those who did not. *Educational level:* In crude analysis, low maternal education was associated with almost three times higher risk of developing ESRD, HR=2.8 (95% CI 1.6-4.8). Low paternal education almost doubled the risk, HR=1.9 (1.1-3.2). When adjusting for sex and age at onset of diabetes, low maternal education was still significantly associated with increased risk, HR=2.8 (1.6-5.0). Stratifying for sex, low maternal educational level was more important in females, HR=3.6 (1.4-9.0), than in males, HR=2.3 (1.1-4.9). Adjusting for maternal educational level, paternal educational was not significant and not included in further analyses. *Income support:* In crude analyses, the risk of developing ESRD was increased if any of the parents had received income support, HR=2.6 (1.8-3.7). Adding age at onset and sex to the model, the effect of parental income support remained significant, HR=3.0 (2.1-4.2). In analyses stratified by sex the parents' need of income support was associated with ESRD in both sexes, females HR=1.9 (1.1-3.3), males HR=3.8 (2.3-6.3). Adding maternal educational level to the model changed the effect of income support, slightly reducing its importance (table 1).
Conclusion: Low maternal education and income support in the family of young patients with type 1 diabetes increases the risk of developing ESRD. Maternal education seems to be the stronger predictor for women, while income support in the family is a stronger predictor for men.

	Total		Male		Female	
Variables in the model	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Sex	1.0 (0.7-1.4)	1.0				
Age at onset	1.2 (1.1-1.2)	0.000	1.2 (1.1-1.3)	0.000	1.2 (1.1-1.3)	0.000
Maternal education	2.4 (1.4-4.3)	0.003	1.9 (0.9-4.0)	0.088	3.3 (1.3-8.2)	0.012
Income support	2.6 (1.8-3.8)	0.000	3.6 (2.2-6.0)	0.00	1.8 (1.0-3.1)	0.044

Table 1. Cox regression model.

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Smoking increases the incidence of diabetic nephropathy and end stage renal disease in patients with type 1 diabetes

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Background and aims: We studied the effect of smoking on the development and progression of diabetic nephropathy (DN) in patients with type 1 diabetes.
Materials and methods: The study included 2,583 patients without DN at baseline participating the FinnDiane (Finnish Diabetic Nephropathy) study. 16 (0.75%) out of 2,135 normoalbuminuric (AER <20µg/min or <30mg/24h) patients and 81 (18.0%) out of 448 microalbuminuric (AER 20-200µg/min or 30-300mg/24h) patients developed DN during the follow-up of 16,112 person years. The AER was based on three 24h or overnight urine collections. Nephropathy was defined as macroalbuminuria (AER >200µg/min or >300mg/24h) or end stage renal disease (ESRD). Smoking status was based on baseline questionnaires and patients were considered as nonsmokers, ex-smokers and current smokers. To evaluate the effect of the cumulative smoking dose, current smokers were further divided in three different groups: less than 5 pack years, 5-19.9 pack years and more than 20 pack years. We also analyzed the cumulative risk of ESRD from the beginning of diabetes and included 4,074 patients with data of smoking history and follow-up data for ESRD. 563 patients developed ESRD during the 122,685 person years of follow-up. Patients were divided into never/ever-smokers and according to the smoked pack years. Cox-regression analyses were used to estimate the HRs for developing DN or ESRD. Sex, duration of diabetes, BMI, hypertension, HbA_{1c}, eGFR, HDL and triglycerides were used as covariates when analyzing the risk of DN. Sex and age at onset of diabetes were used in the multivariate model when estimating the risk of ESRD.
Results: In the univariate analyses, the HR of DN was 2.15 (95% CI 1.22-3.79) for ex-smokers and 4.12 (95% CI 2.60-6.51) for current smokers compared with nonsmokers. In the multivariate model the HR of DN was 1.55 (95% CI 0.85-2.84) for ex-smokers and 3.29 (95% CI 2.02-5.36) for current smokers. Current smokers had significantly higher risk of developing nephropathy compared with ex-smokers (data not shown). Patients who had smoked <5 pack years before baseline had a HR of 1.77 (95% CI 0.73-4.25), 5-19.9 pack years a HR of 4.66 (95% CI 2.77-7.84) and >20 pack years a HR of 5.89 (95% CI 3.11-11.15) compared with nonsmokers in the univariate model and the results remained similar in the multivariate model. Ever-smokers had a significantly higher risk of developing ESRD with a HR of 1.81 (95%1.53-2.15) in the univariate model and 1.75 (95% CI 1.48-2.08) when adjusted for sex and age at onset. The risk of developing ESRD increased with increasing pack years with HR of 1.44 (95% CI 1.08-1.93) for <5 pack years, 1.79 (95% CI 1.44-2.22) for 5-19.9 pack years and 1.98 (95% CI 1.56-2.50) for >20 pack years. When adjusted for sex and age at onset the HRs were 1.36 (95% CI 1.01-1.82), 1.70 (95%CI 1.37-2.11) and 2.05 (95% CI 1.60-2.62), respectively.
Conclusion: Smoking increases the risk of developing DN and ESRD. Smoking cessation has a beneficial effect on the development of DN. Increasing cumulative smoking dose is associated with an increasing risk of DNP.
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Metabolic risk factors according to albuminuria and chronic kidney disease in apparently healthy Koreans

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Background and aims: Both high albuminuria and chronic kidney disease (CKD) represented by low estimated glomerular filtration rate (eGFR) are known risk factors for cardiovascular events and mortality. However, the differences of metabolic phenotypes according to albuminuria and CKD have not been investigated in apparently healthy adults.
Materials and methods: 2963 individuals aged over 40 years (mean age, 58.3 ± 7.2 years) from the Korean Genome and Epidemiology study were included in this cross-sectional analysis. CKD was defined as estimated glomerular filtration rate < 60 mL/min/1.73m^2. Albuminuria was defined as urinary albumin-to-creatinine ratio (ACR) ≥30 mg/g. All participants were divided into four groups according to the presence or absence of albuminuria and CKD. Body mass index (BMI), waist circumference (WC), percent body fat, and metabolic parameters including blood pressure, serum glucose, and lipid profile were all assessed.
Results: The mean eGFR was 74.2 ± 10.6 ml/min/1.73 m^2, and the median ACR was 4.7 mg/g (interquartile range, 2.90-8.86). Obesity measures including BMI and WC, serum triglyceride and high-density lipoprotein cholesterol levels were significantly correlated with both high ACR and low eGFR. Addi-

tionally, fasting plasma glucose and blood pressure were positively associated with ACR. Among four albuminuria/CKD categories, albuminuria with CKD group had the worst metabolic characteristics and the highest prevalence of diabetes. When comparing albuminuria without CKD and CKD without albuminuria groups, the former had higher BMI (26.3 vs. 25.4 kg/m^2, p=0.026), WC (86.1 vs. 83.5 cm, p=0.008), systolic blood pressure (123.3 vs. 113.1 mmHg, p<0.001), fasting plasma glucose (112.3 vs. 98.3 mg/dl, p<0.001), and higher prevalence of diabetes (51.6 vs. 40.3%, p=0.038) after adjusting for age and sex. This finding was similar in subjects with or without diabetes. In the logistic regression analyses, subjects with albuminuria and/or CKD had increased odds of metabolic syndrome compared to those without albuminuria and CKD after adjusting for age, sex, BMI, alcohol, smoking and cardiovascular disease [ORs, 2.32 (95% CI, 1.61-3.33), 1.76 (1.24-2.49), 3.26 (1.71-6.18) in albuminuria without CKD , CKD without albuminuria, albuminuria and CKD groups, respectively).

Conclusion: In apparently healthy population, those with albuminuria and/or CKD had metabolically unhealthier phenotypes than those without albuminuria and CKD. Albuminuria without CKD was more strongly associated with metabolic syndrome and its components compared to normoalbuminuric CKD.

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Genome-wide association scan for diabetes associated chronic kidney disease (CKD) in patients with type 1 and type 2 diabetes

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Background and aims: Diabetes is the leading cause of chronic kidney disease (CKD) and is associated with excessive cardiovascular morbidity and mortality. We conducted a genome-wide association study (GWAS) to identify susceptibility loci for CKD in Patients with type 1 and type 2 diabetes.

Materials and methods: We defined cases as CKD stages 3-5 with an estimated Glomerular Filtration Rate (eGFR), based on MDRD-4 formula, <60 ml/min/1.73m^2 or presence of End Stage Renal Disease (ESRD), defined as dialysis or renal transplantation. Controls had eGFR >=60 ml/min/1.73m^2 and duration of diabetes >= 15 years for T1D, >= 10 years for T2D. GWAS data from 4,207 European samples (1,299 cases, 2,908 controls) with T1D and 4,469 European samples (3,088 cases, 1,381 controls) with T2D were imputed to ~2.4 million single nucleotide polymorphisms (SNPs) with HapMap2 using IMPUTE. To test for association we used a logistic regression model, adjusting for age at onset, gender and duration of diabetes and in addition a meta-analysis using a fixed effects model in GWAMA. We also performed a combined meta-analysis of the T1D and T2D comprising 8,676 individuals (4,387 cases, 4,289 controls).

Results: In T1D CKD we observed the strongest association for rs12632850 (OR = 1.39, P = 1.1 x 10^{-6}, Effect Allele Frequency (EAF) = 0.8) on chromosome 3q25.33 between *SCHIP1* and *IL12A*. The strongest association for the T2D CKD analysis was rs2378013 (OR = 0.60, P = 9.1 x 10^{-8}, EAF = 0.92) a SNP close to the *TGFB2* gene encoding transforming growth factor beta 2. In the combined T1D + T2D analysis the strongest association was rs2231899 mapping to FIGN (OR = 1.25, P = 1.6x10^{-6}, EAF = 0.79). We did not see any significant association (P < 0.05) in previously published signals for non-diabetic CKD (*UMOD*, *PRKAG* or *SOX11*).

Conclusion: We have identified several putative loci for CKD in patients with type 1 or Type 2 diabetes. *TGF-β* has been previously shown to be an important mediator in the pathogenesis of CKD. Non-replication of known loci for CKD from population based GWAS studies in patients with diabetes suggests that the genetic background for non-diabetic CKD disease differs from that of diabetes associated CKD.

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Urinary proteomics predict onset of microalbuminuria in a cohort of normoalbuminuric type 1 diabetic patients in the DIRECT 1 study

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Background and aims: Early prevention of diabetic nephropathy is not successful as early interventions have shown diverging results. Urinary proteomics has shown promise as an early indicator of future development of diabetic nephropathy and could guide need for treatment. In this study we investigate that ability in a large type 1 diabetic cohort with normoalbuminuria.

Materials and methods: In a post-hoc study of the DIRECT-Protect 1 study, a randomized, controlled clinical trial of candesartan for slowing the progression of diabetic retinopathy, we studied patients with type 1 diabetes and normoalbuminuria (n=783), followed for a mean of 4.5 years. We determined a previously defined CKD risk score based on proteomic measurement of 273 urinary peptides (CE-MS), selected from previous cross sectional case-control studies. A Cox regression model for progression of albuminuria was built. The primary endpoint was development of persistent microalbuminuria (MA) (3 out of 4 samples).

Results: Persistent MA developed in 40 patients (5.4%). At baseline the CKD risk score was able to predict development of MA during follow-up, independent of treatment (candesartan/placebo), age, gender, baseline systolic BP, baseline UAER, baseline eGFR, baseline HbA_{1c} and diabetes duration (HR 2.88 (95% CI 1.1-7.6), p=0.032). In the placebo treated group the HR was 4.9 (1.3-18.7) compared to 2.0 (0.5-8.9) in the candesartan group.

Conclusion: In this cohort of patients with type 1 diabetes and normoalbuminuria from a large intervention study, the urinary proteome-based CKD classifier was an independent predictor of MA. This may provide a better opportunity to select normoalbuminuric patients for early prevention of diabetic nephropathy as treatment with candesartan seems to mitigate this risk.

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Alkaline phosphatase is independently associated with renal function in normoalbuminuric type 1 diabetic patients

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is associated with an increased prevalence of chronic kidney disease in patients with type 1 diabetes. The aim of this study was to explore the relationship between markers of NAFLD and renal function in normoalbuminuric type 1 diabetic patients.

Materials and methods: Study included 313 normoalbuminuric type 1 diabetic patients without clinical evidence of cirrhosis or other causes of chronic liver disease and before any interventions with statins, ACE inhibitors or angiotensin II receptor blockers. Normoalbuminuria was defined as a urinary albumin excretion rate (UAE) <30 mg/24h. UAE was measured from at least two 24-h urine samples and determined as the mean of 24-h urine collections. Study included patients with glomerular filtration rate (GFR) >60 mlmin-11.73m-2 estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. Correlations and multiple logistic regressions analysis were performed to identify the relationships between UAE, estimated GFR, serum creatinine and NAFLD associated markers (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK), γ-glutamyltransferase (GGT), ferritin and bilirubin).

Results: The mean age of our patients was 34±11 years, and 51.5% were males. Mean/median values of body mass index (BMI) (24 (15-37) kg/m2), waist to hip ratio (WHR) (0.81±0.07), HDL cholesterol (1.7±0.4 mmol/L), triglycerides (0.91 (0.3-4.1) mmol/L), AST (20 (10-146) units/L), ALT (19 (7-171) units/L), ALK (69 (11-229) units/L), GGT (16 (7-553) units/L), bilirubin (12 (5-103) μmol/L), ferritin (55 (5-697) μg/L), serum creatinine (71±14 μmol/L), UAE (11.0 (1.7-29.8) mg/24h) and eGFR (106±16 mlmin-11.73m-2) were within the normal range for patients with diabetes, with slightly elevated hemoglobin A1c (HbA1c) (7.4±1.6%) and LDL cholesterol (2.8±0.8 mmol/L)

levels. ALT, GGT, bilirubin and ferritin significantly correlated with serum creatinine (r=0.16, 0.18, 0.29, and 0.39, respectively, for all p<0.05), ALK and bilirubin with eGFR (r=0.23, and -0.12, respectively, for all p<0.05) and GGT with UAE (r=0.11, p<0.05). ALT, GGT, bilirubin and ferritin levels were significantly higher in subjects in the highest quartile of serum creatinine (≥80 μmol/L) compared to those in lowest quartile (<63 μmol/L) (21 vs 20 U/L, 18 vs 14 U/L, 14 vs 10 μmol/L, and 103 vs 25 μg/L, respectively, for all p<0.05). ALK levels were significantly higher in subjects in the highest quartile of UAE (≥16.6 mg/24h) compared to those in lowest (<6.8 mg/24h) quartile (71 vs 69 U/L, p=0.03), as well as in hyperfiltrating subjects (eGFR >125 mlmin-11.73m-2) compared to those with normal or mildly impaired eGFR (81 vs 68 vs 64 U/L, p<0.001). In a multiple logistic regression model adjusted for age, sex, duration of diabetes, HbA1c and BMI, only ALK levels were significantly associated with disturbances in serum creatinine and eGFR in our normoalbuminuric subjects (p≤0.007), with odds ratios of 0.98 to 1.02.
Conclusion: NAFLD associated markers, particularly ALK, are associated with renal function in normoalbuminuric type 1 diabetic patients. Whether the detection of elevated ALK levels in normoalbuminuric type 1 diabetic patients has predictive value for development of renal disease needs to be assessed in further follow-up studies.

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Circulating levels of adrenomedullin are elevated in type 1 diabetes with nephropathy and associated with outcome

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Background and aims: Endothelial dysfunction is associated with progression of diabetic nephropathy. Adrenomedullin is a 52 amino acid peptide, which is expressed in various tissues, including vascular endothelium. Circulating levels of the stable midregional fragment of the pro-adrenomedullin peptide (MR-proADM) is measured in plasma using a newly developed assay which reflects adrenomedullin levels. MR-proADM has potential as an endothelial biomarker. The aim of the present study was to examine if MR-proADM levels were elevated in T1D patients with increasing nephropathy, and to evaluate the prognostic value of this biomarker.
Materials and methods: A total of 264 patients with T1D (59.5 % men, mean age 44 years) were enrolled at baseline, and followed for a median of 12 years with respect to all-cause mortality. Measurement of plasma MR-proADM at baseline was available for all the patients. The median duration of diabetes was 19 years (11-28), and 54 (20.5%) of the patients received ACE-inhibitor or angiotensin II antagonist therapy. Twenty (7.6 %) patients had macrovascular complications whereas, 149 (56.4 %) had retinopathy. Mean HbA1c was 8.6 % (1.2 %), and mean BMI 25.1 kg/m^2 (3.7 kg/m^2). During follow-up 39 (14.8 %) patients died, 12 due to cardiovascular disease. Diabetic nephropathy defined as either elevated urinary albumin creatinine ratio (UACR) > 30 mg/g or creatinine levels above reference interval were present in 68 (%) of the patients.
Results: Median (interquartile range (IQR)) MR-proADM plasma levels were elevated in T1D patients with nephropathy 0.38 nmol/l (0.33-0.52 nmol/l) compared to T1D patients without nephropathy 0.35 nmol/l (0.28-0.42 nmol/l), P = 0.009. There was no association between MR-proADM levels and BMI or glycemic regulation measured as HbA1c. Baseline MR-proADM levels tended to be increased in the T1D patients who died during follow-up, 0.40 nmol/l (0.30-0.55 nmol/l) vs. 0.35 nmol/l (0.29-0.43 nmol/l), P = 0.054. Presence of nephropathy was not associated with significantly increased mortality risk (HR 1.8, P = 0.6) in the present population. We could not demonstrate a significant association between MR-proADM levels and all-cause mortality when performing a cox regression analysis, a two fold increase in MR-proADM levels was associated with a HR of 1.3 (0.8-2.2), P = 0.3.
Conclusion: T1D patients with nephropathy have increased plasma MR-proADM levels, which could possess prognostic implications. The relatively limited number of events in the present study should be considered. The prognostic value of MR-proADM in combination with urinary albumin excretion rate with respect to survival and progression of diabetic nephropathy should be evaluated in future studies.

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Renal depletion of the organic osmolyte, myo-inositol, in hypertension, diabetes and insulin resistance: therapeutic implications for chronic kidney disease

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Background and aims: Depletion of the organic osmolyte, myo-inositol (MI), in the kidney is implicated in the pathogenesis of diabetic nephropathy. MI depletion was proposed to arise as a consequence of increased glucose metabolism through the polyol pathway and sorbitol accumulation in the kidney. However, MI depletion in diabetes persists despite normalization of sorbitol levels following treatment with an aldose reductase inhibitor. The origin of MI depletion in diabetes mellitus is therefore currently poorly understood as are the mechanisms linking it to the pathogenesis of renal complications. We hypothesized that renal depletion of MI was due to changes in metabolic pathways regulating its biosynthesis, reabsorption and catabolism in the renal cortex. Of special interest is the catabolism of MI through the renal-specific enzyme myo-inositol oxygenase (MIOX) and the glucuronate-xylulose (GX) pathway, which are implicated in oxidative stress and the pathogenesis of interstitial fibrosis in diabetes. We also surmised that MI depletion might occur not just in diabetes but in other metabolic states characterised by hyperosmotic conditions. We therefore examined the expression of genes regulating MI de novo biosynthesis, reabsorption, and catabolism and extended the analyses to animal models of diabetes, dietary-induced obesity, and hypertension.
Materials and methods: Analyses were investigated in kidneys derived from animal models of streptozotocin-induced diabetes (Wistar rats), dietary induced obesity (C57BL/6 mice), and hypertension (SHR vs. WKY rats). Gene and protein expression were measured using real time PCR, western blotting, and activity measurements. MI content in the kidney was analysed by GC/MS using deuterated MI as an internal standard.
Results: Renal MI depletion occurred in hypertensive and insulin resistant states as well as in diabetes. Compared to their respective controls, renal depletion of MI was most pronounced for hypertension with a 52% decrease ($N = 6$, $p < 0.01$) followed by a 35% decrease in diabetes ($N = 7$, $p < 0.01$) and an 18% decrease in dietary-induced obesity ($N = 6$, $p < 0.05$). Of all the genes and proteins analysed, MI depletion was positively correlated with increased MIOX activity across all three disease states compared with their respective controls. MIOX activity was increased by 144% fold in the hypertensive state, followed by 124% fold in diabetes mellitus ($N = 6$, $p < 0.01$), and 114% for insulin resistance ($N = 7$, $p < 0.05$). We also showed using immunohistochemistry that MIOX and the GX pathway were localized to the proximal tubules in the renal cortex where MI depletion occurs.
Conclusion: Oxidative stress is a common pathological component of chronic kidney disease. Our data indicate that renal MI depletion is a surrogate biomarker reflecting increased catabolism through MIOX and the GX pathway in the proximal tubules. Renal MI depletion is also a common feature of diabetes, insulin resistance, and hypertension, suggesting that the GX pathway could contribute to the respective associated nephropathies by promoting oxidative stress. Inhibition of MIOX and the GX pathway may therefore offer a new therapeutic strategy to target chronic kidney disease.
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Mortality and requirement of renal replacement therapy in people with type 1 diabetes: a 30 years prospective observational study

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Background and aims: We investigated long term mortality and requirement of renal replacement therapy (RRT) in an observational cohort study of 647 patients with type 1 diabetes to study risk factors for late complications and mortality.
Materials and methods: In 1982/83 we recorded the anthropometrical and laboratory data of all 647 patients (47% females) with type 1 diabetes mellitus

who attended the outpatient department of a tertiary referral centre in Vienna for their yearly diabetes review. In 2012 we investigated the two endpoints death and renal replacement therapy by record linkage with national registries. Furthermore all patients still alive were asked questionnaires regarding other endpoints (cardio- and cerebrovascular, blindness, limb amputation).
Results: During the 30-years follow up, 156 patients died [mortality rate: 860 (95%CI: 721-1000) per 100.000 person years] and 55 received renal replacement therapy [incidence rate: 335 (95%CI: 247-424) per 100000 person years]. Mortality was higher in males (p<0.02), but incidence of RRT was equally high in both genders.395 of the still alive patients returned their questionnaires (response rate 86%). These patients reported 56 cardiac events, 24 strokes, 13 limb amputations and 8 blindness due to diabetes. Patients in the highest baseline HbA1c quartile (HbA1c above 8.3%) had the highest mortality rate and incidence of RRT (p<0.05).
Conclusion: In people with established Type 1 diabetes who were observed over 30 years, the overall mortality was 24% and the overall incidence of renal replacement therapy was 8.5%. Patients in the higher HbA1c quartiles were burdened with an increased mortality risk and an increased risk to need renal replacement therapy.
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Pitavastatin improves the estimated glomerular filtration rate (eGFR) in patients with type 2 diabetes and hypercholesterolaemia treated with sitagliptin
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Background and aims: Previous studies have shown that statins improve renal function in patients with chronic kidney disease. In this study, we examined the renal effect of pitavastatin in patients with type 2 diabetes and hypercholesterolemia, who had already been treated with or without the DPP-4 inhibitor sitagliptin.
Materials and methods: The subjects in this study were 81 patients with type 2 diabetes and hypercholesterolemia. Twenty-nine patients were treated with sitagliptin (SITA) and 52 patients were not (no-SITA). The patients were treated with 2mg pitavastatin for 6 months, and we evaluated the effects on eGFR, lipid profile, and glycemic control.
Results: Age (SITA 62±12 vs. no-SITA 63±10 years, p=0.71), male gender (55 vs. 56 %, p=0.96), body mass index (26.2±5.0 vs. 26.1±3.9 kg/m^2, p=0.90), smoking status (17 vs. 8%, p=0.20), systolic blood pressure (135±17 vs. 135±20 mmHg, p=0.88), fasting plasma glucose (FPG; 178±57 vs. 151±57 mg/dl, p=0.05), HbA1c (7.5±1.4 vs. 7.9±1.4%, p=0.21), total cholesterol (TC; 254±39 vs. 243±34 mg/dl, p=0.20), triglyceride (TG; 189±93 vs. 178±101 mg/dl, p=0.62), HDL-C (54±13 vs. 56±16 mg/dl, p=0.68), LDL-C (164±36 vs. 155±27 mg/dl, p=0.22), apolipoprotein B-100 (Apo B; 134±26 vs. 126±20 mg/dl, p=0.15), serum creatinine (Cr; 0.83±0.27 vs. 0.79±0.25 mg/dl, p=0.57), and eGFR (71.3±23.2 vs. 73.3±20.1 ml/min/1.73m^2, p=0.69) were not significantly different between the two groups. A significant increase of the eGFR was observed in the SITA group (71.3±23.2 to 84.2±22.7 ml/min/1.73m^2, p<0.001), but not in the no-SITA group (73.3±20.1 to 76.1±19.7 ml/min/1.73m^2, p=0.15), and the changes of Cr (-0.13±0.16 vs. -0.03±0.15 mg/dl, p=0.0079) and eGFR (+12.9±16.3 vs. +2.8±14.2 ml/min/1.73m^2, p=0.0049) were significantly different in the two groups after 6 months of pitavastatin treatment. Changes of TC (-78±36 vs. -64±37 mg/dl, p=0.09), TG (-25±138 vs. -1±132 mg/dl, p=0.43), HDL-C (+5±13 vs. +4±13 mg/dl, p=0.74), LDL-C (-73±35 vs. -64±34 mg/dl, p=0.26), Apo B(-46±27 vs. -34±19 mg/dl, p=0.05), FPG (-21±73 vs. -2±63 mg/dl, p=0.26) and HbA1c (-0.6±1.5 vs. -0.0±1.2 %, p=0.06) were not significantly different between the two groups.
Conclusion: These findings suggest that combination therapy of pitavastatin and sitagliptin may exhibit a renal protective effect in patients with type 2 diabetes and hypercholesterolemia.

PS 101 Nephropathy basic science: from profiling to targets

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Transcriptional profiling of kidney glomerulus in diabetic mouse models
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Background and aims: Diabetic nephropathy (DN) is the main cause of chronic kidney disease, which is characterised by a gradual decline in renal function, eventually leading to a need for kidney replacement therapy -dialysis or transplantation. Almost 50% of all diabetes patients will develop DN. The pathogenesis of DN is unknown, but likely to be multifaceted, with the involvement of metabolic, endocrine, hemodynamic, and inflammatory factors. DN pathogenesis is thought to onset in the kidney glomeruli.
Materials and methods: Previously, we developed a novel method for the isolation and enrichment of kidney glomeruli in mice, yielding ≈97% purity of glomerular preparations. The high glomerular purity allowed us to generate a glomerular transcriptome profiling. Here we use diabetic mouse model strains such as db/db, Akita, and BTBRob/ob, to extract the kidney glomeruli at different time points (1, 2, 4, and 6 months), and perform microarray in order to compare the transcriptional profiling of the different models analyzed.
Results: Our preliminary data from the db/db model has already identified 14 transcripts that are up- or downregulated in all timepoints analyzed (p<0.05). Upregulated genes are Chi3l3, Ankrd1, Grp, Fn1, Frzb, P2rx1, Fkbp5, Sirpb1. Downregulated genes are Negr1, Cacna2d2, Car3, Keg1, Calca, 2210023G05Rik. Interestingly, we could detect alterations in these transcripts before the onset of hyperglycaemia in the db/db model. We are currently performing pathway analysis as well as preliminary comparisons with public human data. In addition, we are waiting for the microarray results from the BTBRob/ob and Akita models.
Conclusion: Our developed method for high-yield, high-purity glomerular extraction allows us to charting the glomerular transcriptional changes that occur before and during the onset of hyperglycaemia in diabetic mouse models. Our data will prove of high value in validating findings in human samples as well as in monitoring the changes in diabetic mouse models.
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Electron and confocal microscopy of diabetic E1-DN mouse glomeruli revealed thickening of basement membrane and decreased nephrin expression
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Background and aims: Currently available animal models for diabetic nephropathy recapitulate only partially the features of the human disease. The transgenic E1-DN mice express a kinase-negative epidermal growth factor receptor (EGF-R) in their pancreatic islets and are diabetic due to impaired postnatal growth of pancreatic islet β-cells. The mice survive without insulin treatment, and can be used to investigate the effects of long term exposure to hyperglycaemia. The aim of this study was to characterize the renal phenotype of E1-DN mice, focusing on glomerular injury.
Materials and methods: The transgenic mice in FVB background were generated previously. E1-DN homozygous (n=9), heterozygous (n=14) and wild-type (wt) (n=12) mice were followed up to the age of 40 or 50 weeks (wks). Blood glucose, urine volume and albumin excretion were measured. Homozygous male mice were selected for further analysis based on albuminuria. Mesangial matrix expansion was measured using PAS-staining. Thickening of the glomerular basement membrane (GBM) was measured using electron microscopy, and the difference in the glomerular expression level of nephrin was assessed by confocal microscopy.

Results: Increased albumin excretion rate (AER) in homozygous E1-DN mice was detected at both 10 and 20 wks of age ($p<0.01$, Mann-Whitney U-test). At 20 wks, some of the homozygous mice had developed massive albuminuria. In histological analysis, PAS-staining revealed mesangial expansion in E1-DN homozygous mice at 20 wks, and focal glomerular sclerosis was observed in the most albuminuric mice at 50 wks. Measurement of PAS staining intensity per glomerular area showed an increase in homozygous mice compared to wt mice (n = 70 glomeruli from 7 homozygous mice, age 20-50 wks; n=30 glomeruli from 3 wt mice, age 50 wks; $p<0.001$, Student's t-test). Electron microscopy analysis indicated thickening of the GBM in homozygous E1-DN mice at 40-50 wks compared to wt mice at 50 wks (370 µm in homozygous [n= 8 glomeruli] vs. 250 µm in wt [n= 3 glomeruli], $p = 0.025$, Mann-Whitney U-test). In addition, podocyte foot process effacement was observed in homozygous E1-DN mice. The expression level of nephrin was lower in glomeruli of E1-DN homozygous mice (n=15 glomeruli) compared to wt mice (n=10 glomeruli) at 50 wks ($p<0.001$, Student's t-test). Membranous and cytoplasmic localization of nephrin was analyzed by co-staining with ZO-1, but no significant difference in localization between diabetic and control mice was observed.
Conclusion: Hyperglycaemic E1-DN mice develop substantial albuminuria. The histological and structural changes in glomeruli include mesangial expansion, thickening of the GBM and podocyte foot process effacement, and resemble human diabetic nephropathy. In addition, nephrin expression is reduced. Altogether, our data indicate that E1-DN mice are a valuable model to study diabetic nephropathy.
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Characterisation of transcriptional influences on the coagulation protease-activated protein C pathway in diabetic nephropathy
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Background and aims: Recent data demonstrated the prevention of diabetic nephropathy by activation of the serine protease activated protein C (aPC) pathway. In an animal model of diabetic nephropathy nephroprotection was associated with inhibition of glomerular apoptosis and mitochondrial dysfunction. The mechanism how the extracellular protease aPC prevents glomerular apoptosis and mitochondrial dysfunction remains unknown, but in other disease models aPC has been linked with altered gene-expression
Materials and methods: microRNAs (miRNAs) have shown to play a role in diabetic nephropathy. Thus, we performed microarray based mRNA and microRNA expression analysis of kidney samples from mice with and w/o diabetic nephropathy using wild type mice and two different mutants with single mutations affecting aPC availability (TMPro/Pro, low aPC plasma levels; and aPChigh, high aPC plasma levels).
Results: miRNA profiling of the kidney cortex revealed that miR-34a-5p was significantly upregulated in all diabetic mice, regardless of the genetic background (i.e. in both diabetic mutant mice), as compared to controls ($p<0.05$). miR-192-5p was also an indicator of diabetic nephropathy, also less significantly. We did not identify differentially expressed miRs in direct comparison between both diabetic mutants, indicating that no direct post-transcriptional regulation with miR participation is likely to occur within aPC pathway. The analysis also demonstrated the highest number of differentially expressed miRs (n=13) in diabetic TMPro/Pro vs. TMPro/Pro control mice. Seven of the detected miRs showed differential expression exclusively in this comparison: miR-1224-5p, miR-2681, miR-494-3p, miR-5105, miR-5109 and miR-5126, and miR-7e-5p. MiR-26b-5p was the only miR upregulated in diabetic aPChigh vs diabetic WT mice, while miR-148a-3p, miR199a-3p, miR-31-5p and miR-342-3p were all found to be downregulated only in diabetic TMPro/Pro vs. diabetic WT. Validation experiments were used to confirm selected array results. We constructed two networks of genes de-regulated in diabetes, starting with DEGs from comparison between both diabetic mutants. Genes included in the networks interact directly with and are connected with DEGs from comparison of both mutants and are considered particularly important protection against diabetic nephropathy. The most pronounced changes in expression were in diabetic TMpro mice (from 27.8 to -25.3 fold change), followed by diabetic WT mice (from 7.02 to -3.22 fold change), and diabetic aPChigh mice (from 6.32 to -3.25 fold change). Twelve of the aggravations signaling network genes are potentially regulated by four miRs, found specifically deregulated only in diabetic TMPro/Pro mice: let-7a-5p, miR-494, miR-1224-5p and miR-2861. The most pronounced changes in expression were in diabetic TMpro mice (from 27.8 to -25.3 fold change), followed by diabetic WT mice (from 7.02 to -3.22 fold change), and diabetic aPChigh mice (from 6.32 to -3.25 fold change).
Conclusion: These findings indicate that a deregulation of specific miRs is associated with deactivation of the aPC pathway in diabetic nephropathy.
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Experimental podocyte injury leads to reduced expression of CD2AP
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Background and aims: Podocyte injury and loss play a central role in the development of glomerular diseases, such as diabetic nephropathy. Podocyte loss may occur by apoptosis or detachment from the glomerular basement membrane, and it leads to defects in the filtration function and proteinuria. CD2-associated protein (CD2AP) is critical to the structural and functional integrity of the glomerular filtration barrier as mice lacking CD2AP develop nephrotic syndrome with heavy proteinuria and effacement of podocyte foot processes. Here, we studied the role of CD2AP in podocyte injury and apoptosis by using cultured podocytes and a rat model of puromycin aminonucleoside (PA) -induced acute nephrosis.
Materials and methods: Rats and cultured human podocytes were treated with PA to induce podocyte injury and apoptosis. The expression level of CD2AP was analysed by RT-PCR and Western blotting. The localisation of CD2AP in the PA-treated rat glomeruli was analysed by conventional immunofluorescence microscopy and immunoelectron microscopy. Apoptosis of cultured podocytes was measured by flow cytometry, and the involvement of different signalling pathways associated with apoptosis was analysed by Western blotting.
Results: RT-PCR analysis revealed that in 3-day and 10-day PA-treated rats, the mRNA level of glomerular CD2AP decreased to about 50% and 40%, respectively, compared to controls. Immunofluorescence microscopy of 10-day nephrotic kidneys showed a dramatic decrease in glomerular CD2AP signal. Immunoelectron microscopy revealed a dramatic disappearance of CD2AP from the podocyte foot processes 10 days after PA-injection. Occasionally the remaining CD2AP signal associated with cytoplasmic electron dense material, apparently representing disorganized actin cytoskeleton. PA-treatment of cultured human podocytes induced apoptosis, and significantly reduced CD2AP protein level and disrupted the actin stress fibers. Overexpression of CD2AP was able to reduce PA-induced apoptosis in cultured podocytes. Analysis of the activity of different apoptotic pathways revealed that PA-treatment induced the expression of proapoptotic BAX protein and caspase-3 -mediated apoptosis. Additionally, PA-treatment reduced antiapoptotic BCL-2 protein expression and Akt phosphorylation, but overexpression of CD2AP rescued BCL-2 protein expression and Akt phosphorylation and reduced BAX expression in cultured human podocytes.
Conclusion: Our results show that the development of PA-induced acute nephrosis is associated with distinct changes in glomerular expression and distribution of CD2AP, and that these changes are apparent already before appearance of major albuminuria. Disregulation of CD2AP may thus have a pivotal role in the pathogenesis of proteinuria in acute nephrosis. Overexpression of CD2AP may rescue experimentally induced podocyte injury by modulating the expression of both pro- and antiapoptotic proteins.
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Senescence marker protein-30 deficiency accelerates diabetic renal injury through tubulointerstitial fibrosis in a mouse model of type 1 diabetes

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Background and aims: Aging is a risk factor for the progression of diabetic nephropathy leading to end-stage renal failure. However, its mechanism has not been elucidated. Senescence marker protein-30 (SMP30) is abundantly expressed in renal tubular cells and decreases with aging. Our studies using SMP30 knockout mice have revealed that a reduction in SMP30 expression may contribute to age-associated deterioration of cellular function and the enhanced susceptibility to harmful stimuli in aged tissue. In this study, we investigated the effects of SMP30 deficiency on the pathogenesis of diabetic nephropathy.
Materials and methods: Diabetes (DM) was induced using streptozocin in male SMP30 Y/- mice (KO) and wild-type, SMP30 Y/+, mice (WT) at 7 weeks of age. Twelve weeks after the induction of diabetes, the mice were sacrificed for the study.
Results: Urinary albumin excretion was increased in KO compared to WT both in DM and non-DM condition ($P<0.05$). The proportions of cortical tubulointerstitial fibrosis area (Sirius red stain) were increased in KO compared to WT (1.02±0.20 vs 3.00±1.38%, $P<0.05$). In DM, this increase was markedly enhanced in KO (2.02±0.34 vs 19.34±6.10%, $P<0.05$). On the other hand, SMP30 deletion did not affect mesangial expansion (PAS stain). The changes in tubulointerstitial fibrosis were well associated with the expression of HIF-1α, not 4HNE, as assessed by immunohistchemistry. However, increase of HIF-1α mRNA was observed only in KO of non-DM condition ($P<0.05$). Similar to the HIF-1α protein expression, the mRNA expression of CTGF and MCP-1 were increased in KO both in non-DM and DM condition.
Conclusion: These results indicate that SMP30 deficiency deteriorates diabetic nephropathy through tubular injury. Stabilization of HIF-1α and following signaling may be mainly involved in these changes. The decrease of SMP30 could be a factor which explains the cross-talk between pathogenesis of various chronic kidney diseases, including diabetic nephropathy, and aging.

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Methylglyoxal induced insulin resistance in podocytes contributes to diabetic kidney disease

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Background and aims: The plasma concentration of the reactive carbonyl, methylglyoxal (MGO), is elevated in diabetes. Increased accumulation of MGO may contribute to insulin resistance at peripheral sites of glucose uptake. A deficiency in podocyte insulin signalling impairs podocyte function resulting in chronic kidney disease. Hence, we examined the effects of MGO accumulation on glucose trafficking and insulin signalling in podocytes.
Materials and methods: Human podocytes were exposed to MGO or an inhibitor of glyoxalase-1 (GLO-1), an enzyme considered to detoxify MGO. Podocyte glucose uptake was determined using 2-deoxyglucose. Podocyte insulin sensitivity was assessed using pAKT/AKT and membranous GLUT4 protein expression using Western immunoblotting. Male db/db mice (reminiscent of human type 2 diabetes) and db/H control mice were administered with GLO-1 on alternate days from weeks 6 to 9 of life (50mg/kg body weight) and renal function and glycaemic control were assessed.
Results: Human podocytes exposed to MGO or an inhibitor of GLO-1 showed a decline in insulin-dependent glucose uptake and reduced insulin signalling with lower pAKT/AKT ratios and GLUT4 membrane translocation. In the db/db mouse, serum cystatin C was elevated at 9 weeks, and this was exacerbated with GLO-1 inhibition. Peripheral insulin resistance in db/db mice however, was not different under the presence of GLO-1 inhibition. Decreased glucose uptake, insulin signalling and expression of GLUT4 in human podocytes exposed to MGO or an inhibitor of GLO-1 were consistent with the degree of renal dysfunction in diabetic mice.
Conclusion: The accumulation of the reactive carbonyl MGO in diabetes may contribute to renal impairment by adversely affecting podocyte insulin sensitivity.
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Unexpected behaviour of aminoguanidine on human embryonic kidney cell (HEK293) exposed to glycated albumin

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Background and aims: The accumulation of extracellular matrix proteins in the glomerular mesangium and tubular basement membranes is a hallmark of diabetic nephropathy. Advanced glycation end products (AGEs) and TGF-β1 seem to mediate critical steps that lead to this type of pathological modifications. Our aim was to investigate the role of AGEs receptor-RAGE in overexpression of collagen IV in HEK293 cells exposed to glycated BSA (AGE-BSA) and the pathway through which aminoguanidine (AG) could prevent these modifications correlated with diabetic nephropathy development.
Materials and methods: Cultured cells were treated for 12 and 24 hours with AGE-BSA or BSA (control) at concentrations between 50-200 μg/ml in the presence or absence of 10-100 μM AG. The levels of RAGE, TGF-β1 and procollagen α1 (IV) mRNAs were analyzed by quantitative real-time PCR whereas protein expression of RAGE from cell membranes and collagen type IV and TGF-β1 from culture media were assessed by Western blot analysis.
Results: All target genes mRNA relative expression ratios (R) increased with both time exposure and AGE-BSA concentration. After 12 hours of exposure to 200 μg/ml AGE-BSA the R of RAGE increased to 1.21+/-0.06, TGF-β1 increased to 1.25+/-0.1 and procollagen α1 (IV) was upregulated to 1.17+/-0.07. At the same AGE-BSA dose, after 24 hours the target genes RAGE, TGF-β1 and respectively procollagen α1 (IV) were further upregulated to 1.83+/-0.23, 3.4+/-0.09 and 4+/-0.12. The protein levels of RAGE, TGF-β1 and collagen were in good correlation with the mRNA expressions. After the 12 hour co-treatment with AG and 200 μg/ml AGE-BSA, the protein expression for all target proteins showed a proportional increase with AG level compared with the treatment in the absence of inhibitor. The lowest AG dose (10 μM) seemed to amplify the expression of the three genes, as their R reached the highest values of 2.27+/-0.18, 1.85+/-0.05 and 1.53+/-0.08 for RAGE, TGF-β1 and respectively procollagen α1 (IV). At 50 and 100 μM AG, R decreased for RAGE and TGF-β1 and remained nearly unchanged for procollagen α1 (IV), compared to treatment with 10 μM AG. After 24 hours, the treatment with 10 μM AG led to an additional increase of R for all target genes, whereas 50 and 100 μM AG concentrations downregulated the mRNA expression, but only at 100 μM AG, R had subunitary value for RAGE (0.84+/-0.09). In addition, the protein expression for all target proteins, had a downward trend, reaching at 100 μM AG subunitary values, compared to AGE-BSA treatment in the absence of inhibitor.
Conclusion: These results show that AGEs can interfere in collagen IV synthesis through RAGE receptor dependent pathway in which TGF-β1 could have a pivotal role. It seems that at short time intervals (<12 hours) and very low concentrations (<10 μM), not only that AG is unable to block AGEs binding to RAGE, but moreover, due its decomposition, it may have a pro-oxidant effect promoting ROS formations, which in turn, can induce RAGE activation. It is also possible that AG reacted with some albumin-bound dideoxyosones derived from Amadori products competing with arginine residues and thus generated new AGEs products. AG positive effects were observed after 24 hour at higher concentrations, when it could more effectively trap AGEs and inhibit the AGEs-RAGE pathway.
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Anti-fibrotic peptide AcSDKP restores diabetes-suppressed microRNA-let-7 cluster and exhibits therapeutic effect on kidney fibrosis in diabetes

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Background and aims: Fibroblast play vital roles in organ fibrosis process, including kidney. Endothelial to mesenchymal transition (EndMT) has emerged as one of the important origins of matrix producing fibroblast. Endogenous anti-fibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is degraded by angiotensin-converting enzyme (ACE) and ACE-inhibitor increased plasma levels of AcSDKP. microRNA let-7 family exhibits potential anti-EndMT effect.
Materials and methods: In vivo experiments were performed in streptozotocin (STZ)-induced diabetic CD1 mouse, the diabetes-associated kidney fibrosis model. Human umbilical vein endothelial cells (HUVEC) and human microvascular endothelial cells (HMVEC) were stimulated with transforming growth factor (TGF)-β2 or combination of TGF-β2, interleukin-1β and tumor necrosis factor-α with or without AcSDKP incubation. microRNA expression was analyzed by array and qPCR.
Results: Herein we found that AcSDKP inhibited EndMT and exhibited anti-fibrotic effects associated with microRNA let-7 family cluster alterations in vivo and in vitro. When analyzing the kidney fibrosis in STZ-induced diabetic CD1 mice, the conventional ACE-inhibitor plus AcSDKP add-on therapy significantly ameliorated kidney fibrosis in association with EndMT inhibition. In diabetic kidney, mmu-let-7 family cluster was suppressed when compared to non-diabetic kidney; anti-fibrotic and anti-EndMT effects of AcSDKP in diabetic kidney were associated with restored mmu-let-7 expression. TGF-β2 or cytokines combination induced HUVEC/HMVEC cells into spindle shapes and reduced endothelial markers CD31/VE-cadherin expression together with the induction of mesenchymal markers such as αSMA or SM22α expression, suggesting induction of EndMT. Such induced EndMT was restored by the co-incubation with AcSDKP in either HUVEC or HMVEC. In vitro anti-EndMT effect of AcSDKP was also associated with the hsa-let-7 family alterations.
Conclusion: These results suggest that AcSDKP is potential valuable endogenous anti-fibrotic molecule via partially inhibition of EndMT through microRNA let-7 alternations.
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Theobromine cocoa prevents high glucose-induced extracelular matrix accumulation via activation of NAD+-dependent deacetylase sirtuin-1 in human mesangial cells

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Background and aims: Oxidative stress is a critical underlying mechanism for microvascular complications of diabetes, including diabetic nephropathy (DN). Excess oxidative stress causes increased extracellular matrix (ECM) accumulation with progression to fibrosis and end stage renal disease. High polyphenol substances such as cocoa reduce oxidative stress. Surprisingly, we have observed that also cocoa with low amount of polyphenols ameliorated diabetes-induced oxidative stress and ECM accumulation in diabetic rats. HPLC and mass spectrometry analyses revealed that theobromine was an important component of the cocoa extract with low polyphenols. Sirt1 is a class III histone deacetylase with deacetylase activity, depending on intracellular NAD^+ concentrations. Activation or overexpression of Sirt1 is known to protect against oxidative stress, apoptosis and ECM accumulation in the diabetic kidney. The aim of the present study was to assess if theobromine is effective in reducing ECM accumulation induced by high glucose via Sirt1 activation in immortalized human mesangial cells (iHMCs).
Materials and methods: iHMCs were cultured for 24 h in normal glucose (NG; 5 mM), high glucose (HG; 30 mM) or hydrogen peroxide (H_2O_2; 10 μM), with or without theobromine treatment (44 nM), a blocker of NADPH-induced oxidative stress such as diphenyleneiodonium chloride (DPI; 50 nM), NAD+, or Sirt1 activity blocker (EX-527). Oxidative stress was assessed by measurement of reactive oxygen species (ROS) by H_2DCF-DA (2',7'-dichlorodihydro-fluorescein diacetate) and quantification of NADPH oxidase-dependent superoxide (SO) formation. NAD+ and Sirt1 activity levels were determined in total iHMC lysates by colorimetric and fluorometric kits, respectively. ECM accumulation was assessed via western blot for type IV collagen and fibronectin expressions. Immunoprecipitation assay was performed by immunoprecipitating total Smad3 and carrying out western blot for acetylated Smad3.
Results: In iHMCs high glucose or H_2O_2 increase ROS production (P<0.0001), mainly through NADPH oxidase pathway, and decrease NAD+ levels (P=0.0005) and Sirt1 activity (P<0.0001). These abnormalities were prevented by treatment with theobromine. Theobromine did not confer additional rise in NAD+ and Sirt1 activity when co-treated with DPI (P<0.0001) in comparison to DPI treatment alone (P<0.0001). Immunoprecipitation of total Smad3 followed by western blot for acetylated Smad3 showed that theobromine significantly reduced HG-induced acetylated Smad3 (P<0.0001) and this was reversed (P<0.0001) by blockade of Sirt1 with EX-527. Similarly, EX-527 reversed significantly (P=0.018) the ameliorating effect of theobromine in HG-induced rise in type IV collagen and fibronectin (P=0.02).
Conclusion: In summary, theobromine improves HG-induced ECM accumulation via reduction in NADPH-induced ROS production, rise in NAD+ and Sirt1 activity levels and reduction in acetylated Smad3.
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***Cocoa* enriched in polyphenols is renoprotective in experimental diabetes mellitus by reducing transforming growth factor beta-1 (Tgfbeta-1) signalling**

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Background and aims: Oxidative stress (OS) has been implicated in the pathogenesis of diabetic vascular complications, including nephropathy. Cocoa enirched in polyphenols has been reported to be beneficial in endothelial dysfunction due to its antioxidative properties. The aim of the present study was to investigate the effect of cocoa enriched in polyphenols in nephropathy (DN) of diabetic spontaneously hypertensive (SHR) rats.
Materials and methods: Twelve-week-old SHR rats were rendered diabetic by intravenous injection of streptozotocin (60 mg/Kg), whereas control rats received citrate buffer. Blood glucose levels of ≥ 270 mg/L were considered diabetic. Diabetic SHR rats were randomized to receive or not treatment with cocoa enriched in polyphenols (Barry Callebout, Belgium, 24 mg/Kg b.w./day) by gavage. After 16 weeks of treatment, extracellular matrix (ECM) accumulation was evaluated by periodic acid-Schiff (PAS) staining, and western blot and immunohistochemistry for collagen IV (C-IV), fibronectin (F/N), phosphorylated Smad2 and Smad3. OS was determined by quantification of NADPH oxidase-dependent superoxide (SO) generation and by western blot and immunohistochemistry for nitrotyrosine (N/T) expression. For *in vitro* studies, immortalized human mesangial cells (iHMCs) were cultured for 24 h in normal glucose (NG; 5mM) or high glucose (HG; 30mM), or H_2O_2 or TGFβ-1 (5 ng/ml) with or without cocoa treatment (100 ng/ml) and blockers of OS such as N-acetyl cysteine (NAC; 1 mM) and diphenyleneiodonium chloride (DPI; 50 nM). OS was assessed by measurement of reactive oxygen species (ROS) by H_2DCF-DA (2',7'-dichlorodihydro-fluorescein diacetate) and NADPH oxidase-dependent SO generation. TGFβ-1 release was measured in the supernatants of iHMCs by a TGFβ-1 ELISA kit.
Results: Plasma glucose levels (P=0.0001) were higher in untreated and treated diabetic SHR rats than in nondiabetic group. Blood pressure levels and albuminuria were not modified by cocoa treatment. Cocoa treatment significantly reduced diabetes-induced mesangial matrix expansion (P=0.02), C-IV (P=0.0005), F/N (P=0.003), phosphorylated Smad2 (P<0.0001) and Smad3 expression (P=0.04). Cocoa also ameliorated diabetes-induced NADPH oxidase-dependent SO formation (P=0.016), and N/T levels (P=0.0001) in SHR rats. In *vitro* studies showed an inhibition in HG-induced ROS production (P<0.0001) and NADPH oxidase-dependent SO generation (P=0.003) by both treatment of cocoa and its major component, epicatechin, (41 nM) (P=0.0002). Collagen IV, F/N, phosphorylated Smad2 and Smad3 expression (P<0.0001) and TGFβ-1 release were significantly reduced by cocoa and epicatechin treatment under HG levels. Similar results with cocoa treatment were obtained when iHMCs were exposed to H_2O_2 or TGFβ-1 (P<0.0001).

Cocoa did not confer additional reduction when co-treated with NAC or DPI in HG-induced rise in F/N , C-IV, phosphorylated Smad2 and Smad3 and TGFβ-1 release in comparison to DPI or NAC alone (P<0.0001).
Conclusion: In summary, cocoa enriched in polyphenols and particularly epicatechin, improves DN by ameliorating TGFβ-1 signalling-induced ECM accumulation via inhibition of OS and particularly NADPH oxidase activity.
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PS 102 Hypertension: from experimental evidence to bedside

1202

Silencing of a disintegrin and metalloproteinase (ADAM)17 enhances ACE2 protein expression and activity in COS7 cells
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Background and aims: Diabetic Nephropathy (DN) is one of the most common complications of diabetes and a major cause of end-stage renal failure. Angiotensin converting enzyme 2 (ACE2) is conceded to play a renoprotective role by converting Ang II to Ang-(1-7). Administration of recombinant ACE2 is shown to ameliorate albuminuria and kidney function in animal models of diabetes. Indeed, loss of renoprotective ACE2 was observed in diabetic animal models due to increased ACE2 shedding mediated by a Disintegrin and Metalloproteinase (ADAM) 17. COS7, a robust and easily transfectable cell line derived from the kidney of an African green monkey are used to directly demonstrate and assess the role of ADAM17 on ACE2 shedding. The aim of this study was to demonstrate the presence of endogenous and functional RAS and ADAM17 in COS7 cells. We hypothesize that silencing of ADAM17 could be a potential target in the regulation of ACE2 expression and activity.
Materials and methods: RNAi-based silencing of ADAM17 in COS7 cells was done by stably expressing lentiviral constructs encoding short-hairpin RNAs (shRNAs) directed against ADAM17. Silenced cells were then grown to 90-100% confluency and GFP expression was used to screen stable transfectants. Western blot and immunohistochemistry were used to determine RAS components and ADMA17 at the protein level. ACE2 activity was measured using Mca-APK [Dnp] fluorogenic substrate.
Results: Immunoreactive bands for renin (41 kDa), ACE (195 kDa), ACE2 (90 kDa) and ADAM17 (93 kDa) were observed. Silencing of ADAM17 was confirmed using Western blot. Silenced cells demonstrated a significant increase of ACE2 activity in comparison with wild type cells (3.8 pmoles/hr/μg protein vs. 2 pmoles/hr/μg protein, $p<0.05$). In accordance with the activity, expression of ACE2 was also upregulated in the ADAM17 silenced cells.
Conclusion: This is the first report to demonstrate functional and endogenous expression of the RAS in COS7 cells and the crosstalk between ADAM17 and the regulation of ACE2. The transfectable nature of this cell line makes it an attractive *in vitro* model for manipulating components of the renal RAS.
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1203

Deletion of GIPR (glucose-dependent insulinotropic polypeptide receptor) in mice results in reduced blood pressure and changes in vascular contractility
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Background and aims: GIP and GLP-1 (glucagon-like peptide-1) are secreted after a meal to stimulate insulin secretion. In addition to their insulinotropic activity, a plethora of actions have been described in other tissues, including effects on the cardiovascular system. Evidence suggests a cardioprotective role of GLP-1, but less is known about GIP in this context. Previous studies demonstrated effects of GIP on splanchnic blood flow, with increased flow in the superior mesenteric artery and portal vein and decreased pancreatic and hepatic blood flow. These effects were suggested to be achieved by differential production of endothelin-1 (ET-1) and nitric oxide (NO) by endothelial cells. In the work presented here, we study the effect of GIPR deletion in mice on blood pressure and vascular contractility.
Materials and methods: GIPR knockout (KO) and wildtype (WT) littermate mice were used. Blood pressure was measured using tail-cuff plethysmography and vascular contractility was examined in small mesenteric resistance arteries and saphenous arteries by wire myography. Serum levels of ET-1 and

nitrate/nitrite (NOx) levels were determined by commercially available assays.
Results: In GIPR KO mice, we found significantly lower systolic blood pressure when compared to WT control mice (103.1 ± 1.8 vs. 113.5 ± 1.7 mm Hg, p=0.0009). We found no effect of acute stimulation with naturally occurring GIP(1-42) or the stable GIPR agonist D-Ala2-GIP(1-42) on contraction of small mesenteric resistance arteries or saphenous arteries from WT mice, nor did these peptides relax vessels pre-contracted with potassium (30 mmol/L). In contrast, the contractile response to depolarization by potassium (60 mmol/L) was significantly reduced in both small mesenteric arteries and saphenous arteries from GIPR KO mice, when compared to WT mice. Cumulative concentration-response curves using the selective α_1-receptor agonist cirazoline revealed greater force in saphenous arteries from GIPR KO mice compared to WT mice, which could reflect a potential upregulation of α_1-receptor signaling to compensate for the lower blood pressure. Pre-treatment with GIP(1-42) had no effect on cirazoline-induced force in WT mice. No differences in serum ET-1 or NOx levels were found.
Conclusion: Our data demonstrate a role for GIPR in the regulation of blood pressure and vascular contractility. Inhibition of GIPR signaling may have beneficial effects in hypertension and vascular disease.
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1204

Prevalence of hypertension and its determinants among Bangladeshi type 2 diabetic subjects
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Background and aims: Hypertension, an important risk factors for cardiovascular diseases, is known to be more prevalent in diabetic patients; however the prevalence and its determinants vary substantially from population to population. The prevent study was undertaken to investigate the prevalence and determinants of hypertension in a group of Bangladeshi type-2 diabetes mellitus subjects. Aims: This study was aimed to investigate Prevalence of hypertension and its determinants among Bangladeshi type-2 diabetic subjects.
Materials and methods: A cross-sectional study was conducted among 18697 subjects 11917 diabetic and 6780 non diabetic purposively from selected 16 diabetic hospitals health centre located in the capital, Dhaka and in northern part of Bangladesh. Data were collected using a pre-tested, semi-structured questionnaire by face to face interview. Anthropometric measurement and biochemical analysis were done by standard techniques. Hypertension was diagnosed using IDF criteria for this population. Data were analyzed by univariate as well as bivariate analysis. Logistic regression was applied to estimate the odds ratio (OR) and corresponding 95% confidence intervals (CI) for the different explanatory variables.
Results: 42% of the subjects were male and 58% were female. The mean age (±SD), years the non diabetic and diabetic subjects was 46 (±14) and 57% respectively. The corresponding BMI values were 23.7(±3.8) and 25.4 (±3.9). The diabetic group had a substantially higher proportion of hypertensive subjects compare to the non diabetic group (38% vs 7%).On logistic regression analysis, hypertension was found to have strongly significant($p<0.001$) association with increasing age, urban resident, higher socioeconomic status, smoking, higher BMI and west hip ratio and high total cholesterol.
Conclusion: Bangladeshi type 2diabetis mellitus subjects are about 7 times were pron to hypertension compare to their no diabetic counter patient. Irrespective to the percent of diabetic age, urbanization, high-low economic status, generalized and central obesity and hypercholesterolemia an independent risk factors of hypertension in this population.

1205

Normal fasting glucose and HbA_{1c} levels and the development of hypertension in Japanese individuals
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Background and aims: Whether higher fasting plasma glucose (FPG) or HbA1c concentrations within normal range would independently predict hypertension remains unclear. Since the development of high blood pressure would coincide with the development of hyperglycemia, clarifying normal glucose or HbA1c concentrations as risk factors for the development of hypertension among normoglycemic individuals can be considered to be clinically important. We investigated whether FPG or HbA1c concentrations could help detect normoglycemic individuals at increased risk for future hypertension.
Materials and methods: We investigated 8786 individuals without known diabetes and hypertension (hypertension indicated by self-reported history of treatment, systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg). Logistic regression analysis was performed using FPG or HbA1c as a continuous variable (1-SD increment) or quartile (Q) categories for the development of hypertension; odds ratios (ORs) and 95% CIs were calculated.
Results: We documented 1012 incident cases of hypertension at 5 y after the baseline examination. A multivariate model adjusted for age, sex, body mass index (BMI) and pre-hypertension showed a progressively increased risk of hypertension among individuals with FPG 89-93 mg/dL (odds ratio (OR) 1.38 (95% CI 1.11, 1.72)), FPG 94-99 mg/dL (OR 1.43 (1.15, 1.78)) or FPG ≥100 mg/dL (OR 1.87 (1.51, 2.33) than those in the bottom quartile (<89 mg/dL), P for trend <0.001). Further adjustment for parental history of hypertension and metabolic risk factors attenuated the association but the trend remained significant (P=0.001). No significant association was observed across quartile categories of HbA1c concentrations below pre-diabetic ranges and increased risk of hypertension. The joint effect of hyperglycemia and either overweight, older age or pre-hypertension resulted in substantially elevated ORs for hypertension than the absence of such an association.
Conclusion: Higher FPG levels within normoglycemic range rather than HbA1c levels were predictive of future hypertension among Japanese individuals. Elevated glycemic markers may help along with older age, BMI and pre-hypertension to identify apparently healthy individuals at increased risk for hypertension.
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1206

The effect of pre-measurement rest time on ankle systolic pressures in people with and without diabetes
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Background and aims: Systolic ankle pressures are routinely measured as part of an ankle-brachial index to screen for the presence of lower extremity peripheral arterial disease in people aged 50 years and over with diabetes and, in the general population aged 65 years and over. Despite widespread use of this measurement, the effect of pre-measurement duration of rest on the magnitude, or reliability of the ankle systolic pressure measurement is unknown. Similarly although diabetes has been associated with reduced reliability of brachial pressures and altered blood pressure control, possible disease-related effects on ankle systolic pressure and therefore the ankle-brachial index have not been investigated. The aim of this study was to assess the effect of pre-measurement rest duration on systolic ankle pressures on people with and without diabetes.
Materials and methods: One hundred and forty participants meeting current guidelines for screening for peripheral arterial disease including 64 participants with Type 2 diabetes volunteered for this study. Following 5 minutes of rest in the supine horizontal position, ankle systolic pressures from either the dorsalis pedis or posterior tibial artery of the left or right lower extremity

were taken using hand-held Doppler. Measurements were repeated at 10 and 15 minutes. Testing was repeated 7-10 days later.
Results: A significant drop in ankle pressure of 5.02 mmHg occurred between 5 and 10 minutes ($p<0.05$) however no significant change occurred between 10 and 15 minutes (mean change 0.15 mmHg, $p=0.99$). Presence of diabetes was associated with a smaller drop between 5 and 15 minutes (mean change 1.85 mmHg) and predicted 14% of the variance in change in ankle pressure (beta:-3.72, $p>0.05$). In the total population test-retest reliability after 5 minutes was excellent (Intraclass correlation coefficient [ICC]: 0.84, 95% confidence interval [CI]: 0.76 to 0.91) however increased for measurements taken at 10 and 15 minutes (ICC: 0.89 95% CI: 0.83 to 0.94 and 0.89 95% CI: 0.82 to 0.93 respectively). Diabetes was associated with a reduction in test re-test reliability at 10 minutes when compared to those without diabetes (ICC:0.82 95% CI 0.76 to 0.88 and ICC 0.90 95% CI: 0.85 to 0.94 respectively).
Conclusion: Results suggest ankle systolic pressures stabilise after 10 minutes of rest. Longer periods of pre-measurement rest did not improve reliability of the measurement significantly. Presence of diabetes is associated with an altered response of ankle systolic pressure to rest and a reduction in test-retest reliability.

1207

Prognostic value of reading-to-reading blood pressure variability over 24 hours in 584 patients with diabetes from 11 populations

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Background and aims: Information on the predictive value of blood pressure (BP) variability recorded over 24-h in patients with diabetes is scarce.
Materials and methods: We followed health outcomes in 584 patients with diabetes (mean age, 62.0 years; 40.1 % women) randomly recruited from 11 populations. Diabetes was the use of antidiabetic drugs, a fasting blood glucose concentration of at least 7.0 mmol/L, a random blood glucose concentration of at least 11.1 mmol/L, a self-reported diagnosis, or diabetes documented in practice or hospital records. At baseline, we assessed BP variability from the standard deviation (SD) and average real variability (ARV24) in 24h ambulatory BP recordings. We computed standardized hazard ratios (HR), while stratifying by cohort and adjusting for 24h BP and other risk factors.
Results: Over 11.0 years (median), 165 deaths (85 cardiovascular) occurred. The incidence of a fatal or nonfatal cardiovascular event was 155, and 75 experienced a stroke. Higher diastolic ARV24 predicted ($P\leq0.002$) total (HR: 1.28) and cardiovascular (HR: 1.42) mortality as well as the combined cardiovascular endpoint (HR: 1.19, $P=0.040$) and stroke (HR: 1.38, $P=0.006$). Higher systolic ARV24 predicted cardiovascular mortality (HR: 1.27, $P=0.045$) but none of the other endpoints ($P\geq0.08$). Higher diastolic SD was significantly ($P\leq0.042$) associated with total (HR: 1.21) and cardiovascular (HR: 1.31) mortality and fatal and non-fatal stroke (HR: 1.42; $P=0.009$). Systolic SD did not predict any of the endpoints ($P\geq0.086$). While accounting for the 24-h BP level, ARV24 added <1% to the prediction of a cardiovascular event.
Conclusion: In patients with diabetes the BP variability assessed from 24-h ambulatory recordings was an independent predictor of mortality and of cardiovascular and stroke events. However, the proportion of the risk explained by the variability indices was low.

1208

Targeting nocturnal hypertension in type 2 diabetes: bedtime dosing of once-daily antihypertensive drugs reduces night-time BP and 24-h BP

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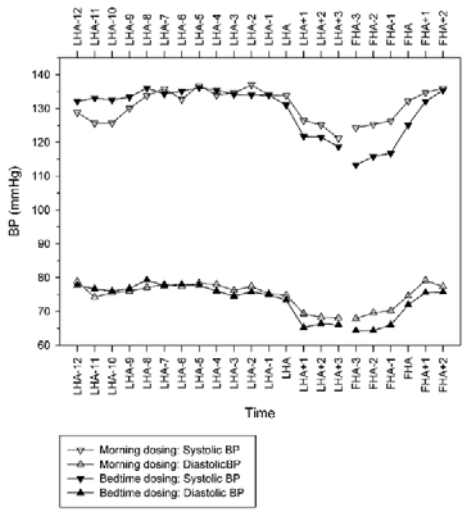
Background and aims: Several studies have suggested nighttime BP as an independent risk factor for cardiovascular disease, stronger than daytime BP. Therefore, new strategies to treat increased nighttime BP are warranted. In a population of type 2 diabetic patients with nocturnal hypertension, our aim was to investigate whether bedtime dosing of once-daily antihypertensive drugs will reduce nighttime BP without an increase in daytime BP.
Materials and methods: We included 41 type 2 diabetic patients with nocturnal hypertension (nighttime systolic BP (SBP) > 120 mmHg) in an open-label, cross-over study. Patients were randomized to 8 weeks of either morning or bedtime dosing of all once-daily antihypertensive drugs, followed by 8 weeks of switched treatment regimen. At baseline and after each of the two 8-week periods, patients underwent ambulatory blood pressure monitoring, measurements of arterial stiffness, and blood and urine testing.
Results: The median number of once-daily antihypertensive drugs was 3 (range 1-6). Nighttime SBP with morning dosing (MD) and bedtime dosing (BD) were 125.3±10.2 mmHg and 117.8±10.7 mmHg, respectively (difference 7.5±10.1 mmHg, $p<0.001$); daytime SBP with MD and BD were 134.2±9.8 mmHg and 133.0±12.1 mmHg, respectively (difference 1.3±8.5 mmHg, $p=0.336$); and 24-h SBP with MD and BD were 131.7±8.7 mmHg and 128.7±10.9 mmHg, respectively (difference 3.1±7.7 mmHg, $p=0.014$). The same pattern of significant decrease in nighttime and 24-h BP and insignificant decrease in daytime BP was found for diastolic BP, mean arterial pressure and pulse pressure. Morning surge was not significantly different between treatment regimens. Figure 1 illustrates the systolic and diastolic BP curves with morning and bedtime dosing for the 41 patients; the hourly averages have been fixed at each individual's last hour awake (LHA) and first hour awake (FHA). Pulse wave velocity and central BP parameters did not differ significantly between MD and BD. Analyses of renin-angiotensin-aldosterone parameters, markers of endothelial dysfunction, and urine albumin/creatinine ratio showed no significant changes. However, with BD urine sodium/creatinine ratio was significantly increased (MD: 14.3±6.7; BD: 18.8±6.4; difference 4.5±7.0, $p<0.001$) and urine osmolality was significantly decreased (MD: 563.5±191.8 mosmol/kg; BD: 509.4±172.2 mosmol/kg; difference 54.1±155.0 mosmol/kg, $p=0.031$).
Conclusion: We found a significant decrease in nighttime BP and, importantly, also a significant decrease in 24-h BP with BD. Despite lower nighttime BP, BD did not result in higher morning surge. Interestingly, the reduced nighttime BP with BD was associated with increased nocturnal natriuresis. In patients with type 2 diabetes and nocturnal hypertension, dosing of antihypertensive drugs at bedtime may be favorable.

Figure 1: Systolic and diastolic BP curves with morning and bedtime dosing for the 41 patients. The hourly averages have been fixed at each individual's last hour awake (LHA) and first hour awake (FHA)



Clinical Trial Registration Number: NCT01158625

1209

Arterial stiffness is associated with diabetic complications in type 1 diabetes and normalisation of albuminuria is associated with reduced arterial stiffness

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Background and aims: We investigate the associations between diabetic complications and arterial stiffness in patients with type 1 diabetes.
Materials and methods: Cross-sectional study including 676 patients with type 1 diabetes. Arterial stiffness was measured by pulse wave analyses (PWA) (SphygmoCor (Atcor Medical, Australia)), as central pulse pressure (CPP), central aortic systolic pressure (CASP), heart rate adjusted augmentation index (AI75), augmentation pressure (AP) and pulse wave velocity (PWV). Patients were classified as normoalbuminuric if the urinary albumin excretion rate (UAER) persistently was <30 mg/24h, and micro- or macroalbuminuric if the UAER had ever been 30-299 mg/24h or ≥300 mg/24h, respectively, in two consecutive measurements in the absence of other kidney or urinary tract disease. History of cardiovascular disease (CVD) was myocardial infarction or stroke. Simple or proliferative retinopathy or blindness collectively defined retinopathy. Cardiac autonomic neuropathy was defined by a heart rate variability (HRV) <11 beats/minute.
Results: PWAs were available in 621 patients (mean age: 54.6 years; 54.8% men). Mean CPP, CASP, AI75, AP and PWV was: 43 mmHg, 118 mmHg, 17, 10 mmHg and 10.4 m/s, respectively. All measures were independently associated with presence of ≥1 diabetic complication (CVD, retinopathy and cardiac autonomic neuropathy) (p<0.05 for all) (adjusted for gender, diabetes duration, mean arterial pressure, heart rate, kidney function, HbA_{1c}, cholesterol and antihypertensive medication). Per SD increase, CASP was strongest associated with CVD and cardiac autonomic neuropathy (HR=3.5 and 4.6, p<0.001 for both) and PWV with retinopathy (HR=1.9, p=0.008). In adjusted analyses, all measures of arterial stiffness were similar in normoalbuminuric patients with low (<15mg/day) *vs.* high UAER at follow up and in previously microalbuminuric patients (30-299mg/day) with normalised *vs.* elevated UAER (≥30mg/day) at follow up (p≥0.083 for all). However, in previously macroalbuminuric patients (≥300mg/day), PWV was significantly lower in patients with normalised (<30 mg/day, n=35) *vs.* elevated UAER (n=129) at follow up (10.0±2.1 *vs.* 11.9±3.3 m/s (p=0.007). Although, all other measures of arterial stiffness were similar in the two groups.
Conclusion: In patients with type 1 diabetes, arterial stiffness is associated with complications. CASP and PWV showed the strongest associations. Patients previously diagnosed as macroalbuminuric with normalised UAER on reno-protective treatment had lower arterial stiffness than non-responders. Thus, maybe responders have lower arterial stiffness than non-responders, or arterial stiffness may be modifiable by reno-protective treatment. However, this needs to be confirmed in prospective studies.
Supported by: NCT01171248

1210

Blood pressure independent effect of renin angiotensin system blockade on soluble klotho levels in patients with type 2 diabetes and albuminuria

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Background and aims: Soluble Klotho is an anti-ageing phosphaturic protein associated with vascular-renal protection. In-vitro and in-vivo studies have demonstrated that renin-angiotensin-system (RAS) blockade increases soluble Klotho levels. The effect of RAS-blockers on soluble Klotho in patients with type 2 diabetes and albuminuria is unknown.
Materials and methods: Plasma soluble Klotho was measured in a secondary analysis of a randomised controlled clinical trial performed at a single university hospital centre. Seventy six patients with type 2 diabetes, systolic hypertension and albuminuria were studied at baseline and at 24- weeks (end of study), following randomisation to valsartan/hydrochlorothiazide (n=37) or amlodipine (n=39) treatment. All patients had an estimated glomerular filtration rate >45 ml/min/1.73 m^2. Aortic-pulse wave velocity (Ao-PWV) by applanation tonometry and albuminuria (from 3 timed urine collections) were also measured at baseline and 24-weeks.
Results: There were no significant baseline differences between the two groups. Valsartan/hydrochlorothiazide treatment significantly increased soluble Klotho mean±standard deviation, from 432.7±179 to 506.4±226.8 pg/ml, p=0.01 and reduced serum phosphate 1.05±0.38 to 0.84±0.31 mmol/l, p=0.04 compared to amlodipine (430.1±145.8 to 411.9±157.6 pg/ml and 0.95±0.18 to 0.87±0.49 mmol/l]. There was a significant between treatment group difference, mean (95% confidence interval), in soluble Klotho, 91.9 (19.9 to 162)pg/ml and serum phosphate levels -0.22 (-0.05 to -0.43)mmol/l with valsartan/hydrochlorothiazide treatment, p<0.05 for both. In the valsartan/hydrochlorothiazide group change in soluble Klotho was negatively correlated with change in phosphate (Pearson correlation R^2 -0.41 p=0.07). Attained blood pressure was similar in the two-groups and levels of soluble Klotho were not associated with Ao-PWV and albuminuria, variables which fell significantly only with valsartan/hydrochlorothiazide.
Conclusion: Treatment with valsartan, a RAS blocker, significantly increases soluble Klotho levels and reduces serum phosphate, effects which appear to be independent of its blood pressure lowering actions. These results suggest that elevations in soluble Klotho levels may at least in part contribute to the blood pressure independent benefits observed with RAS blockers in patients with type 2 diabetes and albuminuria.
Clinical Trial Registration Number: NCT00171561

1211

Effect of direct renin inhibitor aliskiren on circulating endothelial progenitor cells number in patients with type 2 diabetes mellitus and arterial hypertension

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Background and aims: Circulating endothelial progenitor cells (EPCs) derive from bone marrow and have the ability to differentiate into endothelial cells with high angiogenic potential. Diabetes mellitus has proved to have a strong impact to the number of EPCs which contributes to the development of vascular complications in diabetic patients. In the recent years, the ability of several treatments to achieve a favorable modification of EPCs population is under research. The aim of this study was to investigate the effect of renin inhibition on circulating EPCs population in patients suffering from type 2 diabetes mellitus and arterial hypertension.
Materials and methods: This open study enrolled 20 patients aged > 50 years with type 2 diabetes under stable glycaemic control and first diagnosed mild arterial hypertension. In phase A, patients received once-daily aliskiren 300 mg for 3 months. In phase B, once-daily hydrochlorothiazide 25 mg substituted for aliskiren for 3 more months. At baseline and at the end of phase A and phase B, the population of circulating EPCs was estimated by measuring of Dil-AcLDL+ / FITC-lectin+ cells in culture of peripheral blood mononuclear cells and also by quantification of CD34+/CD133+ cells in peripheral blood by flow cytometry. Endothelial function and arterial stiffness were also assessed by determination of brachial artery flow-mediated dilatation (FMD), augmentation index (AIx) and pulse wave velocity (PWV).
Results: After 3 months of treatment with aliskiren (n=18) mean sitting systolic BP (msSBP) and mean sitting diastolic BP (msDBP) were reduced by 8,8 mmHg and 5,9 mmHg respectively. The number of Dil-AcLDL+ / FITC-lectin+ cells and CD34+/CD133+ cells was increased by 40% (p < 0,05) and 31% (p < 0,05) respectively. FMD was increased by 87% (p < 0,001), while PWV and AIx were decresed by 16,8% (p < 0,05) and 10,2% (p < 0,05) respectively. At the end of phase B (n=12), msSBP and msDBP remained unchanged. Despite the similar control of msBP achieved with hydrochlorothiazide, the values of Dil-AcLDL+ / FITC-lectin+ cells and CD34+/CD133+ cells returned to baseline levels, exhibiting a reduction by 21% (p < 0,05) and 33% (p < 0,05) respectively compared to the corresponding values under treatment with aliskiren. In consistence with the effect on EPCs population, treatment with hydrochlorothiazide resulted in loss of the favorable effect of aliskiren on FMD, PWV and Aix.
Conclusion: Treatment with aliskiren resulted in increase of number of circulating EPCs, improved endothelial function and reduced arterial stiffness in type 2 diabetic patients. These effects were not observed with treatment with hydrochlorothiazide despite the similar blood pressure lowering. These results indicate a favorable effect of renin inhibition on EPCs, endothelial function and arterial stiffness beyond lowering msBP.

PS 103 Basic science retinopathy: novel in vitro and in vivo findings

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The C57BL/KsJ-db/db mouse: an appropriate model for investigating diabetes-induced retinal neurodegeneration

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Background and aims: In recent years the C57BL/KsJ-*db/db* mouse has been used as a spontaneous diabetic model of type 2 diabetes to investigate the pathogenesis of diabetic retinopathy (DR). Several authors have reported the presence of retinal neurodegeneration (apoptosis, glial activation and retinal thinning) in this model However, the characterization of the retinal neurodegenerative process and its functional consequences in db/db mice is far from being completed. In addition, whether neurodegeneration could be attributed to genetic factors rather than to diabetes is a question which remains to be elucidated. On this basis the aim of this study was to characterize the sequential events that are taking place in retinal neurodegeneration in this murine model and to examine whether neurodegenerative features could be prevented or arrested by lowering blood glucose levels.
Materials and methods: A total of 72 C57BL/KsJ-*db/db* mice were divided into two groups: non-diabetic (db/+) and diabetic (db/db). To assess the chronological sequence of the abnormalities the analysis was performed at different ages (8, 16 and 24 weeks). The retinas were evaluated in terms of morphological and functional abnormalities [electroretinography (ERG)]. In addition, an interventional study to lowering blood glucose levels by using a GLP-1 agonist was performed. For this purpose db/db mice 8 weeks old received subcutaneous injections (300 µl/50 g body wt) of either vehicle (phosphate-buffered saline, pH 7.3-7.5) (n=8) or liraglutide (400 µg/kg) (n=8) once daily for 15 days. Eight non-diabetic mice matched by age served as control group. Statistical analysis: unpaired Student t-test. Levels of statistical significance were set at $p<0.05$.
Results: Glial activation was higher in diabetic (db/db) than in non diabetic (db/+) mice at 8, 16 and 24 weeks ($p<0.01$). The percentage of TUNEL positive cells in the retinal ganglion cell layer (RGC) was higher in db/db in comparison with db/+ at 8 weeks (30% vs.1%; $p<0.001$), and this differences were even higher at 16 weeks (35% vs. 1%) and 24 weeks (38% vs. 3%). Moreover, a significant reduction of neuroretinal thickness was also observed in db/db ($p<0.05$). Significant ERG abnormalities (increase in b-wave implicit time and decrease of b-wave amplitude; $p<0.01$) were present in db/db mice at weeks 16 and 24 but not at week 8. Overall all these findings are very similar than those found in the early stages of DR in diabetic patients. Diabetic (db/db) mice treated with liraglutide showed a significantly lower GFAP immunofluorescence and rate of apoptosis in GCL than db/db mice treated with vehicle ($p<0.05$). In addition, in treated db/db mice total the neuroretinal thickness (µm) was higher than in those treated with vehicle (215.9 ± 4.7 vs. 195.2 ± 4.0; $p<0.001$), and similar to that observed in age-matched non-diabetic control mice (215.9 ± 4.7 vs. 212.3 ± 3.1). Finally, a significant prevention in ERG abnormalities was also observed ($p<0.01$).
Conclusion: Our results suggest that the db/db mouse is an appropriate murine model for investigating the underlying mechanisms of diabetes-induced retinal neurodegeneration and for testing neuroprotective drugs. In addition, we provide evidence that neurodegeneration is prevented by lowering blood glucose levels and, therefore, diabetes rather than genetic factors is the primary reason accounting for retinal neurodegeneration in this model.
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Connexin 43 (Cx43) upregulation rescues retinal endothelial cells from high glucose-induced apoptosis and excess permeability through amelioration of tight junctions

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Background and aims: Intercellular communication is an essential process for maintenance of cellular function, homeostasis, and cell survival. In the retina, the predominant gap junction protein, Cx43, is abundantly expressed. Downregulation of Cx43 expression has been shown to promote apoptosis in retinal vascular cells, and contribute to the development of vascular lesions in the diabetic retina. In this study, we investigated if upregulation of Cx43 prevents high glucose-induced apoptosis and excess monolayer permeability in rat retinal endothelial cells (RRECs), and whether this is achieved through amelioration of tight junction protein expression.
Materials and methods: RRECs were grown in normal (5 mM glucose; N) or high glucose (30 mM; HG) medium for 7 days. In parallel, cells were grown in either N or HG medium, and transfected with plasmid pEGFPN1 containing full-length rat Cx43 cDNA conjugated to green fluorescent protein (GFP) using a lipofectamine reagent. Transfected cells were subsequently selected for stable colonies. To determine Cx43 upregulation in the transfected cells, Cx43 immunofluorescence microscopy (IF) and western blot (WB) analysis were performed. Additionally, cells were assessed for changes in gap junction intercellular communication (GJIC) using a scrape loading dye transfer (SLDT) assay, and tight junction proteins, ZO-1 and occludin, levels by IF/WB analyses. To identify cells undergoing apoptosis, differential staining using acridine orange/ethidium bromide was used, and an in vitro permeability assay (IVP) was performed to study changes in cell monolayer permeability.
Results: WB analysis indicated a significant increase in Cx43 expression in cells transfected with pEGFPN1compared to untransfected cells (152±13% of N; $p<0.05$), and IF identified most cells to contain the GFP conjugated Cx43 from the plasmid. Importantly, WB analysis showed that the inhibitory effect of HG on Cx43 expression (64±11% of control) was abrogated in transfected cells grown in HG medium (114±10% of N; $p<0.05$). Similarly, SLDT analysis indicated that HG-induced decrease in cell-cell communication (51.2±5%) was returned to near normal levels in cells transfected with pEGFPN1 and grown in HG medium (89±9%). As expected, cells grown in HG medium showed a significant increase in the number of apoptotic cells (225±17% of N). Interestingly, normalization of Cx43 expression resulted in (i) rescue of 50% of the HG cells from apoptosis, (ii) reduction in cell monolayer permeability by 40%, and (iii) upregulation of ZO-1 and occludin expression.
Conclusion: Findings from this study indicate that upregulation of Cx43 may rescue cells from HG-induced apoptosis by improving GJIC, upregulating tight junction protein expression, and reducing cell monolayer permeability in RRECs grown in high glucose condition. Thus, targeting abnormal Cx43 expression may prevent cellular demise and excess permeability associated with diabetic retinopathy.
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1214

Activation of cell proliferation and VEGF transcription by advanced glycation end-products in hydroquinone-treated human retinal pigment epithelial cells

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Background and aims: Age-related macular degeneration (AMD) is the commonest overall cause of irreversible blindness in patients aged 50 or over in the world. The disease has been traditionally classified into early and late stages with its dry and wet (exudative) forms. The dry form AMD was defined as progressive destruction of retinal pigment epithelial (RPE) cells. While, exudative form AMD is characterized by choroidal neovascularization which is led by some angiogenic cytokines such as vascular endothelial growth factor (VEGF). It is known that cigarette smoke and diabetes are important risk factors for both dry and exudative AMD. Advanced glycation end-products (AGE) are generated during diabetes and are correlated with the development of AMD. Hydroquinone (HQ), a toxic substance, is the most abundant quinone in cigarette tar. Here, we investigated effects of AGE on RPE cell proliferation and VEGF expression in HQ-damaged human RPE cells.
Materials and methods: ARPE-19 cells, a human RPE cell line, were treated with bovine serum albumin (BSA) (0-300 µg/ml), HQ (20 µM), AGEs-BSA (0-300 µg/ml), and HQ+BSA-AGE for 12 h. After the treatment, the viable cell numbers were measured by WST-8 cleavage assay. Apoptosis was measured by TUNEL method. Replicative DNA synthesis was measured by 5-iodo-2'-deoxyuridine (IdU) incorporation. Real-time RT-PCR of *VEGF* was performed using HQ, BSA-AGE, or HQ+BSA-AGE treated cell RNA as template. To investigate *VEGF* promoter activation, ARPE-19 cells were transiently transfected with the reporter plasmids consisting of a luciferase gene

under the control of human *VEGF* promoters, and luciferase activities were measured after the treatments of HQ, AGE, or HQ+AGE. VEGF secreted in the culture medium was measured by ELISA.
Results: WST-8 cleavage assay showed that the viable cell numbers were markedly reduced by HQ treatment, and the addition of AGE-BSA increased the HQ-treated cell numbers (P=0.0001) in a dose-dependent manner. We next examined apoptosis of ARPE-19 cells and found that HQ increased apoptosis (P=0.0002) and that the AGE-BSA addition did not reduce the apoptosis of HQ-treated cells (P=0.6112), suggesting that AGE-BSA stimulate cell proliferation. As expected, replicative DNA synthesis was stimulated by the addition of AGE-BSA in HQ-treated cells (P=0.0001), indicating that AGE increases cell number by activating HQ-treated cell replication. Real-time RT-PCR revealed that the level of *VEGF* mRNA was increased by HQ treatment and the addition of HQ+AGE-BSA resulted in a further increment of *VEGF* mRNA (P=0.0025). The reporter gene assay revealed that the *VEGF* promoter was activated by HQ (P<0.01), and further increment was observed in HQ+AGE-BSA-treated cells. The -358~+50 region was revealed to be essential for the *VEGF* promoter activation. The increase of VEGF in the HQ+AGE-BSA treated ARPE-19 cell culture medium (P=0.0064) was confirmed by ELISA.
Conclusion: HQ induces RPE cell apoptosis and AGE-BSA enhances *VEGF* transcription in HQ-damaged cells to stimulate proliferation. The secreted VEGF may act as an autocrine/paracrine growth factor to RPE and/or adjacent vascular cells, causing exudative form of AMD.

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Mesenchymal stem cell-derived microvesicles induce pericyte destabilisation in diabetic-like conditions
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Background and aims: During angiogenesis, the stable association between pericytes and endothelial cells (EC) in pre-existing vasculature is disrupted, leading to EC proliferation. Recent findings suggest the existence of a novel way of intercellular communication, where the 'units' of information are microvesicles (MV), or exosomes, derived from different cells and containing biologically active proteins and RNA, that may promote phenotypic changes in other target cells. MV derived from injured cells may induce dedifferentiation of pericytes allowing their detachment from vessels. The literature on the molecular interactions between EC-derived MV and pericytes for the angiogenic switch is growing, but little is known about mesenchymal stem cells (MSC), a self-renewing cells that can be found in most tissues, to activate pericytes. Our study aimed at evaluating if MV produced by MSC in hypoxia and/or hyperglycaemic-like conditions are able to induce pericyte detachment and influence their survival.
Materials and methods: MSC from bone marrow and human microvascular EC were of commercial origin, while human retinal pericytes (HRP) were immortalized in our laboratory. MV were extracted from the supernatant of MSC or EC cultured in hypoxic and/or high glucose (HG) conditions and added to HRP cultured in physiological conditions both on plastic and EC-produced extracellular matrix (ECM). We evaluated HRP detachment after 2, 4, 6 and 24h exposure to MV by cell counts, as well as viability, cytotoxicity and apoptosis by an ELISA/fluorimetric assay. Motility and cell modifications were analyzed by a MicroImage analysis system.
Results: The number of attached HRP decreased in a time-dependent manner after addition to the culture medium of MSC-derived MV obtained in all the above-described conditions, the higher decrease occurring after 4h of MV exposure. HRP number on plastic after 4h: with MV obtained in physiological conditions (NG) -35.8% vs control (HRP not exposed to MV, p<0.05, n=5); MV in HG -27.1% (p<0.05), MV in NG+hypoxia -27.2% (p<0.05), MV in HG+hypoxia -37.0% (p<0.005). HRP number on EC-produced ECM after 4h: MV in NG -30.1% vs control (p<0.05); MV in HG -52.3% (p<0.005), MV in NG+hypoxia -40.3% (p<0.005), MV in HG+hypoxia -41.0% (p<0.005). Apoptosis and cytotoxicity did not change significantly. Trypan blue staining and TUNEL assay showed that detached cells were alive. Increased cell motility was observed in the presence of MV, reaching its maximum after 1h exposition of HRP to MV (+56.0% vs control, p<0.05), while microscopy observation showed evidence of cell contraction. Interestingly, EC-derived MV had no effects on pericyte viability/destabilization.
Conclusion: We conclude that MSC-derived MV induce HRP detachment, a possible indicator of destabilization. Diabetic-like conditions (hyperglycaemia and hypoxia) may play a synergistic role in influencing vessel destabilization during the stages of retinopathy that precede angiogenesis. Since this role seems to be MSC-specific, EC-derived MV having no effect, further studies are needed to better characterise MSC-HRP interactions.
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$VEGF_{165}b$ reduces blood vessel dysfunction in diabetic retinas
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Background and aims: Diabetic macular edema (DME) is associated with vascular hyperpermeability as a consequence of pro-angiogenic vascular endothelial growth factor (VEGF) upregulation. $VEGF_{165}$ has been connected with the degradation of tight-junction proteins zona occludens 1 (ZO1) and occludin in vitro. This reorganisation of ZO1 and occludin is prevented in retinal pigmented epithelial (RPE) cells by co-treatment with $VEGF_{165}b$, an alternatively spliced anti-angiogenic isoform, suggesting that $VEGF_{165}b$ could abrogate retinal vascular hyperpermeability. The aim of this study was to determine whether $VEGF_{165}b$ reduces retinal vascular hyperpermeability in rats with streptozotocin-induced diabetes.
Materials and methods: Sprague-Dawley male rats (200g) were induced with diabetes using streptozotocin (50mg/kg). After 6 days, 5µl saline was injected into one eye of each diabetic rat, and 5µl 10ng/µl rh$VEGF_{165}b$ injected into the contralateral eye. Control rats had no injection in one eye, and 5µl saline in the contralateral eye. On day 7, Evans blue (45mg/kg) was injected i.v into anaesthetised rats. Plasma was collected every 15 minutes for 2 hours, after which, animals were sacrificed, eyes enucleated and retinas excised. Retinas were weighed and Evans blue was extracted using formamide, with Evans blue solute flux calculated from the amount of Evans blue, per wet weight of tissue, per hour relative to the mean plasma Evans blue level during the 2 hr perfusion.
Results: Increased solute flux in the retinae of rats with streptozotocin-induced diabetes was significantly reduced by intravitreal administration of rh-$VEGF_{165}b$ compared with vehicle-injected controls (P<0.05). Vehicle-treated eyes showed no significant difference in solute flux compared to uninjected eyes in control rats.
Conclusion: $VEGF_{165}b$ reduces hyperpermeability of retinal vasculature in streptozotocin-induced diabetic rats and may offer an effective therapeutic approach to diabetic retinopathy.
Supported by: Diabetes UK

1217

The role of macrophage migration inhibitory factor in a mouse model of diabetic retinopathy
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Background and aims: Diabetic retinopathy is the most common microvascular complication, of which the primary lesion is the formation of acelluar capillaries with no blood flow. The pathogenesis of diabetic retinopathy has not been completely defined. Several studies have shown that diabetic retinopathy shows features of inflammation, such as up-regulation of inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX2), inter-cellular adhesion molecule 1 (ICAM-1), receptor for advanced glycation end products (RAGE), monocyte chemoattractant protein-1 (MCP-1), increased production of nitric oxide, prostaglandin E2 (PGE2), proinflammatory cytokines tumor necrosis factor alpha (TNF-a), interleukin-1 beta (IL-1ß) as well as increased capillary permeability and leucostasis. Macrophage migration inhibitory factor (MIF) is a pleiotropic proinflammatory cytokine involved in the regulation of inflammation and innate immunity. The aim of the study was to analyze the expression of MIF and its role in a mouse model of diabetic retinopathy.
Materials and methods: Wild type (C57BL/6) and MIF-knockout mouse were rendered diabetic by intraperitoneal injection of streptozocin (150mg/kg body weight) and maintained for 6 months. Blood glucose and body weight were monitored regularly and HbA1c was measured at the end of the study. The expression of MIF was assessed by immunofluorescence staining of the retina and western blot analysis of retinal protein extract. Vascular damage was quantitated in retinal digest preparations.

Results: We found that MIF is expressed in the neurons of the inner retina, mainly in the ganglion cell layer and inner nuclear layer. The protein levels of MIF were unchanged in the diabetic retinas. Both diabetic wild type and MIF-knockout animals developed significant increased acelluar capillaries, compared to their non-diabetic controls (Wild type: diabetic 34±7 acelluar segments/mm^2 of retinal area vs control with 24±5 acelluar segments/mm^2 of retinal area, $p< 0.01$; MIF-knockout: diabetic 40±6 acelluar segments/mm^2 of retinal area, vs control with 27±4 acelluar segments/mm^2 of retinal area, $p< 0.001$), while the deletion of MIF aggravated the vascular damage in diabetic retinas compared to their diabetic wild type controls (diabetic MIF-knockout 40±6 acelluar segments/mm^2 of retinal area, vs diabetic wild type 34±7 acelluar segments/mm^2 of retinal area, $p< 0.05$).
Conclusion: Our data demonstrate that MIF is expressed in the retinal neurons and appears to provide partial vascular protection in this mouse model of diabetic retinopathy. Since MIF appears unregulated in diabetes, the mechanism by which MIF deletion affects vasoregression in diabetes needs mechanistic workup.
Supported by: DDG

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S-nitrosoglutathione eye drop inhibits inducible nitric oxide synthase up-regulation by post-translational modification in experimental diabetic retinopathy

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Background and aims: The rise of nitric oxide (NO) production in the retina, as a result of inducible nitric oxide synthase (iNOS) induction, is associated with inflammatory responses and increase of oxidative/nitrosative stress in an experimental model of diabetic retinopathy (DR). It has been shown that high glucose increase iNOS expression in retinal pigment epithelial (RPE) cells. Endogenous NO is unstable and most of its biological actions are mediated via nitrosothiols (SNO), the addition of a NO into thiol groups that contain a sulfhydryl group (SH) with specific cysteine residues. This process, known as S-nitrosylation, promotes post-translational modification of proteins affecting their activities. The purpose of this study was to assess the possible effects of S-nitrosoglutathione (GSNO) eye drop in preventing retinal damage in experimental DR through the post-translational modifications of i-NOS by S-nitrosylation and/or S-glutathionylation.
Materials and methods: Diabetes (DM) was induced by streptozotocin (60 mg/kg) in spontaneously hypertensive (SHR) rats with 4 week-old and randomized to receive vehicle only or a low (900nM) or high (10μM) dose of GSNO eye drop twice daily in both eyes. Similarly, non-DM rats received the same treatments. After 20 days, the animals were submitted to electroretinography (ERG), then euthanized and the retinas were collected for protein extraction or immunohistochemistry. To assess the mechanisms, human RPE cell lines (ARPE-19) were exposed to normal glucose (NG) or high glucose (HG) without or with GSNO 100nM.
Results: In DM animals, GSNO decreased iNOS expression and peroxynitrite formation, thus preventing glial dysfunction (GFAP) and restoring the electroretinogram potentials. In contrast, in the non-DM animals, GSNO induced oxidative/nitrosative stress ($p\leq0.05$). For the *in vitro* studies, ARPE-19 cells under HG+GSNO condition showed a decrease in the total intracellular reactive oxygen species (ROS) and NO levels, accompanied by iNOS down-regulation and decreased peroxynitrite formation ($p\leq0.05$), without any change in reduced glutathione (GSH) levels ($p=0.9$) and showed a tendency to increase GSNO reductase (GSNO-R) expression ($p=0.06$). To assess whether post translational modification could be involved in inhibition of i-NOS by GSNO treatment under HG condition, we addressed the S-glutathionylation of iNOS by immunoprecipitation of GSH/iNOS, which presented an increase of this complex ($p\leq0.05$). In contrast, in the NG condition, GSNO treatment promoted nitrosative stress by NO formation ($p\leq0.05$), but did not increase i-NOS expression ($p=0.7$).
Conclusion: In this study, a new pharmacological therapeutic modality (GSNO eye drop) targeting nitrosative stress by redox post-translational modification of iNOS is efficient against the early retinal damage in experimental DR. In normal conditions, either *in vivo* or *in vitro*, the exogenous GSNO promoted nitrosative stress probably by NO formation. This data shows the potential clinical implications of an S-nitrosoglutathione/glutathione system balance in the treatment of DR. The mechanisms involved in the post-translational modification of iNOS are currently under investigation.
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Neuroprotective effect of fenofibrate on retinal neurodegeneration in an experimental mouse model of type 2 diabetes

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Background and aims: There is now consistent evidence from two major clinical trials (FIELD and ACCORD-Eye) that fenofibrate arrests the progression of diabetic retinopathy in type 2 diabetic patients. However, the underlying mechanisms of this beneficial effect remain to be elucidated. The aim of the study was to evaluate the potential effect of fenofibric acid (the active metabolite of fenofibrate) in preventing retinal neurodegeneration in an experimental mouse model of type 2 diabetes.
Materials and methods: A total of 24 diabetic mice (db/db) aged 8 weeks were randomly assigned to daily oral treatment (by gavage) with fenofibric acid (100 mg/Kg/day) (n=12) or vehicle (n=12) for one week. We measured the body weight, triglycerides and glucose levels. Ten non-diabetic mice (db/+) were used as control group. Retinal neurodegeneration was evaluated by measuring glial activation assessed by immunohistochemistry and western blotting against GFAP (Glial fibrillar acidic protein), and apoptosis was quantified using the TUNEL method. Functional abnormalities were assessed by electroretinography (ERG) before and after treatment. Statistical analysis: paired and unpaired Student t-test and the Mann-Whitney U test.
Results: At 9 weeks diabetic mice presented a significant higher glial activation and apoptosis in ganglion cell layer than non-diabetic mice. Treatment with fenofibric acid significantly prevented glial activation ($p< 0.001$) and neuronal apoptosis ($p< 0.05$). Moreover, db/db mice treated with fenofibric acid showed a dramatic improvement in ERG parameters (b-wave implicit time and oscillatory potentials amplitude). As expected, lower levels of triglycerides were detected in db/db treated with fenofibric acid in comparison with db/db treated with vehicle ($p=0.02$). No differences in blood glucose and body weight were detected between the groups.
Conclusion: Fenofibric acid prevents retinal neurodegeneration induced by diabetes. Our results suggest that neuroprotection could be added to the non-lipidic mechanism by which fenofibrate exerts its beneficial actions in diabetic retinopathy.
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Epi-retinal membranes, pseudoholes and macular holes in diabetes

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Background and aims: Epi-retinal membrane (ERM) may be associated with diabetes and diabetic retinopathy (DR). We report how often ERM and macular holes (MH) were noted in a DR screening programme and their effects on visual acuity (VA) and grading of DR.
Materials and methods: 68,637 patients in a DR screening programme in a single year noting ERM or cellophane maculopathy or MH were reviewed and poor quality photos excluded. Screening involved two digital photographic 45º images, one macula and one disc centred, through dilated pupils. Best corrected VA (Snellen) and the English national grading standards for DR were used. 422 patients had definite ERM that was considered idiopathic with no significant ocular history or pathology in 397 eyes (94.1%). The value of two views was assessed by one senior and one primary grader in a subset of 25 patients. 93 patients had MH confirmed in hospital eye clinics.
Results: 422 patients (457 eyes) with ERM (0.61% of total screened). 213 male, 209 female; mean age 71.6 ±9.5 yrs. ERM was unilateral in 387 (91.7%) (R 181 [46.8%]; L 206 [53.2%]) and bilateral in 35 (8.3%).VA with ERM was 6/6 or better in 161 (35.2%), 6/9 in 172 (37.7%) and ≥6/12 in 124 (27.1%) eyes, with no other cause evident for VA ≥6/12 except ERM in 84 (67.8%) eyes. All eyes but one (456 eyes) had been given a DR grade (R0M0 251, R1M1 123, R2M0 5, R2M1 4, R3M0 11, R3M1 6 and ungradable 1). 251 eyes (55.1%) posed grading difficulties. Both senior and primary graders considered the two views were essential for DR grading in all but one of the subset. In contrast MH was less common (0.14% of total screened) and occurred more often in female patients (73%) than male (27%). Mean age was similar (73.8yrs) but VA more affected: 6/24 or better in 24 eyes (24%), 6/36 in 17(17%), 6/60 in 24 (24%) and worse than 6/60 in 30 (30%). DR grading posed few difficulties, apart from post-operative changes in the minority of surgically treated patients, R0/M0 79, R1/M0 9, R1/M1 5, R2/M1 2 and ungradable 5.
Conclusion: ERM and MH were relatively rare in the elderly as noted within a DR screening programme. However this study is likely to have underestimated prevalence. ERM was associated with some loss of VA and MH with severe loss. Grading of DR was difficult in 55% of ERM but two views enabled adequate assessment of the majority of patients with ERM. MH can be suspected on retinal photography but requires confirmation and assessment of staging in an eye clinic. Both lesions would be better assessed with additional ocular coherence tomography at the time of DR screening. Early detection of these lesions may become important as more effective treatments become available. DR changes were as expected.

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Inflammation and visceral obesity: possible link to pathogenesis of diabetic retinopathy in type 2 diabetes

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Background and aims: Diabetic retinopathy (DR), a microvascular and visually devastating diabetic complication, is the leading cause of new blindness among working-age adults in developed countries. Its pathogenesis is insufficiently understood and presumed to possibly involve chronic, low-grade inflammation. The aim of this study was to investigate the relationship between inflammation markers, other markers of endothelial dysfunction and anthropometric parameters and their association with DR in patients with type 2 diabetes.
Materials and methods: This was a cross-sectional study including 107 patients with type 2 diabetes (67 male / 40 female, mean age 66.74 ± 8.01 years, mean diabetes duration 15.05 ± 5.69 years). Markers of inflammation: C-reactive protein (CRP) and fibrinogen as well as other markers of endothelial dysfunction: glycated hemoglobin value (HbA_1c; HbA_1c_{median}), total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were determined using routine laboratory methods. HbA_1c was evaluated at the beginning of the study from a single venous blood sample, and HbA_1c_{median} was obtained by statistical analysis of data from the National Registry for Diabetes (CroDiabNet). Anthropometric parameters assessed were body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR) and conicity index (C index). Ophthalmologic examination included best corrected visual acuity (BCVA), Goldmann applanation tonometry, slit lamp biomicroscopy of the anterior eye segment, binocular indirect slit lamp fundoscopy and color fundus photography after mydriasis of two fields (macular field, disc/nasal field) of both eyes according to the EURODIAB retinal photography methodology.
Results: According to the EURODIAB standards patients were divided into three groups: group 1 (no retinopathy; n=65), group 2 (mild / moderate nonproliferative diabetic retinopathy; n=19) and group 3 (severe NPDR / proliferative diabetic retinopathy; n=23). The groups did not differ in the levels of inflammation markers, other markers of endothelial dysfunction and anthropometric parameters. C-reactive protein was positively correlated with fibrinogen (p=0.022), HbA_1c (p=0.050), LDL cholesterol (p=0.043), BMI (p=0.038), WC (p=0.000), WHR (p=0.038) and C index (p=0.008). HbA_1c was positively correlated with cholesterol (p=0.022), LDL cholesterol (p=0.010), BMI (p=0.009) and WC (p=0.047). Logistic regression analyses showed that diabetes duration (OR=1.17, 95%CI 1.08-1.27) and prolonged poor glycemic control (HbA_1c_{median}) (OR=1.76, 95%CI 1.08-2.86) were the main predictors of DR in patients with type 2 diabetes.
Conclusion: This study showed that the association between visceral obesity, inflammation and other risk factors plays an important role in endothelial impairment involved in the pathogenesis of diabetic retinopathy. These findings point to the need for testing the effects of treatment aimed at reducing visceral obesity, decreasing inflammatory activity and improving endothelial function as a means of preventing or limiting the progression of retinopathy.

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Diabetic retinopathy is associated with arterial stiffness independent of albuminuric status in patients with type 2 diabetes

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Background and aims: Diabetic retinopathy and abluminuria often develop in parallel with each other and have both been linked to increased risk of cardiovascular disease. This study was conducted to investigate whether diabetic retinopathy is associated with subclinical cardiovascular disease independent of albuminuria in type 2 diabetic patients.
Materials and methods: We conducted a cross-sectional analysis among 290 subjects with type 2 diabetes. Diabetic retinopathy (DMR) was categorized into no DMR, non-proliferative DMR (NPDR), or proliferative DMR (PDR). Albuminuria is defined if urinary albumin-creatinine ratio (ACR) ≥ 30 mg/g. Subclinical atherosclerosis was assessed by measuring arterial stiffness using brachial-ankle pulse wave velocity (PWV) and carotid intima-media thickness (IMT) and plaque score using B-mode ultrasonography.
Results: DMR was diagnosed in 74 patients (25.5%). Patients with DMR had higher PWV compared to those without DMR but no difference in IMT or plaques presence in unadjusted models and in models adjusted for various confounding including cardiovascular risk factors and ACR. In multivariate logistic analysis, the odd ratios for DMR increased across PWV quartiles [1 (reference), 2.35, 7.87, 13.63; P for trend=0.006]. In the subgroup without albuminuria, the association of PWV and DMR was still remained significant [1 (reference), 1.34, 12.08, 33.15; P for trend= 0.025].
Conclusion: DMR was associated with arterial stiffness without structural changes and independent of albuminuric status and cardiovascular risk factors in patients with type 2 diabetes. Our study suggests that arterial stiffness might be an early indicator for DMR, even without combined with albuminuria.

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Digital retinal imaging: community heath workers as imagers in rural areas versus an ophthamology clinic

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Background and aims: Currently, only 50% of U.S. citizens diagnosed with diabetes are screened for retinopathy and in underserved communities, that number is closer to 20%. Therefore, treatable causes of blindness are being missed every day. Digital retinal screening is a cost effective tool developed

to increase screening rates and is particularly beneficial in rural and underserved areas where gold standard ophthalmologic care is not readily available. In this study, we compared adequacy of digital retinal screening conducted by community health workers (CHWs) in rural clinics throughout New Mexico with that of a dedicated ophthalmology clinic.
Materials and methods: CHWs in rural and medically underserved areas of New Mexico were trained in retinal imaging. Training consisted of two days of instruction and practice. Refresher training was offered if problems were encountered. Certification was given if CHWs successfully completed ten images. A community based ophthalmology clinic for underserved patients with a dedicated retinal specialist, served as the control setting. Ophthalmology technicians performed imaging at the clinic. A standardized imaging protocol that acquires two or more images of each eye (macula centered and optic disc centered) was used for this study. A non-mydriatic (no dilation) camera was used to minimize impact on the patient. Images were forwarded via a secure internet connection to a centralized reading center where cases were read and results returned to the patient and primary care clinic within 72 hours. We evaluated number of cases requiring urgent referral, adequacy of images, and reasons for inadequate images between the two settings. Data were reported as absolute and average with standard deviations. Unpaired student's *t*-tests and ANOVA were used to analyze data sets. A $p<0.05$ was considered significant.
Results: Eight hundred and one patients from NM completed imaging studies and 1,645 patients from the clinic. Diabetic retinopathy was detected in 22% of cases in New Mexico and 26% of case at the clinic. Positive findings requiring urgent ophthalmologic intervention (5-10 days) were reported in 3.7% of cases in NM and 4.1% of cases at the clinic. Inadequate readings were reported 12.2±7% of the time in NM and 9.3±10% at the clinic, (p value >0.1, not significant). Reasons for inadequate readings included small pupils, cataracts, and blurred images but were not statistically different between the two sites.
Conclusion: A brief instructional tool for CHWs on retinal imaging with documentation of proficiency was as effective as retinal screening provided at a dedicated ophthalmology clinic with trained technicians. Digital retinal imaging using CHWs as imagers will likely be a cost effective mechanism to increase screening rates for diabetic retinopathy in rural and underserved communities.

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Retinopathy at diagnosis of type 2 diabetes: more or less prevalent at older ages?

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Background and aims: To compare levels of diabetic retinopathy (DR) in patients newly diagnosed with Type 2 diabetes (T2DM) in Gloucestershire in those aged 65 years or below with those 66 and above.
Materials and methods: Data were collected for patients on the screening register of the Gloucestershire Diabetic Eye Screening Service in UK. Retinopathy status as determined from ditigal retinal photographs after mydriasis were extracted from the screening service database and clinical information extracted from primary care records for those newly diagnosed with T2DM between 2005 and 2012. Clinical characteristics and diabetic retinopathy (DR) grading outcomes from the screening records were analysed and tabulated by age group and gender. Classification of DR in the Gloucestershire patients used the English NHS Diabetic Eye Screening Programme (NHS-DESP) categories.
Results: Data were available for 6247 patients within one year of diagnosis of T2DM of whom 6101 had assessable images of both eyes. Minimum age at diagnosis was 25 years, maximum 96. Men and women were of similar age in the younger group (56 (49 to 61) (mean (s.d.) vs 56 (49 to 62)) but in the older group the men were of lower age (72 (69 to 77) vs 74 (70 to 78)). In both men and women older patients were more likely to have unassessable images (0.9% and 3.7% of younger and older men respectively, 1.0% and 4.6% of younger and older women, $p<0.0001$ for both). In women, but not in men, older patients had more DR (32% vs 28% with any DR) and a significant trend to more severe DR (2.8% of older women had referable DR, 2.0% of younger women, $p=0.0076$). HbA1c was available for 5055 patients and was higher in younger than older patients (mean difference 5.2 mmol/mol, 95%Ci 4.2 to 6.2). Within each group HbA1c at diagnosis was positively associated with prevalent DR.
Conclusion: In patients newly diagnosed with T2DM DR is present across the age range. It is therefore important for all newly diagnosed patients to attend for DR screening soon after diagnosis.

Assessable images and DR level at first screen after diagnosis of diabetes

	Men 65 and below	men 66 and above	sig level for difference between older and younger men	Women 65 and below	Women 66 and above	sig level for difference between older and younger women
Number with images	2088	1443		1389	1327	
Not assessable	18 (0.9%)	53 (3.7%)		14 (1.0%)	61 (4.6%)	
Assessable	2070 (99.1%)	1390 (96.3%)	p<0.0001	1375 (99.0%)	1266 (95.4%)	p<0.0001
DR level						
No DR	1403 (67.8%)	948 (68.2%)		990 (72.0%)	860 (67.9%)	
Microvascular damage in one eye	415 (20.5%)	277 (19.9%)		258 (18.7%)	252 (20.0%)	
Microvasular damage in both eyes	202 (9.8%)	124 (8.9%)		100 (7.3%)	118 (9.3%)	
Referable DR	50 (2.4%)	41 (2.9%)	p=0.94	27 (2.0%)	35 (2.8%)	p=0.0076

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Obstructive sleep apnoea predicts the development of preproliferative and proliferative retinopathy in patients with type 2 diabetes: a longitudinal analysis

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Background and aims: Diabetic Retinopathy (DR) is a leading cause of blindness. OSA is associated with increased oxidative and nitrosative stress and endothelial dysfunction in patients with type 2 diabetes (T2DM). Hence, it is plausible that OSA can promote the development and progression of DR.
Materials and methods: An observational longitudinal study in adults with T2DM. Patients with pre-existing OSA, end-stage renal disease and non-diabetic retinopathy were excluded. OSA (apnoea hypopnea index ≥ 5 events/hour) was assessed by a single overnight home-based cardio-respiratory monitoring (Alice PDX, etc.). DR was assesses using retinal images between 2007 and 2012. Sight threatening retinopathy (STR) was defined as pre-proliferative or proliferative DR, maculopathy or photocoagulation. Advanced DR was defined as pre-proliferative or proliferative DR.
Results: 199 patients were included (57.3% men, 47.7% White Europeans). STR and OSA prevalence were 38.7 % and 62.8% respectively. STR prevalence was higher in patients with OSA (OSA+) compared to those without (OSA-) [48.8% vs. 21.6%, p <0.001]. After adjustment for confounders, OSA remained independently associated with STR (OR 3.7, 95%CI 1.6-8.9, p=0.006, maculopathy (OR 4.5, 1.8-11.4, p=0.002) and advanced DR (OR 3.9, 1.02-15.3, p=0.047). Over 4.4±1 years, more OSA+ patients progressed from no or background DR to advanced DR (15.3% vs. 3%, p=0.01). OSA was an independent predictor of advanced DR development after adjustment (OR 6.6, 95%CI 1.2-35.1, p=0.03). OSA did not predict the development of maculopathy. Patients received continuous positive airway pressure treatment were less likely to develop advanced DR
Conclusion: OSA is independently associated with STR and predicts the development of preproliferative and proliferative DR. Interventional studies are needed to assess the impact of OSA treatment on DR.
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High cystatin C level hints severe retinopathy in type 2 diabetic patients

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Background and aims: In order to investigate the relationship between cystatin C and diabetic retinopathy (DR) and clarify the predictive value of cystatin C for diabetic ophthalmopathy.
Materials and methods: A total of 954 type 2 diabetes mellitus patients were recruited and divided into quartiles with respect to their cystatin C levels (≤0.8 mg/L, 0.9-1.0 mg/L, 1.1-1.2 mg/L, ≥1.3 mg/L). The severity of DR was grouped from a bilateral retinal photograph as: no diabetic retinopathy (NDR), non-sight-threatening diabetic retinopathy (NSTDR) and sight-threatening diabetic retinopathy (STDR).
Results: There were significant differences in prevalence of DR, NSTDR and STDR among cystatin C quartiles (all $P < 0.01$). After adjusted for potential confounders, elevated levels of cystatin C were associated with DR and STDR. The multivariable odds ratio (95 % confidence interval) of DR and STDR in the highest quartile was 2.96(1.48-5.91) and 18.10(5.70-57.50) respectively compared to the lowest quartile (both $P < 0.01$). There was also significant difference in cystatin C levels between three DR groups ($P < 0.01$). Multiple logistical regression analysis further revealed that cystatin C was independent risk factors for DR ($\beta = 1.253$, $P = 0.000$) and STDR ($\beta = 3.701$, $P = 0.000$). The receiver operating characteristic (ROC) curve indicated that cystatin C higher than 1.25mg/L predicts high risk of severe retinopathy [odds ratio (OR) =11.36, 95% confidence interval (CI):7.66-16.85].
Conclusion: High cystatin C level closely links the severity of DR and predicts the 11 times increasing morbidity of STDR.
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GLP-1 therapy and diabetic retinopathy: temporary progression and the need to monitor

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Background and aims: Transient worsening of diabetic retinopathy (DR) is well documented during rapid improvement of glycaemic control in instances such as pregnancy and insulin pump therapy. We had already published data on Phase 1 follow up of DR following initiation of GLP-1 therapy. 165 patients on Exenatide therapy were studied in our Phase 1 study after continuation of treatment for at about 12 months based on the lowest HbA1c achieved. 49 patients had progression of DR (New onset of DR: 16 and Worsening of pre-existent DR 33) of which 47 had reduction in HbA1c. We concluded that patients with pre-existent DR had higher risk of progression of DR during the first year of treatment, the risk being higher with greater reduction in HbA1c. The aim of this Phase 2 study was to perform an observational analysis of patients with progression of DR with sustained GLP-1 therapy.
Materials and methods: A retrospective observational interval analysis was made on the 47 patients from the previous study. Patients who discontinued GLP-1 therapy were excluded. Data on DR status was taken from Ophthalmology department records or community retinal screening database. Matched HbA1c done closer to the DR screening data was also recorded and compared against the lowest HbA1c achieved during treatment
Results: N=39 (8 patients stopped Exenatide: 3 ineffective, 2 renal function issues; 2 intolerance;1 bariatric surgery done). The average interval between DR screening between Phase 1 and Phase 2 was 439 days. The mean duration between lowest Hba1c achieved and latest HbA1c was 460 days. Overall Change in DR: 24/39 (62%) patients had improvement in DR compared to Phase 1 study; 17/24 actually had increase in HbA1c (mean increase 1.4%; range 0.2-4.8%); 7/24 had decrease in HbA1c (mean -0.6%; range -0.1 to -1.6%);7 (18%) patients had no change in DR; 6/7 had increase in HbA1c (mean 1.4%; range 0.3-5.0%); 1/7 had decrease in HbA1c; 8 (20%) patients had progression of DR: 6/8 had increase in HbA1c (mean 1.6%; range 0.1-3.1%); 2/8 had decrease in HbA1c (mean -1.5%); 3 patients developed new maculopathy (2 with increasing HbA1c); 3 required new Laser treatments (2 with increasing HbA1c). Subgroup analysis: Of the patients who developed new DR between Baseline and Phase 1 study (n=14 after attrition), DR improved in 10 (71%), no change in 3(21%) and worsened in 1 Of the patients who had progression of pre-existent DR in the previous study (n=25 after attrition), DR improved in 15(60%), no change in 41(16%) and worsened in 6
Conclusion: Progression of DR during GLP-1 therapy, with rapid reduction of HbA1c, appears to be a transient phenomenon, in keeping with other clinical instances. Majority of these changes either remain static or improve their DR with sustained treatment despite the upward trend of the glycaemic control. Worsening of DR should not deter the primary aim of intensifying diabetic control, but close observation of retinal status should be advised, especially in patients with pre-existent DR. Clear protocols based on prospective studies are required to formalize screening process for this cohort of patients with paradoxical worsening of DR.

PS 105 Autonomic neuropathy

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Cardiovascular autonomic neuropathy and lower urinary tract symptoms in men with type 1 diabetes

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Background and aims: The pathophysiology of lower urinary tract symptoms (LUTS) has been reported to involve vascular, hormonal, and neural mechanisms. Data on the relationship between autonomic neuropathy and LUTS in men with type 1 diabetes (T1DM) are limited. We evaluated the association between cardiovascular autonomic neuropathy (CAN) and LUTS in subjects with T1DM participating in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications Study (DCCT/EDIC).
Materials and methods: Measures of CAN (R-R response to deep breathing, Valsalva maneuver, postural change in blood pressure), and LUTS were obtained in 644 DCCT/EDIC male subjects, 16/17 years after DCCT closeout. CAN was defined as:1) R-R variation<15 or 2) R-R variation between 15-19.9 plus either a Valsalva ratio≤1.5 or a supine-to-standing drop of 10 mm Hg in diastolic blood pressure. LUTS was assessed by the American Urological Association Symptom Index (AUASI), a clinically validated, standardized seven item questionnaire that quantifies the presence and frequency of the following lower urinary tract symptoms: nocturia, frequency, urgency, weak urinary stream, intermittency, straining, and the sensation of incomplete emptying. Scores range from 0 to 35. AUASI scores of 0-7 were defined as none/mild LUTS and AUASI of 8-35 as moderate/severe LUTS. Multivariable logistic regression estimated the associations between CAN and LUTS, after adjusting for DCCT cohort, DCCT/EDIC HbA1c, DCCT/EDIC systolic blood pressure, age, smoking and drinking status, and beta blocker use at EDIC year 16/17.
Results: At EDIC year 17, mean age was 52±7 years, mean diabetes duration 29±5 years, and mean DCCT/EDIC HbA1c 7.9±1.0%. Moderate/severe LUTS was reported by 158 (25%) subjects at EDIC year 17. Subjects with moderate/severe LUTS had significantly lower R-R variation at both DCCT closeout and EDIC year 16/17, and significantly lower Valsalva ratio at EDIC year 16/17 compared to men with mild/no LUTS (Table). At EDIC year 16/17, 51% of participants with LUTS also had confirmed CAN, vs. 33% of participants without LUTS (p=0.0001). In adjusted analysis, participants with CAN had 1.64 greater odds of LUTS (95% CI=1.07, 2.50).
Conclusion: The association between CAN and LUTS in the DCCT/EDIC cohort suggests that CAN may be a useful surrogate biomarker of more generalized autonomic neuropathy and may predict development of LUTS in men with long-standing type 1 DM.

CAN measures at DCCT closeout and EDIC year 16/17 by LUTS status

Outcome Measures	No LUTS N=485	LUTS N=158	p-value
At DCCT Closeout			
Confirmed CAN	29 (6)	16 (11)	0.049
R-R Variation	41.1±19.3	34.7±16.2	0.0010
Valsalva Ratio	1.96±0.4	1.91±0.4	0.418
At EDIC Year 16/17			
Confirmed CAN	158 (33)	75 (51)	0.0001
R-R Variation	25.9±17.1	21.5±16.7	0.001
Valsalva Ratio	1.72±0.3	1.66±0.4	0.028

Data are Mean±Std or N (%). P-values based the difference between those with and without FSD or UI using the Wilcoxon rank-sum test for quantitative variables or the Contingency chi-square for qualitative variables.

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Prevalence and risk factors of cardiac autonomic nerve dysfunction in the elderly population with diabetes and prediabetes: the KORA S4 survey

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Background and aims: Cardiovascular autonomic neuropathy (CAN) is associated with an increased risk of mortality, but the glycaemic threshold at which CAN develops and the associated risk factors are unclear. This study aimed to determine the prevalence and risk factors of cardiac autonomic nerve dysfunction in the elderly population with diabetes and prediabetes using a comprehensive set of parameters of heart rate variability (HRV) and QT variability index (QTVI).
Materials and methods: The population-based KORA (Cooperative Health Research in the Region of Augsburg) S4 survey included 2656 subjects aged 55-74 years, who were living in the region of Augsburg, Germany. Of these, 1636 persons participated in the present study, 304 of whom were excluded due to arrhythmias, leaving 1332 subjects aged 64.0±5.4 (mean±SD) years, 51% male, BMI: 28.7±4.3 kg/m², 1202 of whom without known diabetes had an oral glucose tolerance test (OGTT) at baseline, while 130 subjects had known diabetes (k-D). According to the ADA 2003 criteria, 565 persons had normal glucose tolerance (NGT), 336 had isolated impaired fasting glucose (i-IFG), 72 had isolated impaired glucose tolerance (i-IGT), 151 had combined IFG and IGT (IFG-IGT), and 78 had newly detected diabetes (n-D). HRV was computed by 2 linear and 9 nonlinear methods (120 indices) from resting supine 5-min ECGs. QVTI was determined as a measure of sympathetic tone. Normal ranges were defined for each of these measures at the 5th or 95th percentiles adjusted for age, sex, BMI, and medication influencing the autonomic tone.
Results: The prevalence of the most sensitive individual HRV parameters was: k-D: 12-17%, n-D: 12-14%, IFG-IGT: 9-10%, i-IGT: 2-9%, i-IFG: 7-10%, and NGT: 4-5%. Compared to NGT, reduced HRV was more prevalent in k-D, n-D, IFG-IGT, and i-IFG for Renyi entropy at α=4, total power spectrum, and Poincaré plot (all p<0.05). Moreover, abnormal HRV was more frequent in k-D, n-D, IFG-IGT than in NGT for RMSSD and low frequency power spectrum (all p<0.05). Most important risk factors for reduced HRV were heart rate, age, BMI, waist circumference, serum creatinine, hypertension, HbA1c, and medication with known adverse influence on HRV, while for increased QTVI reflecting increased sympathetic activity these were age, hypertension, low physical activity, percentage body fat, and the metabolic syndrome. A simple risk score to predict low HRV in clinical practice (AUC=0.86 for RMSSD) comprised heart rate, age, hypertension, serum creatinine, and medication suppressing HRV.
Conclusion: In the elderly general population, the prevalence of cardiac autonomic nerve dysfunction detected by linear and nonlinear measures of HRV is increased not only in known and newly detected diabetes, but also in combined IFG-IGT and, to a lesser degree, in isolated IFG. A simple risk score including cardiovascular risk factors appears useful to predict cardiac autonomic dysfunction in clinical practice.
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Associations between postural hypotension and neuropathy in type 2 diabetes: the Fremantle Diabetes study phase II

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Background and aims: Due to its association with mortality, it has been recommended that autonomic function be assessed at diagnosis of type 2 diabetes and then annually. The equipment and protocols for such testing are not widely available. A simple surrogate of autonomic dysfunction is orthostatic hypotension (OH) which may also be more frequent in patients with periph-

eral sensory neuropathy. The aim of this study was, therefore, to evaluate the relationship between OH and neuropathic complications of type 2 diabetes.
Materials and methods: We studied 417 unselected type 2 patients from the Fremantle Diabetes Study Phase II who underwent assessment of autonomic neuropathy between 2009 and 2012 using i) the ANS 2000 (DE Hokanson Inc, US) which measures R-R variation with deep breathing (Mean Circular Resultant and expiration/inspiration (E/I) ratio), during and after the Valsalva manoeuvre (maximal heart rate during the manoeuvre/the lowest heart rate after the manoeuvre), and on standing (the 30:15 Stand test or R-R interval at beat 30/R-R interval at beat 15), and ii) EZscan (Impeto Medical, France) which generates autonomic risk and kidney scores based on sudomotor function. OH was defined as a fall of ≥20 mm Hg systolic or ≥10 mm Hg diastolic blood pressure on standing. Peripheral sensory neuropathy was defined using the clinical portion of the Michigan Neuropathy Screening Instrument. Multiple logistic regression (forward conditional stepwise variable selection with ≥0.10 for removal) was used to determine independent associates of OH.
Results: The mean±SD age of the patients was 65.8±10.9 years, 54.2% were male, their median [inter-quartile range] diabetes duration was 10.0 [4.1-17.2] years, and 109/414 (26.3%) had OH (3 could not stand). The characteristics of those with and without OH are summarised in the Table. In logistic regression, OH was independently associated with supine systolic blood pressure (OR (95%CI): 1.28 (1.12-1.46), P<0.001 for a 10 mm Hg increase), diabetes duration (1.21 (1.04-1.40), P=0.012 for a 5-year increase), Log_e(30:15 Stand) (0.35 (0.16-0.75), P=0.007), and the EZscan autonomic risk score (0.97 (0.95-0.99), P=0.011. There was no significant bivariate correlation between the latter two measures (r=-0.002, P=0.96).
Conclusion: In our community-based patients, OH was independently associated with longer diabetes duration and a higher supine systolic blood pressure, but not peripheral sensory neuropathy. Both the 30:15 Stand test (a lower score indicates abnormal cardiovascular parasympathetic function) and the EZScan autonomic risk score (a lower score correlates with sympathetic sudomotor dysfunction) were also independent associates of OH. These data suggest that i) OH is a manifestation of complex dysfunction of parasympathetic and sympathetic systems, and ii) EZscan, a quick and simple procedure, can contribute to prediction of cardiovascular autonomic dysfunction independently of conventional heart rate-based tests.

Baseline characteristics of 414 community-dwelling people with type 2 diabetes by OH status

	OH	No OH	P-value
Age (years)	68.9±11.1	64.6±10.7	<0.001
Sex (% male)	51.4	55.1	0.58
Diabetes duration (years)	12.6 [5.0-19.7]	8.2 [4.0-16.2]	<0.006
HbA_{1c} (%)	7.1 [6.2-7.8]	6.9 [6.2-7.9]	0.75
Supine SBP/DBP (mm Hg)	150±19/81±13	140±19/79±11	<0.001/0.16
Peripheral sensory neuropathy (%)	74.3	66.9	0.19
Paced breathing: Mean circular resultant (geometric mean (SD range))	14.1 (6.8-29.4)	16.9 (7.9-36.2)	0.043
Paced breathing: E/I	1.24 (1.00-1.54)	1.23 (1.00-1.52)	0.74
30:15 Stand	1.31 (0.90-1.92)	1.43 (0.93-2.20)	0.07
Valsalva manoeuvre	1.95 (1.19-3.19)	1.99 (1.13-3.51)	0.75
EZSCAN autonomic risk score	47.5±11.8	53.1±13.0	<0.001

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Gastric emptying, glycaemia, and upper GI symptoms are independent factors in diabetic gastroparesis

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Background and aims: We have previously reported symptomatic improvement in diabetic gastroparesis in a 28-day Phase 2a study of TZP-102, a novel ghrelin agonist, and we have also reported that gastric emptying dynamics show little inter-relationship with symptom severity. For patients with diabetes mellitus and delayed gastric emptying it is important to evaluate these potential inter-dependencies. The aim of this study was to assess if symptoms and glycemia have any correlation with gastric emptying overall in a subgroup of patients with severe symptomatic diabetic gastroparesis and severely delayed gastric emptying.
Materials and methods: Double-blind, randomized, placebo-controlled, parallel-group evaluation of 3 doses of TZP-102 (10 mg, 20 mg, and 40 mg), once daily for 28 days in diabetic patients with gastroparesis. After randomization (1:1:1:1) visits occurred on Days 8, 15, 28 (on-treatment). Gastric half-emptying time (T1/2) was measured pretreatment and at Day 28 and for entry T1/2 ≥150 minutes by Screening Gastric Motility Breath Test (GMBT) was required (6-hour evaluation Metabolic Solutions, Inc.) A 4-symptom PAGI-SYM5 composite score for a subset of 4 main symptoms (nausea, early satiety, bloating, and upper abdominal pain) was analyzed. Overall linear regression (all patients) was performed and the subset of patients with pretreatment T1/2≥168 minutes (healthy volunteer mean +2 standard deviation [SD]) and the subset of patients with pre-GMBT glucose <250 mg/dL were analyzed.
Results: No correlation was observed between the severity of the symptoms and the gastric emptying T1/2 overall or in the subset of patients with severely delayed gastric emptying across TZP-102 active and placebo arms at baseline or at the end of therapy (Figures 1 and 2). Linear regression analyses showed no correlation between absolute blood glucose values at the time of GMBT and gastric T1/2 (Figure 3).
Conclusion: Ghrelin agonist TZP-102, an oral once daily treatment, had a good safety profile in diabetic patients with gastroparesis. In a subset analysis based on blood glucose and gastric T1/2 thresholds the gastric emptying outcomes were similar to the overall study results. There was no correlation between change in gastric emptying during therapy and change in the severity of symptoms, suggesting that these can be independent events in diabetic gastroparesis. Gastric emptying has been correlated with glycemia in healthy subjects, but this correlation was not observed in diabetic gastroparesis patients within the context of the glucose ranges in this study.
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Gastroparesis in adults with type 1 diabetes from the type 1 diabetes exchange clinic registry

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Background and aims: Gastroparesis and its implications are not well characterized in adults with type 1 diabetes (T1D), in part due to small patient cohorts described to date. To better understand the prevalence and impact of gastroparesis, we investigated the clinical diagnosis of gastroparesis in the large T1D Exchange clinic registry database.
Materials and methods: The T1D Exchange clinic registry includes over 25,000 participants with T1D enrolled at 67 centers across the U.S., with data collected through medical chart review and participant questionnaires. This analysis cohort includes 7098 participants who were ≥26 years old, and had diabetes duration of ≥2 years (91% white non-Hispanic, 54% female, mean age 46.7 years, mean duration of T1D 24.7 years). Presence or absence of gastroparesis was obtained from medical records.
Results: Among the 7098 participants, 343 (5%) had the clinical diagnosis of gastroparesis. Participants with gastroparesis were more likely to be female (67% vs. 54%, P<0.001), have longer duration of T1D (mean duration 32.6 years vs. 24.3 years, P<0.001), have higher mean HbA1c (8.1% vs. 7.7%, P<0.001), and were less likely to report CGM sensor use (14% vs. 22%, P<0.001), but were not different with respect to age, race/ethnicity, or use of insulin pump. There was a higher occurrence of severe hypoglycemia characterized by seizure and/or loss of consciousness in participants with gastroparesis (25% vs. 11%, P<0.001). Among the 343 participants with gastroparesis, 87 (25%) were taking one or more of the following medications: metoclopramide, ondansetron, erythromycin, domperidone. Compared with older participants (≥50 years old), a higher proportion of younger participants (26 to <50 years old) with gastroparesis were taking medication (30% vs. 21%, P=0.04). Among the 343 participants with gastroparesis, 192 (56%) had at least one other type of neuropathy. Most of these additional types of neuropathy were diabetic peripheral neuropathy (76%); the remaining types included: erectile/sexual dysfunction (11%), charcot joint (8%), orthostatic hypotension with fixed heart rate (3%), and tachycardia with fixed heart rate (2%).

Conclusion: In adults ≥26 years of age participating in the T1D Exchange registry, 5% had a clinical diagnosis of gastroparesis. Compared with all the participants in this cohort, the subjects with gastroparesis had a longer duration of T1D, higher levels of HbA1c, and a higher frequency of severe hypoglycemic episodes.
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New technologies of fertility restoration in patients with type 1 diabetes mellitus and an autonomic neuropathy
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Background and aims: Retrograde ejaculation (RE) in patients with Type 1 Diabetes Mellitus (DMT1) is a complication of an autonomic neuropathy, which complicated by excretory infertility. RE can be partial (excretion reduction of ejaculate) and total (absence of ejaculate) and it varies from 10% to 20% in men with Type 1 DM. The aim of the study is to assess the effectiveness of new endoscopic method of RE correction and antegrade ejaculation restoration.
Materials and methods: Inclusion criteria: spermatozoa presence in post-orgasmic urine and the disclosure of bladder neck on ultrasonography. 10 Type 1 DM patients, age of 25,7±6,1 years with total RE recruited, diabetes duration - 19±9,6 ys. Mean level of HbA1c before operation 7,1±1,3%. We used conventional irrigated urethrocystoscopy under local anesthesia. During urethroscopy the bladder neck gaping was observed in all cases. Biocompatible material was injected in three points on 14, 18, 22 hours through a special injection needle under mucous layer of a posterior urethra, reaching the closing of the opposite edges of urethra. The spermogramm was examined in 1 week after the operation.
Results: Restoration of antegrade emission of ejaculate achieved at 9 out of 10 patients. The effect of operation maintained during: 6 months at 6 patients; 9 months at 2 patients; 12 months at 1 patient. The spouses of the two men have got pregnancy after surgery. In one case pregnancy completed of spontaneous abortion on the 8th week, in other case pregnancy completed with normal childbirth.
Conclusion: The applying of new method provides highly effective restoration of a physiological passage of the ejaculate. Endoscopic operation is a low-invasive and doesn't disrupt the urination. There is the real opportunity of receiving ejaculate for the artificial insemination. Further investigation is needed.

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Assessment of sudomotor function as a screening tool for microvascular complications in type 2 diabetes
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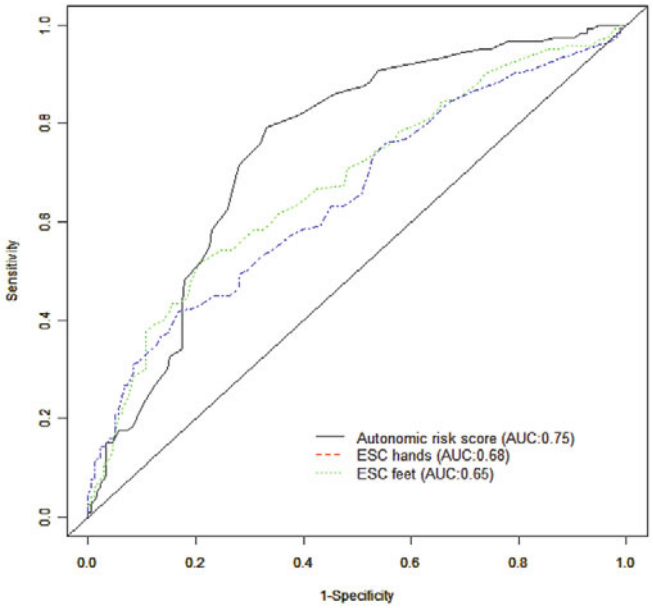
Background and aims: Sweat glands are innervated by small C-fibers, and sudomotor dysfunction is one of the earliest abnormalities to manifest in distal small fiber neuropathy. This study aimed to evaluate SUDOSCAN, a non invasive, quick, simple and quantitative method to measure sweat function, as a screening tool for microvascular complications in type 2 diabetes.
Materials and methods: 309 patients were evaluated for microvascular complications including peripheral neuropathy using a biothesiomether (Vibration Perception threshold > 15 V), nephropathy through measurement of creatinine clearance and calculation of Modification of Diet in Renal Disease (MDRD < 60 ml/mnx1.73 m2), and retinopathy through fundus of the eye examination. Small C-fiber status was assessed through sudomotor function by measurement of hand and foot Electrochemical Sweat Conductance (ESC) and calculation of an autonomic risk score using SUDOSCAN. Patients were required to place their palms and soles - where sweat gland density is the highest - on two large stainless-steel electrodes and then to stand still for 2 minutes. ESC, expressed in microSiemens (μS), is the ratio between current generated and the constant DC stimulus applied on the electrodes.
Results: Hand and foot ESC were lower in patients with at least one microvascular complication as compared to patients without: 49 ± 20 vs 62 ± 17 μS, p <0.001 and 59 ± 21 vs 69 ± 15 μS, p<0.001 respectively. Receiver Operating Characteristics (ROC) curve for detection of at least one microvascular complication is displayed in Figure. Sensitivity and specificity of autonomic risk score using 35% as a threshold were 82% and 61% respectively.
Conclusion: Based on this study that should be completed by a larger clinical study, SUDOSCAN could be used for the screening of microvascular complications in type 2 diabetes and may aid in adhering to follow-up guideline recommendations which are currently unfulfilled.



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Screening for diabetes and its microvascular complications in subjects at risk by a rapid and non-invasive measurement of sweat function
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Background and aims: Several studies have shown a sweat dysfunction, witness of autonomic neuropathy, beginning in people with prediabetes and present in type 2 diabetes patients with microvascular complications. EZSCAN is a new quick and noninvasive technology to assess the sweat function at the palms and soles. It is based on the measurement of conductance resulting from the electrochemical reaction between the chloride ions of sweat and electrodes in contact with the skin. The purpose of this study was to compare this method with conventional tool for screening of diabetes and for screening of microvascular complications of diabetes in patients involved in the AUSDIAB program.
Materials and methods: This study was conducted in 1307 subjects at risk for diabetes. The patients underwent the questionnaire AusDrisk, a fasting plasma glucose, an oral glucose tolerance test (OGTT), an HbA_{1C}, the calculation of Modification of the Diet in Renal Disease (MDRD) index to assess nephropathy and measurement EZSCAN risk score based on measurement of electrochemical sweat conductances of hands and feet. Receiver operating characteristics curve with calculation of the area under the curve (AUC) were performed and odds ratio (OR) were calculated to evaluate screening performances of diabetes or its microvascular complications of this new method.
Results: Based on OGTT2h ≥ 11.1 mmol/l or FPG ≥ 7 mmol/l or HbA_{1C} ≥ 6.5 %, 131 of the subjects involved in the study had diabetes. When looking at screening of diabetes the AUC of the ROC curves was 0.77 for EZSCAN risk while it was 0.75 for AusDrisk score. When focusing on patients with diabetes the OR when compared to patients with no risk according to EZSCAN (4/257) was 5.9 (p<0.001) for patients defined at moderate risk (72/851) and 24.2 (p<0.001) for patients at high risk (55/199) after adjustment on age the OR were 5.4 p=0.002 and 21.1 p<0.001 respectively. When looking at screening of diabetes complications and choosing MDRD < 60 ml/min/1,73m² as reference the AUC of the ROC curve was 0.82 for EZSCAN and 0.73 for AusDrisk.
Conclusion: This study shows that EZSCAN could be used for screening of diabetes or its microvascular in population at risk. These results have to be confirmed on a larger population and with a comparison to other methods of screening.

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Sudomotor dysfunction is associated with increased mortality in patients with chronic kidney disease, with or without diabetes mellitus

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Background and aims: Little is known on the association of sudomotor dysfunction with mortality in patients with chronic kidney disease. Therefore, we carried out a prospective 1-year study to examine this issue.
Materials and methods: We included 153 patients as follows: 97 from dialysis unit [28 with diabetes (DM) and diabetic nephropathy; 37 with nephroangiosclerosis, (NA), 18 with glomerulonephritis (GN), 14 with other conditions (O)]; 32 with type 2 DM and creatinine clearance (CrCl) 30-50 ml/min; and 24 transplanted patients with CrCl>30 ml/min (of these 8 had DM). Somatic sensory neuropathy was documented using the Neuropathy Disability Score (NDS) and the vibration perception threshold (VPT). Sudomotor dysfunction was evaluated by time to colour change of the Neuropad® test. Peripheral vascular disease (PAD) was diagnosed on the basis of Ankle-Brachial Index (ABI) measurement, abnormal waveforms or history of PAD. Investigations were performed twice: 1 year ago and this year. For patients who had died, we only used the data from the first examination.
Results: Among DM patients, 13 exhibited IWGDF risk level 3 diabetic foot pathology (3 critical ischaemia, 3 Charcot foot, 2 ulcer, 2 minor and 3 major amputation). There was 1 patient with critical ischaemia in the NA group and 1 in the GN group. In the 12-month period, 14 patients on haemodialysis had died: 9 in the NA group (mean age 66.8±11 years), 4 among DM (mean age 59±11.9 years), and 1 in the O group (73 years). In univariate analysis, the presence of IWGDF risk 3 level was related to dialysis treatment [odds ratio (OR): 1.18, 95% Confidence Interval (CI): 1.09-1-29, p=0.02]), PAD (OR: 0.55, 95% CI: 0.47-0.64, p=0.01]), VPT (OR: 12.6, 95% CI: 2.7-58.1, p<0.001]), sensory loss (OR: 548, 95% CI: 52.9-5682, p<0.001]). In univariate analysis, death was related to age (OR: 1.06, 95% CI: 1-1.11, p=0.02]), dialysis treatment (OR: 1.17, 95% CI: 1.08-1.27, p=0.003]), VPT (OR: 3.1, 95% CI: 0.99-9.8, p=0.04]), loss of ankle reflexes (OR: 1.7, 95% CI: 1.1-2.8, p=0.007]), and abnormal Neuropad® response (OR: 1.1, 95% CI: 1.03-1.19, p=0.004]). In multivariate analysis, only abnormal Neuropad® test remained significant (p<0.05). Time to complete Neuropad® colour change was 26.4±8.2 minutes in patients who had died and 17.8±9.8 minutes in those remaining alive on dialysis or pre-dialysis renal failure (p=0.002). In univariate ANCOVA test, the association between complete colour change time of Neuropad (dependent variable) and PAD (fixed factor), using age and CrCl as covariates, was significant (p<0.001).
Conclusion: These findings indicate that sudomotor dysfunction is associated with increased mortality in patients with chronic kidney disease, with or without DM. Therefore, we suggest more the widespread use of Neuropad® test, including such population.
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Sexual dysfunction among Sri Lankan adults: effect of diabetes

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Background and aims: Diabetes has become an important public health problem due to its numerous microvascular and macrovascular complications. Diabetes is recognised to be associated with all aspects of sexual dysfunction in men, including desire, erection (arousal), ejaculation (orgasm) and satisfaction. Similarly, diabetes also affects all stages of female sexual response, especially the arousal. Despite diabetes being increasingly recognised among South Asians, there is a paucity of data on sexual dysfunction, especially among the South Asian women. We aimed to determine the prevalence of sexual dysfunction and the effects of diabetes on sexual function in Sri Lankan adults.
Materials and methods: Data on sexual function, relevant socio-demographic factors and diabetic status were obtained from a nationally representative sample of 4485. Recruitment was performed using a multi-stage stratified cluster sampling technique between August 2005 and September 2006. Diabetes was diagnosed using fasting plasma glucose and 75g oral glucose tolerance test according to the World Health Organisation criteria. Anthropometric measurements were performed according to the standard methods using calibrated equipment. All aspects of sexual function were assessed using a modified questionnaire based on the International Index of Erectile Function in men and Female Sexual Function Index in women. A stepwise multiple regression analysis was performed using presence of any form of sexual dysfunction as the dependant variable to identify covariates of sexual dysfunction.
Results: Sample size was 4.485 (response rate -89.7%), 39.5% (n=1,772) were males and 17.6% (n=789) were residing in urban areas. Mean age was 46.1±15.1 years. Crude prevalence of diabetes was 12% (n=536). Overall, 2597 adults admitted to being sexually active. Among the 1109 sexually active males, 73 (6.6%) had a sexual problem whereas among the 1488 sexually active women, 117 (7.9%) complained. Men with diabetes compared to non-diabetic individuals had a higher prevalence of any form of sexual dysfunction (18.4% vs. 5.1%, p< 0.001); lack of desire (6.4% vs. 1.5%, p< 0.001), erectile dysfunction (16.8% vs. 3.7%, p<0.001) and lack or delay in orgasm (3.2% vs. 1.2%, p<0.001). The presence of any form of sexual dysfunction was significantly higher among the women with diabetes (13% vs. 7.3%, p<0.05). Similarly, women with diabetes when compared to the non-diabetic counterparts had higher prevalence of lack of desire (8.9% vs. 3.3%, p<0.01), reduced lubrication (4.1% vs. 1.5%, p<0.05) and absence of orgasm (4.1% vs. 1.1%, p<0.01). In the stepwise multiple-regression analysis, the duration of diabetes (OR: 3.8, 95% CI 2.4-6.1) significantly associated with any form of sexual dysfunction.
Conclusion: We found that sexual dysfunction is significantly common in those with diabetes compared to the non-diabetic adults in Sri Lanka. Primary prevention of diabetes and prevention of diabetic complications by good diabetes care may improve the overall sexual health of the population especially in ethnicities like South Asians with higher prevalence of diabetes.

PS 106 Neuropathy I: correlates and treatments

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Association between distal arterial calcification and diabetic neuropathy in diabetes mellitus

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Background and aims: Medial arterial calcification is common in diabetes mellitus (DM) and is an independent risk factor for major amputation, cardiovascular and all-cause mortality. There is evidence that distal arterial calcification is associated with distal polyneuropathy (DPN) and cardiovascular autonomic neuropathy (CAN) and that the extent of coronary artery calcification (CAC) is closely associated with CAN. The aim of the study was to assess quantitatively tibial arterial calcification (TAC) and CAC in patients with DM and to correlate the extent of calcification with the presence and severity of DPN and CAN.
Materials and methods: 85 patients with DM (54 women, 31 men, type 1 -37, type 2 -48, mean age -51,9±11,9 years) were assessed. Exclusion criteria were: peripheral artery disease (ankle-brachial index<0,9), glomerular filtration rate (GFR)<30 ml/min/1,73 m², DM 1 duration<5 years. Nerve function was assessed by 10-g monofilament detection, vibration threshold, electrophysiological study of peroneal nerves and cardiovascular tests. All patients underwent multislice computed tomography. Calcium scores were determined according to the method described by Agatston et al.
Results: Patients with DM2 had significantly higher CAC scores than DM1 patients (p=0,007) with no difference in TAC scores. There was significant inverse correlation between TAC scores and peroneal amplitude (r=-0,45, p<0,0001), vibration threshold (r=-0,44, p<0,0001), and nerve conduction velocity (r=-0,5, p<0,0001). Significant correlation was found between TAC and CAC scores (r=0,4, p<0,0001). The severity of CAC also correlated with vibration threshold (r=-0,3, p<0,006). In 60% of patients with TAC scores>1000 CAN was diagnosed, but there was no significant association between the quantity of TAC and CAC scores and the presence of CAN.
Conclusion: No association between autonomic neuropathy and artery calcification was found. Medial artery calcification is strongly associated with DPN and coronary calcification. These data suggest that DPN might be an independent cardiovascular risk factor.

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Skin microvascular reactivity relates to advanced glycation end-products accumulation and endothelial activation in patients with diabetes

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Background and aims: The development of diabetic microangiopathy is associated with changes of microvascular reactivity (MVR). Moreover, the advanced glycation end-products (AGEs) accumulation is supposed to be involved in the pathogenesis of diabetic angiopathy. The aim of our study was to measure parameters of skin MVR and skin autofluorescence (AF) reflecting AGEs accumulation, and compare them with markers of endothelial activation in patients with diabetes.
Subjects and methods: Microvascular reactivity (post-occlusive reactive hyperemia /PORH/ and thermal hyperemia /TH/), assessed by laser-Doppler fluxmetry using PeriFlux 4001 (Perimed), was measured on the forearm in 47 patients with diabetes (26 Type 1 /T1DM/, 21 Type 2 /T2DM/; aged 55 ± 14 yrs; diabetes duration 17 ± 10 yrs) and 25 healthy controls (aged 45 ± 10 yrs). Skin AF, expressed in arbitrary units (AU), was measured by AGE-Reader (Diagnoptics BV) on the forearm as well. In all patients, glycated haemoglobin HbA_{1c} (expressed in IFCC units), parameters of endothelial activation (vWF, ICAM, VCAM, E-selectin, P-selectin) and markers of microangiopathy (microalbuminuria, vibration perception threshold estimated by Biothesiometer) were assessed.
Results: Diabetes control expressed by glycated heamoglobin was similar in patients with both T1DM and T2DM (73 ± 20 vs. 67 ± 18 mmol/mol, NS). Microangiopathy (positive albuminuria and/or neuropathy) was present in 41 % of patients with diabetes. Patients with diabetes were evaluated together, since the differences between T1DM and T2DM did not reach statistical significance both in skin AF and microvascular reactivity. Skin AF was significantly higher in patients with diabetes as compared to controls (2.38 ± 0.54 vs. 2.04 ± 0.47 AU, p<0.01). Patients with higher skin AF (AF>2.3 AU) had significantly lower MVR in comparison to patients with lower skin AF (AF<2.3 AU) as expressed by PORH: 353 vs. 526 %, p<0.002; and TH: 908 vs. 1494 %, p<0.02. Significant inverse relationship was found between skin AF and MVR in patients with diabetes (PORH: r=-0.42, p<0.006; TH: r=-0.45, p<0.004). Interestingly, vWF as a marker of endothelial activation was positively associated with skin AF (r=0.61, p<0.001) and inversely associated with TH (r=-0.46, p<0.02) in T1DM and T2DM together. Regarding endothelial activation (EA), if all patients with diabetes were divided according to the activity of vWF into the EA negative (EA-, vWF<110 %) and EA positive (EA+, vWF>110 %) subgroups, significantly lower MVR was observed in EA+ than in EA- patients (PORH: 397 vs. 496 %, p<0.05; TH: 1006 vs. 1776 %, p<0.04).
Conclusion: This is the first study describing significant inverse relationship of the skin microvascular reactivity and skin autofluorescence in patients with diabetes. Our results demonstrate that increased accumulation of advanced glycation end-products in the skin is associated with worsened microvascular reactivity as well as elevated endothelial activity. Causal relationship is suspected but it will be further elucidated in the follow-up study.
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Serum osteoprotegerin levels are associated with peripheral neuropathy in patients with type 2 diabetes

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Background and aims: Recent data suggest that serum osteoprotegerin (OPG) is a useful marker of atherosclerosis and vascular dysfunction in diabetes. The purpose of this study was to determine the relationship of serum OPG levels with peripheral sensorimotor neuropathy (PN) in patients with type 2 diabetes (T2DM).
Materials and methods: A total of 71 patients with T2DM were recruited (mean age 67.7±8.9 years, mean diabetes duration 15.0±10.6 years, male/female 45/26). Diagnosis of PN was based on neuropathy symptom score (NSS), neuropathy disability score (NDS) and vibration perception threshold (VPT). Serum OPG levels were measured using Luminex Multiplex assay. Diagnosis of peripheral arterial disease (PAD) was based on the presence of either biphasic, monophasic or blunted waveforms at the pedal arteries.
Results: Patients with PN (n=39) had significantly higher serum OPG levels in comparison with those without PN (744.8±296.2 vs 555.8±165.5 pg/ml). OPG levels were significantly associated with indices of PN, such as NDS (r=0.390, p<0.001) and VPT (0.412, p<0.001). Univariate logistic regression analysis showed that age (p=0.026), diabetes duration (p<0.001), male gender (p=0.003), smoking (p=0.004), BMI (p=0.024), dyslipidemia (p<0.001), presence of PAD (p<0.001) and OPG levels (p<0.001) were significantly associated with PN. No significant associations were found with HbA1c and arterial hypertension. Multivariate logistic regression analysis, after adjustment for age, gender and BMI, demonstrated that PN was associated significantly with diabetes duration [odds ratio (OR): 1.01, 95% confidence intervals (CI): 1.01-1.11], p=0.023), smoking (OR: 8.2, 95% CI: 1.41-47.92, p=0.019), dyslipidemia (OR: 4.3, 95% CI: 1.50-12.44, p=0.007), presence of PAD (OR: 3.0, 95% CI: 1.17-7.83, p=0.023) and OPG levels (OR: 1.0, 95% CI: 1.001-1.007, p=0.007).
Conclusion: Serum OPG levels are increased in patients with PN and are associated with the presence of PN irrespective of age, diabetes duration, gender, smoking, BMI, dyslipidemia and PAD status.

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Osteoprotegerin, RANKL, RANK genes polymorphisms in diabetic Charcot neuroosteoarthropathy

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Background and aims: Charcot neuroosteoarthopathy(CN) is a rare but devastating complication of diabetes. The etiology is not fully understood but it involves interaction of several factors including abnormalities in bone metabolism. Bone reapsorption is a frequent feature of Charcot foot . Inflammatory condition definitely contributes to the pathogenesis. It is however likely that the cytokines of RANK, RANKL and OPG pathway may contribute to the pathogenesis of osteolysis in Charcot foot. Recently we have suggested an association between two OPG polymorphisms (1181G>C and 245T>G) and diabetic Charcot neuroosteoarthopathy. We have now analysed the frequency of OPG gene polymorphisms in a larger cohort of patients with Charcot neuroosteoarthropathy, diabetic patients with neuropathy (N), diabetic patients without neuropathy(D) and healthy controls(C).
Material and methods: A total of 237 subjects: 64 Charcot neuroosteoarthropathy patients, 44 diabetic patients with neuropathy, 34 diabetic patients without neuropathy and 95 healthy controls were genotyped for 5 different single nucleotide polymorphisms (SNP) within the OPG gene:T245G (rs3134069) G1181C (rs2073618), T950C (rs2073617), C1217T (rs3102734) and A6890C (rs7844539), two polymorphisms in RANK gene C421T (rs35211496) and C575T (rs1805034) and three polymorphisms in RANKL gene:C290T (rs9525641), C643T(rs9533156) and C693G (rs9533155). We have used RFLP and minisequencing to analyze polymorphisms. We have also used ELISA to determine serum levels of OPG and RANKL proteins.
Results: Statistically significant differences between the group of subjects with neuropathy but no Charcot neuroarthropathy and the control group were found only for T245TG (rs3134069) polymorphism. We did not confirm our previous observation of the association of Charcot neuroosteoarthropathy with C1217T(rs3102734) polymorphism . Our analysis showed no significant correlation between studied RANKL gene polymorphisms. With respect to serum OPG concentration statistically significant differences were found only between the patients with neuropathy (N) in which OPG levels were higher and the group with diabetes and no neuropathy (D). Biochemical analysis showed increased levels of RANKL protein in blood serum in patients with neuropathy but not in patients with Charcot's arthropathy.
Conclusion: Genetic factors, particularly T245G (rs3134069) OPG gene polymorphisms, may play a role in the development of diabetic Charcot neuroarthropathy.
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Long-term effect of quinapril or losartan or their combination on diabetic autonomic neuropathy and left ventricular function over a period of 4 years

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Background and aims: To investigate the effect of angiotensin converting enzyme inhibition or angiotensin receptors blockers or their combination on definitive Diabetic Autonomic Neuropathy (DAN) and left ventricular (LVF) systolic and diastolic function over a period of four years
Materials and methods: Sixty patients (30 women, 34 with type 1 DM, aged 51.7±15.7 y, DM duration 20.3±7.0 y) with DAN were studied for a 4 years. No patient with coronary artery disease included. Patients were randomly allocated in 3 groups A (n=24), B (n=18), C (n=18) receiving 20 mg quinapril (Q), 100 mg losartan (L) and 20 mg Q +100 mg L respectively. The presence of DAN was established if 2 or more of the 4 Cardiovascular Reflex Tests (CRT) were abnormal and analyzed with Mean Circular Resultant (MCR), Valsalva index (VALS), 30:15 ratio (POST) and postural hypotension (HYPO). LV function was investigated with radionuclide ventriculography (RNV) at rest. Ejection fraction (EF) was used to assess LV systolic function, while peak filling rate (PFR), first third filling fraction (1/3FF), and atrial contribution to ventricular filling (A/V) were used to investigate LV diastolic function. CRT and RNV performed at baseline and after 4 years. We measured Heart Rate (HR), Systolic and Diastolic Arterial Pressure (SAP,DAP) and HbA1c, also.
Results: In all groups improvement was observed after 4 years of treatment in MCR (A, 16.9± 5.6 vs 29.4± 15.6, p= 0.007, B, 17.3± 9.2 vs 27.2± 17.5 p=0.014, C, 12.9± 10.0 vs 23.0± 13.9, p<0.001). In group C a decrease in SAP and DAP observed at 4 years (142.3±23.7 vs 129.2.0±15.2, 83.2±8.8 vs 77.0±7.9, p<0.05 respectively). In group A a decrease in A/V (%SV) observed 24.4±8.7 vs 20.6±7.3, p<0.05. The rest of CRT and RNV indices were not significantly changed over base line values in 4 years. HR and HbA1c did not change significantly
Conclusion: DAN improved after four years of treatment with quinapril, losartan, or their combination. No deterioration observed in systolic and diastolic LVF except for group A, also. Improved autonomic balance and no decline of LVF may be of clinical importance in long-term prognosis of DM patients

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Amelioration of neuropathic changes with correction of nerve insulin resistance in diabetic Goto-Kakizaki rats treated with DPP-IV inhibitor vildagliptin

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Background: Peripheral insulin resistance is one of the main pathogenetic processes in type 2 diabetes. Recently, insulin resistance in the peripheral nerve has been proposed to contribute to the pathogenesis of diabetic neuropathy (DN) and may be a novel therapeutic target for DN. While there were several investigations that demonstrated that incretin therapy was beneficial for neuropathy in type 1 diabetic animals, the effects on type 2 diabetic models are yet to be examined. The mechanism of how incretin influences on DN was not well addressed in those studies. We therefore investigated whether the treatment with DPP-IV inhibitor, vildagliptin (VG), is beneficial for neuropathy in type 2 diabetic model, Goto-Kakizaki rats (GK) and also explored if insulin signaling in the peripheral nerve is implicated in the incretin effects on DN.
Materials and methods: Male GK 4 weeks of age were divided into 3 groups; untreated, treated with restricted diet (diet group), and treated with DPP-IV inhibitor, vildagluptin (VG) at a dose of 15 mg/kg/day orally twice a day. Age- and sex-matched Wistar rats (W) divided into similar 3 groups served controls. During observation period of 18 weeks, blood glucose, serum insulin, GLP-1 level, motor and sensory nerve conduction velocities (MNCV and SNCV) were monitored. At end, dorsal root ganglia (DRG) and sciatic nerves were extirpated for structural and molecular analyses.
Results: VG-treatment suppressed hyperglycemia, corrected serum level of insulin and GLP-1 in GK (p<0.01). Diet on GK also suppressed hyperglycemia to the extent similar to that in VG-treated GK, improved serum insulin but not GLP-1. Both MNCV and SNCV were significantly delayed in GK. VG treatment on GK improved both NCV near normal levels, but recovery of NCV was equivocal in diet-GK. RT-PCR disclosed an expression of GLP-1 receptor-mRNA in DRG, but not in sciatic nerve, and western blot revealed its protein in both DRG and sciatic nerve. The expression levels were comparable between GK and W. Western blot also demonstrated marked reduction of phospho-CREB, Akt and S6RP expressions in DRG of GK compared to W. They all recovered near 90% of normal in VG-treated GK, but not in diet GK. IRS-2 expression in DRG was similar between GK and W, but immnoprecipitation analysis demonstrated marked reduction of phospho-tyrosine residues of IRS2 in GK compared to W. VG-treatment in GK reverted the phosphotyrosine by 60%, but this was not the case in diet GK. Excessive phosphoserine residue of IRS2 as a marker of insulin resistance was demonstrated in GK, and was normalized in VG-treated GK. The recovery of phospho-serine was partial (30%) in diet GK.
Conclusion: DN in GK was ameliorated by VG-treatment which may have directly exerted GLP-1 receptor signal together with activation of insulin signals. Correction of hyperglycemia by diet alone was not sufficient to fully improve cellular signals of DRG in GK.

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Treatment for chronic pain syndrome of diabetic peripheral neuropathy in type 2 diabetics with thioctic acid and standardised protein-free dialysate of calf blood

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Background and aims: The aim of the study was to reveal the advantages of the combined treatment of pain syndrome with thioctic acid and standardized protein-free dialysate of calf blood in type 2 diabetes mellitus (DM2) patients with diffuse symmetric distal sensorimotor polyneuropathy.
Materials and methods: The study enrolled 62 DM2 patients with diffuse symmetric distal sensorimotor polyneuropathy and chronic pain syndrome (29 females and 28 males). The mean age of the patients was 68.2±5.3 years, the mean duration of DM2 was 17.6±3.3 years, the mean level of glycated hemoglobin (HbA1c) was 7.6±1.2 %. The initial mean intensity of the pain syndrome was 8.07±0.31 points. Blood glucose-lowering therapy, i.e., sulfonylureas (n=16), insulin therapy (n=23), or both (n=18), remained unchanged throughout the whole follow-up period. After signing the informed consent form, patients were randomly assigned to one of the three groups: group I (n=21) included patients who were given 15 intravenous infusions of thioctic acid at a dose of 600 mg/day and then received it orally at the same dose over 24 months; in group II (n=21), daily intramuscular injections of 212.5 mg standardized protein-free dialysate of calf blood over 15 days were added to thioctic acid (15 intravenous infusions of 600 mg/day followed by oral administration at 600 mg/day over 24 months). The control group (n=20) consisted of patients who had initially received 15 intravenous infusions of thioctic acid at 600 mg/day followed by its oral administration at the same dose (600 mg/day) over 2.5 months. HbA1c levels and pain intensity (using a 10-point visual-analogue scale) were evaluated at baseline and at 3, 6, and 24 months. The results were statistically treated using Statistica 6.0 software. The results were considered significant at $p<0.05$.
Results: At 3, 6, and 24 months, pain intensity in group I was reduced on average by 4.3±0.3 points (16.2%; p=0.67), 4.0±0.1 points (33.3%; $p<0.01$) and 4.1±0.1 points (60.0%; $p<0.001$), respectively, as compared to the control. Pain syndrome with the intensity of 4 points relapsed in one patient with an acute exacerbation of chronic bronchitis treated with 3rd generation cephalosporins; in one patient from group I, pain syndrome provoked by cooling of lower extremities persisted. Pain recurrence was relieved by intravenous infusions of thioctic acid at 600 mg in the physiological saline in combination with intramuscular injections of 212.5 mg standardized protein-free dialysate of calf blood for 15 days. In group II, pain intensity was reduced on average by 5.8±0.2 points (56%; $p<0.001$), 5.4±0.1 points (80.1%; $p<0.001$), and 5.1±0.2 points (96.2%; $p<0.001$) at 3, 6, and 24 months, respectively, as compared to the control. The relapse-free period was significantly longer in patients continuing administration of thioctic acid over 24 months (group II) as compared to both the control group and group I (85% vs. 30% and 66%, respectively).
Conclusion: Combined treatment with thioctic acid and standardized protein-free dialysate of calf blood administered through intramuscular injections at a dose of 212.5 mg was significantly more effective in reducing pain syndrome intensity and frequency of relapses in patients with DM type 2 and diabetic peripheral neuropathy as compared to thioctic acid monotherapy for 3 or 24 months.

PS 107 Neuropathy II: large fibres, small fibres, peripheral and central

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The influence of diabetic neuropathy on imbalance in patients with diabetes mellitus

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Background and aims: It is well known that presyncope and syncope due to autonomic neuropathy with orthostatic hypotension are the common cause of falls in patients with diabetes mellitus (DM). Imbalance of diabetic patients unrelated to orthostatic blood pressure reduction are studied less. The aim of this study was to assess posture stability using computerised dynamic posturography in type 1 and type 2 diabetes mellitus patients and to identify the association between diabetic neuropathy and deterioration of balance.
Materials and methods: The 102 (age between 22 and 74 years) type 1 and type 2 DM patients participated. The postural stability was evaluated using sensory organisation test (SOT), motor control test (MCT) of computerised dynamic posturography (CDP). Neurological disability score (NDS) and neuropathy impairment score of the lower limbs (NIS-LL) were used to determinate specific somatosensory loss. Trail making test was used to assess the role of frontal dysfunction in posture stability.
Results: Patients were divided into 3 groups: 1) without reduction of proprioception (kinaesthesia), vibration or tactile sensation, n=27; 2) with reduction of vibration or tactile sensation, but without reduction of proprioception, n=37; 3) with reduction of proprioception, vibration and tactile sensation (large fiber dysfunction), n=38. Equilibrium scores in sensory organisation test (SOT) 1, 2, 3 conditions and the composite muscle response latencies in MCT were significantly worse ($p\leq0.03$) in the group 3 as compared to the group 1. The composite muscle response latencies were 148.5 msec (142.0; 156.0) in the group 3 and 140.0 msec (132.0; 148.0) in the group 1 ($p<0.01$). The patients from group 3 were characterized longer duration of diabetes, more frequent events of imbalance complaints and history of falls as compared to the group 1. There were not significant differences between three groups in age, BMI, levels of HbA1c, in presence of vestibular syndrome and trail making test parameters. Large fiber dysfunction was associated with increased risk of history of falls (1.6, 95% CI 0.9 to 2.8) and with increased risk of imbalance complaints (1.4, 95% CI 1.1 to 1.9).
Conclusion: Peripheral neuropathy with large fiber dysfunction is independent risk factor of postural instability in diabetic patients. The large fiber dysfunction with the reduction of proprioception leads to instability in enviroments of low lighting, visual movement and in case of unexpected external disturbances.

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Function of large nerve fibres and levels of HSP27 in type 1 diabetes: a longitudinal follow-up

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Background and aims: Diabetes mellitus is regarded as the leading causes of peripheral neuropathy. However, there is a lack of a circulating biomarker of peripheral nerve dysfunction. Heat shock protein 27 (HSP27) is proposed as a neuroprotective factor and may even be important for axonal regeneration. Therefore, it is of interest to study whether HSP27 serves as a potential biomarker for nerve dysfunction in individuals at risk. Currently, there are no prospective studies investigating serum HSP27 concentrations and progression of peripheral nerve dysfunction among individuals with type 1 diabetes mellitus or in healthy subject. Thus, our aims were to investigate possible associations between serum HSP27 concentration and measures of peripheral nerve dysfunction, and whether HSP27 concentrations differ between individuals with and without type 1 diabetes (T1DM).
Materials and methods: This longitudinal study is based on patients with T1DM that were regularly treated in our Diabetes Clinic and received their

diagnosis between the ages of 15 and 26. The patients were asked to participate in a study of neuropathy in 1993 and were then followed-up in 2005. Nerve conduction studies, vibration and thermal threshold tests were performed on the lower extremities at baseline and follow-up. Serum HSP27 concentrations were determined at baseline and follow-up as well as for healthy non-diabetic subjects in 2004.
Results: Twenty-three T1DM subjects, including 11 women, and with baseline age of 40±8 years and diabetes disease duration of 22±8 years were included. HbA1c and blood pressure were not significantly different between baseline and follow-up. Serum HSP27 concentrations were not significantly different at follow-up (median 363 pg/ml, IQR 312-1047) than baseline (median 462 pg/ml, IQR 312-969). Healthy non-diabetic controls (n=372) had higher HSP27 concentrations (median 900 pg/ml, IQR 454-1465) than T1DM ($p = 0.014$ at baseline, $p = 0.005$ at follow up). The dynamic change of HSP27 (baseline HSP27 - follow-up HSP27; delta HSP27, median 25 pg/ml, IQR -380-190) significantly correlated to that of large nerve fiber function (delta vibration thresholds, median -11 Hz, IQR 1-51, $r = -0.47$, $p = 0.03$; delta nerve conduction, median 0.7 z-score, IQR -0.2-1.7, $r = 0.44$, $p = 0.04$), but not to small nerve fiber function (cold thresholds $r = -0.33$, $p = 0.14$ and heat thresholds $r = -0.15$, $p = 0.58$). Gender and age did not correlate with delta HSP27. Univariate linear regressions revealed that deteriorating nerve conduction and vibration thresholds from baseline to follow-up were associated with a decrease of HSP27 concentration ($B = 0.001$, SE 0.001, $\beta = 0.44$, $p = 0.038$), and $B = -0.02$, SE 0.01, $\beta = -0.47$, $p = 0.031$, respectively).
Conclusion: A novel finding is that individuals with type 1 diabetes had lower serum HSP27 concentrations than healthy non-diabetic controls. A decrease in large nerve fiber function in individuals with type 1 diabetes is associated with a decrease in serum concentration of HSP27 over time. Despite the small number of type 1 diabetes participants, our preliminary data suggests that HSP27 might potentially be used to follow the progression of peripheral neuropathy.

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Prevalence of chronic use of metformin-related vitamin B12 deficiency is lower in Asian population
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Background and aims: Metformin is widely used in patients with type 2 diabetes but may cause Vit B12 deficiency. The objective of this study was to evaluate the presence of vitamin B12 deficiency and the factors associated with serum vitamin B12 levels in Korean type 2 diabetic patients.
Materials and methods: We conducted a cross-sectional study of 381 type 2 diabetic patients at the university-affiliated diabetes center of St. Vincent's Hospital. Vitamin B12 deficiency was defined as serum B12 concentrations <300 pg/mL. Statistical analysis was performed to evaluate the relationship between metformin and vitamin B12 deficiency.
Results: Vitamin B12 deficiency was present in 14.1% (n=54) of patients using metformin. Mutivariate analysis revealed statistically significant associations between vitamin B12 deficiency and age, metformin use duration and metformin daily dosage. Compared with metformin use of less than 3years, the adjusted OR was 4.107 (95% CI, 1.799-9.375) (P =0.001) for metformin use of at least 3 years. Current dose of metformin was a strong independent predictor of vitamin B 12 deficiency (OR, 2.916 ; CI, 1.363-6.240) (P =0.006) Increased age appeared to be positively related to vitamin B 12 deficiency (adjusted OR, 1.055 ; CI 1.018-1.093) (P=0.003). Significantly increased risks were not found with concurrent use of H2 receptor antagonists, proton pump inhibitors or vitamin B12 supplements.
Conclusion: Our study indicates an increase risk of vitamin B12 deficiency associated with older age, current dosage and duration of metformin use despite adjustment for many potential confounders. The risk factors identified have implications for planning screening or prevention strategies in metformin-treated patients.

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Pain threshold values of small fibres are associated with the severity of diabetic complications
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Background and aims: Nerve conduction study (NCS) is regarded as the "gold standard" for the evaluation of diabetic neuropathy. However, by NCS, it is difficult to evaluate the impairment of small fibers, such as Aδ and C fibers although diabetes mellitus (DM) is the most common identifiable cause of small fiber neuropathy. Previously, Inui et al. reported that intra-epidermal electrical stimulation (IES) can selectively activate Aδ and C-nociceptors. In addition, PNS-7000, which is a portable stimulator specialized for IES and can evaluate small fiber function by measuring pain threshold value of Aδ and C fibers, has been newly developed. Therefore, in this study, we have examined the degree of small fiber neuropathy by using PNS-7000 in type 2 DM (T2DM) and non-DM subjects and evaluated the association between samll fiber neuropathy and the severity of diabetic complications.
Materials and methods: 87 Japanese subjects with T2DM (mean age 58.7 years; 60 men; BMI 26.0; glycated hemoglobin A1c (HbA1c) 9.3% and 36 Japanese subjects without DM (mean age 45.7 years;16 men; BMI 23.4; HbA1c 5.3%) were recruited for the study. The subjects were examined pain threshold values of Aδ and C fibers by using PNS-7000, clinical features, several clinical neurological examinations (neuropathic symptoms, vibratory sensation in foot, and Achilles› tendon reflex (ATR)) and the severity of diabetic neuropathy, retinopathy or nephropathy. The severity of diabetic neuropathy was evaluated by the staging proposed by Diabetic Neuropathy Study Group in Japan.
Results: The pain threshold value of C fibers was 0.061 mA in non-DM subjects, while that of T2DM subjects was 0.149mA ($p < 0.001$). In addition, similarly to C fibers, the pain threshold value of Aδ fibersin T2DM subjects was significantly higher compared with that of non-DM subjects (0.143 vs 0.042 mA, $p < 0.001$). When the T2DM subjects were divided into two groups according to with or without abnormalities of each clinical neurological examinations, the pain threshold values of C fibers were significantly higher in the group with abnormality compared with the group without abnormality (0.294 vs 0.172, 0.254 vs 0.155, 0.262 vs 0.137 mA, for neuropathic symptoms, abnormality of vibratory sensation in foot and abnormality of ATR, respectively, $p < 0.01$). Respect to pain threshold of Aδ fibers revealed a statistically significant difference only between the group with and without neuropathic symptoms (0.193 vs 0.108 mA, p: 0.013). In addition, the pain threshold values of Aδ and C fibers were significantly higher in accordance with the severity of diabetic neuropathy, retinopathy or nephropathy in T2DM subjects.
Conclusion: This study showed that small fiber neuropathy was observed in T2DM subjects, and PNS-7000 was useful to detect small fiber neuropathy. In addition, small fiber neuropathy was closely associated with the abnormalities of clinical neurological examinations, and the severity of diabetic neuropathy, retinopathy or nephropathy. Because PNS-7000 takes shorter time and is less invasive compared to NCS, PNS-7000 could be useful to predict the diabetic complications.
Clinical Trial Registration Number: R000010224
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Retinal nerve fibre layer thickness is associated with measures of small fibre neuropathy in long-term type 1 diabetes
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Background and aims: Corneal confocal microscopy (CCM) can measure small nerve fibers in the cornea and has been shown to correlate with other measurements for early neuropathy in diabetes (small fiber neuropathy). Optical coherence tomography (OCT) can evaluate the retinal nerve fiber layer (RNFL). RNFL thinning is associated with peripheral neuropathy in type 2 diabetes. Little is known about this in type 1 diabetes. Our aims were to investigate the relationship between retinal nerve fiber layer thickness, peripheral neuropathy and small fiber neuropathy in long-term type 1 diabetes by using CCM. We also wanted to evaluate if the advanced glycation endproduct N-epsilon-(carboxymethyl) lysine (CML) ,which has been implicated in oxidative stress and diabetic late complications, was associated with RNFL.

Materials and methods: The level of neuropathy was assessed clinically and by nerve conduction studies (NCS) in 29 patients (53±7 years) with type 1 diabetes of 40 years duration and in 20 healthy controls. Corneal confocal microscopy (CCM)(Confoscan4, Nidek, Italy) was used as a proxy for small fibre damage. Global and sectoral retinal nerve fibre layer thicknesses were measured around the optic nerve head using OCT (Rtvue, Optovue, USA). The advanced glycation endproduct CML was measured in serum ten years prior to these examinations by immunoassay. None of the participants had unstable retinopathy at the time of examination.
Results: NCS were abnormal in 16 patients (59%) and compatible with diabetes neuropathy.Corneal nerve fibre density (CNFD) measured with CCM was significantly lower in the diabetes patients compared to controls, 8.9±2.7 vs. 10.6±2.0 no./mm², p=0.029. CNFD was negatively associated with the presence of peripheral neuropathy (r=-0.42, p=0.032). A thinner inferior retinal nerve fibre layer (RNFL) was observed in the diabetes patients compared to controls (93.5µm±12.3 vs. 102.7±10.2µm, p=0.045). Global RNFL was negatively associated with corneal nerve fibre length and CNFD, r=-0.46, p=0.0011 and r=-0.44, p=0.0017, respectively. No significant association was observed between RNFL and peripheral neuropathy evaluated by NCS. RNFL was negatively associated with the advanced glycation endproduct CML in serum. After adjusting for age, sex and duration of diabetes, CML was still independently associated with RNFL, r^2= 0.37, p=0.016.
Conclusion: RNFL of the inferior retina was thinner in diabetes patients compared to controls. Corneal nerve fiber density was signifcantly lower in diabetes patients and negatively associated with peripheral neuropathy. Measures of RNFL and CCM were positively correlated. These results may suggest that changes in the cornea and possibly in the retina (central nervous system) may parallell changes in the peripheral nervous system. RNFL thinning may be interesting as a novel marker of small fiber neuropathy. This study also indicates that CML may have a prognostic value in RNFL thinning.
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Association of subclinical inflammation with polyneuropathy in the older population: KORA F4 study
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Background and aims: Distal sensorimotor polyneuropathy (DSPN) contributes to significant morbidity and increased mortality among diabetic subjects, and several studies suggest that DSPN is also more prevalent in individuals with prediabetes when compared to those with normal glucose tolerance. Inflammatory processes have been implicated in the pathogenesis of DSPN, but their possible relationship has not been assessed at the population level. Therefore, the aim of our study was to investigate whether circulating concentrations of seven pro- and anti-inflammatory immune mediators are associated with clinically diagnosed DSPN and neuropathic impairments in older subjects from the general population.
Materials and methods: We determined serum concentrations of mediators of subclinical inflammation among 1047 participants aged 61-82 years from the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study (Germany). Logistic and linear regression models were fitted to assess associations between immune mediators (log-transformed) and the presence of clinical DSPN (dichotomous variable) or Michigan Neuropathy Screening Instrument (MNSI) examination score (continuous variable), respectively.
Results: Serum concentrations of the anti-inflammatory interleukin-1 receptor antagonist (IL-1RA) were positively associated with the presence of DSPN (P=0.011) and higher MNSI scores (P<0.001) in age and sex-adjusted analyses, whereas IL-6, IL-18, and soluble intercellular adhesion molecule-1 (sICAM-1) were positively associated only with MNSI scores (all P<0.05). No associations were observed for adiponectin, C-reactive protein (CRP) or tumour necrosis factor-α (TNF-α). Associations for IL-1RA and IL-6 with the MNSI score remained statistically significant (P<0.05) after additional adjustment for waist circumference, height, hypertension, cholesterol, smoking, alcohol intake, physical activity, history of myocardial infarction and/or stroke, presence of neurological conditions and use of non-steroidal anti-inflammatory drugs.
Conclusion: We observed associations between serum IL-1RA, IL-6 and specific manifestations of DSPN and conclude that DSPN is linked to proinflammatory and anti-inflammatory, possibly compensatory, processes in the older general population. Future studies should clarify the temporal sequence and causality of these associations.
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Impact of neuropathy severity on brain volume loss in diabetic peripheral neuropathy
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Background and aims: We have recently demonstrated significant intracranial peripheral grey matter density reduction, attributed to atrophy, in subjects with established diabetic peripheral neuropathy (DPN). This atrophy, which was localised to the sensorimotor cortex, suggests that DPN has significant impact upon the structural integrity and organisation of the brain. It is not fully understood how changes in brain volumes relates to DPN severity. Exploring this relationship is important to understand the pathophysiological significance of brain atrophy in DPN.
Materials and methods: Fifty six patients with type 1 diabetes [No-DPN, n = 22, Established-DPN, n = 34] underwent detailed clinical and neurophysiological assessments. A neuropathy composite score (NCS) was calculated from transformed percentile points for vibration detection threshold, sural (NCV, SNAP), peroneal (NCV, CMAP, DL) and tibial (DL) nerve conduction attributes. Patients with Established-DPN had at least one abnormal nerve conduction parameter in both the sural and peroneal nerves and at least one neuropathic symptom or sign. All subjects underwent T1-weighted volumetric brain MR imaging at 3T. Images were analysed using FSL (fMRIB, Oxford, UK); a library of analysis tools for brain imaging data. The relation between total and peripheral grey matter volumes (adjusted for age, height, weight and HbA1c) and individual attributes of nerve function and NCS was analysed using linear regression analysis.
Results: Both groups were matched for gender [No-DPN vs Established-DPN; males (%): 68.1 vs 67.6], age (42.3 vs 48.0 years; p=0.08) and HbA1c (71.9 vs 77.5 mmol/mol; p=0.24). No-DPN subjects had significantly greater peripheral grey matter volume compared to Established-DPN [632.1 vs 606.0ml; p=0.01). Cortical atrophy was localised to postcentral gyrus, precentral gyrus and cerebellum. Adjusting for age, height, weight and HbA1c, we found that higher peroneal nerve conduction velocity was associated with larger peripheral grey matter volume (R=0.41; p=0.02). The amplitudes in both sural and peroneal nerves were not related to peripheral grey matter volume at a statistically significant level. Peripheral grey matter volume was inversely related to DPN NCS, with each point associated with a volume reduction of 1.55ml (95% CI -2.82 to -0.29). The associations of peripheral grey matter atrophy with vibration detection threshold were not statistically significant.
Conclusions: The significant inverse linear trend and the continuous relations found with NCS are in keeping with a continuing loss of peripheral grey matter volume as the disease progresses. Significant correlations were also found between peripheral grey matter volume and neurophysiological parameters. We postulate that reduction in cortical grey matter volume localised to the primary somatosensory cortex may be induced through reduced cellular activity triggered by peripheral nerve axonopathy. Conversely, atrophy of the motor neurons is most likely indicative of retrograde degeneration due to DPN. Thus we demonstrate clinically eloquent relationships which require future assessments in longitudinal studies.
Supported by: Juvenile Diabetes Research Fund

PS 108 Diabetic foot: epidemiology, risk factors and outcomes

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Reduced incidence of lower-extremity amputations in a Danish diabetes population from 2000 to 2012

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Background and aims: Diabetic foot disease and amputations severely reduce the quality of life and have major economic consequences for patients, their families, and society. Important improvements in diabetes care have occurred over the last decades, but it is not clear to which extent recent advances in diabetes care have reduced the excess mortality in patients with T1 diabetes. The aim was to estimate time trends in the incidence of lower-extremity amputations (LEA) in Danish diabetes patients.
Materials and methods: We studied major and minor LEA from 2000 to 2012 among 11,332 diabetes patients from our Diabetes Center. Amputations were identified by linkage of the electronic medical system with the National Patient Registry. Sex-specific incidence rates of amputations by age, diabetes duration, calendar time, and diabetes type were modelled by Poisson regression.
Results: From 2000-2012, 384 incident LEA (205 major, 179 minor) occurred during 100,495 years. From 2000-2012 the incidence of all LEA decreased by 90.9% among men and 81.8% among women with type 1 diabetes and by 94.7% among men and 85.1% among women with type 2 diabetes (P<0.001). Particularly there was a decline in major LEA. In 2011 the incidence rates of major LEA were 0.25 among men and 0.21 among women per 1000 p.y. at age 50 years and 0.56 among men and 0.41 among women per 1000 p.y. at age 70 years. No significant change in incidence of minor amputations was observed.
Conclusion: The incidence of major LEA reduced significantly from 2000 to 2012 in Danish diabetes patients.

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Pre-hospital delay in patients with diabetic foot problems: influencing factors and subsequent quality of care

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Background and aims: To investigate the proportion of delay in reporting their foot problems in diabetic foot patients, examine the possible risk factors and prognosis.
Materials and methods: In a retrospective study, 270 patients with diabetic foot problems were classified into three groups: those with diabetic foot duration ≤7 days were group 1, those with diabetic foot duration >1month were group 3, the rest were group 2. Compare their demographic and clinical characteristics, laboratory results, treatment data, sequent outcomes (hospital stays, amputation rate and mortality), and analysis the factors influencing patients pre-hospital delay.
Results: 270 patients were enrolled in our study, their mean time to hospital presentation was 46.49 days. A total of 77(28.5%) patients arrived within one week, 106 (39.3%) patients between one week and one month, and 87(32.2%) patients arrived longer than one month after symptom onset. Among numerous factors, we found nine variables were associated with long pre-hospital delay according to univariate analysis (P<0.05), they were no previous ulcer, no health insurance, bad housing conditions, low income level, low education level, seldom foot inspection, few follow up, absence of diabetic foot education and lack of the knowledge of diabetic foot problems. Multivariate logistic regression analysis indicated that never received diabetic foot education (OR 2.70 [95%CI 1.03-7.06], P=0.043) and lack of diabetic foot knowledge (OR 2.14 [95%CI 1.16-3.94], P=0.015) were independent factors for patients pre-hospital delay. Our study also showed that pre-hospital delay was an important risk factor leading to amputation (OR 2.22 [95%CI 1.36-3.64],P = 0.002) and mortality (OR 2.69 [95%CI 1.35-5.33],P = 0.005).
Conclusion: Long pre-hospital delay is most marked in those groups known to: no previous history, low socio-economic status, seldom performance of foot inspection, few follow up, never received diabetic foot education and lack of diabetic foot knowledge; and these delay is likely to be significant contributor to these poorer outcomes, lower extremity amputation and mortality. Considerations should be given to developing a community intervention program targeting at risk communities to encourage earlier assessment directed by the multidisciplinary team service, to reduce disparity and improve foot outcomes.

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The impact of impaired renal function on diabetic foot complications prediction: Should it be included on foot risk classifications?

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Background and aims: Impaired renal function is considered a powerful risk factor for diabetic foot complications; such as peripheral vascular disease (PVD), diabetic foot ulcer (DFU) and lower extremity amputation (LEA). Nevertheless, it was seldom assessed and never included in the diverse diabetic foot risk stratification systems (RSS). Thus, we aim to evaluate the role of impaired renal function in DFU development at 3 years.
Materials and methods: A retrospective cohort study was conducted on consecutive patients with diabetes without active DFU attending our Diabetic Foot Clinic from 01/2007 to 12/2009 [n=551, 47% male, mean age of 65 years (± 11), diabetes duration of 16 years (± 11) and HbA1c of 8% (± 2%); 98% had type 2 diabetes and 40% used insulin]. Baseline characteristics and all variables included in RSS were collected from the clinical file by one investigator. Subjects were followed for at least 3 years or until death. Clinical characteristics and outcomes' comparison was conducted between those with chronic kidney disease (CKD) stage 4 or 5 and the remaining subjects, using the 2013 American Diabetes Association (ADA) classification.
Results: Within a median follow-up of 36 months (range 1-36), 164 subjects (30%) developed a DFU, 33 (6%) required LEA and 75 (14%) died. Those with CKD stage 4 or 5 (n=26, 5%) were more frequently insulin treated, presented longer diabetes duration, physical impairment, foot deformity, diabetic peripheral neuropathy (DPN) using tuning fork and DFU or LEA history. Variables associated with DFU occurrence were older age, longer diabetes duration and higher HbA1c value; male gender; physical and visual impairment; CKD stage 4 or 5; presence of onychomycosis, foot deformity, DPN diagnosis [using Semmes-Weinstein monofilament (SWM) and tuning fork] and symptoms, PVD (diagnosed by pulses palpation), intermittent claudication, previous DFU and previous LEA. In multivariate analysis, only HbA1c value, physical impairment, foot deformity, SWM altered sensation, intermittent claudication and previous DFU maintained statistical significance. Neither considering estimated glomerular filtration rate as a continuous variable nor using different cut-offs of CKD stages [namely stage 5 (dialysis) vs remaining; stages 4 or 5 vs remaining or stage 0 or 1 vs stages 2 or 3 vs stages 4 or 5] an independent association with DFU was maintained.
Conclusion: So far, only 4 studies assessed nephropathy's impact on DFU risk without consensual results. This is the first study using the recently modified CKD's ADA classification and multivariate analysis adjusting the results for other relevant variables. We believe that nephropathy should not be included in foot RSS as it can potentially be a confounding variable. However, prospective studies in inception and larger cohorts should be conducted.
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Diabetic foot and endothelial precursors cells (EPC): preliminary results of an Italian AIL (Italian Leukaemia Association section of Treviso) research project

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Background and aims: Reduction of peripheral mononuclear cells (PMNC) with surface cluster differentiation CD34+, CD133+ common to endothelial

precursor cells (EPC) characterize diabetic vascular complications. Myeloid calcifying cells (MCC) are involved in carotid placques in vitro but not in calcified vascular diabetic lower limb arterial lesions in vivo. Indeed ex novo KDR+ vasculogenesis and/or preexistent precursor cluster CD133+31+34- angiogenesis describe vascular homeostasis.
Materials and methods: We evaluate CD34+, CD133+, KDR+, CD31+, CD45- EPC by citofluorimetry in non coronaropatic patients with acute (timing restitutio until 6 weeks) or remote III-IV TUC diabetic foot lesions and its relationship with coexistent critical vascular calcification. Subject: 9 C controls and 54 type 2 diabetic patients with peripheral somato-sensory neuropathy (positive DNI): 15 N without foot lesions; 16 N1 and 23 NV with foot lesions without or with endovascular or by vascular surgery revascularized critical limb ischemia (CLI: oximetric value < 30 mm Hg).
Results: CD34+ were related with age (p< 0.03; R2=.45) only in C+N and were reduced in NV (239±31 mean± SE for 106 cell counts) versus N1 and C (p< 0.001). CD133+ were reduced in N, N1 and NV versus C (1857±254 mean± SE p< 0.03). In acute lesions, CD34+KDR+ and CD133+31+ were respectively significant reduced in NV and increased in N1 versus remote lesions and correlate with ABI (ankle brachial oscillometric index).
Conclusion: CD34+ peripheral precursor cells are consumed by age and this correlation is lost in diabetes with advanced complications. Neuropatic and vascular foot lesions determine different vasculogenic or angiogenic homeostasis in terms of KDR or CD31+133+ surface cluster pattern. This phenomenon has a rule in worse prognosis of medial distal arterial calcification. It remains to clarify if microvascular neuropathy precedes diabetic distal macroangiopathy.
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Diabetic foot in patients on renal replacement therapy in Lleida health area (Spain)
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Background and aims: To assess the prevalence of diabetic foot (DF) and associated conditions in patients with diabetes mellitus (DM) under renal replacement therapy in Lleida health care area (Spain).
Materials and methods: A cross-sectional study was performed between November 2010 and March 2011. All diabetic patients on dialysis (DL) in our health area were included. Electronic health records were reviewed and a podiatric exam was performed, including vibratory sensitivity with neurotensiometer, inspection for deformities, presence of diabetic foot ulcers (DFU) or lower extremity amputations (LEA). PEDIS classification was used for ulcer classification. Vascular assessment included ankle brachial index (ABI) and carotid ultrasound for the detection of atherosclerotic plaques and measurement of carotid intima-media thickness (IMT). Risk for DF was stratified following the international consensus on DF 2011 (IWGDF). Bivariate and multivariable analysis using logistic regression models were performed to evaluate potential risk factors associated with ulceration and amputation. The time between the start of DL and LEA was also documented.
Results: At the time of the study 92 patients (62% men) with DM (type 2 diabetes: 82%) followed DL treatment (35.8% of the total population in DL), with a mean age 70.9±12.1 years and a mean diabetes duration of 22.3±12.2 years. Mean DL duration was 4.8±4.3 years with 92.4% under hemodialysis treatment. Current or previous smoking was present in 43.5%, and 68.5% had dyslipidemia and 84% hypertension. Diabetic retinopathy (DR) was present in 62% of the patients (blindness: 12%), peripheral neuropathy in 89.1% and peripheral vascular disease in 64.8%. Prevalence of previous ischemic heart disease or stroke was 22.8% and 10.9%, respectively. Prevalence of former DFU was 19.6% and of current DFU was 17.4% (64% neuroischemic). 16.3% of patients had a previous LEA (70.4% minor amputations). Mean IMT was 0.90 ± 0.17mm and 92.5% of patients had non-stenosing carotid plaques (CP). Most patients (67.4%) had 2-3 IWGDF risk stratification. Bivariate analysis showed a relative risk (RR) of DFU of 6.84 for DR and 2.74 for stenosing CP (p < 0.001 and p = 0.05 respectively). RR of LEA was 3.94 (p=0.02) and 4.62 (p = 0.03) for DR and stenosing CP respectively. After multivariate analysis the association remained significant between DR and DFU or LEA (p = 0.004 and p = 0.013, respectively) and also between DR and LEA (p = 0.023). LEA were performed during the first year of DL in 18.8%, and after four years the percentage reached 50% of all LEA.
Conclusion: There is a very high prevalence of DFU in diabetic patients under renal replacement therapy and a high frequency of LEA after starting DL. Presence of DR and stenosing carotid plaques are the main risk factors for DF in these patients.

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Migratory activity of circulating endothelial progenitor cells predicts mortality in patients with diabetic foot
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Background and aims: Prediction of clinical outcome for patients with diabetic foot relies on clinical data. This prospective study investigates if the abundance and functional activity of endothelial progenitor cells (EPCs) predict adverse events, i.e. post-angioplasty restenosis, amputation, and death for cardiovascular causes.
Materials and methods: A consecutive series of 116 diabetic patients with critical limb ischemia (CLI, TASC guidelines) referring to the Diabetic Foot Centre for percutaneous angioplasty (PTA) were enrolled to the study. A blood sample (30mL) was obtained for isolation of mononuclear cells (MNCs). Cell migration was studied in a transwell system using SDF-1α (100ng/ml) as chemoattractant and vehicle as control. Freshly isolated and migrated/non-migrated EPCs were measured by flow cytometry of CD45dim/CD34pos/CXCR4pos/KDRpos cells. Time to event during the 12 months follow-up was defined as the time from patient's hospital admission to the occurrence of the adverse events. The association between basal EPC counts and migratory activity and the risk of an event was evaluated in a multivariable Cox regression analysis.
Results: Fourteen cardiovascular deaths out of 116 diabetic patients were observed during the 12 months follow up. Three occurred after restenosis and 1 after restenosis+amputation. The age and hematocrit (Ht) of patients died for cardiovascular causes were significantly higher than that of event-free subjects (age: 78±4 vs. 70±10 years, p=0.005; Ht: 39.4±2.8 vs. 36.5±5.0%, p=0.018). Percentages of EPCs did not differ in patients with event vs. no event. Surprisingly, the percentage of EPCs migrated towards vehicle (spontaneous migration) or SDF-1α (chemoactrattant-induced) were significantly higher in patients with cardiovascular death compared with event-free subjects (0.13±0.20 vs 0.04±0.06, p=0.018 and 0.08±0.11 vs 0.03±0.04, p=0.017 respectively). These results were confirmed in a multivariable regression model adjusted by age and Ht levels (HR=1.36, 95% CI 1.04 to 1.77, p=0.026 for EPC-vehicle and HR=1.49, 95% CI 1.06 to 2.08, p=0.02 for EPC-SDF). Given that EPCs have been expressed in log 2 scale, this means that for a doubling of these cell counts, the hazard of a cardiovascular death increases by 36% and 49%, respectively. Time to event analysis of restenosis (n=39) and restenosis+amputation (n=12) showed no significant association with the abundance and functional activity of EPCs.
Conclusion: Results newly indicate that unbalanced migratory activity of EPCs is significantly associated to a higher risk for cardiovascular mortality in patients with diabetic foot.
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Lower-limb risk factors for falls in those with and without diabetes mellitus
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Background and aims: Falls are more common in older people with Diabetes Mellitus (DM) than without. Particular lower-limb risk factors for falls have previously been identified in the DM population. This study aimed to identify if the specific lower-limb risk factors associated with falls in DM are different from age/gender matched controls.
Materials and methods: One-hundred and twenty participants over 55 years of age were recruited (n=60 DM; n=60 age/gender matched controls). 'Fall-

ers' were identified as individuals who self-reported at least one fall in the previous year. In addition to DM status and demographic information and the following risk factors generally associated with falls were assessed in all participants: neuropathy symptom score (NSS), neuropathy disability score (NDS) combined algorithm used to classify neuropathy, foot deformity score (FDS), Tinetti performance-oriented assessment of mobility (POMA). Ankle muscle strength: dorsiflexion (DF), plantarflexion (PF), inversion (IN) and eversion (EV), spatial and temporal gait parameters - an average of 3 passes along a 61cm x 366cm walkway [GAITRite© System]. Data from 'fallers' and 'non-fallers' across both control and DM participants were compared using t-tests and binary logistic regression analysis was performed to identify variables predictive of falls.

Results: Thirty-five percent (n = 21) of DM participants had fallen in the preceding year in comparison to 22% (n = 13) of controls. Higher levels of neuropathy existed in the DM group (67%) in comparison to controls (8%). In terms of 'fallers' 86% (n=18) of DM 'fallers' demonstrated neuropathy in comparison to 23% (n=3) of control 'fallers'. The FDS identified that 55% (n=33) of DM participants demonstrated significant foot deformity in comparison to 25% (n=15) of controls (p = 0.002). With regards to ankle muscle strength assessment, within the control group, 'fallers' displayed lower muscle strength values among control 'fallers' in comparison to control 'non-fallers' for all muscle groups assessed; p<0.001). 'Fallers' demonstrated lower muscle strength for all muscle groups assessed in DM and controls. Statistically significant differences were identified between DM and control participants in terms of velocity (p<0.001), double support left and right (p=0.006; p=0.009), step length left and right (p<0.001 bilaterally) and step time left and right (p=0.001, p<0.001). DM 'fallers' had lower walking velocity (p = 0.001), shorter step length left (p=0.002) and right (p=0.001) in comparison to control 'fallers'. Binary logistic regression analysis identified NDS, dorsiflexion strength and gait velocity to significantly contribute to the model and correctly classified 83.9% of cases overall.

Conclusion: Lower-limb risk factors for falls are similar between DM and controls. NDS, DF strength and gait velocity made a significant contribution to the model, and both these modalities were significantly compromised for 'fallers' in comparison to 'non-fallers', irrespective of DM status. These modifiable risk factors for falls should be specifically targeted in falls prevention in both DM and control populations.

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Assessment of optimum transcutaneous oxygen pressure to predict diabetic foot ulcer healing and indicate angiography

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Background and aims: Peripheral arterial disease (PAD) is unquestionably one of the important factors relating to the outcome of a diabetic foot ulcer (DFU). Indication by angiography (AG) based on transcutaneous oxygen pressure measurement (TcPO2) can be useful in clinical practice. According to the international guidelines, the probability of healing based on TcPO2 is usually severely impaired when TcPO2 <30 mmHg, defined as critical limb ischaemia. However, the 40mmHg threshold can also be used for ischaemia detection. The aim of our study was to compare sensitivity of both 30 and 40mmHg TcPO2 thresholds to healing within 6 months as well as to angiographically verified stenosis.

Materials and methods: 53 consecutive patients with DFU and clinical suspicion of PAD treated at our foot clinic between February 2010 and October 2012 were included into the study. The primary outcome was the relationship between basal TcPO2 (measured in the beginning of the follow-up) and healing within 6 months according to the TcPO2 thresholds 30 and 40mmHg. In a subgroup of patients with AG, the sensitivity for TcPO2 levels ≤30 and ≤40mmHg to angiographically verified arterial stenosis were counted.

Results: 45/53 (84,9%) patients completed the 6 months follow-up. 25/45 (55,5%) patients with DFU were healed. Using multivariate stepwise logistic regression, the healed and unhealed groups differed significantly in CRP (11,915.0 vs. 41,644,4 mmol/l, p=0,012). There was significant difference in gender (64,7% healed men vs. 27,3% women, p=0,029). There was no difference in the other characteristics. Mean basal TcPO2 was higher in the healed group vs. unhealed (28,418,3 vs. 19,212,5mmHg, p=0,062). Regarding the primary endpoint, 15/32 (46,8%) patients with basal TcPO2≤30mmHg and 10/13 (76,9%) patients with basal TcPO2>30mmHg were healed (p=0,13 N.S.); 16/35 (45,7%) patients with TcPO2≤40mmHg and 9/10 (90%) patients with TcPO2>40mmHg were healed (p=0,033), respectively. Sensitivity to healing within 6 months was 85% (95%CI 62,1-96,8) for the 30mmHg TcPO2 threshold and 95% (95%CI 75,1-99,9) for the 40mmHg TcPO2 threshold, respectively. In a subgroup of 24 subjects. AG was performed with 22 percutaneous transluminal angioplasty (PTA). Subjects separated by TcPO2 30 vs. 40mmHg thresholds were comparable in number of PTA. Regarding the secondary endpoint, sensitivity of basal TcPO2 to angiographically verified stenosis was 86,7% (95%CI 65,1-97,1) for ≤30mmHg TcPO2 threshold and 90,9% (95%CI 70,8-98,9) for ≤40mmHg TcPO2 threshold.

Conclusion: Results of our study show that the basal 40mmHg TcPO2 threshold has higher sensitivity to DFU healing as well as to the indication of AG compared to the 30mmHg TcPO2 threshold. These data are in accordance with the trend of early vascular intervention in DFU with already mild signs of ischaemia.

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The long-term outcomes of endovascular procedure in diabetic patients with critical limb ischaemia according to chronic kidney disease stage

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Background and aims: To evaluate outcomes after percutaneous transluminal angioplasty (PTA), and the relationship between calcification in tibial arteries and frequency of re-interventions and amputations in diabetic patients with critical limb ischemia(CLI) according to glomerular filtration rate (GFR).

Materials and methods: 93 diabetic patients with CLI were underwent percutaneous transluminal angioplasty of lower limb arteries. 45 patients were underwent non-contrast CT scanning of lower extremities to assess the level of tibial arteries calcification, calculated according to the modified Agatston system, which usually uses for scoring coronary calcium. Follow-up assessment included clinical examination for primary patency, limb salvage (LS) and common survival (CS). After interventional procedure duplex ultrasound (DU) surveillance was performed.

Results: The patients were divided into 2 groups, according to GFR (MDRD) (chronic kidney disease (CKD) 0-2 stage): A-with GFR>60 ml/min/1.73m^2 and B-with GFR<60 ml/min/1.73m^2 (CKD 3-5 stage). In group A were 48 patients (16 males), DM 1/2type -2/46, respectively; mean age-59,8±13,4 years, mean HbA1c -8,0±0,8%; myocardial infarction in anamnesis was in 9 patients (18%), stroke in 5(11%), retinopathy II-III stage was in 19(39%). In group B were 46 patients (22 males), DM 1/2 type - 17/29, mean age-58,9±14,2 years, mean HbA1c -7,9±1,15; myocardial infarction in 11(25%); stroke in12(22%); proliferative retinopathy in 28(61%).CKD obtained in groups A/B: stage 1(8/0), stage 2(14/0), stage 3(0/26), stage 4(0/3), stage 5(0/17).Severe neuropathy in groups A/B was diagnosed in 38(80%)/40(87%), respectively. Chronic limb ischemia according Rutherford classification in group A/B were: 8(16%)/16(35%)patients with 6 category, 28(58%)/30(65%) with 5 category, 12(26%)/4(9%) with 4 category. 21/24 patients from A/B groups were underwent non-contrast CT scanning. In patients with renal insufficiency the level of tibial arteries calcification by CT was significantly higher (8000±1450 vs. 3000±1230, p<0,05). The residual stenosis (>50% remaining diameter) was 12(25%)vs.35(76%)(p<0,05) in groups A/B. During 2-year follow-up 18(38%) in group A and 31(65%) patients in group B were obtained re-occlusions documented by DU (p<0,05). Repeat angioplasty was performed in 15(35%) vs. 22(47%) (p>0,05) patients in groups A/B. In group A vs. group B there were 1(2%)vs.6(13%)major amputations(log-rank, p<0,05); LS rate was 98%vs. 87%(log-rank, p<0,05); mortality was 0(0%)vs.7(15%),CS was 100%vs.85%(log-rank, p<0,05),respectively.

Conclusion: Extensive arterial calcification in diabetic patients with CLI due to CKD is associated with poorer outcome after PTA and high risk of amputations.

PS 109 Pregnancy: treatment, efficacy and safety

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Effectiveness of metformin in gestational diabetes: systematic review and meta-analysis

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Background and aims: Gestational diabetes (GDM) is a common condition and contributes to significant maternal and neonatal morbidity. The prevalence of GDM will triple, from ~5% to 16-18%, if universal screening and the new IADPSG (International Association of Diabetes and Pregnancy Study Groups) cut-off for diagnosis are adopted. Management of GDM is time and resource intensive. Because of the concerns of diluting the existing resources, these guidelines are not yet universally followed. Treatment involves dietary control, followed by insulin therapy if dietary control is ineffective. Oral hypoglycaemic agents, metformin or glibenclamide, are considered safe but used as second line to insulin and are not widely used. Glibenclamide has shown to be effective but does increase the risk of hypoglycaemia. In one large "non-inferiority", open label, randomised controlled trial (RCT) metformin was shown to be as effective as insulin but not routinely prescribed probably due to lack of superiority trials. We conducted a methodologically robust systematic review to evaluate whether metformin is superior over insulin or glibenclamide in GDM.
Materials and methods: Five databases were searched by two independent reviewers without any restriction along with hand searching of relevant references in the primary publications. All primary studies comparing metformin to insulin or glibenclamide and reporting maternal and neonatal outcomes of GDM were included. Nine studies compared with insulin (4 RCTs and 5 non-RCTs/NRCTs) and two RCTs with glibenclamide were identified. Quality assessment of the RCTs and NRCTs used separate risk of bias tools, in line with PRISMA and MOOSE guidelines.
Results: In the meta-analysis of RCTs with insulin, metformin appeared superior to insulin in preventing neonatal hypoglycaemia (odds ratio (OR): 0.67; 95% confidence interval (CI): 0.48,0.94) and minimising maternal weight gain (weighted mean difference (WMD): -1.80kg; CI:-2.57,-1.02). Similar effects were observed in the meta-analysis of NRCTs (neonatal hypoglycaemia: OR: 0.42; CI: 0.27,0.64; maternal weight gain: WMD: -1.77kg; CI: -1.91,-1.64), along with reduction in macrosomia (OR: 0.63; CI: 0.42,0.93) and neonatal intensive care admissions (OR: 0.57; CI: 0.40,0.82). Compared to glibenclamide, metformin reduces the risk of macrosomia (OR: 0.32; CI: 0.08, 1.19) and birth weight (WMD: -249.13g; CI: -355.88,-142.38). There are no differences in the risks of other outcomes.
Conclusion: Metformin in GDM appears to be superior to insulin and glibenclamide in a number of maternal and neonatal outcomes. Metformin is likely to improve the compliance and is less expensive compared to insulin. Therefore, using metformin as a first line therapy for GDM after diet and lifestyle might offer an effective strategy and less strain on the existing resources. This may also enable to manage more mothers with GDM if the new IADPSG guidelines are adopted. However, an adequately powered randomised controlled, superiority trial based on the IADPSG criteria, to demonstrate the differences in the key maternal and neonatal outcomes is urgently warranted.
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Study of the safety and efficacy of the use of insulin and metformin in combination in gestational diabetes

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Background and aims: Analogue insulin, NPH insulin and metformin have been shown to be safe in pregnancy. This study aims to determine the safety and efficacy of the use of insulin and metformin in combination in gestational diabetes.
Materials and methods: A retrospective observational study of all women diagnosed with gestational diabetes in a District General Hospital from 2008 to 2012. Women were screened using a 75 gm oral glucose tolerance test (OGTT) between 24 and 28 weeks gestation. A positive OGTT was defined as a fasting glucose >5.8mmol/L, 1 hour >10.0mmol/L and 2 hour >7.8mmol/L.
Results: 287 pregnancies with gestational diabetes during the study period with 3 foetal losses (27, 27, 31 weeks). There was no difference at baseline (antenatal booking) in mean body mass index (BMI, 30.54±0.43, p=0.16), systolic blood pressure(121±0.7,72±0.5mmHg, p=0.14), previous foetal losses (0.44±0.05, p=0.23), or maternal age (33.52±0.3 years, p=0.09). Diagnosis was made earlier in the metformin alone group (26.18±0.48 weeks, p=0.024) compared to the diet alone group (27.42±0.26 weeks). Diastolic blood pressure was lower at baseline in the diet alone compared to the metformin group (71.1±0.6mmHg vs 71.7±1.4mmHg p=0.001) and lower in the metformin compared to the insulin alone group (73.6±1.3 vs71.7±1.4mmHg, p<0.001). There was no difference in the mean Hba1c after 20 weeks gestation (p=0.7) in any group (insulin group was 5.61%±0.84, metformin 5.56±0.72%, insulin and metformin 5.68±0.1%, diet 5.47±0.18%. BMI at 32 weeks gestation was significantly higher in the metformin group than the diet groups (31.92±0.97, 30.64±0.58, p=0.001). There was no difference seen between any other groups. There was no difference between systolic BP at 34 weeks between the groups (p=0.054). Diastolic BP is higher in the metformin group compared to the insulin group (78.3±0.77,72.3±1.2, p<0.001) and compared to diet only (78.3±0.77, 73.83±0.75 p=0.001). There was a correlation between BMI and diastolic BP at 34 weeks (p=0.038, R^2 =0.031) in these groups on regression analyses. There was no significant difference in gestation at delivery (39.3±0.8 weeks p=0.22), birth weight (3.55±0.31kgs, p=0.64) or Apgar score at 0 or 5 minutes (8.78±0.6, 9.81±0.3, p= 0.58, p=0.14) between the groups. 47 received insulin alone ((12 received insulin glargine alone, 1 glulisine alone, 12 aspart alone, 6 glargine and glulisine, 13 glargine and aspart), 58 received metformin alone, 30 received metformin in combination with insulin and 154 were treated with diet alone). The mean total dose during pregnancy of metformin used in the metformin only group was 659.4 mgs/kg/day for 7.58 weeks and commenced at 31.29 weeks gestation. The mean total dose of metformin in the metformin and insulin group was 1123.67 mgs/kg/day for 8.22 weeks and commenced at 28.97 weeks gestation and the total dose of insulin used in this group was 4.16 units/kg/day of glargine, 4.5 units/kg/day of Aspart, 2.43 units/kg of Lispro and 0.79units/kg of Isophane for a mean of 7.41weeks and commenced at a mean of 30.91 weeks gestation. The mean total dose of insulin was 3.92 units/kg/day of glargine, 6.43 units/k/day of glulisine, 6.54 units/kg/day of aspart for 6.67 weeks, commenced at 31.94 weeks gestation.
Conclusion: This study shows the non-inferiority of insulin in combination with metformin in foetal and maternal outcomes. It also demonstrates the link between maternal BMI and diastolic blood pressure during pregnancy.
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Breaking down misclassifications: not all gestational diabetes needs treatment as not all children need insulin

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Background and aim: The new criteria suggested by the HAPO Study for the diagnosis of gestational diabetes determinated a doubling of the pregnant women diagnosed with this disease. Not all the gestational hyperglycemias have the same etiology, as demonstrated in pediatric diabetology where, before the discover of the existence of monogenic forms of not autoimmune diabetes, all patients with hyperglycaemia were classified as "type 1 diabetes" and treated with insulin. The objective of the present study was to distinguish the diseases on yet by their phenotypes but by their pathogenesis providing a "*taylored medicine*".
Materials and methods: Two hundred and sixty four women with gestational diabetes for HAPO study criteria (fasting glycemia ≥ 92 mg/dl, and/or OGTT T60 ≥ 180 mg/dl and/or T120 ≥ 153 mg/dl) were recruited. 110 were overweight or obese (BMI >25) and were excluded by the study because patients with monogenic diabetes have low levels of insulin and generally are not overweight. One hundred and fifty four women had normal weight and continued to be enrolled. Gynecologist and Diabetologist treated all the women with low amount of CHO diet and/or insulin if the glycaemic control was not

obtained. We have typed for MODY-2 and MODY-1 mothers who gave birth newborns with birthweight <2.500 Kg and their children.
Results: The women who gave birth newborns with birthweight <2.500 Kg were 5/154 (3.24%) and were all positive for mutation for monogenic, autosomic dominant, diabetes: four with heterozygous mutation of the GCK gene (MODY-2) and one for HNF4alpha gene (MODY-1). All the newborns were heterozygous for the same mutation of their mother.
Conclusions: The treatment of a mother affected by MODY2 or 1 is the same of a mother with gestational diabetes to avoid the birth of a LGA child, due to the maternal hyperglycemia. It's far different if both mother and child carry a MODY2 mutation: in this case the inborn has a poor insulin secretion that, if not increased by the exposure to maternal higher glycaemic levels, may determinate a Small for Gestational Age (SGA) baby. Intra uterine growth of the fetus has to be investigated by familial anamnesis to suspect monogenic diabetes. We are confident that wider research of monogenic diabetes in pregnancy will avoid not necessary and sometimes *dangerous* therapies. This is the real *cutting edge* perspective of the modern medicine that has to pull down the categories in order to *tailor* the therapy on the patients and not on their disease.

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Preliminary data on pregnancy outcome of diabetic mothers treated with basal insulin lispro-protamine in a multicentre study in northeast of Italy

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Background and aims: The occurrence of gestational diabetes or pre-gestational diabetes worsen maternal-fetal outcomes in terms of morbidity-mortality.Insulin treatment could improve glycemic control and pregnancy outcome. Aim of the study is to evaluate pregnancy outcome in women affected by type 1, type2 or gestazional diabetes treated during pregnancy with Insulin LisproProtamine(L-P) .
Materials and methods: 606 women treated with L-P basal insulin were retrospectively evaluated and compared with a control groups(793women) treated with NPH basal insulin. The results are shown in table1.
Results:

	GDM		Type 1		Type2	
	L-P (508)	NPH (125)	L-P (37)	NPH (504)	L-P (67)	NPH (164)
Age (yrs)	36.3 (16.2)	24.9 (4.7)	35.5 (4.4)	29.9 (4.8)	33.2 (5.6)	33.2 (4.8)
Prepregnancy BMI	27.2 (6.0)	26.0 (6.7)	24 (6.0)	23.3 (3.4)	28.7 (5.7)	28.1 (6.4)
Pregnancy hypertension(%)	6.9	6.9	8.1	12.8	8.9	9.4
Cesarean section(%)	48.7	48.8	46	73	46.5	69.3
Birthweight (G)	3295 (558)	3423 (533)	3550 (743)	3300 (670)	3235 (820)	3200 (760)
Macrosomia (%)	5.3	6.9	21	13.3	8.9	11.9
Congenital malformation(%)	0	0	0	5.9	0	1.9
Ketoacidosis (%)	0	0	0	5.4	0	0
Severe hypoglycemia(%)	0	0	5.4	15	1.5	1.3

HbA1c improved in the same way in both prepregnancy diabetic groups during pregnancy from 7.46% in preconception period to 7.1% in the 1st trimester,6.5% in the 2nd trimester and 6.4% in the 3rd trimester in type 1 L-P treated diabetic mothers, from 7.5% to 7.2,7.2,6.4 in NPH group ; in type 2 women from 6.7% in preconception period to 6.4 in 1st trimester,5.8 in the 2nd trimester and 6.2 in the 3rd trimester in L-P group and from 6.6% to 6.1,6.4,5.7 in NPH group. A reduction in severe hypoglycemic events and ketoacidosis in L-P treated type 1 women was found. In GDM group maternal anf fetal outcome was not different. In type 1 and type 2 L-P groups a lower frequency of cesarean Section was observed with respect to NPH: Macrosomia was not significant higher in type 1 L-P group with respect to NPH ones but was lower in type 2 group
Conclusion: Our preliminary data suggest that the use of insulin lispro-protamine to be safe and effective in pregnancy.
Clinical Trial Registration Number: 67916

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Insulin treatment in women with gestational diabetes mellitus: Is there a label effect on delivery characteristics?

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Background and aims: Maternal diabetes mellitus is associated with spontaneous preterm birth and abnormal myometrial contractility. On the other hand, the "label" of gestational diabetes mellitus (GDM) has been associated with an unexplained rate of cesarean delivery (CS) in some series. We aimed to evaluate delivery characteristics in women with GDM in relation to insulin treatment.
Materials and methods: We conducted a retrospective cohort study of all singleton pregnancies of women with GDM progressing to >22 weeks and delivering in a tertiary care center between 1986 and 2008. Potential predictive variables included anthropometrics, obstetric history, GDM diagnosis characteristics (gestational age, blood glucose values) , HbA1c (after diagnosis and in the third trimester) and insulin treatment. Outcomes variables were gestational age at delivery, preterm birth (total/ spontaneous/ induced), elective delivery and cesarean section. Statistics: descriptive, bivariate and multiple logistic regression analysis (backwards method).
Results: A total of 2302 pregnancies were included. Insulin treatment was used in 48.4% pregnancies and the results of outcome variables were as follows: gestational age at delivery 39 (38, 40) weeks, 10% preterm birth, 5.3% spontaneous preterm births, 31.3% elective deliveries and 24.4% cesarean sections. In women receiving insulin treatment, baseline maternal characteristics and glycemic control were more unfavourable. As to delivery characteristics in women receiving insulin treatment vs those without were as follows: gestational age at delivery 38.6 vs 38.8 weeks, p <0.05; preterm birth 11.2 vs 8.9%, p <0.05, spontaneous preterm birth 5.5 vs 5.1%, elective delivery 34.3 vs 28.6%, p <0.05 and CS 26.7 vs 22.4%, p <0.05. After adjustment for othe potential predictors, the logistic regression analyses revealed an independent negative association between insulin treatment and preterm birth (OR 0.583 , CI95 0.373, 0.913) and spontaneous preterm birth (OR 0.411, CI95 0.228, 0.745), a positive association with elective delivery (OR 1.293, CI95 1.034, 1.618) and no significant association with iatrogenic preterm birth or CS.
Conclusion: In these women with GDM, insulin treatment did not have a consistent label effect on delivery characteristics. Contrariwise, insulin treatment was associated with a protective effect against spontaneous preterm labour, independent of other predictors.

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Type 1 diabetes and pregnancy: continuous subcutaneous insulin infusion systems versus multiple daily injection therapy

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Background and aims: Intensive insulin therapy through multiple daily doses of insulin (MDI) or subcutaneous insulin infusion (CSII) contributes to obtain good metabolic control and thus decrease the risk of maternal and fetal complications during pregnancy in DM1. This study aims to evaluate and compare the CSII and MDI therapy during pregnancy.
Materials and methods: Retrospective analysis of data of pregnancies in women with type 1 diabetes followed in Endocrinology-Obstetrics Department since 2005 treated with CSII and MDI. We evaluated metabolic control (A1C), maternal and fetal outcomes. Statistical analysis program SPSS 18.0 was used.
Results: We followed 18 pregnant women (19 pregnancies) treated with CSII and 65 with MDI, mean age 30.4±4.3years and 29.3±4.6years, respectively. Mean duration of diabetes 17±6.7years, with CSII, and 11.7±6years with MDI (p=.006). The pre-conception counseling was higher in the group with CSII (84.2% versus 51.6%, p=.02). No differences were observed in diabetic chronic complications (nephropathy and retinopathy). Prepregnancy A1C was

similar in both groups (8%±1.5 in pump group and 7.9%±1.5 in MDI). The metabolic control was similar in the 2 groups, except for the 2nd trimester, when a significant improvement in the pump group was observed (7.1%±0.8 versus 7.3%±1.2, 6.2%±0.5 versus 6.7%±1, 6.7%±0.7 versus 6.6%±1). The pregnancy-induced hypertension was higher in pregnant women with pump (27.8% versus 5.3%, p=.007), the occurrence of preeclampsia was similar. Preterm delivery occurred in 52.6% of pregnant women with CSII versus 27.9% with MDI (p=.045). The percentage of caesarean sections was high in both groups and related to the longer duration of diabetes (p=.01); CSII 73.7% versus 60.7% (p=ns). Birth weight did not differ between groups (3563g±675 versus 3514g±513). Birth weight >4000g occurred in 26.3% in the pump group versus 13.1% (p=ns). These differences remained regardless of the duration of diabetes. The morbidity and neonatal malformations were similar in both groups.
Conclusion: These data show that the metabolic control and fetal prognosis did not differ significantly with these two modalities of intensive insulin therapy. Both were effective in improving maternal glycemic control. However pregnancy-induced hypertension and preterm delivery were higher in women with CSII. The use of infusion pump in pregnancy should be decided on an individual basis taking into account not only the glycemic balance as well as other factors that may determine the maternal-fetal prognosis.

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Real-time continuous glucose monitoring in pregnant women with type 1 diabetes at high risk of severe hypoglycaemia
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Background and aims: Severe hypoglycaemia occurs in up to 45% of pregnant women with type 1 diabetes, especially before 20 gestational weeks and in women with a history of severe hypoglycaemia. Given severe hypoglycaemia the year prior to pregnancy, 70% of the women followed in our center also experience severe hypoglycaemia during pregnancy, and these women can therefore be selected as women at specifically high risk of severe hypoglycaemia during pregnancy. The aim of this study was to evaluate whether continuous use of real-time continuous glucose monitoring (CGM) prevents severe hypoglycaemia in this subpopulation of women at specifically high risk of severe hypoglycaemia in pregnancy.
Materials and methods: In the study period of February 2011-November 2012, pregnant women with type 1 diabetes at estimated high risk of severe hypoglycaemia were offered initiation of continuous real-time CGM (Guardian® Real-time Continuous Glucose Monitoring System mainly with the Enlite Sensor; Medtronic Minimed) from early pregnancy on. A questionnaire on real-time CGM alarms and satisfaction was collected median 3 (range 1-7) times during the intervention period. Episodes of severe hypoglycaemia were recorded prospectively. Pregnancy outcomes were collected from hospital records.
Results: Continuous real-time CGM was initiated in 11 (8%) of 140 referred pregnant women with type 1 diabetes, all 11 with episodes of severe hypoglycaemia either before pregnancy (n=10) or early in the current pregnancy (n=1). HbA1c at first pregnancy visit was 6.7% (5.8-11.5) (IFFC 50 (40-102) mmol/mol) and the duration of diabetes was 13 (6-21) years. Nine women were on multiple daily injections and two women were on insulin pump therapy. Real-time CGM was used from 10 (6-18) gestational weeks for 11 (3-32) weeks and was generally well-tolerated. The real-time CGM hypoglycaemic alarm was set at 3.8 (3.0-4.5) mmol/l and these alarms occurred 11 (1-38) times weekly, whereof 4 (1-12) during night time. From onset of real-time CGM to 38 (32-39) gestational weeks, two (18%) women experienced one episode of severe hypoglycaemia each. Firstly, one woman had a severe hypoglycaemic event at 18 gestational weeks during ongoing real-time CGM, but the sensor did not alarm for hypoglycaemia. Secondly, another woman experienced severe hypoglycaemia at 22 gestational weeks after termination of real-time CGM. Real-time CGM was reinitiated without any additional severe hypoglycaemic event until now (32 gestational weeks). As of April 3rd 2013, 10 women have given birth at 38 (34-39) gestational weeks to 10 infants weighting 3,548 (2,516-4,248) grams.
Conclusion: In this subset of women with type 1 diabetes at specifically high risk of severe hypoglycaemia during pregnancy, continuous use of real-time CGM seems to be an efficient tool in preventing these episodes.

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Serum levels of the myokine and adipokine irisin are decreased in pregnancies complicated by preeclampsia
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Background and aims: Preeclampsia (PE) is a serious cardiovascular complication in pregnancy which is associated with an increased life-time metabolic and cardiovascular risk for mother and newborn. Irisin has very recently been introduced as a novel myokine and adipokine that drives brown-fat-like conversion of white adipose tissue, reverses obesity, and improves glucose metabolism in mice. Therefore, we investigated circulating levels of irisin in women with and without preeclampsia during pregnancy and after delivery.
Material and methods: We measured serum irisin levels in patients with PE (n=51) as compared to healthy, age-matched controls (n=51) during and 6 months after pregnancy. Furthermore, association of irisin with markers of renal function, glucose and lipid metabolism, as well as inflammation, was elucidated in all individuals.
Results: Median maternal irisin serum concentrations adjusted for body mass index and gestational age at blood sampling were significantly, almost 1.4-fold decreased in PE patients (499.3 ng/l) as compared to healthy, age-matched pregnant women (668.8 ng/l) ($p < 0.001$). Furthermore, irisin concentrations were independently correlated with low density lipoprotein (LDL) cholesterol in pregnant women. Interestingly, median irisin serum concentrations adjusted for body mass index remain significantly 1.4-fold decreased in former PE patients (266.8 ng/ml) compared to healthy controls (372.6 ng/ml) 6 month after pregnancy. Furthermore, multiple regression analysis showed that PE, time between delivery, and systolic blood pressure (SBP) were independent variables influencing serum irisin levels at 6 months postpartally (p=0.001). Moreover, irisin serum levels significantly decreased more than 2-fold in former pregnant women (614.7 ng/ml) to 6 months after pregnancy (291.8 ng/ml) ($p < 0.001$) in the study population in which pre- and postpartal serum specimens were available.
Conclusion: Maternal irisin serum concentrations are significantly decreased in PE during and 6 month after pregnancy. Furthermore, LDL cholesterol is an independent predictor of circulating irisin in pregnant women, while PE, time between delivery, and SBP were independent associated with irisn serum levels 6 month after pregnancy suggesting that irisin may play a crucial role in future metabolic and cardiovascular risk after pregnancy complicated by preeclampsia.
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GDm-Health: telehealth for remote monitoring and treatment of gestational diabetes
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Background and aims: Widening screening criteria, lowering diagnostic thresholds and underlying demographic changes threaten to overwhelm resource-limited services for women with gestational diabetes mellitus (GDM). We have developed a mobile phone-based telehealth system (GDm-Health) with the aims of supporting treatment of GDM, reducing clinic visits, improving response time to abnormal BG readings and increasing women's satisfaction with the care they receive. This pilot study aimed to demonstrate the feasibility of the GDm-Health system in clinical practice, to assess blood glucose (BG) control and user satisfaction and to inform the design of a randomised controlled trial of the technology.
Materials and methods: Pre- and post-meal BG results are transferred wirelessly in real-time from a blood glucose meter to a smartphone app (where they can be annotated by the patient) and then to a server. The data are tabulated and presented on both the server and the smartphone in graphical formats with out-of-target colour codes. Diabetes specialist midwives and doctors review the automatically-prioritised data and recommend dose changes by text messages or phone calls. Women can request a call from their

care team to discuss their diabetes, which the diabetes midwife can schedule around her other tasks. The technology has been used by 50 women with GDM from two Oxfordshire hospitals.
Results: To date, 41 women have finished using the system, all of whom have had live births. 15,609 BG results have been transmitted. The women tagged 98% of these readings with annotations indicating whether they were taken before or after a meal. Free text comments from the women about diet and other factors were attached to 16% of the readings. The clinical care team sent 403 text messages to the women, including 110 medication adjustments. Initial prescriptions of metformin or insulin were given in clinic but dose adjustment to manage the progressively rising glucose levels were also given by phone in response to regular inspection of the automatically-prioritised results. Women made 56 requests for calls from their diabetes midwife, primarily to obtain prescriptions, lancets and strips (39% of calls) or discuss their diet and medication (18%). Of 32 women who have returned questionnaires to date, 31 (97%) agreed that the system was convenient (27 strongly agreed) and 31 women (97%) agreed that the method of monitoring fitted with their lifestyle (20 strongly agreed). All 32 women reported a good relationship with their diabetes care team.
Conclusion: The GDm-Health system allowing remote delivery of care works well in clinical practice and has been positively received by patients and healthcare professionals. A randomised controlled trial will be conducted to establish whether its use can maintain effectiveness while improving quality and efficiency of care, with fewer clinic visits and time and cost savings for both patients and the health service.
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PS 110 Pregnancy outcomes in GDM

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Pregnancy outcome in gestational diabetes mellitus compared to the Swedish birth register
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Background and aim: Gestational diabetes mellitus (GDM), depending on the population studied, affects 1-14% of all pregnant women. In Sweden 2% of pregnancies are complicated by GDM. GDM is often more common in populations with a high frequency of type 2 diabetes, and it is well known that women with GDM have a substantial risk of developing type 2 diabetes later in life, but the risk of developing type 1 diabetes is also increased. Known risk factors for developing GDM are higher age, overweight, and heredity for diabetes. It is of general belief that an intrauterine environment complicated by maternal diabetes increases the risk for overweight and obesity in the offspring. However, overweight among women with GDM per se can also increase risk for overweight and obesity in their offspring. The aim of this study was to compare pregnancy outcome of GDM pregnancies against Swedish general population pregnancies, from the Swedish Birth Register.
Materials and methods: In our region in Sweden, all pregnant women are tested with a 2-hour oral glucose tolerance test (OGTT) consisting of 75 g glucose in solution as a general screening for GDM in the 28th gestational week. The cut off value for GDM in Sweden is ≥10.0 mmol/l in capillary plasma glucose and is based on the European Association for the Study of Diabetes (EASD) recommendations. Women who receive a GDM diagnosis are transferred to our specialised maternity unit with a team of obstetrician, diabetologist, midwife and dietician. Between 2000 and 2010 we had 704 women with at least one GDM pregnancy. The women's medical journals from their GDM pregnancy were studied retrospectively and the data was compared to all pregnancies (n=1102729) in the Swedish Birth Register from this time period.
Results: Age of the mother was 32yrs ±5.5 (n=704) in GDM pregnancies and 30yrs ±5.2 (n=1099161; p=<0.0001) in the general population. First weight of the mother during GDM pregnancy was significantly higher than the weight of the general pregnant women, 72.9 kg ±17.5 (n=688) and 68.0 kg ±13.2 (n= 990604; p=<0.0001) respectively. However, there was no significant difference in weight of the mother at delivery. For women with GDM weight at delivery was 82.63kg ±17.2 (n=602) and for the general population 81.9 ±14.1 (n=366046; p=NS). Birth weight of the child in GDM pregnancies was 3468.8g ±546.4 (n=708) and for the general population 3520.1g ±591.2 (n=1112381; p=0.02). Gestational length in GDM pregnancies was 38.8weeks ±2.4 (n=652) compared to 39.3weeks ±2.0 (n=1114513; p=<0.0001). The percent of caesarean delivery was 21% in the GDM pregnancies and 17% of the general population (p<0.005).
Conclusion: Even though women with GDM have a higher initial weight during pregnancy, there was no significant difference in weight at delivery compared to the general population. This is probably due to a combination of intense dietary advice at our clinic to achieve good metabolic control during pregnancy and shorter duration of the pregnancy. The higher frequency of caesarian section terminates the pregnancies earlier and birth weights of the newborns were similar in our patients compared to the general population in Sweden. The prevention of large infants is crucial to avoid complications during delivery.

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Maternal prepregnancy body mass index but not gestational diabetes determines postnatal growth from birth to 4 years
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Background and aims: Maternal hyperglycaemia and obesity determine in utero environment and have been associated with postnatal overgrowth. Some controversy exists about the capacity of gestational diabetes (GDM) treatment to mitigate such. The aim of the study was to analyse the influ-

ence of GDM and prepregnancy body mass index (BMI) on birth weight and postnatal growth in a cohort of children born to GDM and normal glucose tolerant (NGT) women.
Materials and methods: This is a longitudinal study in which we analyzed postnatal growth in infants born to women with GDM and NGT and who were delivered between January 2006 and February 2009. Their mothers were recruited during pregnancy and outcome data were recorded prospectively in a database. Postnatal weight and height were also recorded. We analysed maternal age, prepregnancy body mass index, BMI gain during pregnancy, gestational week of delivery, glucose tolerance status, neonatal sex, birth weight and length. From an initial cohort of 484 children, in this study we included 243 children whose weight and height were recorded at birth and at 6, 12, 24 and 48 months. Weights and BMIs were transformed into a Z Score using gender-specific references of children's growth. Student's t-test, the chi-square, ANOVA and ANOVA of repeated measures were used to evaluate differences between groups.
Results: Ninety-two infants born to GDM and 151 born to NGT women were included in the study. No differences were observed in birth weight and in postnatal growth between infants born to GDM compared to those born to NGT women. According to maternal prepregnancy BMI, children were divided in three groups: 140 infants in the normal weight group (<25), 61 in the overweight group (25 to <30) and 42 in the obesity group (≥30). Gender distribution (p=0.727) and glucose tolerance status (p=0.284) were similar among the three groups. BMI gain at the end of pregnancy, birth weight, birth weight Z score and birth BMI Z score were significantly different among groups (p<0.01 for all). BMI gain was lower in the obesity group compared to the normal weight group (p<0.05), whereas neonates born to obese mothers were significantly heavier than those born to normal weight and overweight mothers (p<0.05). No differences among the groups were observed in weight and BMI Z Score at 6 and 12 months, but they were significantly different at 24 and 48 months' evaluation (p<0.01). Children born to obese mothers were significantly heavier than those born to normal weight women at 24 (<0.05) and at 48 months (p<0.01) months. These differences persisted after adjusting for glucose tolerance status and BMI gain during pregnancy. See figure 1.
Conclusion: The offspring of treated GDM women have similar postnatal growth compared to those born to NGT women, whereas maternal pregnancy BMI is an independent determinant of birth weight and postnatal growth.

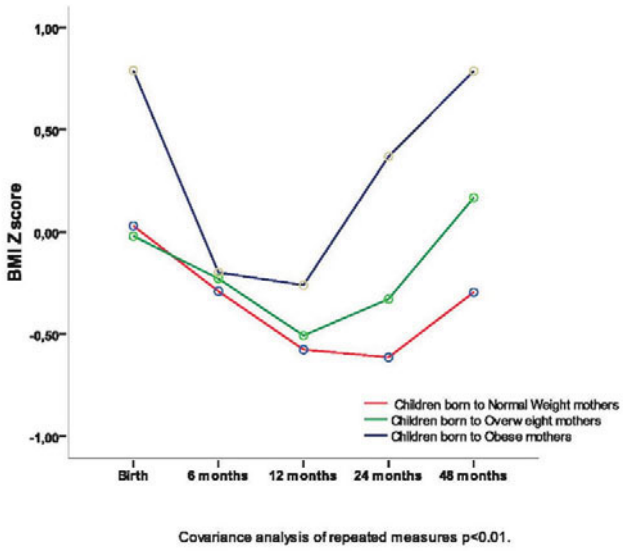

Figure 1. Postnatal BMI Z score according to prepregnancy maternal weight adjusted for glucose tolerance status and BMI gain during pregnancy.

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Effects of prepregnancy weight on foetal growth in patients with well controlled gestational diabetes mellitus

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Background and aims: The relative impact of prepregnancy body mass index and maternal glycemia during pregnancy on adverse maternal and perinatal outcomes is difficult to tease apart. Moreover, excess gestational weight gain complicates a large number of pregnancies and is highly correlated with maternal overweight and obesity, as well as the development of Gestational Diabetes Mellitus. Many studies examining the effects of maternal obesity and/or glucose levels have not accounted for this important factor. The aim was to determine effect of prepregnancy weight on the risk of fetal macrosomia in women with well controlled gestational diabetes mellitus (GDM) attending in Outpatient Endocrinology Clinic
Materials and method: Retrospective cohort study of the association of prepregnancy weight in women with well controlled GDM and term (≥37 weeks) singleton live borns and macrosomia (birthweight ≥4000 g) between January and December 2012. Anthropometric and analytical variables were determined for each patient, including body mass index (BMI), HbA1c and thyroid function. Multivariate logistic regression models were used to adjust for covariates and test for interactions.
Results: Of 150 women studied, the mean HbA1c (±standard deviation) was 6.1% ± 0.3. Prepregnancy body mass index (BMI) categories were: normal (39.6%), overweight (28.5%) and obese (31.9%). The mean (±standard deviation) weight gain (kg) for these groups was: 12.5 ± 4.9, 11.0 ± 5.9 and 8.5 ± 7.1 (P<0.0001), whereas the occurrence of macrosomia was 8.3, 12.5 and 21.2%, respectively. Women with an obese BMI were twice as likely to have a macrosomic infant compared with women in the normal BMI group (odds ratio, OR 2.0; 95% CI 1.4-3.0; P<0.005). Independently, women who exceeded recommended gain weight during pregnancy were two times more likely to have a macrosomic infant (OR 2.5, 95% CI 2.2-3.1 , P<0.0001).
Conclusion: Maternal prepregnancy weight and weight gain during pregnancy appear to be significant and independent risk factors for macrosomia in women with well controlled GDM

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Maternal glucose levels in early pregnancy, gestational diabetes, prepregnancy BMI and the risk of childhood obesity: the mother child cohort, RHEA study in Crete, Greece

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Background and aims: Obesity in childhood is indicated to have its origin in early life. A hypothesis is that hyperglycaemic environment in utero, apart from leading to f0etal hyperinsulinaemia and increased growth, may also programme biological regulatory mechanisms with long term effects in offspring, like increased risk of overweight and obesity. Many studies, in diverse populations, have examined the association between maternal hyperglycaemia/gestational diabetes mellitus (GDM) and childhood body size in different ages, but the results are not conclusive. The aim of this study was to assess the associations of maternal glucose and insulin levels in early pregnancy, GDM, and prepregnancy BMI with the risk of obesity in offspring at 4 years of age.
Materials and methods: The mother-child "Rhea" study in Crete is a prospective cohort examining pregnant women (Greek and immigrants) residents at the prefecture of Heraklion that became pregnant during one year starting in February 2007. Eight hundred sixty three (863) mother-child pairs, were available for the present analysis after excluding twin pregnancies and women with pre-gestational diabetes. Maternal fasting serum samples were collected at the time of the first major ultrasound (Mean: 12 weeks, SD: 1,5). Insulin sensitivity was calculated by a homeostasis model assessment approach (HOMA index). Pregnant women were screened for GDM between 24 and 28 weeks of gestation, and GDM was defined by the criteria proposed by Carpenter and Coustan. Weight, height, abdominal circumference, and thickness

of triceps, subscapular, suprailiac, and subscapular were measured at 4 years of age. Multivariable linear and log-binomial regression models were used to estimate the effect of maternal glucose and insulin levels in early pregnancy, and GDM on the risk of obesity in preschool children after adjusting for maternal age, education, parity, and pre-pregnancy BMI.
Results: The most prominent risk factor for childhood obesity was maternal pre-pregnancy BMI. Maternal obesity prior to gestation increased significantly the risk of overweight/obesity in preschool children (RR: 1.7, 95% CI: 1.2-2.5), while almost doubled the risk of abdominal obesity (waist circumference >90th percentile: RR: 2.1, 95% CI: 1.3-3.3). An elevation of 20 mU/ml in fasting insulin levels in early pregnancy was associated with elevated levels of fat mass as measured by the sum of skinfolds measurements (b coef 0.9, 95%CI 1.3-4.4), while a per unit increase in HOMA index was associated with increased fat mass (b coef 0.15, 95% CI 0.01-0.28), and waist circumference in preschool children (b coef 0.08, 95% CI 0.01-0.15). Gestational diabetes, and maternal fasting glucose levels in early pregnancy were not associated with increased risk of obesity in offspring after adjustment for potential confounders.
Conclusion: Maternal obesity and f0etal exposure to high maternal insulin concentration in early pregnancy may contribute to the development of adiposity in the offspring, while GDM was not found to be significantly associated with childhood obesity. Further follow up of this cohort will allow to determine whether maternal hyperglycaemia/diabetes in pregnancy is associated with a broader range of cardiometabolic risk factors in childhood.
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1273

Predictors of newborn and placental weight and placental ratio in women with gestational diabetes mellitus
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Background and aims: Excessive fetal growth is the most common complication in diabetic pregnancy. Placental is also disproportionately enlarged with an increment in the placental to birth weight ratio. We aimed to analyze, in women with gestational diabetes mellitus (GDM) and singleton pregnancies, the predictive ability for birth weight, placental weight and placental to birth weight ratio of acknowledged birth weight predictors.
Materials and methods: Retrospective cohort study. Setting: Tertiary hospital. Diagnosis of GDM: universal screening with 50 gram oral load, National Diabetes Data Group criteria. Placental to birth weight ratio (PWBW) was defined as (placental weight/ birth weight) x100. Quantitative variables: expressed as median (P25, P75) or mean ± SD according to their distribution. In the multiple regression analyses; dependent variables were: birth weight, placental weight, PWBW. Potential predictors: maternal ethnicity, anthropometrics, smoking habit, family history of DM, personal history of prior glucose intolerance, prior pregnancy, prior macrosomia, gestational age and glucose values at GDM diagnosis, insulin treatment, mean HbA1c in the third trimester, chronic and pregnancy-induced hypertension, gestational age at delivery and fetal sex. Inclusion criteria: singleton pregnancy, GDM, delivery between 1982 and 2009.
Results: We evaluated 2431 women; placental weight was available in 85.4% of deliveries. Birth weight was 3250 g (2963, 3560), placental weight 620 g (540, 720) and PWBW 19.27 (17.20, 21.47). Of the 12 significant predictors of birth weight, 13 predictors of placental weight and 11 of PWBW, most were coincident. In Table 1 we display the coefficients of significant predictors of PWBW and the corresponding ones for birth weight and placental weight. Most anthropometric and metabolic parameters had a disproportionate influence on placental weight, both when promoting growth (maternal weight or 3rd trimester HbA1c) or when restricting it (insulin treatment). However smoking habit restricted fetal growth with a disproportionate effect on the fetus.
Conclusion: In women with GDM and singleton pregnancies, predictors of birth weight, placental weight and PWBW are largely concordant. The influence of different predictors has a disproportionate influence on the fetus and the placenta.

Coefficients of significant predictors of PWBW

Predictors	Birth Weight	Placental Weight	PWBW
Maternal weight	7,973	3,174	0,043
Smoking habit	-127,841	-15,525	0,788
Prior history of GDM		1,318	0,022
Family history of DM			0,333
Prior pregnancy	74,623		-0,351
Gestational age at diagnosis	4,596		-0,031
3 hours blood glucose at diagnosis	11,548	4,729	0,130
Mean HbA1c in the 3rd trimester		17,893	0,505
Insulin Treatment		-17,921	-0.642
Gestatinal age at delivery	120,433	8,718	-0,492
Weight gain	20,991	6,176	0,055

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ATLANTIC DIP: gestational weight gain and pregnancy outcomes in women with pregestational and gestational diabetes mellitus
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Background and aims: The aim of this study was to assess if excessive gestational weight gain (GWG) in pregnancies complicated by diabetes mellitus results in higher adverse obstetric outcomes when compared to pregnancies with non-excessive gestational weight gain.
Materials and methods: The ATLANTIC DIP database identified 802 women with diabetes in pregnancy who had a booking and predelivery weight and height recorded. The cohort comprised 543 (68%) women with gestational diabetes mellitus (GDM) and 259 (32%) with pregestational diabetes mellitus (PGDM), Type 1 and Type 2. GWG was categorized as excessive or non-excessive according to the maternal booking BMI and using Institute of Medicine guidelines. Maternal and neonatal outcomes were assessed based on the additional effect of GWG. Adjusted odds ratios (aOR) were calculated using logistic regression.
Results: Over 50% of women had excesive GWG and this was noted more frequently in women who were overweight (BMI 25-29.9kg/m2) at first antenatal visit (p=0.0001). In women with PGDM, excessive GWG resulted in higher adjusted odds overall for large for gestational age (LGA) [aOR 3.972 (1.849, 8.530); p<0.001] and macrosomic [aOR 3.583 (1.773, 7.238); p=0.000] infants. Similar results were found in women with GDM [aOR 2.008 (1.241, 3.248); p=0.005 for LGA and aOR 2.166(1.321, 3.550) for macrosomia]. In addition, in women with GDM, treatment with insulin further increased the adjusted odds for LGA [aOR 2.802 (1.231, 6.379); p=0.014] and macrosomia [aOR 5.630 (2.158, 14.685); p<0.0001]. Excessive GWG in women with GDM also increased aOR of pregnancy induced hypertension (PIH) [aOR 1.719 (1.037, 2.852); p=0.036].
Conclusion: In a population already at high risk of LGA and macrosomic infants, excessive GWG further increases this risk. LGA and macrosomic infants are more likely to succumb to neonatal morbidities and birth trauma. Macrosomic infants are also at risk of adolescent obesity and diabetes. Thus, this study highlights the importance of intensive weight management throughout pregnancy to prevent excessive GWG in women with GDM and PGDM. Prevention of excessive GWG is likely to translate into meaningful improvements in neonatal outcomes which may translate into long term benefits for these offspring in adult life.
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Serum interleukin-1-receptor antagonist levels in women with prior gestational diabetes mellitus and in control women
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Background and aims: Soluble interleukin-1-receptor antagonist (IL1Ra) is the natural competitive inhibitor of interlukin-1, an independent predictor of type 2 diabetes. IL1Ra levels are elevated in prediabetic individuals however its potential role in gestational diabetes (GDM - a prediabetic state) has

not yet been investigated. Thus our aims were to investigate the association (1) between current IL1Ra levels and gestational glucose tolerance status 3 years before IL1Ra measurement, (2) and between IL1Ra levels and current glucose intolerance. (3) We also determined the independent covariates of serum IL1Ra levels.
Materials and methods: We report results from a case-control study nested in a cohort: early GDM (n=46, diagnosed between 16-20 weeks of gestation), late GDM (n=43, diagnosed between 24-28 weeks of gestation), controls (n=64). Women with known diabetes were excluded. IL1Ra levels were measured by. Quantikine Human IL-1Ra Immunoassay. Glucose intolerance (GI, n=28) was defined as impaired fasting glucose or impaired glucose tolerance based on a 75g oral glucose tolerance test (WHO 1999). Other covariates were lifestyle measures, anthropometrics, blood pressure, blood lipids, and C-reactive protein (CRP). IL1Ra levels were modelled using multiple linear regression.
Results: The severity of GDM (early GDM > late GDM > control) was positively associated with age, blood pressure, 2-hour blood glucose, frequency of current GI, and negatively with height (all p for trend<0.05). Serum IL1Ra concentration was positively related to the severity of GDM (median [interquartile range]): control 329 [266] vs. late GDM 353 [338] vs. early GDM 384 [225] ng/l (p for trend=0.028). Elevated IL1Ra levels were found in women with current GI compared to controls (728 [740] vs. 518 [569], p=0.03). In a model adjusted for current GI status prior GDM remained an independent predictor of current IL1Ra levels (β-attenuation: 21%). The independent covariates of IL1Ra levels were higher BMI, higher CRP, lower HDL-cholesterol levels (all p<0.05).
Conclusion: The anti-inflammatory process observed 3 years after a GDM pregnancy (independent of current metabolic status) may be a compensatory response to the inflammatory effects associated with the earlier GDM event.
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Metabolic characterisation of women with prior gestational diabetes maintaining normal glucose tolerance up to five years postpartum
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Background and aims: Women with prior gestational diabetes (priorGDM) display a high-risk population for the development of overt type 2 diabetes, and the majority of priorGDM convert to diabetes within the first five to ten years postpartum. Previous reports have shown that conversion to overt glucose intolerance is linked to declining beta-cell-capacity to compensate for increased insulin resistance (=disposition index), which is associated with weight gain, decreasing adiponectin and HDL-cholesterol and rising markers of subclinical inflammation. However, the fact that some women are able to maintain normal glucose tolerance (NGT) over the years raises the question whether metabolic disturbances in these women have only been temporary (during pregnancy) and may disappear by time. Therefore, the current study aimed to investigate the metabolic characteristics of priorGDM with maintained glucose tolerance five years after delivery by comparing them to women without a history of GDM (healthy controls).
Study population and methods: Until now, data of 43 priorGDM with NGT and 10 healthy controls (parous women without a history of GDM) five years postpartum, who participated in the Viennese Post Gestational Diabetes Project, a longitudinal follow-up study, were analysed. All women underwent oral and intravenous glucose tolerance tests three to six months after the index pregnancy (baseline) and five years thereafter (follow-up). PriorGDM and CON were matched for age (37.5±4.3 and 35.0±5.6 years, respectively, p=ns) and BMI (25.9±4.3 and 24.7±3.2kg/m2, respectively, p=ns). In addition, blood samples for the measurement of metabolic and inflammatory parameters were taken.
Results: At baseline, only waist circumference and the 1 hour-glucose concentrations during the OGTT were increased in priorGDM compared to CON. At five years postpartum, priorGDM showed significantly increased serum concentrations of CRP (0.46±0.35 vs. 0.21±0.18mg/dl, p=0.03) and decreased concentrations of HDL-cholesterol (55.2±10.1 vs. 63.1±12.8mg/dl, p=0.04) and adiponectin (7.6±2.8 vs. 12.3±6.2 μU/ml, p=0.02). In addition, in priorGDM insulin sensitivity (5.4±2.9 vs. 9.0±4.9 10-4min-1/(μU/ml), p=0.009) and the disposition index (2.46±2.39 vs. 4.43±2.67 10-2min-1, p<0.05), both derived from IVGTT, were significantly lower compared to CON. Waist circumference -despite comparable BMI- remained higher in priorGDM.
Conclusion: The metabolic disturbances, which have recently been reported to characterise priorGDM with impaired glucose metabolism, can also be found in those who were able to maintain NGT up to five years postpartum. Hence, it seems that these changes may not be solely responsible for metabolic deterioration in this population. It furthermore highlights the importance of regular screening for glucose intolerance/diabetes in priorGDM.
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A multiplatform metabolomics study of gestational diabetes mellitus
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Background and aims: Metabolite fingerprinting has been successfully used for diagnosis of many diseases and becomes a powerful tool for biomarkers discovery. The combination of different untargeted metabolomics methods allows for simultaneously measuring as many metabolites as possible from a single biological sample that offers important advantages for global metabolome profiling. Metabolite fingerprinting approach by using multiplatform analytical techniques (LC-QTOF-MS, GC-Q-MS and CE-TOF-MS) has been applied to identification of clinically relevant changes in circulating metabolites in the plasma and urine samples of women with Gestational Diabetes Mellitus (GDM). Metabolite fingerprinting is nowadays one of the best available tools for understanding the molecular bases of the onset and progression of GDM. In addition the platform can help to understand the highly associated risk of diabetes mellitus type 2 development.
Materials and methods: Plasma and urine of 20 women with GDM and 20 pregnant women with normal glucose tolerance (after 2-h 75-g OGTT) matched according to week of gestation and age, were enrolled into the study. Liquid chromatography/mass spectrometry (LC-QTOF-MS; Agilent 6550), gas chromatography/mass spectrometry (GC-Q-MS; Agilent 7890A) for plasma and capillary electrophoresis/mass spectrometry (CE-TOF-MS; Agilent 6224) for urine analysis followed by matrix data alignment, data filtration as well as univariate and multivariate statistical analysis have been applied to the study.
Results: 309 masses in positive and 346 in negative LC-QTOF-MS ionization mode were found to have statistically significant differences according to univariate (*t* test) and multivariate (jack-knifed confidence interval) statistic. Identification of metabolites was performed by searching for possible identity of accurate masses in several databases including CEU Mass Mediator database and by final confirmation according to LC-MS/MS. By GC-Q-MS analysis we determined 12 relevant compounds and 6 out of 59 significant masses obtained by CE-TOF-MS were confirmed by available standards.
Conclusion: Most of identified compounds are related to carnitine, aminoacids, glycerophospholipids, fatty acids and sphingolipids metabolism and metabolic pathways perturbations. The importance of metabolites as alpha-hydroxybutyric acid or sphingosine-phosphate related to the biochemical mechanism of insulin resistance and impaired glucose regulation should be emphasized. Meaningful information obtained by metabolomics fingerprinting can help to detect early prognosis markers or contribute to the development of new diagnostics strategies for impaired glucose in diabetes mellitus.

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Gestational diabetes mellitus after gestational diabetes mellitus

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Background and aims: Gestational Diabetes Mellitus (GDM) in an index pregnancy increases the risk of recurrent GDM in subsequent pregnancies. The recurrence rate of GDM has been reported to range between 30% and 84%. Factors identified as predictive of GDM recurrence include advanced maternal age, multiparity, obesity, weight gain between pregnancies, requirement of insulin therapy and macrosomia during the initial pregnancy. Although previous studies have reported important data regarding GDM recurrence rates, there are no evidences about clinical and matabolic features of GDM arising in women with prior GDM. The aim of this study was to assess GDM recurrence rates in a cohort of pregnant women with prior GDM, who came on the occasion of a following pregnancy, and to compare clinical and metabolic features recurrent GDM with those of previous GDM (G1 vs G2).
Materials and methods: We carried out a longitudinal observational study of the main clinical and metabolic features in 30 women (age: 32,88±4,6 yy in G1 and 36,27±4,8 yy in G2) with GDM in an index pregnancy (G1), at least a subsequent pregnancy (G2) and normal glucose tolerance between the two. GDM was diagnosed, until 2010, according to Carpenter and Coustan criteria (glycaemia 0'>90, 60'>180, 120'>155, 180'>140) and, from 2010, according to IADPSG criteria (glycaemia 0'≥92, 60'≥180, 120'≥153). Insulin resistance was defined as HOMA-IR, pancreatic function was defined as HOMA-B% and insulin secretion was defined as AUC IRI/AUC BG. Statistics: χ^2, *t-test*, Mann Whitney, Multilevel mixed model multivariate analysis, $p<0,05$.
Results: GDM recurrence rate: 93,3% (*n* 28/30); pre-pregnancy BMI not significantly increased in G2 (22,26 vs 23,05 *ns*); pre-pregnancy BMI≥30 in 12,5% (G1) vs 8,3% (G2); earlier GDM diagnosis in G2 (gestational week of diagnosis: 24,8±6,3 vs 20,27±8,12, $p=0,022$), due to the early OGTT execution in a gestational age in wich the insulin resistance degree is lower (BG0': 88,47±15,86 vs 88,76±11,69 ns; BG60': 196,82±42,84 vs 160,65±37,4, $p=0,002$; BG120': 169,31±43,07 vs 123,81±33,2 mg/dl $p=0,001$); insulin resistance not significantly reduced in G2 (HOMA-IR: 2,19±1,15 vs 1,1±0,33, ns); pancreatic function reduced in G2 (HOMA-B%: 120,52±44,48 vs 102,42±40,19, $p=0,018$); increased insulin therapy use in G2 (70,4% vs 96,3%, $\chi^2=0,011$); insulin therapy started earlier in G2 (29,37±5,7 vs 21,9±7,6 weeks, $p=0,003$). Maternal and neonatal outcomes are statistically comparable between the two pregnancies: macrosomic infants number reduction in G2 (*n* 1 vs 0) and LGA number reduction in G2 (*n* 2 vs 1); maternal hypertension rate reduction in G2 (26,1% vs 4,3%, $\chi^2=0,04$).
Conclusions: High GDM recurrence rate in normal weight/overweight women; slight deterioration of pancreatic function in the second pregnancy.

Women clinical and metabolic features in G1 and G2

	G1	G2	p-value
GDM	100% (n 30)	93,3% (n 28)	
GDM diagnosis (gestational week)	24,8±6,3	24,8±6,3	0,022
BG0' (OGTT)	88,47±15,86	88,47±15,86	ns
BG60' (OGTT)	196,82±42,84	160,65±37,4	0,002
BG120' (OGTT)	169,31±43,07	123,81±33,2	0,001
HOMA-IR	24,8±6,3	24,8±6,3	ns
HOMA-B%	120,52±44,48	102,42±40,19	0,018
Insulin therapy	70,4%	96,3%	0,011
Start of insulin therapy (gestational week)	29,37±5,7	21,9±7,6	0,003
Maternal hypertension	26,1%	4,3%	0,04

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Genetic background of gestational diabetes mellitus in the Czech population

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Background and aims: As part of our extensive study mapping the genetic background of selected polygenic endocrinopathies, in this communication we have focused on gestational diabetes mellitus (GDM). GDM is defined as glucose intolerance that is first detected during pregnancy and subsides spontaneously after the childbirth. Its prevalence increases due to better screening but also owing to rise in obesity as well as on account of higher age of pregnant women. It is well known that GDM has a strong genetic component. Our aim was to provide broad genetic information regarding GDM in the Czech population.
Materials and methods: Our cohort of studied subjects consisted of 987 women, 514 of them were diagnosed with GDM (age 34,0±5,97 years; BMI 24,3±4,98 kg/m2) and 473 were control non-diabetic women without history of GDM (age 34,8±13,97 years; BMI 24,1±4,58 kg/m2). We used the ABI TaqMan SNP Genotyping Assays to genotype 128 single nucleotide polymorphisms (SNPs) in 65 genes, which have been described in association with type 2 diabetes mellitus (T2DM), insulin resistance, insulin secretion, obesity, energy metabolism, or lipid spectrum. Statistic analysis was conducted using the NCSS 2004 software (Chi-squared test).
Results: Concerning the allelic distribution between the GDM group and controls, the most significant association with GDM showed the C/G SNP rs738409 in the obesity-associated gene adiponutrin (minor allele G frequency 27.8 % in GDM group compared with 21.9 % in controls; $p<0.01$). Furthermore, strong association with GDM was found in the intergene blood lipid-associated T/C SNP rs4942296 (minor allele C frequency 32.8 % in GDM group compared with 42.8 % in controls; $p<0.01$). Also the C/T SNP rs7903146 in the TCF7L2 gene showed association with GDM: the minor T allele, which confers risk for T2DM, was represented by 33.3 % in the GDM group compared with 26.9 % in controls ($p=0,02$). In addition, statistical analysis of genotype distribution of the A/G SNP rs7498665 in the obesity-associated SH2B1 gene revealed higher proportion of gestational diabetics among the G allele carriers (72.0 %) compared with only 63.6 % of controls ($p=0.01$).
Conclusion: Of the 128 SNPs in 65 genes conferring risk for T2DM, insulin resistance, obesity, and metabolic syndrome, we confirmed the strongest association of GDM with SNPs in genes TCF7L2, adiponutrin, SH2B1, and the intergene SNP rs4942296. According to genome-wide association studies, the TCF7L2 gene was found to be significantly associated with T2DM. Our results confirm also in GDM that the risk is linked to the minor allele T of the rs7903146 in this gene. The two SNPs in adiponutrin and SH2B1 represent obesity-susceptibility loci. Considering almost identical BMI in the compared groups of gestational diabetics and controls in this study, described associations of the two SNPs with GDM suggest that obesity and diabetes share the common etiopathological roots operating prior to the obesity manifestation.
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Daily arsenic contamination associated with gestational diabetes and low Apgar score

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Background and aims: Much evidence suggest that populations exposed to high level of arsenic in water, increases the risk of diabetes, but little is known about exposers during pregnancy and to Gestational Diabetes (GD) and poor pregnancy outcome, such as Low Apger Score (LAS). The study was undertaken to investigate the arsenic level of daily water usage associated with GD and low apger score babies.

Materials and methods: The study was observational case-control design. Total of 263 pregnant women (age, M±SD, 21±3.7 and BMI, 25.1±4.1 kg/m^2 were matched) were included in this study. All women were 28-38 wk of gestation. Clinical and anthropometric parameters were measured by standard techniques. Serum glucose level by glucose oxidase method. Arsenic level measured by Standard Methods. Arsenic exposer assessed by daily using (drinking, cooking, washing and bathing) water of each household. Statistical analysis was performed mean±SD and median(range), univariate and multivariate was also analyzed where as appropriate.
Results: Arsenic concentration was significantly higher in GD subjects [µg/l, median (range), 62(22-306)] compared to non-GD subjects [µg/l, 3.6(1-99)]. Significantly higher level of 2 hr 75g glucose (mg/dl, 12,2±1.7) in GD as compare to non-GD subjects (6.3±0.6). The apger score is significantly lower in GD subjects (4.7±0.8) when the compared to non-GD subjects (6.4±0.7) (p<0.001). The Pearson' correlation showed daily arsenic contamination is a significantly positive correlation with Glucose level (r=0.638, p=0.034) and lower apger score (r=0.892, p=0.041). Linear regression also showed arsenic concentration was strongly associated with higher glucose level and lower apger score in GD subjects (p<0.001).
Conclusion: The above findings imply that Arsenic contamination may play a role in glucose intolerance and may associated with an increased risk of GD; it may be contributed to lower apger score in babies.
Supported by: BADAS

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Gestational diabetes screening: new considerations on the recent Italian recommendations

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Background and aims: Recent recommendations from the Italian Ministry of Health indicate that screening for gestational diabetes mellitus (GDM) must be performed in all pregnant women ≥35 years old or in women <35 years old, in the presence of risk factors such as BMI ≥25, previous GDM, previous newborn with macrosomy, familiarity for type 2 diabetes. Our aim was to determine the effectiveness of these recommendations compared with the criteria established in 2010 by the International Association of the Diabetes and Pregnancy Study Groups, recommending GDM screening in all women not previously diagnosed with overt diabetes.
Materials and methods: This is a retrospective cohort study of 2448 pregnant women admitted to the University of Catanzaro outpatient clinics, and to the Struttura Operativa Complessa Endocrinologia-Diabetologia, Ospedale Pugliese-Ciaccio, Catanzaro, Italy, from May 1, 2010 to December 31, 2012 for GDM screening. Out of these, 893 (36.5%) pregnants were ≥35 years old and 1555 (63.5%) <35 years old. In this latter group, 34.6% (538/1555) showed no risk factors for GDM. GDM was diagnosed with 75 g oral glucose tolerance test (OGTT) at 24-28 weeks of gestation, following the IADPSG 2010 cut-offs. Different maternal and neonatal outcomes were considered. Effect of GDM was analyzed by multiple regression analysis.
Results: Overall, 674 (27.5%) women were diagnosed with GDM. In particular, GDM was diagnosed in 31.8% (171/538) of <35 years old pregnants without risk factors for GDM, who would have not been tested according to the Italian recommendations. Interestingly, diagnosis was made at baseline (55.5%), at 1 hour (39.8%), or at 2 hours (4.7%) during OGTT. Despite of the appropriate treatment, GDM represented a risk factor for primary caesarean section [adjusted odds ratio (AOR)=1.92, 95% confidence interval (CI) 1.21-3.06; P=0.006]; polyhydramnios (AOR=4.48, 95% CI 1.20-16.73; P=0.025); increased birth weight (P<0.001); admission to the neonatal intensive care unit (AOR=4.39, 95%CI 1.44-13.37; P=0.009); and large for gestational age (AOR=3.53, 95%CI 1.34-9.34; P=0.011).
Conclusion: Our findings indicate that over 30% of pregnant women <35 years old, without risk factors for GDM, would miss diagnosis of GDM by adopting the recent Italian recommendations. The fact that these women have increased risk for adverse pregnancy outcomes suggest to extent the screening to all pregnants. To limit costs for GDM screening (with a ~5% drop in sensitivity), OGTT could be restricted to two steps, basal and 1 hour.

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Prediction for development of gestational diabetes mellitus in the 1st trimester of gestation based on levels of HOMA-IR index

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Background and aims: As is well know gestational diabetes mellitus (GDM) are diagnosed in the 2nd trimester of gestation (after 24 week of fetation). Prediction for development of GDM in the 1st trimester of pregnancy is a very important problem. The aim of the study was to determine a cut-off level of HOMA-IR index for prediction of development of GDM in 1st trimester of gravidity.
Materials and methods: We estimated and compared the HOMA-IR indices in 1st, 2nd and 3rd trimesters in 160 women with GDM and in 39 pregnant healthy subjects (control group). Using ROC-analysis we determined cut-off level for prediction of developing of GDM in 1st trimester of gestation.
Results: The HOMA-IR index values were significantly higher in women with GDM compared with control group in 1st (Me [P25; P75] 3,50 [1,16; 9,13] vs. 1,32 [0,59; 2,30], p<0,001), 2nd (Me [P25; P75] 4,96 [1,99; 38,88] vs. 1,99 [1,33; 3,21], p<0,001) and 3rd (Me [P25; P75] 4,92 [1,55; 34,72] vs. 2,64 [1,68; 4,38], p<0,001) trimesters. The predictive cut-off level of HOMA-IR index in the 1st trimester of pregnancy for development of GDM is above 1.89 Sensitivity of ROC-analysis is 90,0% (95%Cl 84,3-94,2), Specificity - 89,7% (95% Cl 75,8-97,1); Positive Predictive Value - 86,8%, Negative Predictive Value - 79,4%. Area under curve is 0,963± 0,01 (95% CI 0,92-0,98), p< 0,001.
Conclusion: The HOMA-IR index level above 1.89 in 1st trimester of pregnancy is a prognostic value for development of gestational diabetes mellitus that require more frequent control of blood glucose concentration and reduction for consumption of refined carbohydrates.

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Insulin secretion in addition to insulin resistance predicts gestational diabetes mellitus

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Background and aims: Insulin resistance is a common implication of gestational diabetes (GDM). Although maternal glucose levels and fetal insulin have often been investigated, maternal insulin levels during the oral glucose tolerance tests (OGTT) were not studied in larger trials. The aim of this ongoing study was to evaluate insulin levels of pregnant women during OGTT for the prediction of GDM.
Materials and methods: 256 women (age 31±6 years) were screened for GDM in a prospective study at tertiary care unit during 24-32 (28±2) week of gestation. A 75g - OGTT was performed, glucose and insulin levels were measured at 1-hour, 2-hours and fasting. In addition, HOMA-IR and Matsuda Index were calculated for estimation of insulin resistance and insulin sensitivity. Non-parametric valuables were log10 transformed. Statistics included student's t-test, univariate and multivariate logistic regressions including confounding analysis and calculations of odds ratios (OR) per 1 STD (OR-STD).
Results: 38.7% (n=99) of the women were diagnosed with GDM. Women with GDM were significantly more obese (BMI: 30.0±5.4 vs 27.8±5.9 kg/m^2, p=0.014), older (32±6 vs 20±6 years, p=0.013) and had a higher HbA1c (5.3±0.4 vs 5.1±0.3%, p=0.001) than women without GDM (nonGDM). Fasting insulin levels were as followed: GDM: 11.2 (7.7,17.1), nonGDM: 10.6 (7.5,14.1), p=0.040; 1-hour insulin levels, GDM: 108.5 (68.2,151.4), nonGDM: 86.8 (58.2,129.4), p=0.145; and 2-hours insulin levels, GDM: 94.5 (65.6,131.7), nonGDM: 63.2 (46.1,100.9), p<0.001. Since the variance of the predictors of GDM was highly different, OR-STD were calculated. After elimination of confounders seven variables remained. In univariate fashion, OR-STD were as followed: 2-hour glucose: 2.15 (p<0.001), 2-hour insulin: 1.63 (p<0.001), HOMA-IR: 1.58 (p=0.004), Matsuda Index: 0.50 (p<0.001), HbA1c: 1.90 (p=0.001), BMI: 1.40 (p=0.017), triglycerides: 1.28 (p=0.025). After multiple stepwise backward regression, 2-hours glucose (OR: 1.036, p<0.001) and Matsuda-Index (OR: 0.842, p=0.011) were identified as final model. Thus a change in glucose of 1 mg/dl resulted in an increase of 3.6%, a change in Matsuda Index for 1 unit in a decrease of 15.8% for GDM risk. OR-STDs of the final model: 2-hours glucose: 2.15; Matsuda Index: 0.50. Thus the Magnitude of 2-hours glucose (2.15) was 7.5% stronger than the magnitude

of the Matsuda Index (2.0). However, as confounding analysis demonstrated, the Matsuda Index was independently significantly associated with GDM, whereas the significant association of 2-hours glucose was confounded by the Matsuda Index.
Conclusion: Women with GDM are significantly more often insulin resistant, as is often demonstrated by calculations of the OGTT. Our findings indicate, that first reduced insulin sensitivity might be more relevant for the establishment of GDM than previously anticipated (HOMA-IR is eliminated from the model). Second, 2-hours glucose and the Matsuda Index were identified as predictors of GDM. 2-hours glucose is just natural, however, the strong preventive power of the Matsuda Index is new. Third, our multivariate analysis revealed that the Matsuda Index was independently associated with GDM, whereas 2-hours glucose was confounded by the latter. Altogether our findings strongly argue for future determinations of insulin levels during the OGTT in pregnancy.

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Serum adiponectin levels in women with previous gestational diabetes
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Background and aims: Gestational diabetes (GDM) is a risk factor for cardiovascular disease and metabolic syndrome later in life. As both decreased adiponectin levels and GDM are important predictors of abnormal glucose metabolism, insulin resistance, and metabolic syndrome, we sought to investigate (1) the association between prior GDM status and current adiponectin levels and (2) the determinants of adiponectin levels 3 years after delivery.
Materials and methods: Our nested case-control study included 97 women with previous GDM and 44 with normal glucose tolerance during pregnancy (age [mean±SD]: 35.4±3.9 vs. 33.6±3.6 yrs, P=0.011; BMI: 25.7±5.6 vs. 24.0±4.3 kg/m2, P NS). Thirty-four women(24.1%) had glucose intolerance (GI - diabetes mellitus, impaired fasting glucose, or impaired glucose tolerance) at follow-up. We collected data on lifestyle measures (questionnaire), anthropometry, and blood pressure (physical examination). Blood samples were drawn in the fasting state and during a 75g OGGT for the determination of serum glucose, insulin, creatinine, HbA1c, sex hormone-binding globulin (SHBG), and blood lipids. Insulin sensitivity and insulin secretion was determined using homeostasis model assessment (HOMA) calculator. Serum adiponectin concentrations were measured by Quantikine Human Total Adiponectin Immunoassay (R&D System Minneapolis, USA; 4.5 hour solid-phase ELISA).
Results: Serum adiponectin levels were lower in prior GDM women compared to controls (9.44±4.41 vs. 12.33±6.83 μg/ml, P=0.013) however they were similar among GI and normal glucose tolerant women (9.19±4.81 vs. 10.71±5.58 μg/ml, P NS). Negative correlations were found between serum adiponectin and BMI (r=-0.298), waist circumference (r=-0.349), systolic and diastolic blood pressure (r=-0.210/-0.215), HbA1c (r=-0.184), fasting and 2-hour insulin (r=-0.248/-0.329), HOMA-2B (r=-0.283), serum triglycerides (r=-0.245), LDL-cholesterol (r=-0.319) and GDM status (r=-0.172). HOMA-2S (r=0.210), HDL-cholesterol (r=0.296) and SHBG (r=0.296) correlated positively with serum adiponectin. In multiple linear regression, waist circumference, serum triglycerides, LDL-cholesterol were positively, while hip circumference and SHBG were negatively related to serum adiponectin (all P<0.05).
Conclusion: The opposing direction of association between adiponectin and waist or hip circumference in the multivariate model raises the hypothesis that subcutaneous fat may play a protective role in the development of insulin resistance.
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Estimation of the postpartum glucose intolerance risk in women with gestational diabetes mellitus using routine glycaemic indices assessed in the mid-trimester of pregnancy
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Background and aims: Incidences of T1DM and T2DM as well as gestational diabetes mellitus (GDM) are increasing worldwide. Given that women with previous GDM have increased risk of developing diabetes in the future compared with those normoglycaemic during pregnancy, however their participation in the postpartum screening is low the aims of our study were (1) to ascertain a frequency of early (up to 12 months post-delivery) postpartum conversion of GDM into permanent diabetes (DM) or impaired glucose tolerance (IGT), (2) to test whether the degree of glucose intolerance diagnosed by standard criteria at the time of GDM diagnosis correlates with the degree of early postpartum glucose (in)tolerance and (3) to find eventual significant predictive factors for early postpartum conversion of GDM to DM/IGT from glycemic indices routinely measured in the mid-trimester of gravidity.
Materials and methods: We carried out a retrospective epidemiological analysis of electronic health records data of an ethnically homogenous, central European population cohort of women with GDM diagnosis followed in a single medical centre during the period 2005 - 2011 that underwent repeated oGTT up to 1 year after the index delivery (n=1090 for the purpose of incidence analysis). A subgroup of 305 GDM subjects with compete anthropometric, clinical and biochemical data that underwent both mid-trimester and postpartum 3-point oGTT test in the central laboratory of the University Hospital Brno was chosen for more detail analysis of risks and predictive factors.
Results: DM/IGT was detected in a total of 11.7% subjects, of those 4.1% had DM (2.8% T2DM and 1.3% T1DM). Glycaemia in all three time-points of mid-trimester oGTT, area under the curve (AUC_{oGTT}) and mid-trimester HbA1c were significantly associated with the postpartum disorder (P<0.05, Mann-Whitney). A highly statistically significant trend was identified in the number of above threshold values during mid-trimester oGTT and the postpartum DM/IGT conversion ($P<1x10^{-12}$): 8% of patients with one oGTT value above cut-off, 25% with two and 65% with all three were diagnosed with DM/IGT within the 1 year postpartum. Uni- and multivariate regression models were used to identify the best predictive parameters (FPG and AUC_{oGTT}) for the construction of ROC.
Conclusion: Parameters of glucose metabolism measured between 24-28th week of pregnancy exhibit - apart from diagnostic value for GDM diagnosis - significant predictive potential for early reoccurrence or postpartum persistence of glucose intolerance. Considering generally low-compliance of GDM women in postpartum screening, risk-based stratification of GDM population could improve efficiency of the screening for diabetes after delivery.
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Changes of serum vaspin, adiponectin, MCP-1 and BDNF during course of pregnancy and their association with glucose metabolism
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Background and aims: Pregnancy is characterized as having a diabetogenic effect on normal carbohydrate metabolism. Different factors were suggested to have additional time-dependant impacts on the manifestation of gestational diabetes (GDM). The objective of the present study is to assess longitudinal changes in Vaspin, Adiponectin, MCP-1 and BDNF during the course of pregnancy until 3 months postpartum and their association with insulin sensitivity and pregnancy outcome in women with GDM compared to healthy women.
Materials and methods: In a longitudinal study comprising five visits, 68 pregnant women (36 with GDM and 32 with normal glucose tolerance) were included before the 22th gestational week (GW, Visit 1) and underwent a 75g-2h-oral glucose tolerance test. In case of a negative result the test was

repeated in the GW 24-28 (V2) for final diagnosis according to the IADPSG criteria. An additional blood drawn was performed for determination of circulating Adiponectin, Vaspin, BDNF and MCP-1 levels in ≤ 22th GW (Visit 1 (V1)) as well as during GW 24-28 (V2), GW 30-34 (V3), GW 36-40 (V4) and 8-12 weeks after delivery (V5). The oral glucose insulin sensitivity index (OGIS) was calculated by using data of the diagnostic OGTT to accurately estimate insulin sensitivity. Neonatal data including length, weight and gestational age (GA) at delivery were used to calculate the Ponderal Index (PI) as well as the percentiles for adjusted birth weight in order to define small-for-GA (SGA) and large-for-GA (LGA) outcomes.
Results: While we found significant differences between GDM and NGT in Adiponectin (7.42±3.13 vs. 10.09±3.80 μg/ml, p=0.002), there were no differences in Vaspin (p=0.986), BDNF (p=0.931) and MCP-1 (p=0.375) at V1 between GDM and NGT women. However, a strong linear association of Adiponectin (r=0.464, p<0.001) with OGIS was observed already in early pregnancy. In the follow-up there appeared no time-dependant interactions in BDNF (pg/ml) between both groups with a significant decrease in V3 and V4 as compared to V1 (V3: mean difference 0.765, p<0.001; V4: mean difference 0.637, p=0.004 for log transformed data). However, Adiponectin levels in the GDM group remained constantly low during pregnancy and after delivery. At postpartum there was a relevant change regarding Vaspin and MCP-1 levels, as Vaspin significantly decreased and MCP-1 was ascending compared to the respective levels during pregnancy. There was no association of these parameters with adjusted birth weight or PI at any time point.
Conclusion: The strong predictive value of Adiponectin for glucose intolerance in women affected by GDM can be affirmed independently from point of measurement with constantly low serum concentrations beginning from early pregnancy to the first months postpartum. However, changes in time-depended courses of Vaspin, MCP-1 and BDNF are observable but were not associated with glucose tolerance status in pregnant women. Thus, during pregnancy time of blood sampling should be taken into account in analyses with group based comparisons.
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The effect of prior gestational diabetes on the shape of the glucose response curve during an oral glucose tolerance test 3 years after delivery
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Background and aims: Monophasic glucose response during an oral glucose tolerance test (OGTT) is associated with decreased insulin sensitivity and β-cell function. Furthermore, it is an independent predictor of type 2 diabetes (T2DM). Since gestational diabetes (GDM) is also associated with an increased lifetime risk of T2DM, we aimed to investigate the association between the shape of the glucose response curve and (1) glucose tolerance status during a pregnancy 3 years before and (2) current glucose tolerance status. We also sought (3) other determinants of the shape of the glucose response.
Material and methods: We report of a case-control study nested in a cohort of women delivered at a Hospital in Budapest, Hungary in 2005-2006. Participants are women with early GDM (n=46, diagnosed between 16-20 weeks of gestation), late GDM (n=43, diagnosed between 24-28 weeks of gestation), and controls (n=64, normal glucose tolerance [NGT] during pregnancy). Women with known diabetes at follow-up were excluded. We defined monophasic glucose response curve if it followed an inverted U-shape and biphasic if there was a second rise in plasma glucose after the first rise. Glucose tolerance was defined by WHO 1999.
Results: At follow-up 3.5±0.7 (mean±SD) years after delivery participants were 35.1±4.0 years old, their fasting plasma glucose was 5.3±0.4 mmol/L, 2-hour glucose was 6.1±1.7 mmol/L, HbA1c 5.5±0.3%, BMI 25.2±4.9 kg/m^2, systolic blood pressure (SBP) 119±15 mmHg. Twenty percent had NGT, 5% impaired fasting glucose (IFG), and 15% impaired glucose tolerance (IGT). Thirty-seven percent had the early GDM, 32% had the late GDM, and 31% NGT during pregnancy. Women with a biphasic response had a lower hip circumference (100±9 vs.104±10 cm), lower levels of triglycerides (median [IQR] 0.9 [0.6] vs. 1.0 [0.9] mmol/L), higher levels of adiponectin (10.9 [7.6] vs. 8.1 [5.5] μg/ml), lower number of previous pregnancies (2 [1] vs. 3 [2]), and were less frequently diagnosed with early GDM (44.9 vs. 23.3%, all p<0.05). Women with biphasic response tended to be younger (34.3±4.0 vs. 35.7±3.9 year), to have a lower SBP (116±13 vs. 121±16 mmHg), and C-reactive protein (1.05 [1.85] vs. 1.10 [3.35] mg/l, all p<0.1). There was no difference between women with biphasic and monophasic curves in fasting and 2-hour glucose or insulin levels, or body mass index (all p>0.1). According to a multiple logistic regression model, a monophasic response was independently associated with a prior early GDM (OR 2.85 95% CI 1.05-7.73), elevated triglycerides (OR 1.74 95%CI 0.97-3.10 /mmol/l), and a higher number of previous pregnancies (OR 1.64 95%CI 1.03-2.51 /pregnancy).
Conclusion: We found that although the shape of the glucose response curve is independent of current glucose and insulin levels, it is strongly associated with 'severe' (early onset) glucose intolerance during pregnancy 3 years before the OGTT test. These results may suggest that the shape of the OGTT curve captures abnormal glucose regulation independent of plasma glucose and may be an earlier indicator of an elevated diabetes risk than fasting or post-load glucose however further studies are required to confirm this hypothesis.
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The role of HbA_{1c} as a risk predictor for overt diabetes after pregnancy with gestational diabetes mellitus
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Background and aims: Risk stratification after pregnancy with gestational diabetes mellitus (GDM) is actually based on screening with the 2h-OGTT performed in the first months after delivery. However, the main limitation of this examination is the necessity of a certain time expenditure, which is possibly contributing to the non-adherence of many affected women. The assessment of HbA1c was proposed as an alternative with the advantages of independent performance at any time without requiring a fasting state and the little biological variability. Thus, this report aimed to compare the predictive accuracy of both examinations to detect pathologies in carbohydrate metabolism early after GDM pregnancy.
Materials and methods: 75 women with previous gestational diabetes (pGDM) and 41 controls participating in the "Vienna Post-Gestational Diabetes Project" were included 3-6 months after delivery and underwent a detailed metabolic characterization: frequently sampled 3h-OGTT with measurements of glucose, insulin, C-peptide and proinsulin, as well as a frequently sampled intravenous glucose tolerance test (FSIGT) to estimate insulin sensitivity and insulin secretion. pGDMs were annually invited for routine diabetes screening to a maximum of 10 years.
Results: Although, pGDM women showed higher HbA1c levels as compared to females with uncomplicated pregnancy (5.50±0.42 vs. 5.17±0.27, p<0.001), univariate analysis revealed only moderate associations of HbA1c with area under the curve (AUC) of glucose measurements during the OGTT (r=0.30, p=0.001). Moreover, no associations were found between HbA1c and OGTT dynamics of insulin, proinsulin and C-peptide. Also with regard to glucose disposition, HbA1c showed only modest correlation with FSIGT insulin sensitivity index (r=-0.26, p=0.008) and disposition index (sensitivity x secretion: r=-0.25, p=0.010), while no association was reported with first phase of insulin secretion. A multivariate linear model including fasting as well as 1h and 2h post load glucose levels showed a good predictability for the FSIGT disposition index explaining 21% of the variance. The additional inclusion of HbA1c had no further impact to improve the predictive ability of this model. Graphical tests of interactions indicated comparable effects between women after GDM and controls. During follow-up, 16 cases of overt diabetes were reported in the pGDM subgroup. Of those only 9 females (56%) were sufficiently detected by HbA1c≥5.7% at baseline, while 44% of subjects under highest risk were not detected.
Conclusion: It is clearly suggested that the predictive ability of HbA1c for the detection of subclinical alterations in glucose metabolism is inferior to that of the OGTT. As a surrogate marker of the mean plasma glucose of up to 3 months, HbA1c fails to predict latent alterations of insulin sensitivity and β-cell function before manifestation of overt diabetes. These alterations could be unmasked only by performing a standardized dynamic test as the OGTT.
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Fertility in affected and non-affected siblings in families with type 1 diabetes: results from the type 1 diabetes genetics consortium (T1DGC)

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Background and aims: A recent Finnish study described reduced fertility in patients with childhood-onset type 1 diabetes (T1D). The T1DGC is an international effort aimed at the study of the genetics and pathogenesis of T1D where families with at least 2 affected siblings have been included from all over the world. The aim of our study was to assess fertility and its distribution in the T1DGC dataset available on 1st October 2009.
Materials and methods: Clinical information, including family history, was obtained using questionnaires at each of the participating centres. Only subjects aged 18 or more at the time of examination were included in the present analysis. Affected and unaffected siblings were compared regarding the number of offspring (chi squared and Wilcoxon's test) and stratified by gender. Non-parametric adjustments were performed for age of birth and age of onset. Additional analyses were performed in those families comprising both affected and unaffected siblings, and the number of offspring was compared within each family. Statistical analysis was performed using "R".
Results: A total of 2929 affected and 759 unaffected adult siblings were assessed, belonging to 1748 families. Mean age was 32.3 (11.0) years and 51% were women. Offspring were distributed as shown in the table: chi-squared= 64.33, p-value = 3.563*10-13.This distribution was skewed at the expense of women, whereas men did not show a difference. Birth year did not significantly affect the differences seen between groups. However, there was a positive correlation between the age of onset and the number of offspring: 0.28 (0.25-0.31), p<2.2 10-16. When only families with at least one unaffected sibling were included (N=1153), the difference between the affected and unaffected sibling ranged between 0.14-0.15 children (p=7.6*10-5).
Conclusion: Women with T1D have fewer children than their unaffected siblings, but such an effect is not seen in men with the disease. Birth cohort does not seem to affect this observation. Later age of onset of diabetes is associated with a higher number of offspring.

Nº of offspring	0	1	2	3	≥4
Affected (%)	60.61	16.52	16.56	4.97	1.34
Non- Affected (%)	54.35	12.01	19.79	9.50	4.35

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Circadian variation in glucose during diabetic pregnancy is associated with macrosomia in the newborn

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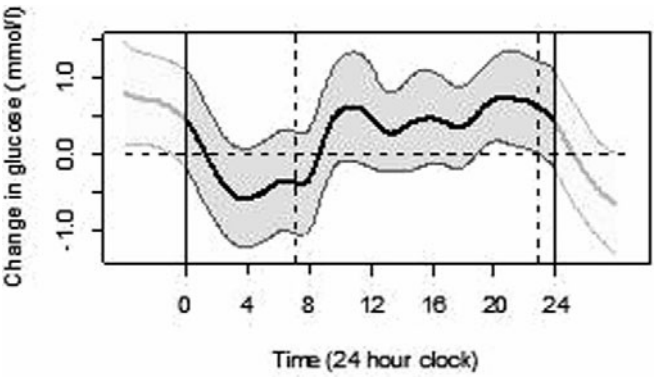
Background and aims: Macrosomia is the commonest complication of diabetic pregnancy, often occurring despite achieving good glucose control as measured by HbA1c and home blood glucose monitoring. Although it is commonly attributed to hyperglycemia, the actual continuous glucose profile across the 24 hour day/night cycle that is associated with its development has not been described. The purpose of this study was to determine the circadian glucose profile associated with macrosomia in well controlled diabetic pregnancy, utilising functional data analysis of glucose obtained by CGMS (Continuous Glucose Monitoring System) from a previous observational study.
Materials and methods: We developed functional data analysis to analyse the numerous and complex data obtained by CGMS in 35 Type 1 and 14 Type 2 diabetic pregnancies. 256640 glucose measurements were made over 171 measurement sessions. Knot placement was fixed at equally-spaced points every 30 minutes over the 24 hour time period, with extensions by 4 hours either side of midnight to remove edge effects. Mean functions were calculated for subgroups of mothers, divided by Type of diabetes, or by those with or without macrosomia. A functional regression model was fit with the outcome glucose as a function and trimester, Type of diabetes and macrosomia as scalar variables. Pointwise 95% confidence intervals were calculated. Macrosomia was defined as above the 90th centile for gestation-adjusted birth-weight.
Results: Of the 49 live births, 23 (47%) pregnancies resulted in a baby with macrosomia, and 26 (53%) babies did not have macrosomia. Mothers who developed macrosomic babies had a greater circadian variation in glucose with significantly higher levels of glucose during the daytime and evening (by 0.8 mmol/l), but lower levels from 1am until 9am (by 0.6 mmol/l), compared to mothers who did not develop macrosomia (see figure).
Conclusions: This study is the first to demonstrate, using functional data analysis, the circadian variation in glucose that is associated with the development of macrosomia in diabetic pregnancy. It confirms established evidence that postprandial hyperglycemia during the day is associated with macrosomia, but gives novel information about the contribution of nocturnal glucose control and suggests that relative hypoglycemia has an important role to play. This information is important to enable development of more accurate diagnostic and prognostic assessments, and optimize the application of new therapeutic technologies in insulin administration. Glucose levels associated with macrosomia with 95% pointwise confidence intervals derived from a functional regression model.



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In pregnancy maternal active ghrelin levels predict positively neonatal birth waist and negatively cord blood insulin levels

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Background and aims: During pregnancy maternal appetite-dependent mechanisms contribute to energy intake for mother and fetus. Neonatal waist is associated to its future growth and metabolic health while it reflects adipose tissue and hence, energy deposits. We aimed to investigate, during pregnancy, the association of fasting maternal appetite-related hormones levels [the gut-derived ghrelin (active), GLP1(active) and total PYY and the adipocytokine leptin] with neonatal waist, percent total body fat and insulin levels at birth.
Materials and methods: 80 non-obese non-diabetic primigravidae women (mean±SD; age: 26.9±2.5 years; pre-pregnancy BMI 23.5±2.2 kg/m^2) were seen at each of the three trimesters. At each visit they had blood sampling for hormones measurements and a 2hr 75gr oral glucose tolerance test. At birth, neonates were submitted to anthropometry and cord blood sampling for c-peptide, glucose, insulin, adiponectin and leptin measurements.
Results: Second trimester maternal active ghrelin levels correlated positively with neonatal waist (p=0.04, r=0.75) while the former, by using stepwise multiple regression, were the best positive predictor of the latter (p=0.03, beta=0.84)among all fasting maternal appetite related hormones studied. Third trimester maternal active ghrelin levels correlated negatively with percent total neonatal body fat (p=0.04, r=-0.94), fetal HOMAR (p=0.021, r=-0.829) and cord blood insulin levels (p=0.04, r=-0.829) while maternal active ghrelin levels were by multiple regression analysis, the best

negative predictor of cord blood insulin levels (p=0.02, beta=-0.99)among all fasting maternal appetite related hormones. Third trimester maternal leptin levels correlated negatively with neonatal waist (p=0.027, r=-0.81). **Conclusion:** During a non-diabetic non-obese pregnancy circulating maternal active ghrelin, a pro-appetite hormone, is associated with neonatal visceral energy storage (as expressed by neonatal waist).By inhibiting glucose-driven maternal insulin secretion, ghrelin ensures adequate fasting glucose and nutrient supplies to the fetus while limiting overall fetal adipose tissue deposition.

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Risk factors of macrosomia in type 1 diabetes pregnancy: one centre analysis between 1999 and 2012

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Background and aims: Type 1 diabetes mellitus (T1DM) in pregnancy is associated with increased risk of maternal and fetal complications. Macrosomia is one of the most frequent complications of pregnancy in T1DM women. Its risk remains high in spite of recent advances in diabetes care during pregnancy and improvement in glycemic control observed in many European countries. We aimed to examine the impact of maternal glycemic control on birth weight and to identify risk factors of macrosomia in T1DM women.
Materials and methods: We accessed records of 502 T1DM pregnancies, treated at the Department of Metabolic Diseases between 1999 and 2012. We excluded women who experienced miscarriages (n=40), preterm deliveries (n=71) or with missing outcome variables (n=16). In 375 women, linear regression models were used to examine the impact of glycemia assessed by HbA1c and mean of measurements in daily glucometric profiles in each trimester on birth weight. We also evaluated maternal age, BMI, weight gain and pregnancy planning. Logistic regression was used to model risk of macrosomia (> 4 kg). The models were adjusted for gestational age.
Results: The mean age (±SD) of the women was 28.2±4.7 years, diabetes duration - 11.9±7.6 years. Their pregestational BMI was 24.0±4.4 kg/m2. The mean birth weight was 3.57±0.58 kg at 39.3±1.5 gestation week. There were 85 (22.7%) macrosomic newborns. HbA1c in the 1st, 2nd and 3rd trimester was 6.8±1.3, 5.8±0.9 and 5.7±0.8 %, respectively, while mean glucose in daily profiles was 6.1±1.3, 5.8±0.9 and 5.8±0.9 mmol/l. There was no association between 1st trimester HbA1c or glucose and birth weight. Birth weight was higher by 74 g per 1% increase in 2nd trimester HbA1c (p=0.024) and by 157 g per 1 mmol/l increase in mean glucose (p<0.001). The corresponding effects in the 3rd trimester were 219 g per 1% and 155 g per 1 mmol/l (p<0.001 each). Third trimester HbA1c and glucose were independent predictors of birth weight and macrosomia, as was maternal age. The analysis restricted to women who reached the recommended criterion of HbA1c<6.0% in the 3rd trimester (n=210) revealed similar risk factors.
Conclusion: Macrosomia in children of T1DM mothers was prevalent (> 20%) in this one-center cohort despite excellent metabolic control. Hyperglycemia during the 3rd trimester was predominantly responsible for this condition, other risk factors were 2nd trimester glycemia and maternal age.

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1, 5-Anhydroglucitol (1, 5 AG) and neonatal complications in pregnancy complicated by diabetes

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Background and aims: Macrosomia is characterized by disparate rates of fetal growth and abdominal obesity. Large for gestational age (LGA) babies are at risk of neonatal hypoglycemia (NH) despite excess abdominal fat, attributed to poor maternal glycemic control; mainly postprandial hyperglycemia (PH). Low levels of 1,5 AG are associated with PH in non-pregnant diabetic patients. We investigated associations of 1,5 AG on neonatal birth weight (NBW) and parameters of fetal growth (FG) by ultrasound (US) that may help predict LGA and NH and compared to HbA_{1c}.
Materials and methods: Retrospective cohort of 102 consecutive pregnancies: 17 with gestational diabetes (GD), 48 with Type 1 and 37 with Type 2 diabetes seen at the University of Washington Medical Center. To determine the association of 1,5 AG with parameters of FG and NBW, we extracted monthly simultaneous 1,5 AG and HbA_{1c} values throughout pregnancy until delivery. From the infants' records, NBWs were evaluated by standardized z-scores, controlling for gestational age at delivery and gender. Generalized Estimating Equations were used to examine the association of 1,5 AG with FG measurements: length, abdominal (AC) and head circumference (HC), after adjusting for gestational week. Linear regression was used to assess the association of 1,5 AG and HbA_{1c} with NBW z-scores. NH was defined by a note in the infant record, capillary glucose in mmol/l of <1.7 in the first 24 h or <2.5 in the first 48 h after birth. A t-test or Welch's approximate t-test was used to compare the mean 1,5 AG of mothers with babies with NH vs those without. Data were analyzed by type of diabetes and trimester.
Results: A negative association was found between 1,5 AG and NBW z-scores; and 1,5 AG and AC. With 1,5 AG modeled as a linear function, increasing values were associated with decreased z-scores, R=-.28, p=.008. There was also evidence that the association was non-linear, with a larger negative association for lower 1,5 AG values relative to larger values. In contrast the association between HbA_{1c} and z-scores was NS. Results were similar across type of diabetes and trimester, independent of BMI. Similarly as 1,5 AG increased, AC decreased, p=.0002. 1,5 AG's association with length or HC was NS. The mean 1,5 AG (μg/ml) in all women was lower in the NH group vs the non-NH: 4.68 (*n 54*) vs 6.13 (*n 47*) respectively, but NS, p=.08. In women with GD and NH, the mean 1,5 AG vs the no-NH GD group was significant: 6.08 (*n 7*) vs 10.86 (*n 10*), p=.01; more so in the 3rd trimester: 5.62 (*n 7*) vs 12.54 (*n 7*), p=.006; but not for other types of diabetes.
Conclusion: AC is the most reliable prenatal parameter to assess the risk of LGA. Our preliminary analysis demonstrates an association of 1,5 AG with AC by US and NBW. 1,5 AG's lack of association with length and HC is in agreement with previous observations on the disproportionate size of the abdomen girth described in diabetic fetopathy but normal HC and length. The mean 1,5 AG was lower in the NH group but statistically significant only for GD. Further examination of this is called for, as the *n* in this cohort provides limited statistical power. 1,5 AG's association with known complications related to PH, superior to HbA_{1c}, support the notion of its utility in all types of diabetes during pregnancy and across trimesters. 1,5 AG can help predict risk of LGA early in pregnancy and alert aggressive glycemic interventions to reduce its occurrence.

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Pregnancy outcomes in diabetic type 1 patients of an academic centre combining endocrine and obstetric care from before conception to delivery; still not good enough

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Background and aims: Numerous studies in the last decades have shown that pregnancies in women with type 1 diabetes remained associated with an increased incidence of adverse outcomes, such as congenital malformations, perinatal mortality and fetal macrosomia. Not only optimal glycaemic control, from preconception period until and including delivery, is of paramount importance but also careful planning of pregnancy, preconceptional counselling and adequate timed obstetrical interventions at the end of pregnancy are very important issues. Therefore, close collaboration between the endocrinologist and the obstetrician is of prime importance in the management of the diabetic pregnancy. We report the pregnancy outcomes during the past 18 years of working with a structured clinical collaborative scheme extending from the preconceptional to the post-delivery period in an attempt to optimise treatment with special emphasis on the patients who were treated from preconceptional period until and including delivery.
Material and methods: This is an observational, cohort study. All women with type 1 diabetes mellitus with a singleton pregnancy progressing beyond 16 weeks of gestational age who delivered at our center from January 1993 until January 1, 2011 were included. Baseline characteristics: maternal data and diabetes-related parameters. Outcome measurements: HbA1c**,** perinatal mortality (PNM), congenital malformations, Large for Gestational Age(LGA) infants ,preeclampsia, preterm delivery, low Apgar score, obstetric interventions.
Results: 243 pregnancies in 156 women fulfilled inclusion criteria. Pregnancy outcomes were still unfavourable with 9.2% of congenital malformations and 40.9% of LGA children. Overall PNM was 2.1%, but only 0.6% in women

treated from before conception till delivery. Median HbA1c before pregnancy in the latter group was 7.0% and 1st, 2nd, 3rd trimester values were 6.8, 6.1 and 6.3, respectively. Preterm delivery in this group occurred in one-third of the pregnancies, almost exclusively between 35 and 37 weeks. More than half of the pregnancies in group 1 ended in a Caesarean section (56.9%).
Conclusion: Despite our multidisciplinary efforts in a combined and specialised outpatient clinic the aims of the St Vincent declaration were still not met. Although perinatal mortality was reduced, at the cost of high intervention rates, the incidence of malformations and LGA infants remained unchanged. Apparently at this moment we are not able to meet the specific demands of a diabetic pregnancy. Further improvements may prove difficult. A radical rethinking is maybe needed with more emphasis on the periconceptive period as well as innovative steps to meet social as well as medical social requirements. We must pose the important question how much patients themselves as well as the general society are willing to invest in overcoming the final gap to the goal of the St Vincent declaration.

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Obesity and gestational weight gain both contribute to the risk of large for gestational age babies in women without gestational diabetes mellitus
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Background and aims: The aim was to retrospectively evaluate, in a highly-selected cohort of pregnant women universally screened and treated for gestational diabetes mellitus (GDM), the association between prepregnancy overweight (OW) or obesity (OB) and pregnancy complications.
Materials and methods: We selected 15.551 women without prepregnancy diabetes or hypertensive disorders, neither from Asia India Pakistan Sri Lanka origins nor underweighted, who delivered between 2002 and 2010 of singleton babies. Women were classified according to BMI (normal (N: BMI 18.5-24.9) n=9.317; OW n=4.075; OB (BMI≥30 kg/m^2) n=2.159), GDM and gestational weight gain (GWG <7: 32%; 7-11.5: 37%; 11.6-16: 23%, >16kg: 8%).
Results: GDM (12.8, 14.4, 14.9%, p<0.01), GWG (9.1±5.6, 8.8±5.6, 8.5±5.8 kg, p<0.001), small for gestational age babies (14.3, 12.7, 12.3%, p<0.01), preeclampsia (2.0, 2.2, 2.9%, p<0.05) but neither large for gestational age babies (LGA: 8.4, 8.9, 9.4%, p=0.27) nor caesarian section (20.9, 21.2, 21.2%, p=0.97) were associated with N, OW and OB status, respectively. The higher the BMI (N, OW, OB) and GWG classes, the more frequent LGA in the women with GDM (overall effect p<0.001, BMI effect NS, GWG effect NS, interaction NS) or without GDM (overall p<0.001, BMI effect p<0.05, GWG effect p<0.05, interaction p=0.051). For example, in women without GDM, the prevalence of LGA babies was 5.5% in women with normal BMI and GWG <7 kg, and 20.3% in those with OB and GWG >16 kg. The respective prevalences in the women with GDM were 14.3 and 29.2%.
Conclusion: OW/OB is associated with more GDM and preeclampsia. The risk of LGA babies associated with OW/OB is amplified by GWG particularly in women without GDM. This suggests that dietary advice should be given to OW/OB women to control GWG.

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Outcome of pregnancies in women with type 1 diabetes
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Introduction and aims: The outcome of pregnancies in women with type 1 diabetes has improved significantly during the last decades. The previous high frequencies of miscarriages, malformations, premature births, and other complications have declined due to better glycaemia control. In this study we have compared outcome of pregnancies among type 1 diabetic women in our Hospital to the outcome for all pregnancies in the general population in Sweden. Our model for taking care of pregnant women with type 1 includes the following: optimising glucose control prior to pregnancy, a specialised unit for gestational care including obstetrician, diabetologist, midwife and dietician, intensified self monitored glucose measurements with daily seven point curve of p-glucose, weekly phone communication with diabetologist to adjust insulin dose after sending glucose values by Diasend, possibility to direct contact with diabetologist 24 h a day for both pregnant women, midwifes and obstetricians, extra ultrasound late in pregnancy to estimate growth of foetus, liberality to start labour if rapid growth and continuous contact with diabetologist during labour.
Materials and methods: During the years 2000-2010, the pregnancies of 202 consecutive women with type 1 diabetes have been studied. A team of obstetrician, diabetologist, midwife and dietician took care of these women at a specialist unit. The goal was to normalise the glucose levels by tight control with frequent self monitored glucose measurements and weekly contact with diabetologist to adjust the insulin dose.
Results: These women had have diabetes for 15.5±8.0 yrs, their initial daily dose of insulin increased from 50.0±17.5 E to 86.3±35.4 E, weight increased from 70.1±10.3 kg to 85.0±11.1 kg. HbA_{1c} decreased from 55.2±10.5 to 39.2±4.7 mmol/mol at the end of the pregnancy. The mean weight of the newborns was 3566±721 g. The frequency of preeclampsia was 25/201 (12.3%), instrumental delivery 26/200 (12.7%) and prematurity 40/201 (19.6%). The frequencies of newborns having Apgar ≥7 after 5 min was 98%, neonatal hypoglycaemia 44/200 (22%), any type of malformation 16/202 (7.9%), and neonatal death was 3/202 (1.5%).
Conclusion: The outcome of pregnancy in women with type 1 diabetes is comparable with normal pregnancies due to an extreme good metabolic control, which requires a multidisciplinary team and high compliance of the patients.

Deliveries - mother with T1D compared to background population

	Deliveries in women with Type 1 in Lund N=203	Reference population Sweden N=1102729	P-value
Gestational length (weeks)	37.7±2.1	39.3±2	<0.001
Birth weight (g)	3566,19±720,81	3507±591	<0.001
Frequency of LGA ≥4500 g (%)	6.9	3.6	<0.001
Apgar<7 at 5 min (%)	2.0	1,3	<0.001
Sectio (%)	44	17	<0.001
Hypoglycaemia in infant (%)	22	NK	NA
Intensive care unit for infant (%)	43,8	NK	NA
Stillbirth (%)	1,5	0.35	<0.001

Supported by: Region Skåne Foundation

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Maternal high fat diet alters weight regulation and blood glucose prior to mating but not during gestation, and reduces offspring weight at weaning
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Background and aims: We have previously reported elevated abdominal adiposity together with reduced hepatic insulin signalling proteins in 15 week old offspring of dams fed a high fat (HF) diet during gestation. We have also reported that physical activity during pregnancy reduces birth weight of offspring born to HF mothers. What is currently unknown is the specific maternal characteristics that may be driving disturbed metabolic development in the offspring and if these disturbances are present in offspring from birth or develop in the post-weaning environment. These are important questions to answer in aid of informing targeted interventions at the developmental origins of disease. We therefore aimed to characterise our high fat fed maternal model in both sedentary and exercise trained mothers as well as investigating offspring outcomes at weaning.
Materials and methods: 24 females (150g) were fed either a HF diet (34% omega-6 PUFA, 26% Protein, 22.3 kJ/g n = 12) or a standard chow (C) diet (11% fat, 23% Protein, 13.9 kJ/g n = 12) for 28 days. On day 24 fasting blood was collected and analysed for glucose, insulin and leptin. On day 28, mating pairs were established. Following conception, six females from each dietary group were randomly allocated to a training intervention. Training consisted of 30min of treadmill running at a moderate intensity for 10 days of the 16 between mating and spontaneous delivery. A fasting blood sample was collected from all females on the 19th day of gestation. Following delivery, all pups were weighed and litter sizes standardised to 8 (4m, 4f) per dam. During lactation all mothers were fed standard laboratory chow. Body weight was recorded throughout lactation and a fasting blood sample was collected from offspring and mothers at weaning (day 25 post-partum).

Results: Maternal body weight prior to mating was similar between all groups as was plasma insulin and leptin. However, maternal blood glucose was significantly elevated in HF mothers prior to mating ($p = 0.002$). During pregnancy, blood glucose normalised between groups. Plasma insulin was similar between all groups, however insulin was significantly elevated compared to pre-mating values ($p < 0.004$) for all groups. While exercise training did not significantly affect weight gain during pregnancy, HF did result in lower weight gain in the HF sedentary group compared to sedentary controls ($p = 0.02$). Offspring of HF mothers displayed lower body weight ($p = 0.03$) compared to controls throughout lactation. No significant effect of maternal diet or activity was observed for offspring glucose, insulin or leptin. Interestingly, fasting induced weight loss was greatest in C mothers prior to mating and during gestation.
Conclusion: HF feeding resulted in maternal hyperglycaemia prior to mating and disturbances in weight regulation during fasting. Metabolic outcomes were normalised however during gestation. Furthermore, offspring of HF mothers were smaller than controls despite comparable metabolic outcomes. Our data suggest that the developmental origins of disease in our model are more likely related to an interaction between maternal diets *per se* and offspring post-weaning environment.
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Excretion of amino nitrogen and ammonia with the urine in patients with type 1 diabetes, according to the gestational age and the severity of nephropathy

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Background and aims: To study excretion of amino nitrogen and ammonia with a daily urine for the early detection of tubular dysfunction in women with diabetes mellitus type 1, depending on gestational age and stage of diabetic nephropathy.
Materials and methods: We monitored 100 pregnant women with type 1 diabetes: 58 women - in the first trimester up to 12 weeks, 58 pregnant women - in the 2nd trimester, 22-24 weeks, 53 patients - in the 3rd trimester, 32-34 weeks. Age of women was from 19 to 37 years [26 (23, 29) years], diabetes duration of 1 month to 20 years [9 (2.75, 12.5) years]. Stage of diabetic nephropathy was defined by the level of albumin in the urine. The function of the proximal tubules was assessed by daily excretion of amino nitrogen (nitrogen of amino acids that are in the test substrate), of the distal tubules - the ability to maintain the acid-base status - daily ammonia excretion (indicator of reabsorbed amino acids metabolism). The control group consisted of 18 healthy women in different trimesters of pregnancy at the age of 20 to 32 years, an average of 27 (23, 32) years. Statistical analysis was performed with nonparametric methods of statistics.
Results: The study showed an increase of excretion of ammonia and amino nitrogen in the urine of pregnant women with type 1 diabetes in all trimesters of pregnancy compared to healthy pregnant women. In the study of excretion of ammonia and amino nitrogen with the daily urine in patients with normoalbuminuria showed its increase in 2 and 3 trimesters of pregnancy, which is more pronounced in the 2nd trimester. In patients with microalbuminuria showed a reduction in excretion of ammonia and amino nitrogen with increasing of gestational age. In patients with macroproteinuria we found increased excretion of ammonia and amino nitrogen in the 2 trimester with a reduction of these values in 3rd trimester which are even lower than in 1 trimester. Regardless of the trimester, with the progression of nephropathy showed a reduction in ammonia excretion in the urine and increased excretion of amino nitrogen. We revealed high correlation between ammonia and amino nitrogen during all trimesters of pregnancy in all groups of patients, with complete direct link in the group of macroproteinuria in 1 trimester (r=1) and the complete reverse link of the same group in the 3rd trimester of pregnancy (r=-1). A strong correlation was also found between the level of ammonia and glomerular filtration rate in the group of microalbuminuria in the 3rd trimester of pregnancy (r=0,9), and in all three trimesters in macroalbuminuria group (r=1, r=0,77 and r=1, trimesters, respectively). A strong correlation was also observed between the excretion of ammonia and ethanolamine in the urine in the normoalbuminuria group in 1 trimester (r=0,75), microalbuminuria group 1 (r=0,75) and 2 (r=0,75) trimesters of pregnancy, macroproteinuric group 1 (r=-1), 2 (r=0,77) and 3 (r=1) trimesters.
Conclusion: In patients with type 1 diabetes with increasing of gestational changes ammonia and urinary amino nitrogen excretion with a daily urine indicate involvement of the both distal and proximal tubules. Differences in rates depending on the stage of nephropathy and pregnancy can point to different compensatory potentials of kidneys.

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Endothelial dysfunction induced by dipeptidyl peptidase-4 in isolated mice mesenteric microvessels

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Background and aims: Cardiovascular diseases are the main cause of morbidity and mortality in obese and diabetic patients. Dipeptidyl peptidase-4 (DPP-4) is a multifunctional glycoprotein playing a major role in the regulation of glucose homeostasis. At present, DPP-4 inhibitors are used to prolong the insulinotrophic effects of incretins. Recently, DPP-4 has been identified as a novel adipokine over-secreted in obesity where it promotes insulin resistance and may represent a link between adipose tissue and the metabolic syndrome (Lamers et al. Diabetes 60: 1917-25, 2011). The aim of this study was to analyse the ability of DPP-4 to directly impact on endothelial function with special focus on endothelium-derived relaxation.
Material and methods: Mesenteric microvascular segments isolated from 3 month-old female B57BL/6 mice were mounted on a small vessel wire myograph to determine vascular reactivity.
Results: In the mice mesenteric microvessels pre-incubated with DPP-4 (20 to 500 ng/mL), the contractility to noradrenaline (1 nmol/L to 1 μmol/L) was not altered. However, DPP-4 impaired the endothelium-dependent relaxation to acetylcholine (1 nmol/L to 1 μmol/L) in a concentration-dependent manner by a 75%, without modifying endothelium-independent relaxations to sodium nitroprusside (1 nmol/L to 10 μmol/L). Moreover, the co-incubation of DPP-4 (200 ng/mL) with its enzymatic inhibitor K579 (100 nmol/L) prevented the impaired endothelium-dependent relaxation by DPP-4. Similarly, the cyclooxygenase inhibitor indomethacin (10 μmol/L) and the thromboxane A2 receptor antagonist SQ29548 (10 μmol/L) did abrogate the impairing action of DPP-4. The NADPH-oxidase inhibitor apocynin (10 μmol/L) did not restore the relaxation impaired by DPP-4.
Conclusion: DPP-4 directly impairs endothelium-dependent relaxation, through a mechanism that involves cyclooxygenase activation and likely the release of a vasoconstrictor prostanoid. Over-production of DPP-4 in obesity and diabetes might therefore contribute to endothelial dysfunction associated with both metabolic diseases.
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Hyperglycaemia-induced resistance to GLP-1 in the endothelium: the possible involvement of NRF2 and PKC pathway

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Background and aims: Hyperglycaemia induces endothelial dysfunction, which contributes to the development of cardiovascular disease (CVD) in diabetes. Activation of transcription nuclear factor (erythroid-derived 2)-like 2 (NRF2) is associated with its translocation into the nucleus stimulating the expression of a great number of genes responsible for the synthesis of antioxidant proteins, including heme oxygenase-1 (HMOX-1) and NAD(P)H dehydrogenase quinone-1 (NQO-1). Recently, GPL-1 has been proposed as an antioxidant agent, because it increases intracellular antioxidant gene expression. Furthermore, we have recently reported, in vivo, that hyperglycaemia induces endothelial resistance to the action of GLP-1, in terms of reducing GLP-1 effects on endothelial function, inflammation and oxidative stress. It has been also reported that PKCβ overexpression, induced by hyperglycaemia, is able to reduce GLP-1 receptor expression. The aim of this study is to asses if hyperglycaemia induced PKCβ activation is involved in the appearance of GLP-1 resistance and test if other molecular mechanisms are involved.
Materials and methods: HuVEC cells were cultured for 21 days in normal (5mmol/L, NG) or high glucose (25mmol/L, HG), and treated with GPL-1 at 50nmol/L for 1h before being harvested. Differences on gene and protein expression of the antioxidant enzymes HMOX-1 and NQO-1, of the ER stress/UPR genes (CHOP and PERK) and of the proliferation/apoptosis-related genes (BCL-2 and CDKN1A) were evaluated by quantitative real time PCR and immunobloting. In parallel, GLP-1 receptor and PKCβ protein levels were also tested, as well as NRF2 cytoplasm-nucleus translocation.
Results: High glucose, as well as GLP-1 added in normoglycaemia, induced the expression of antioxidant genes HMOX-1 and NQO-1, (NG vs HG or vs NG+GLP-1, HMOX-1 $p<0.01$ and $p<0.05$; NQO-1 $p<0.05$ and $p<0.05$, respectively). GLP-1 induces NRF2 translocation into the nucleus in normoglycaemia, allowing antioxidant response, but this was not happening when GLP-1 was added after sustained high glucose exposure. The same effect was observed with UPR markers CHOP and PERK (NG vs HG or vs NG+GLP-1, CHOP $p<0.05$ and $p<0.05$; PERK $p<0.01$ and $p<0.05$, respectively). Moreover, proliferation related genes, CDKN1A and BCL-2 were induced and repressed by hyperglycaemia, respectively (NG vs HG $p<0.05$ CDKN1A and $p<0.01$ BCL-2), as well as GLP-1 increases its expression in normoglycaemia in both cases (NG vs NG+GLP-1, $p<0.01$ and $p<0.05$, CDKN1A and BCL-2 respectively). Interestingly, a complete loss of GLP-1 capacity was observed after HuVECs were exposed to high glucose (NG vs HG p=NS in all cases). All these effects were accompanied by a reduction of GLP-1 receptor protein levels (NG vs HG $p<0.05$) and by an increase in PKC isoform βI expression (NG vs HG $p<0.05$).
Conclusion: These findings suggest that endothelial cells, after the exposure to high glucose, lose their ability to normally respond to the GLP-1 action, in terms of activating their antioxidant capacity, in a possible NRF2-responsive dependent manner. HuVECs also lose GLP-1 capacity in regulating ER function and UPR response to GLP-1 under hyperglycaemia. This resistance to GLP-1 during chronic high glucose exposure could be due to an increase in PKCβ activity that reduces GLP-1 receptor pathway.
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1301

The phosphodiesterase-5 inhibitor tadalafil decreases ET-1 expression in HUVECs and suppresses circulating ET-1 levels type 2 diabetes mellitus patients

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Background and aims: Impaired insulin action reduces synthesis of nitric oxide (NO) and elevates expression of endothelin-1 (ET-1) in vascular endothelial cells, i.e. promotes endothelial dysfunction and vascular inflammation. Tadalafil is a selective phosphodiesterase-5 (PDE-5) inhibitor that enhances NO signaling pathway by augmenting formation of cyclic guanosine monophosphate (cGMP) through reduction of its hydrolysis by cGMP-degrading PDEs. Thus, PDE-5 inhibitors may induce anti-inflammatory effects through an enhanced NO signaling in endothelial cells. However, it is unknown whether acute administration of tadalafil may decrease circulating ET-1 levels in type 2 diabetes (T2DM). Therefore, the aim of this study was to evaluate whether tadalafil attenuates ET-1 expression in HUVECs in vitro and if tadalafil 20 mg compared with placebo decreases ET-1 levels after a mixed meal in T2DM patients.
Materials and methods: TNF-α was used to mimic inflammatory activation in HUVECs. Cells were treated with 1 μM tadalafil and after 12 hours medium from cell culture was collected and RNA was extracted and analysed with quantitative PCR. Twenty-six T2DM patients who met the following criteria: (1) Age 40-70 (men) and 50-70 years (postmenopausal women); (2) BMI 27-40 kg/m2; (3) HbA1c <60 mmol/mol, were recruited to a study with parallel groups, and after a double blind randomisation they received either 20 mg tadalafil or placebo 30 min prior to a mixed meal consisting of 74 g carbohydrates, 25 g protein and 42 g fat. Arterial blood sampling was conducted at baseline, three and five hours after intake of the meal. Differences in vitro were analysed using paired t-test, while in vivo we used Mann-Whitney U-test, p-value < 0.05 was considered significant.
Results: After TNF-α stimuli tadalafil was able to significantly attenuate mRNA expression of ET-1 by 48 % in HUVECs compared to cells stimulated by TNF-α alone. Similarly, when measuring ET-1 in conditioned medium, ET-1 levels were decreased by 17 %, a moderate but significant reduction. Three hours after a mixed meal circulating ET-1 levels were significantly decreased in patients treated with tadalafil compared with placebo treated patients (Fig 1, $p<0.05$). No significant differences appeared in Gender (F/M): 5/9 vs 4/8; Age (years): 60±2.3 vs 62±1.7 BMI (kg/m2): 29.8±1.0 vs 30.9±1.2; HbA1c (mmol/mol): 45±1.8 vs 45±2.3; Glucose AUC300: 2465 vs 2525; Insulin AUC300: 9158 vs 10689, between the tadalafil- and placebo treated groups, respectively.

Conclusion: In summary, our data show that tadalafil decreases ET-1 expression in human vascular endothelial cells in vitro and suppresses circulating ET-1 levels in vivo after a mixed meal. We propose that tadalafil may act as a potent drug against endothelial dysfunction induced by ET-1 in T2DM patients.

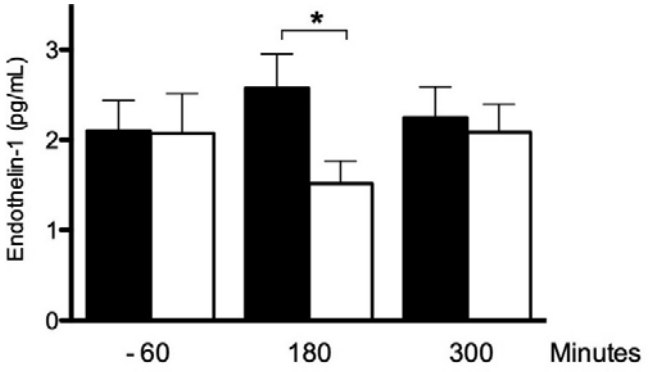

Fig 1. Arterial concentrations of Endothelin-1 before and after a mixed meal in T2DM patients randomised to tadalafil 20 mg or placebo. Black bars placebo n = 12; white bars tadalafil n = 14. *, $p < 0.05$, data presented as mean ± SEM.

Clinical Trial Registration Number: NCT01238224
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1302

The new role of PDK4 in vascular calcification

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Background and aims: Vascular calcification is a well-established risk factor of cardiovascular events associated with aging, diabetes, renal failure, vitamin D intoxication, and hyperphosphatemia. Osteogenic switch of vascular smooth muscle cells (VSMCs) triggered by procalcific stimuli such as ROS and excess inorganic phosphous (Pi) was recognized as a key step in mechanism of vascular calcification. We observed that pyruvate dehydrogenase kinase 4 (PDK4), a mitochondrial protein to regulate of glucose oxidation, increased its expression in human VSMCs cultured mineralization medium. We assessed whether inhibition of PDK4 could decrease vascular calcification and further investigated the molecular mechanism in details.
Materials and methods: We evaluated the mineralization induced by Pi in vitro using von Kossa staining and quantitative analysis of calcium in VSMCs and isolated aorta. Vitamin D was injected to PDK4 deficient mice for inducing vascular calcification in vivo.
Results: PDK4 overexpression by using PDK4 containing adenoviral vector, even without BMP2 pre-treatment, increased vascular calcification. PDK4 deficient mice showed profound attenuation of aortic calcification induced by Vitamin D in vivo. The VSMCs cultured from PDK4 deficient mice also presented a decrease of mineralization and mRNA expression of osteogenic markers. Furthermore, DCA, known PDK inhibitor, attenuated the mineralization of VMSCs and aorta cultured in Pi-treated medium. We found that PDK4 directly bound R-smad and further enhanced phosphorylation of R-Smad confirmed by GST-pull down assay and in vitro kinase assay. In consistent with these results, vascular calcification induced by Vitamin D in vivo was also decreased in DCA-treated mice by gavage (20mg/kg/day for 3weeks).
Conclusion: In this study, we showed that PDK4 augmented osteogenic switch of VSMCs and vascular calcification through the direct phosphorylation of R-Smad. This study suggests that PDK4 might be a novel target to inhibit vascular calcification.

1303

FoxO1 compartmentalisation results in different metabolomic profiles in human endothelial cells

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Background and aims: FoxO1 is a pivotal element in the regulation of endothelial activation. Compartmentalization and activity of FoxO1 is regulated by post translational modifications, but the implication in endothelial dysfunction and atherosclerosis remain controversial. Our aim was to identify FoxO1 post translational modifications and related metabolic signatures associated to improved endothelial function.
Materials and methods: RT-PCR and western blot were performed on mRNA and protein extracted from human atherosclerotic plaques (n=70). Human umbilical endothelial cells (HUVECs) were infected with adenoviral vector expressing the wild type FoxO1 (WT), the acetylation defective mutant (KR), the unphosphorylated nuclear localized mutant (ADA) and the GFP control vector. Cell lysates and supernatants as well as mRNA and proteins were collected for GC/MS and LC/MS/MS metabolomics, RT-PCR and western blot.
Results: Preliminary data in human atherosclerotic plaques indicated that the acetylated form of FoxO1 is associated with increased markers of inflammation and cellular activation (VCAM-1, ICAM-1, TLR4, MMP9), whereas the phosphorylated form of FoxO1 acts inversely. In HUVECs overexpressing different FoxO1 mutants we confirmed the upregulation of VCAM-1 and ICAM-1 in ADA cells. Next, in HUVECs we analyzed metabolite markers associated to distinct FoxO1 activated proteins. In ADA cells, consistently with reduced NO production, we identified increased levels of dymethilarginine (SDMA+ADMA), the endogenous inhibitors of endothelial nitric oxide synthase (eNOS). ADA expression is also associated to higher levels of lysophosphatidic acid (LPA), suggesting that constitutive dephosphorylation of FOXO1 may lead to activation of angiogenic, inflammation or cell death programs in endothelial cells. The levels of 7-alpha and 7-beta-hydroxycholesterol, lipid peroxidation products from cholesterol increased in in response to all three alleles of FOXO1 over-expression compared to the GFP control which would be consistent with FoxO1 dependent increased lipid peroxidation Next, we analyzed by RT-PCR, the three major enzymes involved in ADMA generation, protein arginine methyltransferase 1 and 2 (PRMT1, PRMT2) and dimethylarginine dimethylaminohydrolase (DDAH1), founding that DDAH1 was significanlty downregulated in ADA cells. Moreover ADA expression resulted in an inhibition of Srebp2 mRNA that, in turn, is involved in the regulation of DDAH1 expression, suggesting a mechanism linking FoxO1 and Srebp2.
Conclusion: Our data suggest that FoxO1 affects endothelial cell function through several metabolic pathways potentially implied in endothelial activation and dysfunction, two processes that are associated to increased incidence of atherosclerosis.

1304

Monoamine oxidase activity promotes type 1 diabetes-induced mitochondrial dysfunction and cardiac damage

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Background and aims: Oxidative stress is well known to contribute to the development of diabetic cardiomyopathy. Mitochondrial respiratory chain and NADPH oxidase are described as major sources of reactive oxygen species (ROS) in this setting. However, this accepted concept overlooks the potential contribution of other ROS sources. Monoamine oxidases (MAOs) are mitochondrial enzymes that catabolize neurotransmitters such as norepinephrine, generating hydrogen peroxide in this process. These mitochondrial flavoenzymes are responsible for oxidative stress and cell death in failing and ischemic/reperfused hearts. Here we assessed whether MAO contributes to the onset and progression of cardiac alterations occurring in type 1 diabetes (T1D).
Materials and methods: Mitochondrial ROS production (Mitotracker Red CM-H_2XRos) and membrane potential (TMRM) were measured in neonatal

rat cardiomyocytes cultured in normal glucose (NG, 5 mM), high glucose (HG, 30 mM) or high mannitol (HM, 30 mM) containing media in the absence or presence of MAO inhibitor pargyline. T1D was induced by single streptozotocin injection (STZ, 150 mg/kg) and mice were randomized into vehicle- or pargyline-treated groups. After 4 weeks, cardiac tissue was analyzed for oxidative stress (malondialdehyde levels), protein oxidation and apoptosis (TUNEL assay).
Results: Mitochondrial ROS production was significantly higher in HG myocytes (+30%, p<0.005) as compared to other groups. Pargyline completely prevented this increase. When myocytes were co-incubated with ATP synthase inhibitor oligomycin, HG-treated cells started depolarizing immediately whereas NG- or HM-treated cells were able to maintain their membrane potential longer (>50 minutes). Pargyline prevented this suggesting that MAO activation triggered by HG induces a latent mitochondrial dysfunction causing mitochondria to hydrolyze ATP. In in vivo model of T1D, hearts of STZ-treated mice displayed high oxidative stress (+70%, p<0.05) accompanied by oxidation of contractile proteins such as tropomyosin (p<0.005) and a 3-fold increase in apoptosis (p<0.05). Pargyline administration to STZ-treated mice completely prevented these changes, suggesting that MAO activation provides a major contribution to cardiac dysfunction in diabetes.
Conclusion: These data provide the novel evidence of a pivotal role of MAO in diabetes-induced oxidative stress and mitochondrial abnormalities that are responsible for contractile derangements and loss of cardiomyocyte viability. Based upon the present findings MAO inhibition should be exploited as a novel therapeutic approach to counteract diabetic cardiomyopathy.

1305

Repetitive selective breeding for susceptibility to glucose intolerance accelerates atherosclerotic lesion formation in mice
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Background and aims: Epidemiological evidence demonstrates that postprandial hyperglycemia in people with impaired glucose tolerance (IGT) is an independent risk factor for atherosclerotic cardiovascular disease. However, there are few suitable animal models for understanding the involvement of IGT in the pathogenesis of atherosclerosis in vivo. We recently established two lines of mice with different susceptibilities (prone and resistant) to high fat diet (HFD)-induced glucose intolerance by selective breeding (designated SDG-P and SDG-R, respectively). Here, we examined intergenerational changes in atherosclerotic lesion formation in SDG-P and SDG-R during the selective breeding for different glucose tolerance.
Materials and methods: The selective breeding of SDG-P and SDG-R was performed with C57BL/6J, C3H/HeJ and AKR/N mice as background strains. After 5-week HFD feeding (32% energy as fat; 5-10 weeks of age), mice showing high and low 120-min blood glucose levels ($BG_{120\ min}$) in OGTT (2.0 g/kg body weight) were selected and bred repetitively over 20 generations to produce SDG-P and SDG-R, respectively. During the selective breeding, we evaluated atherosclerotic lesion formation in female SDG-P and SDG-R. After 20-week atherogenic diet feeding (1.25% w/w cholesterol, 0.5% w/w sodium cholate, 36% energy as fat; 8-28 weeks of age), serial frozen sections (10 μm thickness, spanned 450 μm) of aortic sinus were prepared and the lesion size was quantitated from oil red O-stained area (mean of 9 sections, each separated by 50 μm). Data are expressed as mean±SEM. Intergenerational changes in glucose tolerance and atherosclerotic lesion size were examined by Pearson's correlation analysis. Differences between SDG-P and SDG-R were assessed by Student's *t* test.
Results: As the breeding generations proceeded, female SDG-P mice became more susceptible to HFD-induced glucose intolerance (p=0.0004; $BG_{120\ min}$ during 5th-20th generations (G5-G20)); meanwhile, female SDG-R mice maintained normal glucose tolerance during the generations. At G20, $BG_{120\ min}$ in SDG-P was more than 2-fold higher than that in SDG-R (9.6±0.5 vs 4.3±0.2 mmol/l, n=15-17, p<0.0001). Male SDG-P and SDG-R showed similar intergenerational trends in glucose tolerance. After the atherogenic diet feeding in female mice, atherosclerotic lesion size of SDG-P was greater than that of SDG-R in all paired experiments (p<0.05; 4 paired experiments during G12-G19). At G18-G19, the lesion size in SDG-P was 4-fold greater than that in SDG-R (37.6±5.5 vs 9.4±3.0 ×10^3 μm^2, n=4-6, p=0.0020). In addition, the differences in lesion size tended to increase as the generations proceeded (p=0.0519).
Conclusion: Glucose intolerance-prone mice (SDG-P) are more susceptible to atherosclerotic lesion formation relative to glucose intolerance-resistant mice (SDG-R). Furthermore, as the breeding generations proceeded, the lesion formation in SDG-P was accelerated concomitantly with worsening glucose intolerance. Thus, the differences in atherosclerotic lesion formation may reflect different glucose tolerance status. The newly established two lines of mice with different glucose tolerance may therefore serve as suitable animal models for investigating pathogenesis, prevention, and treatment of atherosclerotic complications in IGT.
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1306

Histone methyltransferase EZH2 regulates glucose induced VEGF production through H3K27 methylation in retinal endothelial cells
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Background and aims: Glucose induced augmented vascular endothelial growth factor (VEGF) production is a key event in diabetic retinopathy. We have previously demonstrated that downregulation of miR-200b causes overexpression of VEGF, mediating structural and functional changes in the retina in diabetes. However, regulation of miR-200b is not known. Histone methyltransferase, enhancer of the zeste homolog (EZH2), has been demonstrated to repress miRNAs in neoplastic process. We hypothesized that, in diabetes, EZH2 represses miR-200b through its H3K27 methylation mark.
Materials and methods: Human retinal microvascular endothelial cells and endothelial cells of dermal origin, isolated from both type 1 and type 2 diabetic and non-diabetic individuals, were treated in high glucose (25mM) or normal glucose (5mM) for 24 hours. Expression of EZH2, VEGF and various miRNA were measured by qPCR. Loss-of-function experiments were also performed using a chemical inhibitor for EZH2, 3-Deazaneplanocin A (DZNep).
Results: When treated with high glucose, all cell types showed significantly increased VEGF expression (p<0.05). Retinal endothelial cells showed increased expression of EZH2 with decreased expression of miR-200b. Dermal endothelial cells isolated from diabetic patients showed increased EZH2 and decreased miR-200b expression as well. Furthermore, inhibition of EZH2 in retinal endothelial cells produced increased miR-200b expression with parallel decreased VEGF, demonstrating a causal link.
Conclusion: This research has demonstrated a repressive relationship between EZH2 and miR-200b. These data further provide evidence of a novel mechanism of miRNA regulation through another epigenetic pathway, i.e, histone methylation. Understanding such pathways will potentially yield new treatment strategies.
Supported by: CDA, CIHR

1307

Impaired glucose tolerance in rats is associated with a reduced expression of hepatocyte growth factor
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Background and aims: Hepatocyte growth factor (HGF) contributes to organ protection and repair and its alterations were suggested to play a role in the development of diabetic end-organ damage. However, little is known about its function in the state of impaired glucose tolerance.
Materials and methods: In male Wistar rats, impaired glucose tolerance was induced by administration of low doses of streptozotocin (25mg/kg/d i.p.) for 3 consecutive days (STZ). Controls (CON) received vehicle. After 8 weeks, oral glucose tolerance test (OGTT) was performed. Additionally, we tested endothelial function of isolated aorta by relaxation to acetylcholine. Expressions of HGF were analyzed in aortas and kidneys by qRT-PCR. In addition, endothelial NO-synthase (eNOS) as a key factor regulating vascular relaxation was analyzed in aortas by Western blotting.
Results: Both groups of rats showed a normal fasting glucose (CON: 6.2 ± 0.3 mmol/l vs. STZ: 6.2 ± 0.9 mmol/l) but STZ rats exhibited significantly elevated plasma glucose concentration in OGTT (Glc levels after 1 h - CON: 8.1 ± 0.4 mmol/l vs. STZ: 15.2 ± 2.2 mmol/l, p<0.01; total AUC - CON: 1835 ± 55 vs. STZ: 3079 ± 415, p<0.01). Additionally, STZ rats showed a blunted endothelium-dependent relaxation to acetylcholine (pD2 - CON: 6.71 ± 0.16

vs. STZ: 6.27 ± 0.08, p<0.001; Emax CON: 37.1±3.1 % vs. STZ: 23.6±1.4 %, P<0.01). Endothelium-independent relaxation to sodium nitroprusside was not altered. Accordingly, we observed a significant downregulation of eNOS protein in STZ aortas (CON: 1.00 ± 0.13 vs. STZ: 0.39 ± 0.07, p<0.001). STZ rats had a significantly lower expression of aortic HGF mRNA in comparison to controls (CON: 1.00 ± 0.10 vs. STZ: 0.71 ± 0.07, p<0.05) and a trend of lowered HGF expression in kidney (CON: 1.00 ± 0.28 vs. STZ: 0.77 ± 0.19, NS).
Conclusion: In summary, we observed a significant decrease of HGF expression in aortas and a tendency to decrease in kidneys of rats with glucose intolerance. Our data suggest that HGF downregulation could contribute to the early development of renal injury and endothelial dysfunction in impaired glucose tolerance.
Supported by: EFSD New Horizons

PS 114 Sleep apnoea, cognitive function and musculoskeletal complications

1308

Obstructive sleep apnoea is independently associated with arterial stiffness and in-hospital early clinical outcome after first-ever ischaemic stroke in type 2 diabetes

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Background and aims: Obstructive sleep apnaea (OSA) syndrome significantly increases the risk of stroke independently of other risk factors. Pulse wave velocity analysis (PWV), a marker of aortic stiffness, has independent predictive value for cardiovascular morbidity and mortality, especially fatal stroke, and for long-term functional stroke prognosis. We determined the early (7 days) prognostic significance of OSA in relation to arterial stiffness in patients with type 2 diabetes (T2DM) and functional disability after acute first-ever ischemic stroke.
Materials and methods: In a 90 days after the stroke, prospective study, we enrolled 270 patients with T2DM and acute first-ever ischemic stroke (122 women/148 men), aged 58.0 ± 11.2 years, mean ± SD. National Institutes of Health stroke scale (NIHSS) scored 7.1 ± 5.5 at admission. Patients enrolled were defined a priori as having an apnoea-hypopnea index ≥5 (≥five events per hour of sleep) during 2-hours of attended sleep monitoring. Patients with an apnoea-hypopnea index ≤5 constituted the comparison group. Carotid-femoral (CF) PWV and central augmentation index (cAIx) were measured by applanation tonometry (SphygmoCor, Atcor, Australia) 7 days after stroke onset. At hospital discharge, favourable outcome was defined as a ≥4-point improvement from baseline NIHSS or NIHSS of 0-1. Data were analyzed with logistic regression.
Results: The mean apnoea-hypopnea index among the OSA patients was 35±29 compared to 3.0±1.5 in the comparison group. Baseline characteristics of patients included in the two groups were similar. CF-PWV and cAlx were significantly different between patients with and without OSA (11.6±3.1m/s vs. 9.5±1.4m/s, and 34.6±21.6% vs. 12.4±15.6%, respectively, p<0.001). In multivariate analysis, after adjustment for age, gender, body-mass index, NIHSS score, blood glucose, lipids on admission, as well as heart-rate, systolic and mean blood pressure, high sensitivity C-reactive protein and homocysteine values measured at day 7±2, both low CF-PWV (p<0.001), and cAIx (p=0.002), were significantly associated with early favourable outcome. Moreover, a 40% decrease of both CF-PWV and cAlx values on admission and at day 7 was significantly correlated with baseline NIHSS score and favourable outcome. Adjusting for age, gender, smoking and alcohol-consumption status, body-mass index, and presence/absence of hyperlipidemia, atrial fibrillation, and hypertension resulted in a significant association between OSA and clinical outcome, including death as end-point (1.92; 95%CI, 1.80 to 3.16; p<0.001). The risk of death in patients in the most severe quartile of OSA was three times that in the comparison group.
Conclusion: In patients with T2DM and acute first-ever ischemic stroke, aortic stiffness predicts early stroke functional outcome, independent of other known prognostic factors. Presence of OSA is significantly associated with increased arterial stiffness and functional disability, as well as death, in these patients.

1309

Characterising sleep apnoea syndrome in type 2 diabetes (Sweet Sleep study)

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Background and aims: Sleep apnoea is highly prevalent in patients with type 2 diabetes mellitus (T2DM). However, it is unknown whether or not diabetic patients share the same sleep breathing pattern than non-diabetic patients.
Materials and methods: Case-control study between 119 T2DM patients and 238 non-diabetic subjects closely matched by age, gender, BMI, waist and neck circumferences, and smoking status. The exclusion criteria included chronic respiratory disease, neuromuscular and cerebrovascular disease, alcohol abuse, use of sedatives, and pregnancy. Examination included a respiratory polysomnography, oxygen saturation measures as the cumulative percentage of time spent with oxygen saturations below 90% (CT90), and the degree of sleepiness using the Epworth Sleepiness Scale (ESS). Studies with less than 5 hours of correct signal recording were ruled out. An apnoea was defined as cessation of airflow with duration of at least 10 seconds, and differentiation between obstructive and central apnoeas was made. The apnoea-hypopnea index (AHI) was defined as the sum of apnoeas plus hypopneas divided by time in bed.
Results: A higher AIH was observed in T2DM patients (34.0±3.4 vs. 26.0±2.8 events/hour; p=0.017). When sleep events were evaluated separately, a similar incidence of hypopnea events was present in diabetic and non-diabetic patients (18.3±1.1 vs. 16.7±3.9 e/h; p=0.951). However, in T2DM patients, a significantly increase in the number of apnoea events (15.2±8.2 vs. 10.3±3.2 e/h; p=0.048) and in the CT90 (18.0±33.5 vs. 10.7±10.4 %; p=0.042) were detected. No differences between obstructive and central apneas were observed. In addition, diabetic patients also showed higher daily sleepiness (7.5±3.6 vs 6.0±3.2, p=0.012) than non-diabetic subjects.
Conclusion: T2DM adversely affects breathing during sleep, becoming an independent risk factor for higher rates of sleep apnoeas, as well as severe nocturnal hypoxemia and daily sleepiness.
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1310

Association between glycaemic control and impaired lung function in Japanese adults

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Background and aims: Several studies have reported that diabetes is associated with impaired lung function. However, evidence on the dose-response relationship between measures of glycaemic control and impaired lung function is still scarce. In the present study, we hypothesized that there could be a continuous and inverse association between glycaemic control and lung function and examined the hypothesis in a large health checkup population which included both diabetic and non-diabetic individuals.
Materials and methods: This was a cross-sectional study performed in the medical checkup unit of one general hospital in Japan. All participants(n=3,611) who were apparently healthy and underwent a medical checkup focusing on metabolic syndrome from May 2008 to December 2011 were invited to the study. Information on lifestyle was obtained by a questionnaire. Blood samples and anthropometric data were obtained under a fasting condition. Lung function tests were performed by trained personnel. A total of 361 participants were excluded because of missing anthropometric, laboratory or lifestyle data. Finally, 3,250 participants (mean age, 56±12 years; 69% men; 15% diabetic individuals) were included in the analysis. The differences in forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) were estimated across HbA1c categories (<5.5%, 5.5-5.9%, 6.0-6.4%, 6.5-6.9% and 7.0-%). Multiple linear regression was used to adjust the differences for age, sex, body mass index, waist to hip ratio, smoking status, hypertension, dyslipidaemia and white blood cell count.
Results: FVC decreased steadily as HbA1c increased. Compared with individuals with HbA1c <5.5%, the adjusted differences in mean FVC among those with HbA1c of 5.5-5.9%, 6.0-6.4%, 6.5-6.9% and 7.0-% were -14.6ml, -118.6ml, -195.4ml and -239.9ml, respectively. HbA1c was also analysed as a continuous variable, yielding a significant decrease in FVC of -77.3ml for a 1% increase in HbA1c levels. Similarly, FEV1 was inversely associated with HbA1c levels. Compared with those with HbA1c of <5.5%, the adjusted differences in mean FEV1 among those with HbA1c of 5.5-5.9%, 6.0-6.4%, 6.5-6.9% and 7.0-% were 1.1ml, -57.4ml, -122.8ml and -140.8ml, respectively. A 1% increase in HbA1c was significantly associated with a 40.9ml reduction in FEV1.
Conclusion: The present study found that there was an inverse and continuous relationship between glycaemic control and lung function in Japanese adults. The association was observed in a wide range of HbA1c levels which included both diabetic and non-diabetic individuals. The result suggests that the lung could be another organ to be affected by hyperglycaemia.

Association of HbA1c levels with lung function (FVC and FEV1)

	Adjusted difference in FVC (ml) (95%CI)	Adjusted difference in FEV1 (ml) (95% CI)
HbA1c (%)(categorical)		
- 5.4	reference	reference
5.5 - 5.9	-14.6 (-60.6 to 31.4)	1.1 (-36.2 to 38.4)
6.0 - 6.4	-118.6 (-188.0 to -49.1)	-57.4 (-113.8 to -0.9)
6.5 - 6.9	-195.4 (-300.7 to -90.0)	-122.8 (-208.3 to -37.4)
7.0 -	-239.9 (-328.2 to -151.7)	-140.8 (-212.4 to -69.2)
P for trend	<0.001	<0.001
HbA1c (%) (continuous)		
Increase in 1%	-77.3 (-104.2 to -50.3)	-40.9 (-62.7 to -19.0)

1311

Type 2 diabetes is an independent risk factor for dementia conversion in mild cognitive impairment patients

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Background and aims: Mild cognitive impairment (MCI) is a clinically heterogeneous syndrome associated with an elevated dementia conversion rate, in particular the Alzheimer´s disease. It has been reported that diabetes is a risk factor for MCI. However, there is no information whether the rate of dementia conversion in MCI patients is higher in type 2 diabetic patients. In order to shed light on this issue a case-control study aimed at exploring whether type 2 diabetes is a risk factor for dementia conversion in MCI patients was designed.
Materials and methods: Design: longitudinal nested case-control study. Cases: 101 type 2 diabetic patients older than 60 years with MCI. Controls: 101 non-diabetic patients with MCI matched by age and gender. All patients were functionally literate, without severe auditory and visual abnormalities. A neuropsychological, neurological and psychiatric evaluation, as well APOE genotyping was performed. The mean of follow-up was 28 months (SD± 18) without any significant difference between cases and controls. Statistical analysis: Chi-square, Student's t test. In order to evaluate the risk factors independently associated to dementia conversion from MCI a logistic regression analysis was performed taking into account as independent variables the following: age, presence of diabetes, hypertension and hypercholesterolemia, APOE genotype and time of follow-up. Data were analyzed using SPSS for Windows v.18.
Results: The dementia conversion rate was 57, 4% in type2 diabetic patient vs. 42, 6% in non-diabetic patient (p= 0, 02). We did not found any difference in either the type of dementia (Alzheimer´s disease, vascular dementia, mixed dementia [Alzheimer´s disease and vascular], frontotemporal dementia and dementia with Lewy´s bodies) or *APOE* genotype between cases and control subjects. The logistic regression analysis shown that type 2 diabetes (OR: 2.09; CI 95%: 1.15-3.79) and APOE ε4 allele (OR: 2.56; CI: 1.32-4.96) were independent risk factors for developing dementia in MCI subjects.
Conclusion: Type 2 diabetes is an independent risk factor for dementia conversion in MCI patients. Further studies addressed to explore the underlying mechanisms are warranted.

1312

Selective cognitive decline is related to focal brain volume loss in type 1 diabetes patients with microangiopathy: a 4 year follow-up

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Background and aims: Cross-sectional studies showed cognitive and structural brain changes in type 1 diabetes (T1DM) patients, predominantly in those with peripheral microangiopathy. Whether these brain changes progress over time is not well known.
Materials and methods: In 25 T1DM patients with microangiopathy (baseline age: 46.1±7.8yrs and HbA1c: 7.9±1.0%; 40% male; baseline estimated IQ: 112±12.7) and 25 matched controls (baseline age: 44.3±8.5yrs and HbA1c: 5.4±0.3%; 52% male; baseline estimated IQ: 109.4±11.0) we assessed general cognitive ability, memory, information processing speed, executive functions, attention, motor and psychomotor speed at baseline and follow-up. A 3D-T1 structural MRI-scan at both time points was used to determine whole-brain volume loss, analysed with FSL-SIENA. Group differences and associations over time were analysed with regression analyses corrected for mean follow-up time, baseline age, sex and baseline cognitive performance when assessing change in cognition, baseline total brain volume when determining brain change or both when determining associations between change in cognition and brain volume.
Results: After 4 years, patients versus controls showed significantly greater decline in executive functions (patients: δz=-0.402±0.63sd vs. controls: δz=-0.007±0.39sd; β=-0.397; P=0.006). Furthermore, patients versus controls had a larger percentual whole-brain volume loss (patients: -1.34±1.0% vs. controls: -0.68±0.63%; β=-0.314; P=0.022), most marked in the right frontal and central areas. In all participants, larger loss of right frontal and central brain volume was related to accelerated executive functions decline (β=-0.330; P=0.025). In patients, higher baseline HbA1c was associated with larger executive performance decline (β=-0.407; P=0.043) and higher baseline systolic blood pressure was correlated to frontal brain volume loss at follow-up (β=-0.501; P=0.011).
Conclusion: At 4-years follow-up, loss of executive performance and brain volume was significantly greater in T1DM patients with microangiopathy compared with healthy controls. Poorer glycaemic control and higher systolic blood pressure at baseline predicted both cognitive and brain alterations over time.
Supported by: EFSD/ Lilly

1313

Hypoglycaemia related events due to falls in elderly diabetics (H.E.A.L.E.D) study using a US commercial database

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Background and aims: Falls are a major cause of concern in the elderly population resulting in increased morbidity and mortality. The goal of this project was to examine the association of hypoglycemia with falls and fall-related events in elderly patients with type 2 diabetes mellitus (T2DM).
Materials and methods: A retrospective cohort study was conducted in patients with T2DM. Records were obtained from Truven Health MarketScan® Commercial Database for patients (N= 1,147,937) with at least 2 records of T2DM diagnosis from 1/1/2008 to 12/31/2011. The first date of a recorded hypoglycemia diagnosis (ICD-9-CM codes: 250.8, 251.0, 251.1 and 251.2) was defined as the index date for a case, ensuring that there is a one-year baseline and one-year follow-up. The control cohort (no hypoglycemia record) was 1:1 randomly matched by age and gender. Patients were required to be ≥ 65 years at cohort entry with a continuous enrolment and pharmacy benefits throughout one year prior to cohort entry. Fall-related outcomes of fractures and head injuries were defined as ICD-9-CM codes between 800.x -995.x, with a fall being the external cause defined as ICD-9-CM E-codes between E880 - E888; which were recorded within ±2 days of each other in any order. Unadjusted chi-square tests were done. Conditional logistic regression models were used to compare the post-index fall-related events within 30 days, 90 days, 180 days and 365 days between the two cohorts. Adjusted odds ratios (aOR) and corresponding 95% confidence intervals (95% CI), controlling for baseline characteristics and co-morbidities, were estimated from logistic regression models. Two subgroup analyses (age categories <75 years and >=75 years) were also conducted.
Results: A total of 21,613 cases were matched with 21,613 controls. Patients with hypoglycemia consistently had higher fall-related events: 235 events (1.09%) among the cases and 37 events (0.17%) among the controls within 30 days; 373 events (1.73%) and 118 events (0.55%) within 90 days; 520 events (2.41%) and 204 events (0.94%) within 180 days; and, 720 events (3.33%) and 351 events (1.62%) within 1 year. All frequency differences between cases and controls were statistically significant; p-values <0.0001. Conditional logistic regression analyses showed an elevated risk of fall-related events over 365 days (aOR=2.10, 95% CI=1.85-2.39). The sub-group analyses showed elevated risk for both groups: <75 years group (aOR=2.53, 95% CI=2.06-3.09) and >=75 years group (aOR=1.83, 95% CI=1.54-2.17).
Conclusion: The risk of fall-related events increased by 2-fold among elderly diabetic patients who experienced hypoglycemia. Physicians treating elderly diabetes patients need to make treatment decisions that minimize the risk of hypoglycemia and thereby avoiding the ensuing complications of falls. In addition, patients with hypoglycemia may need additional education and other preventive measures to reduce the risk and clinical impact of falls.

1314

Incidence and predictors of hip fracture in type 2 diabetes: the Fremantle Diabetes study

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Background and aims: Most studies that have examined the relationship between diabetes and hip fractures have utilized administrative databases and/or have had limited/incomplete data including recognized hip fracture risk factors. The aim of this study was to determine the incidence and predictors of hip fracture in well-characterized community-based patients with type 2 diabetes.
Materials and methods: We studied a cohort of 1,296 type 2 diabetic participants (mean±SD age 64.0±11.3 years, 49% male, median diabetes duration 4.0 years) in the longitudinal observational Fremantle Diabetes Study Phase I (FDS1) together with 5,159 age-, gender- and postcode-code-matched non-diabetic residents. All deaths and hospitalisations in the state of Western Australia (WA) are recorded in the WA Data Linkage System which was used to provide outcomes from 1982 until end-December 2010. The main outcome measure was incident hospitalization for/with hip fracture between FDS1 study entry in 1993-6 and end-2010. In the diabetic cohort only, mobility was assessed by self-administered questionnaire and peripheral sensory neuropathy was defined using the clinical portion of the Michigan Neuropathy Screening Instrument. Cox proportional hazards modelling was used to identify independent predictors of first hospitalization for/with hip fracture during follow-up.
Results: During 12.0±5.4 years of follow-up, 86 (6.6%) patients were hospitalized on 135 separate occasions for a fractured hip (8.7/1,000 patient-years). This compared with 285 matched non-diabetic residents fracturing their hip on 471 occasions during 13.3±5.0 years' follow-up (6.8/1,000 patient-years). The incidence of hip fracture amongst those with type 2 diabetes was thus 28% higher than in matched non-diabetic residents (incident rate ratio (IRR) (95% CI): 1.28 (1.05-1.55), P=0.011). Amongst the diabetic patients, older age, female sex, lower BMI, peripheral sensory neuropathy (hazard ratio [95% CI]: 2.10 [1.31-3.36]), and any reduction in mobility (1.98 [1.18-3.32]) were independently associated with risk of first hospitalization for/with hip fracture (P≤0.010).
Conclusion: The risk of hip fracture is moderately increased among community-dwelling type 2 diabetic patients compared with matched non-diabetic residents. Peripheral sensory neuropathy and reduced mobility both double the risk of hip fracture in patients with type 2 diabetes. Further research is required to determine if hip fractures can be prevented in diabetic patients, especially older females, through interventions aimed at optimal detection and management of mobility and peripheral neuropathy.
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1315

Diabetes is not a risk factor for the carpal tunnel syndrome: a case-control study

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Background and aims: The Carpal Tunnel Syndrome (CTS) seems to occur more frequently in patients with Diabetes Mellitus (DM) and might be associated with specific diabetic related factors. Previous studies described a relationship between duration of diabetes, microvascular complications, glycaemic control and CTS but many of these studies were in small cohorts. Moreover, many studies investigated diabetes as a risk factor for musculoskeletal disorders of the hand and shoulder in general, but not for CTS in particular. The aim of the present study was to determine if DM can be identified as a risk factor for CTS. Furthermore we investigated the influence of duration of DM, microvascular complications and glycaemic control on the development of CTS.
Materials and methods: Retrospective case-control study using data from electronic patient charts from a Dutch clinic complemented by data of a diabetes specific database of the Diabetes Centre of that clinic. All patients who were diagnosed with CTS in the period from January 2011 to July 2012 were included. These patients were compared with a control group of herniated nucleus pulposus patients who went for surgery. Primary outcome was the prevalence of DM in both groups. Secondary outcomes were duration of DM, known microvascular complications and glycaemic control in both groups. The sample size required for the control group to detect a difference in DM prevalence of 5%, with an estimated CTS group size of 900 patients, a power of 0.8 and an alpha of 0.05 was 459. Statistical analysis was carried out using logistic regression, adjusting for confounding variables using three different models. Multiple imputation was used for missing data.
Results: A total of 1014 patients with CTS and 594 controls were included. In 92.2% of the cases the diagnosis CTS was confirmed by nerve conduction tests. Prevalence of DM was 12.3% in the CTS group versus 7.2% in the control group (Odds Ratio (OR) 1.80 (95% confidence interval (CI) 1.25-2.59)). Mean (±SD) age (55.7 ± 15.2 year vs. 49.3 ± 13.0 year), BMI (28.4 ± 5.4 kg/m^2 vs. 26.7 ± 4.6 kg/m^2) and percentage of female patients (71.1% vs. 49.3%) were higher in the CTS group compared to the control group (all p <0.001). In multivariate analyses with correction for gender, age and BMI, DM was not significantly associated with CTS (OR 1.12 (95% CI 0.75-1.66)). There were no differences in duration of diabetes, microvascular complications or glycaemic control between groups.
Conclusion: Although DM was more frequently diagnosed among patients with CTS, it could not be identified as a separate risk factor. Furthermore, no associations were found between the duration of diabetes, complications of diabetes or glycaemic control and the development of CTS.

PS 115 Other diabetic complications

1316

Urologic complications and health-related quality of life in type 1 diabetes: long-term follow-up in the DCCT/EDIC study

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Background and aims: Limited data exist on the influence of urologic complications on health-related quality of life (HRQOL) in patients with type 1 diabetes (T1DM). We used biomedical information gathered during the Diabetes Control and Complications Trial and its Epidemiology of Diabetes Interventions and Complications follow-up (DCCT/EDIC) to examine the relationship between urologic complications and HRQOL.
Materials and methods: We studied 664 men and 580 women, mean (SD) ages 51.6±6.9 and 50.6±7.2; BMI 28.9±4.8, 28.6±5.9; duration of T1DM 29.5±4.8, 29.8±5.1; mean time-weighted HbA_{1c} 7.9%±1.0, 8.0%±0.9, respectively. Assessments made at EDIC year 17 (an average of 23.5 years after DCCT baseline) included patient reports of sexual dysfunction (International Index of Erectile Function and abbreviated Female Sexual Function Index), lower urinary tract symptoms (LUTS) in men using the American Urological Association Symptom Index and urinary incontinence (UI) in women with the Sandvik Scale. General HRQOL was assessed using the Medical Outcome Survey Short Form 36 (SF-36), which generates 9 sub-scales from 0-100 and perceived value of health with the EuroQol5 (EQ5D) utility index (scale 0-1). Diabetes-related quality of life was assessed using the Diabetes Quality of Life Scale (DQOL) (scale 0-100). Analyses were adjusted for age and education level.
Results: Among men, 31% reported very low to low confidence in maintaining an erection and 25% reported moderate to severe LUTS. Among women, 26% reported sexual dysfunction; 30% reported moderate to severe UI. Each urologic complication was consistently associated with lower general and diabetes-specific HRQOL in both men and women. For example, in women moderate to severe UI compared to minimal symptoms, was associated with lower mean EQ5D health utility scores (0.78 vs. 0.88, p<0.0001), SF-36 social functioning scores (67.9 vs. 76.4, p<0.0001) and total DQOL score (69.7 vs. 74.8, p<0.0001). In men, ED led to lower mean EQ5D utility score (0.82 vs. 0.90, p<0.0001), SF-36 social functioning (73.1 vs. 80.8, p<0.0001), and DQOL score (70.2 vs. 78.3, p<0.0001). In both men and women there was a cumulative negative effect of having both sexual dysfunction and urinary symptoms after adjusting for age, education and HbA_{1c}. For example, in men the odds (95% CI) of a low total DQOL (≤25th%tile, or score ≤70.00) were 3.18 (1.88-5.36) times greater in men with ED and 2.58 (1.43-4.65) times greater in men with LUTS. For those men with both symptoms the odds were 7.20 (3.96-13.11) times greater. In women the odds of a low DQOL (score ≤67.26) was 2.77 (1.53-5.01) and 2.88 (1.68-4.97) times greater among those with sexual dysfunction and UI, respectively. For those women with both symptoms, the odds were 4.20 (2.18-8.08) times greater. Similar effects were observed for the SF-36 and EQ5D measures.
Conclusion: Sexual and urinary complications in men and women with T1DM are associated with lower HRQOL. Since these complications may be embarrassing to discuss, their impact may not be fully appreciated by clinicians. HRQOL inquiries can guide such discussions.
Clinical Trial Registration Number: NCT00360893
Supported by: NIH DK083927

1317

Prevalence of urinary tract infection among type 2 diabetes mellitus patients vs non-diabetic patients in Germany

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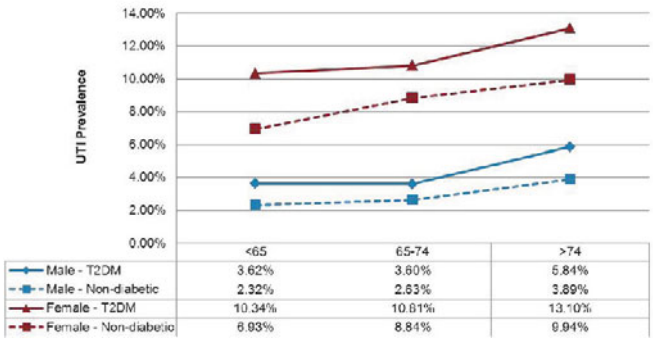
Background and aims: Urinary tract infections (UTI) commonly occur in patients with type 2 diabetes mellitus (T2DM). However, its prevalence has not yet been reported in a European patient population other than UK. Thus,

this study aims to evaluate the prevalence of UTI in a T2DM patient population compared to non-diabetic patients in Germany.
Materials and methods: This was a retrospective cohort study using the IMS Disease Analyzer database. Patients age 18 years or older with a T2DM diagnosis between 1/1/10 and 12/31/11 (index period) were identified. Patients were excluded if they had type 1 diabetes mellitus (T1DM), gestational diabetes mellitus, or other forms of secondary diabetes. Eligible patients had an existing medical record 1 year before (baseline) and after (follow-up) the index date (first observed T2DM diagnosis during the index period). Patients with no diagnosis of diabetes during the study period were individually matched 1:1 to the T2DM patients based on age, gender, and location. UTI events were identified using ICD-10 codes for UTI, cystitis, pyelonephritis, and trigonitis during baseline and follow-up periods. Charlson comorbidity index (CCI) score was calculated based on the patient information from the baseline period, and used as the indicator for patient health status.
Results: After matching, a total of 40,337 pairs of T2DM patients and non-diabetic subjects (49.17% male, mean age = 65.35 years), were identified as the study cohort. Compared to the non-diabetic cohort, the T2DM cohort had significantly higher prevalence of all comorbidities included in the CCI calculation, except for acquired immunodeficiency syndrome (AIDS) and metastatic solid tumor. Among the T2DM cohort, 12.25% were diagnosed with diabetes related chronic complications. The 1-year prevalence of UTI was 7.80% in T2DM cohort vs. 5.65% in non-diabetic cohort (p<0.0001). The age and gender specific prevalence was reported for those <65, 65-74, and ≥75 (Figure 1). The overall risk of UTI was higher for those with a history of UTI during the baseline period than those without (crude relative risk [RR] = 5.84, [95% CI: 5.54, 6.14]). After adjusting for age and gender, the adjusted RR of UTI was 4.68 [4.44, 4.92], which suggested potential confounding effect. After controlling for comorbidities, T2DM (odds ratio [OR] = 1.13, [1.06, 1.20]), age ≥75 vs. age <65 (OR = 1.50, [1.32, 1.71]), female gender (OR = 2.66, [2.41, 2.95]), and UTI during baseline period (OR = 6.09, [5.68, 6.52]) were significantly associated with UTI in the follow-up period. However, female gender showed a moderate but yet significant protective effect in patients aged ≥75 (OR = 0.81, p=0.0059).
Conclusion: In Germany, T2DM patients have a higher prevalence of UTI as compared to non-diabetic patients. The increased infection risk was greater for T2DM, female, elderly patients, and those with previous history of UTI.

Figure 1 UTI 1-year prevalence by age and gender (T2DM vs. non-T2DM)



	<65	65-74	>74
Male - T2DM	3.62%	3.60%	5.84%
Male - Non-diabetic	2.32%	2.63%	3.89%
Female - T2DM	10.34%	10.61%	13.10%
Female - Non-diabetic	6.93%	8.84%	9.94%

1318

Disease burden of urinary tract infections among type 2 diabetes mellitus patients: a US database study
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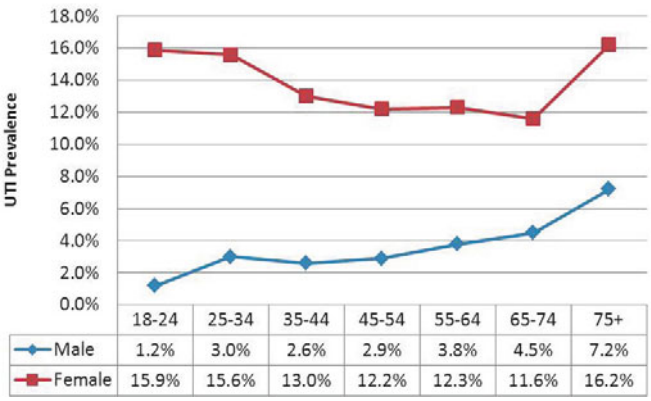
Background and aims: While the association between type 2 diabetes mellitus (T2DM) and urinary tract infection (UTI) is known, the associated economic burden has not been adequately quantified. Healthcare resource utilization and costs for T2DM patients with (prevalent cohort [PC]) and without (incident cohort [IC]) a history of UTI were assessed in this study.
Materials and methods: This was a retrospective cohort study using a US commercial health insurance claims database (MarketScan). T2DM patients aged ≥18 years with continuous enrollment for ≥1 year prior to, and after, the first observed T2DM diagnosis (between 1/1/2009-9/30/2010) were included. Patients with type 1 diabetes or gestational or other forms of secondary diabetes were excluded. Cases of UTI, cystitis, and pyelonephritis were identified using ICD-9 codes, which were further used to identify UTI-related inpatient and outpatient visits. Antibiotic prescription claims for treating UTI within 7 days after inpatient and outpatient visits were used to calculate the pharmacy cost.
Results: The final study cohort comprised 93,919 T2DM patients. UTI prevalence was 7.6% (n=7,143) and 8.3% (n=7,835) at baseline and follow-up year, respectively. The age and gender specific 1-year prevalence is reported in Figure 1. During the follow-up period, 33.9% of the PC had ≥1 UTI while 6.2% of the IC had ≥1 UTI (p<0.0001). After controlling for patient characteristics and baseline comorbidities, age 75+ (odds ratio [OR]=1.36 vs. age 55-64, [95% CI: 1.26, 1.47]), female gender (OR=2.9, [2.75, 3.06]), and history of UTI (OR=5.81, [5.48, 6.15]) were the strongest predictors of UTI. Compared to the IC, the PC was more likely to have UTI related outpatient visits (96.9% vs. 95.2%, p=0.0005), more outpatient visits per patient (2.60 vs. 1.62, p<0.0001), UTI-related antibiotics use (65.8% vs. 60.9%, p<0.0001), and a greater number of UTI-related prescriptions per patient (4.97 vs. 3.30, p<0.0001). The associated outpatient cost per patient per year was also higher for the PC than IC ($630.8 vs. $415.9, p=0.001 for payers, $62.92 vs. $48.54, p=0.0006 for patients). The PC also had higher pharmacy cost as compared to IC ($36.51 vs. $23.82, p<0.0001). However, the PC had lower UTI-related inpatient cost for patients ($319.0 vs. $481.6, p=0.036). No significant difference in inpatient cost was observed for payers ($16,425 vs. $16,866, p=0.87) or in total inpatient cost ($16,744 vs. $17,347, p=0.82).
Conclusion: Among T2DM patients, UTI was associated with increased healthcare utilization and higher costs, especially for those with previous UTI.

Figure 1 UTI prevalence during 1-year follow up



	18-24	25-34	35-44	45-54	55-64	65-74	75+
Male	1.2%	3.0%	2.6%	2.9%	3.8%	4.5%	7.2%
Female	15.9%	15.6%	13.0%	12.2%	12.3%	11.6%	16.2%

Supported by: Merck Sharp & Dohme Co.

1319

Statin therapy is associated with development of new onset diabetes after transplantation (NODAT) in liver recipients
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Background and aims: The metabolic complications including new onset diabetes after transplantation (NODAT) and dyslipidemia become important in organ transplantation recipients, since it is associated with graft survival as well as patient survival. The statins are most widely used for dyslipidemia treatment in general population, but it is associated with increased risk of diabetes. The aim of this study is to investigate whether statin therapy is associated with NODAT development in liver transplant (LT) recipients.
Materials and methods: A total of 154 liver transplanted recipients, who underwent surgery in the age of 20-75 without previous history of diabetes were included. We evaluated the incidence of NODAT according to the statin use and associated risk factors. The fasting blood glucose and lipid profiles were examined at baseline (before transplantation) and 6, 12, 24, 36 months after transplantation.
Results: The incidence of NODAT was significantly higher in stain group (9/29, 31.0%) compared to control group (14/125, 11.2%) (p=0.007). There were no significant differences in age, body mass index, corticosteroid cumulative dose, prevalence of hypertension and impaired fasting glucose be-

fore LT, fasting serum glucose and total cholesterol levels between the groups at baseline. The mean follow up duration was 34.46±23.56 months in statin group and 33.34±22.23 months in control group (p=0.972). Statin use over 6 months was the principal factor related to the development of NODAT after LT (OR, 4.49; 95% CI, 1.49-13.54, p=0.008)
Conclusion: Statin treatment over 6 months could contribute to the development of NODAT in liver transplantation recipients.

Table. Independent Predictors of NODAT development in LT patients

	OR (95% CI)	p-value
Sex	1.08 (0.39 to 3.02)	0.883
BMI	1.16 (0.97 to 1.39)	0.103
Age	0.99 (0.93 to 1.05)	0.696
Duration of f/u	1.00 (0.97 to 1.02)	0.721
Cummulative dose of steroid for 6months	1.00 (0.99 to 1.00)	0.253
Statin use	4.49 (1.49 to 13.54)	0.008
IFG before LT	2.82 (1.03 to 7.75)	0.044
Hypertension	0.72 (0.25 to 2.13)	0.561

Multivariate analysis was performed using multiple logistic regression.

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Sex hormone-binding globulin: a new marker of non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus
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Background and aims: Recent data suggested that increased serum SHBG levels, an independent predictor of type 2 diabetes mellitus (T2DM) and metabolic syndrome, resulted from decreased liver fat. Thus we aimed to investigate the association of serum sex hormone-binding globulin (SHBG) with metabolic variables and to evaluate whether it could be a biomarker of non-alcoholic fatty liver disease (NAFLD) in T2DM patients.
Materials and methods: 438 type 2 diabetic patients who visited our university hospital were enrolled in the study. Subjects were assigned to the NAFLD group (n=181) and non-NAFLD group (n=257) according to liver ultrasound. Fasting serum SHBG, fasting plasma glucose (FPG), liver enzymes, lipids, insulin and C-peptide were measured. The correlations between serum SHBG levels and metabolic parameters as well as the presence of NAFLD were analyzed.
Results: Serum SHBG levels were significantly lower in patients with NAFLD than that in patients without NAFLD (23.86 ± 10.98 vs. 42.06 ± 17.69 nmol/L, $P < 0.001$). In the multiple linear regression model with SHBG as the dependent variable, age (β = 0.316, $P < 0.001$), waist (β= -0.175, $P = 0.033$), triglycerides (TG) (β = -0.243, $P = 0.005$), HDL cholesterol (β = 0.200, $P = 0.021$), and homeostasis model assessment of insulin resistance (HOMA-IR) (β= -0.163, $P = 0.036$) were independent contributors that influenced the levels of SHBG. With the increased quartiles of serum SHBG, age and HDL cholesterol were significantly increased (P for trend < 0.001), and levels of BMI, waist, alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GT), TG, insulin and c-peptide, and also HOMA-IR were significantly decreased (all P for trend < 0.05). However, levels of aspartate aminotransferase (AST), total cholesterol, LDL cholesterol, FPG, and HbA_{1c} showed no any tendency of change. Besides, Elevated levels of SHBG were obviously and consistently associated with reduced presence of NAFLD. After adjustment for age and sex, the odds ratio (ORs) and 95% confidence interval (CI) for NAFLD from the first to the fourth quartile of serum SHBG were 1.00 (reference), 0.26 (95% CI, 0.14 - 0.47), 0.14 (95% CI, 0.07 - 0.26), and 0.03 (95% CI, 0.01 - 0.07), respectively (P for trend < 0.001). When further adjusted for potential coviables such as smoking status, alcohol use, duration of diabetes, BMI, waist, γ-GT, TG, FPG, and HOMA-IR, the inverse association between SHBG and NAFLD was slightly attenuated but still significantly existed (P for trend < 0.001).
Conclusion: Serum SHBG levels were significantly associated with age, waist, TG, HDL cholesterol, and HOMA-IR. Low serum SHBG may be a good marker in helping predict presence of NAFLD in type 2 diabetic patients.

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Low relative skeletal muscle mass is independently associated with NAFLD in Korean women with type 2 diabetes
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Background and aims: Diabetic patients tend to have age-related skeletal muscle loss (sarcopenia), which is associated with an increased risk of adverse health outcomes. Non alcoholic fatty liver disease(NAFLD) is highly prevalent in patient with diabetes and metabolic syndrome and the poor prognosis is associated with female sex. In this study, we investigated whether skeletal muscle mass was associated with NAFLD in women with type 2 diabetes.
Materials and methods: We enrolled 1926 women (mean age 58±12 yr) with type 2 diabetes, who visited our Diabetes Center from 2006 to 2010, who gave complete information on all covariates; who had no history of viral or other liver diseases; whose alcohol consumption was < 140 g/week; and who had undergone both bioimpedance body composition analysis and liver ultrasound. NAFLD was defined as the presence of hepatic steatosis. The skeletal muscle mass was expressed as skeletal muscle mass index (SMI = skeletal muscle mass/total body mass x 100).
Results: In the subjects, 984 (51.5%) patients had NAFLD. HbA1c, waist, triglyceride, systolic and diastolic blood pressure were higher in those with NAFLD. HDL-cholesterol and SMI were lower in those with NAFLD (SMI : 40.1±9.7 vs 41.3±9.1, p<0.01). The frequency of NAFLD was significantly decreased in higher SMI quartile(15.8%, 13.5%, 11.9%, 9.9%, p<0.01). The odds ratio for NAFLD was 2.25 (95% CI: 1.66, 3.04) in women with lowest quartile of the SMI compared with women with highest quartile of the SMI after adjusting for age, HbA1C, waist, triglyceride, systolic blood pressure and HDL-cholesterol.
Conclusion: The low relative skeletal muscle mass indicative of sarcopenia is associated with NAFLD in women with type 2 diabetes. Interventions leading to skeletal muscle build-up such as resistance training and/or amino acid supplementation may need to be investigated in diabetic women with NAFLD.

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Insulin resistance and hepatic steatosis relate to increased cardiac oxidative stress in mice
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Background and aims: Diabetic cardiomyopathy has been related to lower oxidative capacity and increased oxidative stress in cardiomyocytes. Non-alcoholic fatty liver (NAFL) and insulin resistance are associated with increased cardiovascular morbidity and mortality. Here, we examined how NAFL and insulin resistance relate to cardiac oxidative stress and mitochondrial oxidative capacity.
Materials and methods: Female mice, aged 18 and 36 weeks (w), with adipose tissue-specific overexpression of the sterol regulatory-element binding protein-1c (aP2-SREBP-1c: AP2), a model of NAFL, and wild-type controls (CON) underwent hyperinsulinemic-euglycemic clamps to assess insulin sensitivity (n=5-7). Cardiac mitochondrial respiration and reactive oxygen species production from isolated mitochondria were assessed by high-resolution respirometry and Amplex Red, respectively. Cardiac morphology and function were measured in vivo by NMR imaging in 36-weeks old mice (n=8).
Results: Whole body insulin sensitivity was 71% and 70% lower in both 18- and 36-weeks old AP2 mice than in aged-matched CON (p<0.05). Ex vivo cardiac mitochondrial oxidative capacity on tricarboxylic acid cycle-derived substrates was unchanged in 18 w old, but almost doubeld in 36 w old AP2 mice (state u respiration: 2.9±0.1, CON: 1.5±0.4 nmol/mg protein/s; p<0.05). Oxidative capacity on β-oxidation-derived substrates was 60% and 125% greater in 18 w old (2.2±0.2, CON: 1.4±0.2; p<0.05) and 36 w old (2.5±0.7, CON: 1.1±0.2 nmol/mg protein/s; p<0.05) AP2 mice, respectively. H2O2 production by mitochondrial complex III was 51% higher (p<0.05) only in 36 w old mice than in age-matched CON. AP2 mice also featured 34% increase in left ventricular mass and 21% increase in wall thickness (p<0.001) suggesting myocardial hypertrophy. Finally, older AP2 mice had 24% greater stroke volume and 29% higher cardiac output (for both p<0.05 vs. CON).
Conclusion: Insulin resistance in a mouse model of hepatic steatosis associates with increased cardiac mitochondrial respiration and oxidative stress,

which develop progressively with rising age. These changes relate to left ventricle hypertrophy and increased cardiac output, which could reflect adaptation to either higher blood pressure or to increased substrate flux. Insulin resistance and steatosis may therefore lead to higher myocardial energy turnover and oxidative stress rendering the hearts vulnerable for ischemic intolerance and impaired myocardial function.

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Increased accumulation of skin advanced glycation end products is associated with microvascular complications and insulin resistance in type 1 diabetes

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Background and aims: Advanced glycation end products (AGEs) are involved in the pathogenesis of atherosclerosis and reflect the risk of cardiovascular disease. Skin autofluorescence (AF) measured with Autofluorescence Reader (AR) device is a noninvasive tool to measure the tissue accumulation of advanced glycation end products. The aim of the study was to assess the association between AF and microvascular complications and insulin resistance in type 1 diabetes (DM1).
Materials and methods: Study population consisted of 416 type 1 diabetic patients, aged 32 years (IQR: 25-42), 227 women, with disease duration 14 years (IQR: 10-22). We evaluated the presence of late diabetic complications and indirect parameters of insulin resistance (waist circumference, estimated glucose disposal rate - eGDR, visceral adiposity index - VAI). We used AGE Reader (DiagnOptics, Groningen, the Netherlands) to measure skin autofluorescence phenomenon, which occurs because of fluorescent properties of AGEs.
Results: The median AF was 2.1 (IQR: 1.8-2.5). We found retinopathy in 188 patients (45%), diabetic kidney disease in 62 (15%) and neuropathy in 83 (20%). We found positive correlation between skin AF and diabetes duration (R=0.46, p<0.001), waist circumference (R=0.20, p<0.001), systolic blood pressure (R=0.16, p<0.001), LDL cholesterol level (R=0.13, p=0.005) and VAI in women (R=0.10, p=0.03) and negative correlation with eGDR (R=-0.14, p=0.006). There was no correlation between the AF and current measurement of HbA1c. In the univariate logistic regression AF was significantly associated with retinopathy (OR 3.4; 95%CI: 2.2-5.1, p<0.001), diabetic kidney disease (OR 2.5; 95%CI: 1.6-3.9, p<0.001), neuropathy (OR 4.4; 95%CI: 2.8-6.9, p<0.001) and with any microvascular complication (OR 4.1; 95%CI: 2.7-6.4, p<0.001). Multivariate logistic regression showed that skin AF was associated with diabetic neuropathy (OR 2.4; 95%CI: 1.3-4.3, p=0.002) and diabetic kidney disease (OR 2.2; 95%CI: 1.2-4.2, p=0.01) in connection with diabetes duration, smoking, history of hypertension and HbA1c level.
Conclusion: The tissue accumulation of AGEs is significantly associated with microvascular complications and insulin resistance in DM1. The assessment of skin AF might provide important clinical information for the risk of microangiopathy in diabetic patients.
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Activated Notch1 signalling has repressive effects on wound healing in diabetes

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Background: Diabetic wounds are characterized by impaired coordination of several cellular processes such as angiogenesis and cell differentiation. Notch signaling is a major player in cell differentiation and angiogenesis being a cell-cell system activated upon the interaction between the membrane-bound Notch receptors (Notch1-4) and their ligands (Jagged 1-2, delta 1, 3, 4). Binding of the ligands is followed by proteolytic cleavage of the receptor by γ-secretase complex which results in activation of the signaling with specific outcome depending on the members of the Notch system involved. We decided to study the potential role of Notch signaling in diabetic wound healing.
Materials and methods: The modulation of Notch system by hyperglycemia was studied *in vitro* in human dermal fibroblasts (HDF), human endothelial cells and *in vivo* in several animal models (db/db mice and Goto-Kakizaki (*GK*) *rat*) using the corresponding technique (western blot, transitory transfections with reporter gene assay or evaluation of target genes by quantitative RT-PCR). The functional consequence of the notch system modulation was studied *in vitro* by assessment of the migration of HDF and by angiogenesis assay. Notch pathway inhibition was induced either nonspecific by γ-secretase inhibitors (DAPT, L-685,458) or by specific siRNA silencing of the Notch receptors (1-4). Using cre-lox system we have generated mice that lack Notch 1 in the skin. Wound healing rate was evaluated both in db/db mice and in skin specific Notch1 knock-out mice in which diabetes was induced by streptozocin (STZ).
Results: Notch signaling is activated in the skin of several animal models of diabetes. Hyperglycemia activates Notch pathway at multiple levels and has repressive effect on fibroblasts migration and angiogenesis. Blocking Notch signaling with γ-secretase inhibitors improves wound healing rate just in diabetic (db/db mice) but not in control non-diabetic animals. Using loss-of-function genetic approaches we demonstrate both at the cellular level (fibroblasts, endothelial cells) as well as in an animal model that the Notch1 activation is the key player of the repressive effects of Notch on wound healing in diabetes, which is confirmed in the biopsies from patients with diabetic foot ulcers.
Conclusion: We propose specific targeting of Notch1 signaling as potential therapy for diabetic wounds.
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PS 116 Cerebrovascular and peripheral artery disease

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Impact of established and undiagnosed type 2 diabetes mellitus on acute ischaemic stroke severity and outcome

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Background and aims: It is unclear whether undiagnosed type 2 diabetes mellitus (T2DM) has the same prognostic significance as established T2DM in patients with acute ischemic stroke. We aimed to assess the impact of established and undiagnosed T2DM on acute ischemic stroke severity and outcome.
Materials and methods: We prospectively studied 342 consecutive patients (37.4% males, age 78.8±6.4 years) who were admitted for acute ischemic stroke. Undiagnosed T2DM was defined as fasting serum glucose levels at the second day of hospitalization ≥ 126 mg/dl in the absence of history of T2DM. Stroke severity was evaluated with the National Institute of Health stroke scale (NIHSS) at admission and the outcome was assessed with the modified Rankin scale (mRS) at discharge.
Results: Undiagnosed and established T2DM were present in 7.4 and 35.1% of the patients, respectively. At admission, the NIHSS was 8.0±9.9, 16.0±11.8 and 9.6±9.4 in non-diabetic patients and in patients with undiagnosed and established T2DM, respectively (p=0.006). In post-hoc tests, the NIHSS was higher in patients with undiagnosed T2DM than in non-diabetic patients and patients with established T2DM (p=0.005 and p<0.05, respectively) but did not differ between non-diabetic patients and patients with established T2DM. At discharge, the mRS was 2.2±2.1, 3.3±2.4 and 2.8±2.2 in non-diabetic patients and in patients with undiagnosed and established T2DM, respectively (p<0.05). In post-hoc tests, the difference in mRS approached significance only in the comparison of non-diabetic patients and patients with undiagnosed T2DM (p=0.089). Patients with established T2DM had lower serum low-density lipoprotein cholesterol levels but higher serum triglyceride and lower high-density lipoprotein cholesterol levels than non-diabetic patients. Other cardiovascular risk factors did not differ between non-diabetic patients, patients with undiagnosed T2DM and patients with established T2DM.
Conclusion: In patients admitted for acute ischemic stroke, undiagnosed T2DM is associated with more severe stroke and worse outcome than established T2DM. It is possible that the aggressive management of cardiovascular risk factors in patients with established T2DM prior to stroke negates their increased risk whereas patients with undiagnosed T2DM remain at risk.

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Falling or static national rates of diabetes-related vascular and renal outcomes from 2006 to 2011 despite a 50% increase in diabetes prevalence

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Background and aims: Despite rapid and substantial increases in the national prevalence of diabetes (DM) in New Zealand (NZ) over more than 20 years, we have not seen the anticipated rise in adverse outcomes for renal and vascular morbidities.
Materials and methods: We therefore assessed the number of lower-limb amputations, renal replacement therapy access procedures, cardiovascular (CV) events and strokes occurring throughout NZ over the 6 year period 2006-2011 inclusive. All such events, identified by ICD-9 or ICD-10 codes using standard data collection and coding methods. can be linked by a unique national patient identifier available for >98% of people. We use a detailed algorithm, the NZ Virtual Diabetes Register (VDR), to define the population with DM; this defines individuals as having DM based on their specific healthcare facility and therapy usage and has ≥ 90-95% sensitivity and specificity.
Results: Diabetes prevalence using the VDR rose from 138,200 (end-2005) to 208,076 (end-2011), an increase of 50.6% over 6 years - 7.0% compound per annum (p.a.); while total national population rose only 7.3% (4.12 to 4.42 million). Lower limb amputations in people with DM remained roughly static between 648 and 782 events annually; as a rate per 1000 people with DM this fell from 0.47% to 0.39% p.a. (-17%; $p<0.05$). Renal access procedures similarly remained static (between 422 & 458 events annually, 0.24-0.33% p.a.; p=NS) with no clear trend. The absolute number and the rate of CV events fell from over 13,000 to below 11,000 (9.48 to 5.74% p.a.), -39% ; r=-0.97; $p<0.005$). Stroke events rose in absolute numbers from 2600 to 3300 but fell as a rate from 1.90 to 1.73% p.a., -9%; r=-0.90, $p<0.02$).
Conclusion: We conclude that, despite a continuing long-term increase in diabetes prevalence, national major end-point rates are either static or falling, particularly so for CV events.

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Diabetes mellitus and chronic kidney disease amplify accumulation of tissue advanced glycation end products in patients with peripheral artery disease

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Backgrounds and aims: Diabetes mellitus (DM) and chronic kidney disease (CKD) are important risk factors for peripheral artery disease (PAD) and associated with a severely increased cardiovascular (CV) risk in these patients. DM increases production of AGEs and CKD decreases their clearance, while chronic low grade oxidative stress induces AGEs formation in both. However, human data on the role of tissue AGEs in PAD are scarce. We aimed to study the effects of DM and CKD on accumulation of tissue AGEs in patients with PAD.
Materials and methods: We performed a cross-sectional study of 486 PAD patients at the outpatient clinic of Vascular Surgery. PAD was confirmed by angiography or duplex ultrasonography. CV risk factors and CV comorbidity (coronary artery disease [CAD], cerebrovascular disease [CVD], abdominal aortic aneurysm [AAA]) were assessed. Hypertension and hypercholesterolemia were defined as the use of blood pressure and lipid lowering drugs, respectively. Patients were divided into four groups based on the presence of DM and severity of CKD (> or < 60 ml/min per 1.73 m^2). Tissue AGEs were noninvasively assessed by skin autofluorescence (SAF) with the AGE Reader™. Data are shown as mean ± standard deviation, number (%), geometric mean (95% confidence interval), or as median (Q1-Q3). ANOVA and logistic regression analysis were performed.

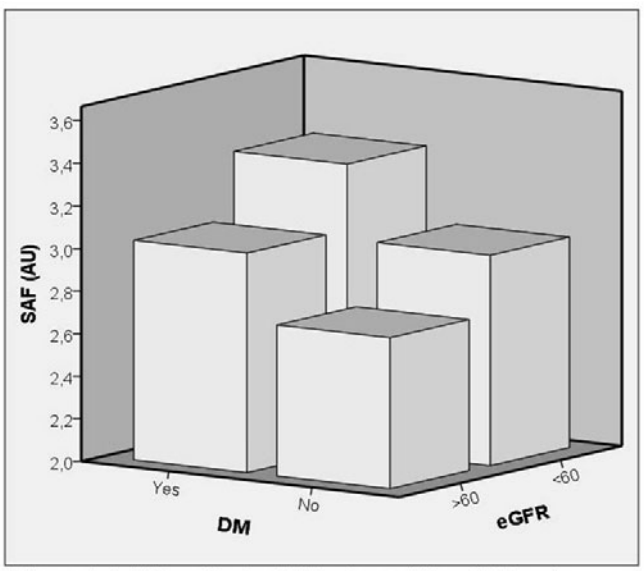

Figure 1. Additive effects of diabetes mellitus (DM) and chronic kidney disease on skin autofluorescence (SAF) in peripheral artery disease.
eGFR: estimated glomerular filtrating rate, AU: arbitrary units.

Results: Mean age of the 339 men and 147 women was 66 ± 11 years. DM was present in 123 (25%) patients who had a median HbA1c of 6.0 (5.7-6.5) % (42 [38-47] mmol/mol). Mean eGFR was 79 ± 28 in DM and 82 ± 26 ml/min per

1.73 m^2 in non-DM patients. 30 (24%) DM patients and 65 (18%) non-DM patients had eGFR< 60 ml/min per 1.73m^2. Figure 1 shows the additive effect of DM and CKD on accumulation of tissue AGEs: SAF was significantly different among the groups: DM and eGFR<60: 3.27 (95% CI: 3.02-3.52); DM and eGFR>60: 2.96 (2.81-3.10); non-DM and eGFR<60: 2.90 (2.73-3.08); non-DM and eGFR>60 ml/min per 1.73 m^2: 2.64 (2.56-2.71) arbitrary units, *P*<0.0001. SAF was associated with the presence of CV comorbidity, independent of age, gender, DM and eGFR; odds ratio 1.71 (95% CI: 1.26-2.33) per unit increase of SAF. The presence of CV comorbidity was: 70% in DM and eGFR<60, 66% in non-DM and eGFR<60, 54% in DM and GFR>60 and 41% in non-DM and eGFR>60 ml/min per 1.73 m^2, *P*=0.0001.

Conclusion: DM and CKD have additive effects on accumulation of tissue AGEs in PAD and are associated with the presence of CV comorbidity. Moreover, SAF predicts presence of CV comorbidity, independent of age, gender, DM and eGFR. Accelerated accumulation of AGEs may promote atherosclerosis and contribute to the severely increased CV risk in PAD patients with DM and CKD.

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Skin autofluorescence as a measure of tissue advanced glycation end products deposition is elevated in diabetic patients with peripheral artery disease

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Backgrounds and aims: Diabetes mellitus (DM) is an important risk factor for peripheral artery disease (PAD) and associated with a particularly poor prognosis in these patients. Increased glycemic and oxidative stress in DM enhance the accumulation of advanced glycation end products (AGEs), which play an important role in the development of atherosclerosis and cardiovascular (CV) disease. Skin autofluorescence (SAF) is a validated noninvasive method to measure tissue AGEs. Earlier, we showed that SAF is increased in DM, associated with vascular complications and predictive for mortality. However, accumulation of tissue AGEs in DM patients with PAD has not been studied. We aimed to study whether tissue AGEs are increased in DM patients with PAD and to establish the determinants of tissue AGEs deposition.

Materials and methods: We performed a case control study. PAD was confirmed by angiography or duplex ultrasonography. CV risk factors and CV comorbidity (coronary artery disease [CAD], cerebrovascular disease [CVD], abdominal aortic aneurysm [AAA]) were assessed. Hypertension and hypercholesterolemia were defined as the use of blood pressure and lipid lowering drugs, respectively. SAF was measured with the AGE Reader™. Independent sample t-tests, Mann-Whitney-U tests, Chi-square tests, backward linear and logistic regression analysis were performed. Data are shown as mean ± standard deviation, median (Q1-Q3), or as number (%).

Results: A total of 127 DM patients with PAD were compared with 127 DM patients without PAD, matched for age. Characteristics are shown in table 1. Patients with PAD had more CV risk factors, but a higher estimated glomerular filtration rate (eGFR). SAF was higher in diabetic patients with PAD compared to diabetic patients without PAD: 3.11 ± 0.69 versus 2.71 ± 0.68 arbitrary units, *P*<0.0001. In the complete group (DM patients with and without PAD), the odds-ratio for presence of PAD was 2.55 (95% CI: 1.43-4.54) per 1 unit increase of SAF, adjusted for CV risk factors and CV comorbidity. In the group of DM patients with PAD; sex, age, smoking and a history of AAA were the independent determinants of SAF while hypertension, hypercholesterolemia, HbA1c, eGFR, type 1 or 2 DM and a history of CAD or CVD did not contribute to the model.

Conclusion: Skin autofluorescence is elevated in DM patients with PAD, independent of glycemic control, CV risk factors and CV comorbidity. Therefore, increased accumulation of tissue AGEs may promote atherosclerosis and contribute to the poor prognosis of DM patients with PAD.

Table 1. Baseline characteristics of DM patients with and without PAD.

Characteristics	DM with PAD	DM without PAD	*P* value
N	127	127	
Age (years)	69 ± 10	68 ± 10	0.973
Male gender	91 (72%)	62 (49%)	0.0002
Diabetes mellitus Type 1 Type 2	8 (6%) 119 (94%)	- 127 (100%)	0.004
HbA1c* (%) (mmol/mol)	7.1 (6.4-7.9) 57 (46-62)	6.6 (6.0-7.6) 48 (42-59)	0.027
eGFR (ml/min per 1.73m^2)†	76 (60-100)	65 (57-74)	<0.001
Ankle-brachial index‡	0.57 (0.49-0.63)	-	N/A
Current smoker	47 (37%)	20 (16%)	0.0001
Hypertension Hypercholesteremia	119 (94%) 103 (81%)	73 (58%) 33 (26%)	<0.0001 <0.0001
CAD CVD AAA	51 (50%) 26 (21%) 10 (8%)	22 (17%) 4 (3%) -	<0.0001 <0.0001 N/A

Data as mean ± standard deviation, number (%), median (Q1-Q3). PAD = peripheral artery disease; DM = diabetes mellitus; eGFR = estimated glomerular filtrating rate; CAD = coronary artery disease; CVD = cerebrovascular disease; AAA = abdominal aortic aneurysm; N/A = not applicable.
* Data missing for 13 DM patients with PAD.
† Data missing for 4 DM patients with PAD.
‡ Data missing in 34 patients; 13 patients had non-compressible arteries and in 21 patients angiography was directly performed for diagnosis.

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Relationship between peripheral vascular calcification and accuracy of ankle brachial index and pulse palpation to screen peripheral arterial occlusive disease

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Background and aims: Accuracy of ankle brachial index and pulse palpation is discussed for screening peripheral arterial occlusive disease in patients with diabetes. Vascular calcification may indeed be associated with false negative tests. The main aim of this study was to objectively assess the association between peripheral vascular calcification and false negative screening tests for peripheral arterial occlusive disease in patients with diabetes. We also compared improvement of screening by combining pulse palpation and ankle brachial index, and assessed variables associated with peripheral vascular calcification.

Materials and methods: This was a cross-sectional study. The main exclusion criterion was severe renal disease. Peripheral arterial occlusive disease was diagnosed by color duplex ultrasonography. Vascular calcification was assessed by below-knee arteries calcification score measured by computer tomography scan. Linear regression analysis was used to study the variables associated with calcification. Lower limbs with false negative and true negative tests for pulse palpation and ankle brachial index were compared.

Results: We included 200 patients with diabetes (400 lower limbs). Accuracy of ankle brachial index and pulse palpation was low (69.5-72.5%) and not improved by combining both. Calcification score was positively associated with age, diabetes duration, male gender, neuropathy disability score, abnormal monofilament test, peripheral arterial occlusive disease, abnormal pulse palpation and ankle brachial index ≥1.30 or ≤0.90 (p<0.01 for all). Below-knee arteries calcification score was significantly higher in patients with false negative tests, than in those with true negative tests, for both pulse palpation and ankle brachial index (p<0.0001 for all) (see table).

Conclusion: False negative results of ankle brachial index and pulse palpation for screening peripheral arterial occlusive disease is associated with below-knee vascular calcification in patients with diabetes. Combination of both tests does not improve screening accuracy.

	Normal ankle brachial index, n=323 (80.5)			Normal pulse palpation, n=234 (58.5)		
	False negative, n=84 (21.0)	True negative, n=239 (59.8)	p-value	False negative, n=40 (10.0)	True negative, n=194 (48.5)	p-value
Calcification score	725 (0-6326)	102 (0-7205)	< 0.0001	625 (0-6326)	71 (0-6452)	<0.0001

Limbs with normal ankle brachial index and limbs with normal pulse palpation. Comparison of calcification scores in patients with false negative and true negative results for ankle brachial index and pulse palpation respectively.
Data are median (minimum-maximum) or n (%).

Supported by: Lilly Company and Lausanne University

1330

Statin therapy successfully maintains low LDL-C in type 2 diabetes mellitus and prevents the age-related LDL-C increase

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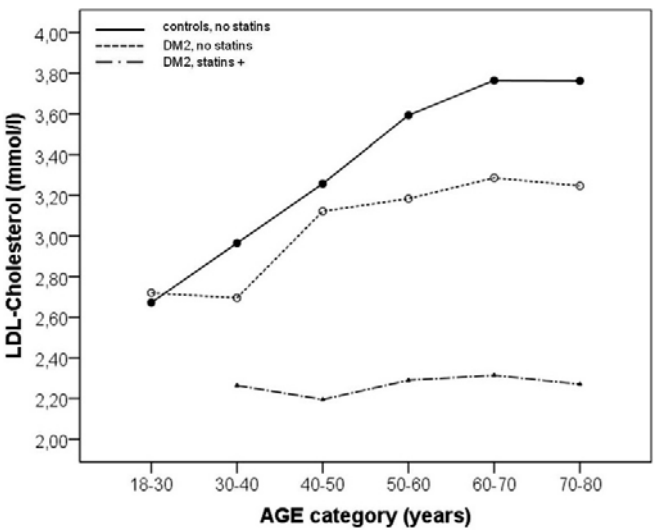
Background and aims: Lowering LDL cholesterol (LDL-C) level has been shown to reduce cardiovascular morbidity and mortality in type 2 diabetes patients (DM2). Several international guidelines therefore recommend to aim for an LDL-C of at least below 2.5 mmol/l in all DM2 patients. Statins are the drugs of choice to reach this goal. In the present study, we evaluated LDL-C levels per age category (decades) in DM2 patients with and without statin therapy, and compared this to non-statin users from the general population.
Materials and methods: Study participants were derived from 60,320 subjects in the LifeLines Cohort Study, a large population-based study on the role of gene-environment interaction in the development of frequently occurring diseases. 56,233 of the 58,712 (96%) non-DM participants did not use statins (group 1). All 967 DM2 participants were included in this analysis. DM2 was defined as diagnosed by a medical professional or treatment with oral blood glucose lowering agents. Among DM2 participants, there were 376 not on statin therapy (group 2) and 591 participants taking statins (group 3).
Results: Mean age in group 1,2 and 3 were: 44, 56 and 59 years, mean BMI: 26, 31 and 31 kg/m2, mean cholesterol: 5.1, 5.0 and 4.1 mmol/l, mean LDL-C: 3.2, 3.2 and 2.3 mmol/l, mean HDL: 1.48, 1.27 and 1.24 mmol/l, median triglycerides: 0.97, 1,46 and 1.44 mmol/l, mean HbA1c: 5.5, 6.8 and 7.0%, mean systolic BP: 125, 134 and 135 mmHg, respectively. In both group 1 and 2, there was a clear age-related rise in LDL-C (Figure 1). LDL-C in DM2 on statins (group 3) was similar in all age categories and was significantly lower compared to the other 2 groups (p<0.001).
Conclusion: Statin therapy adequately reduced the LDL-C level in DM2 participants below 2.5 mmol/l and prevented the age-related rise in LDL-C. The successful reduction of LDL-C in DM2 patients may be an explanation for the trend to a decrease in excess mortality in DM2 compared to the general population.



Supported by: BioSHaRE-EU, Netherlands Consortium Healthy Ageing

1331

The effect of HMG-CoA reductase inhibitor of ischaemic heart disease and cerebrovascular attack in elderly diabetic individuals: difference in risk by age

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Background and aims: LDL-cholesterol (LDL-C) is a risk factor for ischemic heart disease(IHD) and agents for dyslipidemia like HMG-CoA reductase inhibitors (statins) can reduce that risk in middle-aged diabetic individuals. We reported the importance of HDL-C levels for IHD and cerebrovascular attack(CVA) in the very elderly diabetic individuals (older than 75y.o.). However, the effect of statins on IHD or cerebrovascular attack(CVA) are not known at all in very elderly. The aim is to identify factors and the effect of agents for IHD and CVA in the elderly and to investigate their differences by age.
Materials and methods: We performed a prospective cohort study (Japan Cholesterol and Diabetes Mellitus Study) with 5.5 years of follow-up. 4,014 patients with type 2 diabetes and without previous IHD or CVA (1,936 women; age 67.4±9.5 years, median 70 years; <65 years old, n=1,261; 65 to 74 years old, n=1,731; and ≥ 75 years old, n=1,016) were recruited on a consecutive outpatient basis from 40 hospitals throughout Japan. Lipids, glucose, and other factors related to IHD or CVA risk were investigated. We also registered 405 sub-cohort patients among 4014 patients in the beginning of the study. The detailed information of medicine and the laboratory data on the change of medication on sub-cohort patients as well as patients suffered from IHD or CVA have been recorded. Statin users are divided into prevalent users, new users, and non-users.
Results: One hundred fifty-three cases of IHD and 104 CVAs (7.8 and 5.7/1,000 people per year, respectively) occurred over 5.5 years. Lower HDL-cholesterol (HDL-C) were correlated with IHD in patients ≥75 years old. In contrast, systolic BP(SBP), HbA1C, LDL-C and non-HDL-C were correlated with IHD in subjects <65 years old, and the LDL-C/HDL-C ratio was correlated with IHD in all subjects. HDL-C was correlated with CVA in patients ≥75 years old. In sub-cohort study, among patients prescribed no agents for dislipidemia, prevalence of both IHD and CVA increased by age dependent manner. In IHD, the rate of incidence was higher in prevalent and new users of statins than that in non users (Hazard ratio,HR 1.558,1.782 respectively), especially in non-elderly(HR:2.25, 2.05), but not in very elderly (HR:0.58,1.35) However, in CVA, the rate of incidence was lower in prevalent and new users of statins than that in non users (HR:0.460,0.523), especially in very elderly(HR:0.51,0.21). These data are same after adjustment various factors such as glucose and blood pressure which was different by age.
Conclusion: IHD and CVA in late elderly diabetic patients were predicted by HDL-C. Statin treatment may prevent IHD and CVA, however the effect may change by age. These age-dependent differences in risk are important for developing individualized strategies to prevent atherosclerotic disease.
Clinical Trial Registration Number: UMIN-CTR, UMIN00000516
Supported by: Ministry of Health

1332

Vitamin D and carotid intima media thickness in 416 Danish patients with type 2 diabetes mellitus at entry into the CIMT trial

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Background and aims: A number of studies indicate a high prevalence of vitamin D insufficiency in the general population, and several epidemiologi-

cal studies have reported an association between vitamin D insufficiency and risk of cardiovascular disease (CVD). Low vitamin D status may have an impact on the degree of osteopenia, osteoporosis and cardiovascular risk factors, including thickness of carotid intima media. The aim of the present study was to investigate the association between vitamin D, risk factors for osteopenia and osteoporosis, and carotid intima media thickness (carotid IMT) in the Copenhagen Insulin Metformin Treatment Trial (CIMT).

Materials and methods: We investigated 416 patients with T2D, recruited from 8 diabetes outpatient clinics in the greater Copenhagen area. Inclusion criteria were age>30 years, HbA_{1C}>7.5 % (>58 mmol/mol), eGFR>60 ml/min and duration of T2D >1 year. Vitamin D, carotid IMT, bone mineral density (BMD), trabecular bone structure (TBS) and anthropometric measures were determined at baseline. Vitamin D was measured by immunoassay ECLIA and insufficiency was defined as 25(OH)D <50nmol/l and deficiency as 25(OH)D <25nmol/l. An ultrasound scan was performed to determine carotid IMT. BMD and TBS were measured by Dual Energy X-ray Absorptiometry (DXA) (Hologic, Discovery A). Osteopenia was defined by any T score (spine, neck and hip-neck) between -1 to -2.5 and osteoporosis as any T score<-2.5. BMD was measured in (g/cm^2).

Results: A total of 416 patients (68% men), age 60 ± 9 years [mean ± SD], BMI (kg/m^2) 32 ± 4 [mean ± SD], and HbA_{1c} 8.5 ± 1.0% [mean ± SD] were included. The prevalence of vitamin D insufficiency was 51% and 17% were deficient. Patients had a carotid IMT of 0.8 mm ± 1.7 [mean ± SD] and 70 % have plaques. Vitamin D levels correlated positively with carotid IMT (R = 0.1, P = 0.02, unadjusted and adjusted for gender and BMI) (in men R = 0.04, P = 0.02). We found no correlation in relation to carotid IMT and TBS or BMD, and similar no correlation between vitamin D and TBS or BMD.

Conclusion: A high prevalence of vitamin D insufficiency and deficiency as well as increased thickness of carotid intima media, plaques and osteopenia was found in 416 Danish patients with T2D.We found a paradoxical positive correlation between vitamin D and carotid IMT, and no correlation between vitamin D status and measures of bone mineral contents.

Clinical Trial Registration Number: NCT00657943

1333

Improved cardiovascular function after prolonged caloric restriction in obese patients with type 2 diabetes mellitus and cardiac complications

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Background and aims: Obese patients with type 2 diabetes mellitus (T2DM) have a higher incidence of cardiovascular disease. These patients have increased ectopic fat accumulation, which is associated with cardiovascular disease. In addition, myocardial triglyceride (TG) content is increased in patients with T2DM, leading to altered myocardial function. In obese T2DM patients without cardiac complications prolonged caloric restriction decreases myocardial TG content and improves diastolic heart function. We hypothesize that dietary interventions decrease ectopic fat accumulation, including myocardial TG, and improve cardiovascular function in patients with T2DM with cardiac complications.

Materials and methods: 27 obese T2DM patients with cardiac complications, i.e. myocardial infarction in medical history and/or coronary artery occlusion ≥ 50%, were studied before and after a 16 week low calorie diet (LCD) (450-1000 kcal/day) to achieve substantial weight loss. Cardiovascular function, ectopic fat accumulation and pulse wave velocity (PWV), a marker for aortic stiffness, were assessed with magnetic resonance (MR) imaging and myocardial TG content with MR spectroscopy. In addition, plasma hemoglobin A1c (HbA1c), and body mass index (BMI) were measured.

Results: BMI decreased from 32.2 ± 0.9 (mean ± SEM) to 26.8 ± 0.8 kg/m^2, p < 0.001, and HbA1c from 6.9 ± 0.2 to 5.8 ± 0.1 % , p<0.001. Systolic cardiac function (Left ventricular ejection fraction) increased from 54.8 ± 1.7 to 56.2 ± 1.5 %, p=0.016. PWV decreased from 7.9 ± 0.4 to 7.2 ± 0.2 m/s, p 0.016, reflecting a more compliant aorta. Myocardial TG content decreased from 1.21 ± 0.13 to 0.81 ± 0.08 %, p<0.001. In addition epicardial and paracardial fat volumes decreased from 5.5 ± 0.3 tot 4.7 ± 0.3 ml, p<0.001, and from 8.0 ± 0.7 to 5.7 ± 0.6 ml, p<0.001, respectively.

Conclusion: 16 weeks caloric restriction in obese T2DM patients with cardiac complications decreases BMI and improves glucoregulation associated with decreased ectopic fat depots and improved cardiovascular function. Therefore, therapeutic intervention by caloric restriction is an efficient and attractive intervention for improving cardiovascular function in patients with T2DM with cardiac complications.

Clinical Trial Registration Number: NTR 2897

Supported by: Funding by the Netherlands Heart Foundation (Project UL 2009-4548)

PS 117 Risk factors for cardiovascular disease

1334

Diabetes duration rather than the level of metabolic control is a determinant of endothelial damage in type 1 diabetes

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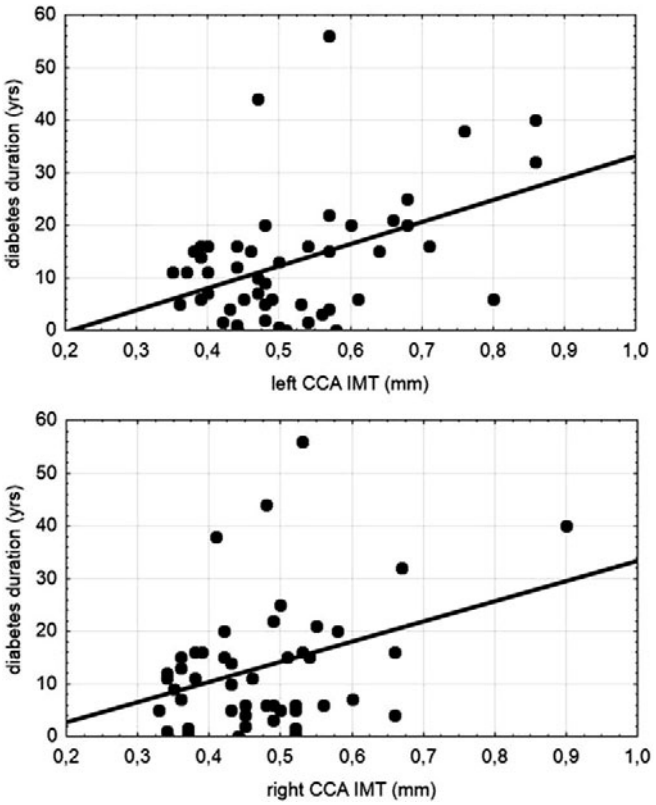
Background and aims: Besides clear susceptibility to developing microvascular complications, type 1 diabetes subjects are also at increased risk of cardiovascular and macrovascular complications. Recently, in a group of type 1 diabetes subjects without any vascular complications, we have documented the positive relationship between insulin resistance and QT interval. In this study we aimed at assessing arterial elasticity with the use of newly developed non-invasive two-dimensional strain imaging for estimation of vascular tissue motion and deformation (strain) during the cardiac cycle using speckle tracking. This technique enables angle-independent calculations of deformation arterial variables.
Materials and methods: Two-dimensional long- and short-axis grey-scale cine loops of the left and right common carotid arteries (CCA) were acquired in the group of 50 type 1 diabetes subjects (mean [±SD] 7.1±14.1 years, diabetes duration 13.0±12.0 years, BMI 23.8±1.6 kg/m^2, HbA_{1c} 9.1±2.2%) and 30 healthy age-, gender- and BMI-matched controls (38.6±10.8 years, BMI 24.9±2.1 kg/m^2). Circumferential strain (CS, %) and strain rate (CSr, strain per time unit, 1/s) as well as intima media thickness (IMT, mm) in CCAs were measured.
Results: Type 1 diabetes subjects presented with similar values of elasticity to the controls in left and right CCA (CS 6.04±2.50 vs 5.22±1.84; 6.01±2.60 vs 5.16±1.77%; CSr 0.97±0.55 vs 0.80±0.26; 0.90±0.32 vs 0.77±0.25 1/s, respectively). However, there was a significant positive relationship between age and IMT in left and right CCAs, and this relationship was stronger in diabetes type 1 subjects than in the controls (Pearson's correlation coefficient r=0.68 and 0.57 vs 0.59 and 0.55, all p<0.05, respectively) and negative one between age and CS or CSr (r values ranged from -0.35 to -0.64, all p<0.05), weaker in diabetes type 1 subjects than in the controls, where r values ranged from -0.58 to -0.70, p<0.05. We have also noted (figure) a positive correlation between diabetes duration and both IMT (r=0.45 and 0.34, p<0.05), but not arterial wall elasticity indices. Also, smokers with type 1 diabetes had thicker both IMT than non-smokers (0.58 vs 0.50 in left, and 0.53 vs 0.45 mm in right CCA, p<0.05). Interestingly, no relationship between HbA1c or blood lipid profile parameters were found in patients with diabetes.
Conclusion: Type 1 diabetes seems to affect intima media complex i.e. endothelium to a much greater degree than arterial wall elasticity, and diabetes duration is a more significant contributor to endothelium damage than the level of blood glucose or lipid profile control. Moreover, arterial wall strain assessment does not seem to be of high diagnostic value in vascular studies conducted in subjects with type 1 diabetes.



Supported by: Medical University of Lodz Grant No 502-03/8-072-03/502-64-053

1335

Prognostic impact of haemoglobin A_{1c} in high-risk type 2 diabetic patients: the Rio de Janeiro type 2 diabetes cohort study

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Background and aims: The prognostic importance of glycated hemoglobin (HbA_{1c}) in type 2 diabetes for cardiovascular outcomes after full adjustment for potential confounders remains debatable. The aim was to evaluate the prognostic impact of HbA_{1c} for cardiovascular morbidity and all-cause mortality in a cohort of 569 high-risk type 2 diabetic patients.
Materials and methods: Clinical, laboratory, and ambulatory blood pressure (BP) monitoring data were obtained at baseline. The primary endpoints were a composite of fatal and non-fatal major cardiovascular events and all-cause mortality. Multiple Cox survival analysis assessed the associations between HbA_{1c}, as a continuous variable and categorized at 3 stages (<7%, between 7 and 8%, >8%) and the endpoints. HbA_{1c} values were analyzed as the baseline value, as the mean value obtained during the first year of follow-up, and updated as time-varying covariate during the entire follow-up. In a sub-analysis, patients with pre-existent cardiovascular complications were excluded.
Results: After a median follow-up of 6.25 years, 96 total cardiovascular events and 92 all-cause deaths occurred. After adjustments for potential cardiovascular risk factors (age, sex, BMI, diabetes duration, smoking status, physical activity, number of anti-hypertensive drugs in use, presence of macrovascular and microvascular complications at baseline, 24-hour ambulatory BPs, HDL- and LDL-cholesterol, statins and aspirin use), HbA_{1c} was predictive of the composite endpoint and of all-cause mortality. First-year mean HbA_{1c} was a predictor of the composite endpoint as a continuous variable (HR: 1.20; 95% CI: 1.03-1.38; p=0.017, for increments of 1%). For all-cause mortality, both categorized baseline (HR: 1.98; 95% CI: 1.16-3.40; p=0.013; for HbA_{1c} between 7-8%; and HR: 1.83; 95% CI: 1.04-3.23; p=0.037, for HbA_{1c} >8%) and first-year mean values (HR: 1.93; 95% CI: 1.12-3.30; p=0.017, for HbA_{1c} between 7-8%; and HR: 1.85; 95% CI: 1.04-3.32; p=0.038, for HbA_{1c} >8%) were predictors in relation to the reference group with HbA_{1c} <7%. In the analysis excluding patients with cardiovascular complications at baseline, first-year mean values and time-varying HbA_{1c}, as continuous variables, were independent predictors of the composite endpoint. As a categorized variable,

only the subgroup with mean first year HbA_{1c} values > 8% showed an increased risk for the composite cardiovascular endpoint (HR: 2.82; 95% CI: 1.22-6.53; p=0.015).
Conclusion: HbA_{1c} provides cardiovascular risk prediction independent of standard risk factors, degenerative complications and ambulatory blood pressures, and improves cardiovascular risk stratification in high-risk type 2 diabetes. Achieved HbA_{1c} values during follow-up were particularly important risk predictors in patients without macrovascular complications at baseline.
Supported by: FAPERJ, CNPq

1336

Glycaemic variability is related to duration of diabetes rather than cardiovascular risk factors
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Background and aims: The role of glycemic variability in the development of cardiovascular diseases remains controversial. We investigated the relationship between indices of glycemic variability and cardiovascular (CV) risk factors in diabetic patients.
Materials and methods: Two hundred sixty three diabetic patients performed a seven-point self-monitoring of blood glucose during each month for 3 consecutive months. From these records, glycemic variability indices (standard deviation (SD) and M-value) were calculated. HbA1c was measured on the last day of the third month. Body mass index (BMI), waist circumference (WC), blood pressures, duration of diabetes, hsCRP, fibrinogen, alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), creatinine, uric acid, total cholesterol, triglyceride (TG), HDL, LDL, Apo B and Apo AI levels, urine albumin: creatinine ratio (UACR) and ankle-brachial pressure index (ABI) were assessed.
Results: The SD was significantly correlated with duration of diabetes (r=0.281; p<0.001) and UACR (r=0.131, p<0.05), but not with BMI, WC, blood pressures, hsCRP, fibrinogen, ALT, GGT, creatinine, uric acid, lipid profile and ABI. The M-value was correlated with duration of diabetes (r=0.288; p<0.001), UACR (r=0.198; p<0.01), TG (r=0.132; p<0.05) and ABI (r=-0.212; p<0.001), but not with other parameters. Using multiple linear regression to adjust for HbA1c and other covariates, only diabetes duration (β=0.184; p<0.01 and β=0.134; p<0.01, respectively) remained independent correlate of the SD and M-value. CV risk factors failed to maintain its independent association.
Conclusion: In this study, duration of diabetes rather than CV risk factors was an independent variable of indices of glycemic variability. These findings suggest that glycemic variability is largely determined by β-cell function which deteriorates with increasing duration of diabetes, but not cardiovascular complication.

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Restenosis after percutaneous transluminal coronary angioplasty in individuals with different categories of glucose tolerance: a 1-year follow-up study
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Background and aims: Patients with diabetes (DM) are at high risk for restenosis after coronary stenting. Data on the outcome of percutaneous transluminal coronary angioplasty (PTCA) in individuals with prediabetes is, however, limited. The study objective was to investigate the relationship between rate of restenosis after coronary stenting and baseline glucose status.
Materials and methods: We retrospectively analyzed data of 1003 patients (mean age 58.5±4.2 yrs, 61.5% of men, mean history of coronary heart disease 9.5±1.1 yrs) who undertook coronary stenting through PTCA at Emergency Department between Jan 2008 and Dec 2011. Baseline measurements included blood pressure (BP), fasting plasma glucose, lipid profile, HbA1c, C-reactive protein (C-RP), and 2-hour capillary glucose. All patients had follow-up coronary angiography 1 year after PTCA. Restenosis was defined as ≧ 50% stenosis in stent or within 5 mm adjacent to stent. The rate of restenosis was compared among patients with normal glucose tolerance (NGT, n=436), impaired glucose regulation (IGR, n=275), and DM (n=292) according to their baseline glucose levels. The diagnosis of NGT, IGR and DM was made according to 1999 WHO criteria.
Results: Patients with DM had highest levels of body mass index (BMI), BP, triglycerides (TG), and C-RP, followed by those with IGR and NGT (P<0.01). At baseline, the number of lesion was 1.6, 1.8, and 2.5 in patients with NGT, IGR, and DM, respectively (p<0.05). At the end of year 1, the rate of restenosis were 4.2%, 5.3%, and 12.0% in individuals with NGT, IGR, and DM, respectively (P<0.05). In the DM subgroup, compared with patients with HbA1c<8%, those with HbA1c>8% had increased rate of restenosis (14.6% vs. 11.2%, P<0.05). In the logistic regression model, the odd ratio (OR) of having restenosis was 1.46 (95%CI: 1.05-1.87) for DM and 1.10 (95% CI: 1.01-1.25) for IGR, after adjusting for age, history of CHD, BMI, BP, LDL-C, TG, and C-RP.
Conclusion: Restenosis occurs more frequently in patients with compared those without DM. Prediabetes is associated with increased risk of restenosis after PTCA during a follow-up period of 1 year. The data indicates that intervention of hyperglycaemia should be addressed in patients receiving PTCA.

1338

Delay in treatment intensification increases the risks of cardiovascular events in patients with type 2 diabetes
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Background and aims: The results from the large clinical trials evaluating the benefits of intensified glucose lowering treatment on cardiovascular risks in patients with T2DM have been inconclusive. Also, the effects of the delay in treatment intensification in newly diagnosed patients with T2DM have not been explored at population level. Using large longitudinal data on newly diagnosed patients with T2DM from the UK Clinical Practice Research Datalink, the aims of this study were to explore the effects of delay in treatment intensification, and of remaining under poor glycaemic control on long term risk of cardiovascular events (CVEs).
Materials and methods: A cohort of 110,543 patients with diagnosis of T2DM between May 1990 and January 2010 were selected for this study under the conditions: complete measures of age, sex, smoking status and HbA_{1c} at the time of diagnosis, minimum two years of follow-up before death or censoring, and completeness of dates for prescriptions. The longitudinal data on HbA_{1c} were organised on a 6-monthly window. Treatment intensification was defined under two conditions: (i) taking at least 2 OADs simultaneously (OAD2), and (ii) taking at least 1 OAD plus insulin simultaneously (OAD+INS). Time to initiation of OAD2 and OAD+INS was calculated from the date of diagnosis. The effects of the delay in treatment intensification in interaction with glycaemic control (HbA_{1c} below or above 7%) on the risks of myocardial infraction (MI), stroke, heart failure (HF) and composite of these CVEs were evaluated using Stratified Cox Regression Models, adjusted for age, sex, smoking status, and history of CV disease.
Results: Patients were 56% male and 19% smokers; mean(SD) of age, BMI and HbA_{1c} at diagnosis were 61 (13) yr, 32 (7) kg/m2, and 8.1 (2.2)% respectively; 12% had a history of CVE before diagnosis. During median (Q1, Q3) 5.3 (3.5, 7.7) years of follow-up, 2.3% had MI, 2.2% had stroke, 3.3% had HF, and 7% experienced at least one of these CVEs, 44% took OAD2, and 11% took OAD+INS. In patients with HbA_{1c} > 7% during 1 year post diagnosis (29%), at least 31% did not receive any intensified treatment within 1 year. In patients with HbA_{1c} above 7% during 1 year post diagnosis, a 6-month delay in treatment intensification by OAD2 / OAD+INS regimen would significantly increase the risks of MI, stroke, HF and composite CV events by 26% / 26%, 15% / 15%, 24% / 25% and 20% / 21% respectively (Table). Patients with HbA_{1c} > 7% during 1 year post diagnosis had 25% increased risk of CVEs (Hazard Ratio: 1.25, 95% CI: 1.19-1.31, p<0.001). Potential limitations of this analysis include lack of information on medication adherence and residual confounding.
Conclusion: This primary care data based retrospective cohort study with more than 5 years of follow up clearly suggests the cardiovascular benefits of early treatment intensification in patients with poor glycaemic control.

CVE HR (CI) associated with delay in treatment intensification in patients with poor glucose control

	MI HR(95% CI)	Stroke HR(95% CI)	Heart Failure HR(95% CI)	Any CVD HR(95% CI)
6-month delay in Intensification to OAD2	1.26 (1.13, 1.40)	1.15 (1.03, 1.29)	1.24 (1.12, 1.36)	1.20 (1.13, 1.28)
6-month delay in Intensification to OAD+INS	1.26 (1.13, 1.40)	1.15 (1.03, 1.28)	1.25 (1.14, 1.37)	1.21 (1.13, 1.29)

Supported by: Novo Nordisk A/S

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Secondary prevention in patients with type 2 diabetes and previous myocardial infarction in Dutch primary care

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Background and aims: Patients with type 2 diabetes and a previous myocardial infarction are at risk for future cardiovascular events; secondary prevention is of high importance. The aim of this study is to evaluate secondary prevention therapy in patients with T2DM and previous MI.
Materials and methods: Retrospective record study including anonymous data of 11,267 patients with T2DM from 56 general practices in the Netherlands. In 743 patients with T2DM and previous myocardial infarction, the proportion of patients with a prescription for an anti-thrombotic agent (acetylsalicylic acid, carbasalate calcium, clopidogrel or vitamin K antagonists), a beta-blocker and/or a statin was calculated. Furthermore the association between patient characteristics (i.e. age, gender, socio-economic status, duration of T2DM and time since MI) and prescription were determined by logistic regression analysis.
Results: Of all 743 patients, 78% had a prescription for an anti-thrombotic agent, 61% had a prescription for a beta blocker, 70% had a prescription for a statin and 46% had a prescription for all three types of drugs. Men were more likely to receive an anti-thrombotic agent (OR 1.57, 95% CI 1.11-2.23; p=0.01), a beta blocker (OR 1.37, 95% CI 1.01-1.84; p=0.04) and the combination of all three (OR 1.50, 95% CI 1.10-2.04; p=0.01). Older people were less likely to receive a statin (OR 0.98, 95% CI 0.97-1.00; p=0.03) and the combination of all three (OR 0.99, 95% CI 0.97-1.00; p=0.05). We found no significant independent association between socio-economic status, duration of T2DM and time since MI and the prescription of an anti-thrombotic agent, a beta blocker, a statin and all three types of drugs.
Conclusion: A substantial proportion of patients with T2DM and previous MI were insufficiently treated for secondary prevention. Our findings confirm the need for a different approach to increase the proportion of prescription in patients with T2DM and previous MI, with special attention to women.

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Significant impact of gender on the association of HbA_{1c} with angiographically diagnosed coronary atherosclerosis

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Background and aims: The association of HbA1c with angiographically determined coronary atherosclerosis is unclear. In particular, it has not been investigated so far whether gender modulates the association of HbA1c with angiographically diagnosed coronary atherosclerosis. We therefore aimed at clarifying this issue.
Materials and methods: We enrolled a large consecutive series of 1449 patients, 484 women and 965 men, who did not have previously known diabetes and who underwent coronary angiography for the evaluation of stable coronary artery disease. Significant coronary atherosclerosis was diagnosed in the presence of significant coronary stenoses with lumen narrowing ≥ 50%.
Results: HbA1c values of <5.7% (normal according to ADA criteria), 5.7-6.4% (at risk of diabetes according to ADA criteria), and ≥ 6.5% (diabetes according to ADA criteria) were found in 36.4%, 56.2%, and 7.4% of women and in 44.2%, 46.6%, and 9.1% of men, respectively. The prevalence of angiographically diagnosed coronary atherosclerosis in these HbA1c categories was 31.2%, 38.2%, and 47.2% among women (ptrend=0.041) and 63.2%, 65.3% and 64.8% among men (ptrend=0.589). In logistic regression models, HbA1c as a continuous variable was a strong predictor of coronary atherosclerosis among women (adjusted OR for a 1% increase in HbA1c 1.61 [95% CI 1.07-2.43]; p=0.024) but not among men (OR 0.92 [0.74-1.13]; p = 0.416). The interaction HbA1c by gender was significant (p=0.022), indicating that HbA1c was a significantly stronger predictor of coronary atherosclerosis among women than among men.
Conclusion: We conclude that gender has a significant impact on the association of HbA1c with angiographically diagnosed coronary atherosclerosis among subjects without previously known diabetes.

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Gender differences in cardiovascular events, cardiovascular and all-cause mortality in type 2 diabetes: lesson from the San Luigi Gonzaga Diabetes study

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Background and aims: Whether type 2 diabetes attenuates or abrogates the cardiovascular (CV) protection conferred by female gender in the general population is a matter of debate. Therefore, we aimed to evaluate the gender differences in CV events, CV and all-cause mortality in an Italian cohort of type 2 diabetic patients.
Materials and methods: We investigated 786 type 2 diabetic patients (362 women and 424 men), belonging to the prospective study "San Luigi Gonzaga Diabetes Study", which in 1995 consecutively enrolled 786 type 2 diabetic patients followed-up at our clinic. At the enrolment, gender, age, known diabetes duration, body mass index (BMI), smoking habit, systolic and diastolic blood pressure (SBP and DBP), serum creatinine, albumin excretion rate (AER), total and HDL cholesterol, triglycerides and HbA_{1c} were assessed. Outcomes were all-cause mortality, CV mortality and the first CV event occurring during the 14-year follow-up. Univariate statistical analysis was carried out with Student's t-test for unpaired data and multivariate statistical analysis with the Cox proportional hazard model (backward method).
Results: At baseline women and men differed for age (64.03±10.04 vs 60.85±9.22 years, p=0.0001), known diabetes duration (11.22±8.77 vs 8.9±7.47 years, p=0.0001), BMI (29.54±5.01 vs 28.74±4.88 Kg/m2, p=0.025), smoking habit (previous/present) (18% vs 71%, p=0.0001), SBP (148.09±18.7 vs 145.32±17.92 mmHg, p=0.034), serum creatinine (0.79±0.24 vs 0.97±0.30 mg/dl, p=0.0001), total cholesterol (226.0±42.0 vs 212±42 mg/dl, p=0.0001), HDL cholesterol (54.0±16.0 vs 46.0±14.0 mg/dl, p=0.0001), HbA_{1c} (7.96±1.54 vs 7.62±1.26%, p=0.001). During the 14 year follow-up: a) 273 patients (W/M: 124/149) died; b) 88 (W/M: 35/53) died as a direct consequence of a CV event (defined "CV mortality"); b) 313 (W/M: 121/192) presented a first CV event (acute myocardial infarction, unstable angina, stroke, transient ischemic attack, ischemia-related lower limb amputation, sudden death and revascularization at any site). HRs for outcomes were evaluated by building three models: Model A, including gender, age and known diabetes duration; Model B, as model A + BMI, SBP, serum creatinine, total cholesterol, HDL cholesterol, HbA_{1c}; Model C: as model B + smoking habit. The HRs conferred by female gender were: a) for all cause mortality, in Model A: 0.691 (CI 0.540-0.884, p=0.003), in Model B: 0.707 (CI 0.548-0.912, p=0.008), in Model C: 0.851 (CI 0.620-1.168, p=0.318); b) for CV mortality, in Model A: 0.570 (CI 0.366-0.885, p=0.012), in Model B: 0.614 (CI 0.389-0.970, p=0.036), in Model C: 0.614 (CI 0.389-0.970, p=0.036); c) for the first CV event, in Model A: 0.589 (CI 0.466-0.744, p=0.0001), in Model B: 0.537 (CI 0.423-0.681, p=0.0001); in Model C: 0.672 (CI 0.508-0.890, p=0.005). Beside gender and age, significant predictors in Model C were: a) for all-cause mortality, AER (p=0.0001), HbA_{1c} (p=0.0001) and smoking habit (p=0.051); b) for CV mortality: AER (p=0.0001) and HbA_{1c} (p=0.002); c) for CV events: HbA_{1c} (p=0.0001), smoking habit (p=0.003), total cholesterol (p= 0.013) and SBP (p=0.037).
Conclusion: Even in the presence of type 2 diabetes, female gender confers a protection against CV events and CV mortality.

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Evidence for sex-specific effect of serum total adiponectin on cardiovascular death in patients with type 2 diabetes mellitus

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Background and aims: Cardiovascular disease is the first cause of death in patients with type 2 diabetes mellitus (T2DM). Unraveling new pathogenic factors involved in cardiovascular (CV)-death in diabetic patients is, therefore, urgently needed. Adiponectin has insulin-sensitizing, anti-inflammatory, and antiatherogenic effects. No data are available, so far, on the relationship between serum adiponectin and CV-death in T2DM. Our aim was to address this issue in several cohorts of diabetic patients of European ancestry.
Materials and methods: Three prospective studies of patients with T2DM, were investigated. 1) The Gargano Heart Study (GHS-prospective design; 359 patients; 7 year follow-up). 2) The Nurses' Health Study (NHS; 722 female patients, 20 year follow-up). 3) The Health Professional Follow-up Study (HPFS; 751 male patients, 16 year follow-up). In all studies the end point was CV-death.
Results: In the GHS-prospective design total serum adiponectin predicted CV-death in a model comprising age, sex, smoking habit, BMI, HbA1c, insulin therapy, hypertension, total cholesterol, HDL-cholesterol and triglycerides: HR per SD increment = 1.44, 95% CI: 1.14-1.82. This association was evident and significant among males (n = 242, HR = 1.58, 95% CI: 1.21-2.05), but not females (n = 117, HR = 0.84 , 95%CI 0.43-1.66), thus pointing to a sex-specific effect of adiponectin on CV-death. Total adiponectin levels were significantly associated with CV-death in HPFS males (HR = 1.43, 95%CI 1.20-1.71), but not NHS females (HR = 1.00, 95%CI 0.80-1.25). In a pooled analysis of all studies, a clear sex-specific association was observed: cumulative adjusted HRs being 1.50 (95%CI 1.27-1.71) in 993 males and 0.98 (95%CI 0.79-1.22) in 839 females (p for HRs heterogeneity=0.009).
Conclusion: To the best of our knowledge, this is the first report of a sex-specific counterintuitive deleterious effect of high serum adiponectin on increased CV-death in patients with T2DM of European ancestry.
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Risk stratification with plasma-NT-proBNP and coronary calcium score predicts all-cause mortality in type 2 diabetic patients

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Background: The burden of coronary artery disease (CAD) is significantly increased in type 2 diabetic patients and is associated with mortality. An effective screening tool for subclinical CAD is needed to predict and prevent cardiovascular mortality in these patients.
Methods: Plasma-NT-proBNP, Agatston coronary calcium score (CCS) and echocardiography were performed in 200 asymptomatic type 2 diabetic patients with elevated urinary albumin excretion rate (>30mg/24 h) and without prior history of CAD. Patients with P-NT-proBNP >45.2 ng/L and/or CCS ≥400 at baseline were stratified as high risk patients for CAD and were further examined for significant CAD by myocardial perfusion imaging and/or CT-angiography and/or coronary angiography. Following these investigations, patients were stratified into 3 groups, low risk (n=67), high risk without CAD (n=63) and high risk with CAD (n=70) and were followed prospectively. After 5.3 years of follow-up, vital status was assessed in all subjects, and echocardiography was re-performed in available patients (n=130) (65%).
Results: At baseline, patients were 59±9 years, 152(76%) male, with eGFR: 96±26 ml/min/1.73m^2 and diabetes duration of 13±7 years. Of 130 patients with follow-up echo-data available, 117 had normal (≥50%) left ventricular ejection fraction (LVEF) at baseline. Of these, 2(5%) and 9(12%) patients with low *vs.* high risk had reduced LVEF (<50%) at follow-up (p=0.324). During follow-up, 22(11%) patients died from all-causes, of which 1(1%) low risk, 9(14%) high risk without CAD and 12(17%) high risk with CAD (log rank p=0.012). In Cox regression analysis comparing low risk *vs.* high risk no CAD patients, the latter had significantly higher mortality (p=0.041), as was the case for low risk *vs.* high risk CAD patients (p=0.017). In Cox regression analysis comparing low risk *vs.* all high risk, the latter had significantly higher mortality (HR: 8.7, p=0.047, adjusted for gender, age, HbA1c, cholesterol, systolic blood pressure, smoking and creatinine). However, comparing the two high risk groups, mortality was similar (p=0.5).
Conclusion: Risk stratification with P-NT-proBNP and CCS predicts all-cause mortality in asymptomatic type 2 diabetes patients with microalbuminuria and normal kidney function. Additional cardiovascular evaluation did not improve risk prediction. Deterioration from normal to impaired LVEF was not significantly different between groups although this may be a power issue.

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Stress cardiac magnetic resonance imaging reveals hypoperfusion in asymptomatic type 1 diabetes patients, missed by the routine non-invasive evaluation

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Background and aims: Stress myocardial perfusion cardiac magnetic resonance (stress CMR), by measuring myocardial perfusion rate index provides a reliable index to assess myocardial hypoperfusion. We tested the hypothesis that stress CMR can detect silent ischemia in asymptomatic type 1 diabetes (T1DM) patients with normal routine non-invasive cardiac profile and secondly we tried to clarify correlations of CMR indexes with other clinical and laboratory findings.
Materials and methods: A cohort of 20 (10F/10M) asymptomatic T1DM aged 33±15 yrs without history of coronary artery disease or heart failure and

with normal routine nonivasive cardiac assessment - including 12-lead ECG, echo study and exercise testing - were studied. CMR data were correlated with clinical and laboratory profile of T1DM and were compared with those of age and sex-matched controls. Stress CMR was performed in 1.5T using 140 mg/kg/min adenosine for 4 minutes based on international established protocols. All measurements were expressed as mean±SD. Statistical significance of the differences was investigated using unpaired Student's T-test. Correlation between variables was sought with Pearson's correlation coefficient. Statistical significance was considered for $p<0.05$.

Results: No difference in left ventricular (LV) volumes and ejection fraction was identified between T1DM and controls. However, MPRI was significantly lower in T1DM compared to controls (0.82±0.3 vs 3,00±0.2, $p<0.001$). A negative correlation was identified between MPRI and patients' age ($p<0.05$). Reversely, no correlation was found between MPRI and disease duration, HbA1c or other metabolic parameters. Left ventricular diastolic dysfunction, albuminuria and diabetic retinopathy were present at the 10%, 35% and 20% of study population respectively. MPRI was severely impaired in all of them, representing the earliest abnormal cardiac finding in T1DM patients. The quantitative analysis revealed regional impairment of myocardial blood flow in all T1DM patients, although late gadolinium enhanced images were normal. X-Ray coronary angiography, available in the majority of subjects, was normal; therefore, abnormal MPRI was attributed to microcirculation disease.

Conclusion: MPRI detects perfusion impairment in asymptomatic T1DM, missed by the usual non-invasive evaluation, irrespectively of disease duration and represents the earliest abnormal parameter in asymptomatic T1DM, before the development of diastolic dysfunction, albuminuria and/or retinopathy. However, the clinical implications of these findings need further evaluation in larger patients' cohorts.

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Impaired left ventricular diastolic function and increased systolic contractions in type 2 diabetes compared to healthy controls: a case-control study

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Background and aims: Left ventricular (LV) myocardial dysfunction is prevalent in type 2 diabetes (T2D). We used advanced echocardiographic imaging techniques to compare myocardial systolic and diastolic function in T2D with matched healthy controls (CTRs).

Materials and methods: This case-control study involved a clinical examination and a detailed echocardiographic assessment, incl. tissue doppler imaging (TDI) in patients with T2D. CTRs matched for age, gender, weight and systolic blood pressure were obtained from a population based health survey (HUNT). Myocardial tissue velocity and deformation were obtained off-line from stored loops of colour tissue Doppler. Diastolic function was evaluated from the conventional Doppler variables E/A-ratio, deceleration time (DT) and mitral doppler early (MVE) and late (MVA) diastolic velocities, in addition to the longitudinal myocardial velocities in early (e') and late (a') diastole. Systolic function was assessed from biplane ejection fraction (EF) and peak systolic longitudinal strain and strainrate utilizing data from 2 apical LV imaging views.

Results: One hundred patients with T2D (29% females, age/diabetes duration (mean±SD) 58±10/6±6 yrs, BMI 30.1±5.5 kg/m², hypertension 68 %, coronary artery disease 12%, self-reported dyspnoe 53%, HbA1c 7.6±1.6%, LDL 2.9±0.9 mmol/L, blood pressure 141±18/83±9 mmHg), and 100 matched healthy CTRs were included. Patients with T2D had higher resting heart rate (72±12 vs 67±10 bpm; $p=0.001$) and diastolic blood pressure (83±9 vs 79±8 mmHg; $p=0.005$). LV cavity diameters were similar between the groups while interventricular septum was thicker in T2D (1.1±0.2 vs 1.0±0.2 cm, $p<0.001$). Diastolic function differed adversely in T2D with significantly lower E/A-ratio, deceleration time and MVA, as well as reduced e' and a' (Table). E/e', a marker of LV end diastolic filling pressure, was also higher in T2D (12.1±4.5 vs. 10.4±3.3, $p=0.001$). Patients with T2D had evidence for increased contractions with higher EF, longitudinal strain, and strain rate.

Conclusion: We observed that T2D was characterized by *impaired* LV diastolic function combined with *augmented LV myocardial contractions*. This finding supports that the acknowledged subclinical LV diastolic dysfunction in T2D is in part compensated by a raised contractile state.

Echocardiographic findings in T2D and CTRs. Data given as mean±SD, p obtained by T-tests.

	Type 2 diabetes (n=100)	Controls (n=100)	p
Diastolic parameters			
MVA (m/s)	0.71±0.13	0.62±0.18	<0.001
E/A-ratio	0.91±0.27	1.12±0.38	<0.001
Deceleration time (ms)	195±49	242±72	<0.001
e' (cm/s) *	5.7±2.0	6.6±1.8	0.001
a' (cm/s)*	6.5±2.0	7.5±1.5	<0.001
Systolic parameters			
EF (%)	62.8±7.7	55.2±7.6	<0.001
Peak systolic strain (%)**	16.4±3.9	15.6±2.4	0.065
Peak systolic strain rate (1/s)**	1.22±0.32	1.04±0.21	<0.001

*= mean of 2 measures: basal septal, basal lateral (4 chamber view)
**= mean of 8 measures: basal and mid septal, basal and mid lateral, basal and mid inferior and basal and mid anterior (4 and 2 chamber view).Abbreviations: T2D= type 2 diabetes, CTR= controls, MVA=late mitral doppler flow velocity, e'= early diastolic myocardial velocity, a'= late diastolic myocardial velocity, EF= ejection fraction

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Diabetes and the spectrum of hyperglycaemia adversely affects left ventricular diastolic function and is particularly toxic to South Asians

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Background and aims: Diabetes increases heart failure risk, via mechanisms that are currently unclear. Study of risk factor associations with subclinical disease can be informative. Diastolic dysfunction precedes the development of heart failure (HF) and predicts increased mortality. South Asians (SA) and African Caribbeans (AC) may be at particular risk due to increased prevalence of diabetes. We compared LV diastolic function in a community-based cohort of older SA, AC and White Europeans (EU) and investigated the role of established cardiovascular risk factors in ethnic differences.

Materials and methods: A population based sample of 1295 individuals (609 EU, 477 SA and 209 AC), age 69.6±6yrs (mean±SD) underwent conventional and Tissue Doppler echocardiography. Peak velocity of early diastolic relaxation (e') was used as a relatively, load-independent indicator of LV diastolic function. Subjects underwent CT to image coronary calcium (CAC), had detailed fasting blood tests, a spot urine test and blood pressure (BP) measurements. Hypertension was physician diagnosed or participant reported accompanied by BP lowering medication. Diabetes was defined according to the WHO 1999 guidelines. BP and echocardiography measurements were used to calculate total arterial compliance (TAC) and total peripheral resistance (TPR).

Results: e' was consistently poorer with advancing glucose intolerance in all ethnic groups. People with diabetes had lower e' than normoglycaemic individuals by 0.43 cm/s in EU ($p=0.04$), 0.73 cm/s in SA ($p<0.0001$) and 0.24 cm/s in AC ($p=0.3$). This adverse glycaemic effect extended into the normal range. e' was significantly lower for a unit increase in HbA_{1c} in SA and AC (Table, model 1). The association between HbA_{1c} and e' was significantly different between EU and SA (p ethnicity x HbA_{1c} interaction = 0.008). After adjustment for key cardiovascular risk factors (model 2) this ethnicity/HbA_{1c} interaction persisted ($p=0.001$) and a significant ethnicity x HbA_{1c} interaction was found between EU and AC ($p=0.04$). Additional adjustment for haemodynamic variables (Model 3) did not explain these interactions (EU and SA ($p=0.001$),EU and AC ($p=0.03$)).

Conclusion: LV diastolic function is poorer in people with diabetes, but is also adversely affected across the hyperglycaemic spectrum. This adverse association could not be accounted for by associated cardiovascular/haemodynamic risk factors, or prevalent co-morbidity. Moreover the adverse effects of

hyperglycaemia on diastolic function are more prominent in SA and AC than EU. Explanations for the effect of hyperglycaemia on LV function, and the greater susceptibility of SA and AC to this effect, need to be sought if we are to combat the increasing burden of heart failure in association with diabetes.

Change in Diastolic function (e') for a unit change in HbA_{1c}

	European		South Asian			African Caribbean		
	β±SE	p value	β±SE	p value	Interaction	β±SE	p value	Interaction
Model 1	0.3±0.7	0.7	-2.0±0.5	<0.0001	0.008	-1.5±0.7	0.03	0.1
Model 2	1.0±0.8	0.2	-1.3±0.5	0.01	0.001	-0.9±0.7	0.3	0.04
Model 3	1.2±0.8	0.1	-1.0±0.5	0.06	0.001	-1.1±0.7	0.1	0.03

Data are β±SE. Model 1: Age, sex. Model 2: Model 1+ hypertension, microalbuminuria, estimated glomerular filtration rate, coronary artery disease, smoking, HOMA-IR, total:HDL cholesterol and LV mass. Model 3: Model 2 + total arterial compliance, total peripheral resistance and heart rate.

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Comparison of left ventricular filling pressures in male patients with type 2 diabetes with normal and low testosterone levels

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Background and aims: Heart failure is a complication of patients with type 2 diabetes frequently left ventricular filling pressures are increased before symptoms appear. Male patients with type 2 diabetes have low total testosterone in 30% of cases. The relationship between low testosterone levels in male diabetic patients and ventricular filling pressures is still unknown. Aims: to compare in male patients with type 2 diabetes the left ventricular filling pressures in those with normal and low total testosterone levels.
Materials and methods: We assessed diabetic patients with tissue Doppler imaging for evaluation of left ventricular filling pressures by passive transmitral left ventricular inflow velocity to tissue Doppler imaging velocity of the lateral mitral annulus during passive filling (E/e' ratio). Patients with history of heart failure, myocardiopathies, left ventricular hypertrophy, diastolic dysfunction (E/e´>15) and arrhytmias were excluded. We compared them depending on low (< 3,5 ng/ml) or normal (> 3,5 ng/ml) total testosterone levels. All patients were asymptomatic and without history of cardiovascular disease. We report preliminary analysis. Data were analyzed using Spearman correlation coefficient, Mann Whitney U test and test. Odds ratio [OR] and 95% confidence intervals (CI) were calculated using simple and multiple logistic regression
Results: 148 male diabetic patients were included. Mean age was 58±5,8 years, mean time of diabetes duration 7±3,1 years, 47(32 %) patients have low Testosterone (Group A). There was no difference between groups regarding age, time of diabetes evolution, hypertension, weight, heart rate, BMI or echocardiographic parameters .The E/e' ratio in group A was 8,05±1,9 vs. group B 6,1±1,7 p < 0,0001. Group A compared with group B, had six fold increased risk of E/e' ratio >5 (91.67% vs. 64.56%; OR=6.04 CI=1.69-21.47; p=0.005). Differences remained significant after adjust for age, time of diabetes evolution, BMI, lipids, HbA1c and drug treatment.
Conclusion: Patients with type 2 diabetes and low total testosterone levels have higher E/e' ratio, showing pre clinic increased left ventricular filling pressures compared with diabetic patients with normal testosterone levels. This finding is independent of time of diabetes evolution, hypertension, or other echocardiographic parameters.

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Advanced glycation endproducts are associated with higher pulse wave velocity and 24-hour pulse pressure: preliminary results from the Maastricht study

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Background and aims: Advanced glycation endproducts (AGEs) are thought to play a role in the development of cardiovascular disease (CVD). Certain AGEs, like pentosidine, form cross-links between proteins within the arterial wall, which may lead to arterial stiffness, and subsequently, an increased CVD risk. Therefore, we evaluated the association of skin autofluorescence (SAF), as a possible reflection of tissue AGEs, and plasma AGEs with arterial stiffness (AS) in individuals with normal (NGM) or impaired (IGM) glucose metabolism or type 2 diabetes mellitus (T2DM).
Materials and methods: We studied a cohort of 740 individuals from The Maastricht Study (413 NGM, 124 IGM and 203 T2DM) with mean age of 60y, 45% female. SAF was measured with the AGE reader (DiagnOptics, Groningen). Plasma levels of protein-bound pentosidine, Nε-(carboxymethyl)lysine (CML) and Nε-(carboxyethyl)lysine (CEL) were measured with HPLC or UPLC and fluorescence detection or MS/MS, respectively. Carotid to femoral pulse wave velocity (cfPWV) and 24-hour pulse pressure (aPP), as measures of AS, were measured with applanation tonometry (SphygmoCor, Atcor Medical, Australia) and ambulatory 24-hour BP monitoring (WatchBP O3, Microlife AG, Switzerland), respectively. aPP was calculated by subtracting 24-hour diastolic from systolic BP. All associations of AGEs with AS were analysed with linear regression analysis, and adjusted for age, sex, glucose metabolism status, waist, smoking, use of anti-hypertensive medication, MAP, eGFR, total-to-HDL-cholesterol ratio and triglycerides. The associations of SAF and AGEs with PWV were additionally adjusted for heart rate.
Results: Our preliminary results show that with deteriorating levels of glucose metabolism, mean SAF (AU) (2.56 vs. 2.70 vs. 2.94), CEL levels (nmol/mmol LYS) (32.9 vs. 34.1 vs. 35.9), PWV (m/s) (8.4 vs. 9.3 vs. 9.9) and aPP (mmHg) (42 vs. 47 vs. 50) were higher, and plasma CML levels (nmol/mmol LYS) (77.6 vs. 72.0 vs. 69.3) were lower (p<0.01). Higher SAF and plasma pentosidine levels were associated with higher cfPWV (table); these associations were different in individuals with T2DM (sβ 0.11 for SAF and 0.12 for pentosidine) compared to NGM (sβ 0.04 and 0.05, respectively) (p for interaction 0.02 and 0.01, respectively). In addition, SAF (borderline), plasma pentosidine and CML levels were associated with higher aPP (table).
Conclusion: These preliminary results support the hypothesis that AGEs, in particular the cross-linking AGE pentosidine, are involved in the development of AS.

	cfPWV			aPP		
	sβ	95%-CI	p	sβ	95%-CI	p
SAF	0.09	0.02-0.16	0.01	0.06	-0.003-0.13	0.06
Plasma pentosidine	0.08	0.02-0.14	0.01	0.09	0.03-0.16	<0.01
Plasma CML	0.01	-0.05-0.08	0.68	0.09	0.02-0.15	<0.01
Plasma CEL	0.01	-0.05-0.07	0.79	0.03	-0.03-0.09	0.26

Standardized Bèta (sβ) indicates the change in cfPWV or aPP (in SD) per 1 SD higher SAF or AGE level
Supported by: CTMM, the NHF, DDRF and DKF. PREDICCt.

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Postprandial haemodynamic responses are altered in uncomplicated type 2 diabetes

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Background and aims: While postprandial changes in haemodynamic variables have been well described in healthy subjects, the effects in subjects with type 2 diabetes (T2DM) or those with metabolic syndrome (MetS) remain

largely unknown. The aim of this study was to identify the effects of a standardised mixed meal on haemodynamics in a cohort of controls and subjects with MetS or T2DM without autonomic neuropathy (defined by normal Ewing-tests). In addition, the role of autonomic nervous system (ANS) balance was investigated.
Materials and methods: After an overnight fast, a standardised high-fat mixed meal (900 kcal; 75g carbohydrates, 50g fat (60% saturated), 35g protein) was given to 44 age-matched males (15 controls; 12 MetS; 17 T2DM, total mean age 55.4 yrs). Blood pressure was continuously monitored using digital finger photoplethysmography (Portapres). From the pulse wave recordings, systolic (SBP) and diastolic (DBP) blood pressure, heart rate (HR), stroke volume (SV), cardiac index (CI) and total peripheral resistance (TPR) were derived using dedicated software (Beatscope). The beat-to-beat intervals were analysed using spectral analysis to calculate ANS or sympathovagal balance (LF/HF-ratio) from low frequency (LF, sympathetic) and high frequency (HF, parasympathetic) domains. Glucose and insulin levels were measured prior to and 2 hours after the meal.
Results: Subjects with T2DM and MetS had higher BMI, baseline SBP/DBP and fasting insulin levels compared to controls, whereas subjects with T2DM had higher fasting plasma glucose and HbA1c compared to controls and MetS ($p \leq 0.001$). At baseline, 30% of T2DM and 50% of MetS had BP >140/90 mmHg, compatible with hypertension, but no individuals received antihypertensive drugs. As expected, T2DM vs controls was associated with elevated postprandial levels of glucose and insulin, whereas no differences were seen between T2DM and MetS. All groups showed a comparable significant increase in HR following the meal compared to baseline ($p \leq 0.05$). T2DM patients demonstrated significant postprandial decreases in DBP (from baseline $p=0.039$), which was accompanied by a significant decrease in TPR ($p=0.01$) and increase in SV and CI ($p=0.03$ and $p=0.011$, respectively). In MetS subjects only CI increased after the meal ($p=0.041$), while TPR and SBP/DBP remained unaltered. Controls showed no changes in postprandial BP or other haemodynamic markers compared to baseline. T2DM showed a postprandial decrease in LF/HF-ratio ($p=0.022$), whereas MetS demonstrated a decrease in HF ($p=0.038$). No changes from baseline in postprandial ANS balance were seen in controls. No correlations were found between the AUCs (baseline to 2-hours postprandial) of any of the haemodynamic or ANS parameters, glucose and insulin.
Conclusion: Postprandial haemodynamic variables show more marked changes with deteriorating states of metabolic control. While all subjects experienced a meal-related increase in HR, only patients with uncomplicated T2DM, who had no clinical signs of autonomic dysfunction, demonstrated a fall in DBP and TPR, with concomitant increase in SV and CI. Additionally, our findings suggest that meal-induced changes in haemodynamic variables in uncomplicated T2DM are independent of postprandial metabolic and ANS responses.

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The co-existence of endothelial dysfunction and deteriorating glucose metabolism or insulin resistance synergistically increases incident CV event risk: the Hoorn study

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Background and aims: It has been suggested that endothelial dysfunction (ED) on the one hand and deteriorating glucose metabolism status and insulin resistance (IR) on the other act synergistically in the development of cardiovascular disease (CVD). Epidemiological evidence for this hypothesis, however, is scarce. We therefore investigated the interaction between ED and deteriorating glucose metabolism and IR on incident (non-)fatal cardiovascular (CV) events.
Materials and methods: In a population-based cohort (n=445, 69 years/51% women; 23% type 2 diabetes (DM2) and 28% impaired glucose metabolism (IGM) (by design)), IR was determined by the homeostasis model assessment (HOMA2-IR), and ED by flow-mediated dilatation (FMD). In addition, a morbidity and mortality registry was kept.
Results: After a follow-up of 7.6 (range 0.2; 8.9) years, 107 participants had had a CV event. After adjustment for age, sex and CVD risk factors, the results showed that FMD was associated with CV events in DM2 and IGM (HRs, per one SD smaller FMD, 1.65 [95%CI 1.10; 2.46]) and 1.42 [0.92; 2.19], respectively), but not in normal glucose metabolism (NGM) (HR 0.84 [0.62; 1.14]). Similarly, FMD was associated with incident CV events among those with IR (highest HOMA2-IR tertile; HR 1.86 [1.36; 2.53]), but not among those with normal insulin sensitivity (lower HOMA2-IR tertiles; HR 0.87 [0.62; 1.23] and 0.82 [0.57; 1.21], respectively). The interaction between FMD and DM2, IGM or IR was present on both a multiplicative (P-interaction <.05) as well as an additive scale (relative excess risk due to interaction >0). These results did not materially change when we mutually adjusted glucose metabolism status and IR for each other.
Conclusion: FMD was associated with incident CV events in individuals with DM2, IGM or IR, but not among individuals with NGM or normal insulin sensitivity. The present results support the hypothesis that ED on the one hand and deteriorating glucose metabolism and IR on the other act synergistically in the pathogenesis of CVD.

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Metabolic predictors of CVD in type 2 diabetes

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Background and aims: Our aim was to use a high-dimensional metabolomics platform to identify novel metabolites associated with and predictive of cardiovascular disease (CVD) in type 2 diabetes (T2D). Our aim was also to develop and apply new analyses methods appropriate for such high dimensional data.
Materials and methods: The study included baseline samples from T2D patients from Go-DARTS (n=1200), Scania Diabetes Registry (n=655), IMPROVE (n=44) and 60-year Old Stockholm Study (n=20) with incident MI or stroke (n=953 cases) or free of CVD at end of follow up (n=966 controls), matched for age and diabetes duration. Using the Metabolon mass spectrometry analysis platform UPLC-MS/MS and GC-MS, 612 metabolites were measured. Clinical factors included in the analysis were age, sex, diabetes duration, BMI, height, blood pressure, smoking, LDL, HDL, Triglycerides, HbA1c, eGFR, study centre, and medication (including anti-hypertensives, aspirin, lipid-lowering agents and insulin use). Forward selection using logistic regression, and sparse classification using L1-regularized approaches were applied and evaluated by using 50/10 nested cross-validation to select non-redundant sets of predictive biomarkers. We report those metabolites that were consistently selected by these models as being predictive of CVD. Descriptive statistics were produced by logistic regression using the identified biomarker panel.
Results: When used for predictions of CVD, the best metabolomics panel resulted in the moderate increment in the area under the ROC curve on test data from 0.67 for the clinical model to 0.70. Of 612 metabolites, 9 metabolites were consistently selected by all the models as independent predictors. The most strongly and consistently associated metabolites were L-histidine ($p<1e-5$, OR=0.77 [0.69, 0.86]) and Tryptophyl-Asparagine ($p<1e-4$, OR=1.33 [1.17, 1.52]). We also identified several metabolite CVD associations where further structural characterization of the metabolite signal is required.
Conclusion: Overall the addition of this metabolite panel to clinical factors produces a modest improvement in the prediction of CVD. However we identified several associations, some novel, of metabolites with CVD, though further validation of this work is needed. Whilst the overall improvement in prediction due to the metabolite panel is limited understanding the mechanism of these associations may lead to new insights into the pathogenesis of CVD in T2D and thereby target discovery.
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PS 119 Diabetes in childhood

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DKA at diagnosis of type 1 diabetes predicts poor long-term glycaemic control

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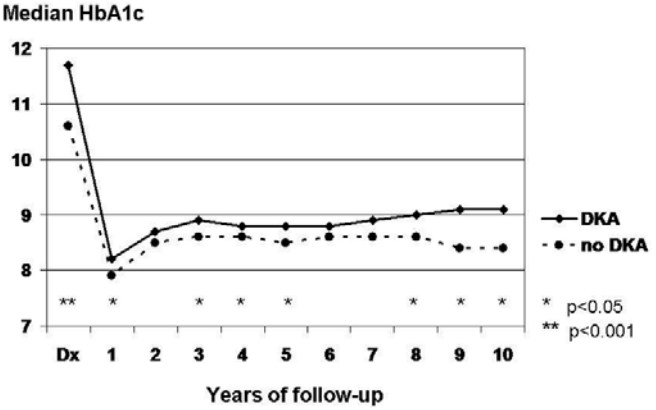
Background and aims: The diagnosis of type 1 diabetes in children is often associated with diabetic ketoacidosis (DKA). We tested the hypothesis that DKA at diagnosis is an independent predictor of poor glycemic control later in the course of the disease.
Materials and methods: A cohort of 631 Colorado residents diagnosed with type 1 diabetes before the age of 18 yrs, in 1998-2001, was followed for up to 14 years. Of those, 224 (35%) had DKA (venous pH<7.3 or bicarbonate<15mEq/l) at diagnosis. HbA1c was measured using the DCA 2000 Analyzer at each visit (3.4 visits/yr, on average; median 24 visits/patient). Individual average HbA1c was calculated annually, starting 60 days after the diagnosis. Medians were compared using Wilcoxon rank sum test (Figure). Multiple linear regression was used to evaluate DKA at diagnosis as an independent predictor of long-term HbA1c levels.
Results: Longitudinal HbA1c levels tracked significantly higher in children diagnosed in DKA, compared to those with a less severe presentation (Figure). DKA at diagnosis was a significant (p<0.01) predictor of long-term higher HbA1c levels, independent of the age at diagnosis, sex, ethnicity and insurance status (none, Medicaid, or private) and controlling for the duration of follow-up. The difference in HbA1c between the DKA vs. no-DKA groups appeared to increase over time, from initially 0.3% to 0.7% at 10 years of follow-up.
Conclusion: DKA at diagnosis of type 1 diabetes in children predicts chronic worse glycemic control, independent of demographic factors and access to diabetes care. Lower residual insulin secretion in children presenting with DKA may be at fault and could be preventable with earlier diagnosis.

Figure. Presence of DKA at diagnosis (Dx) of T1D in children predicts worse long-term glycemic control.



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The effect of treat-to-target insulin regimen on glycaemic control in overweight/obese type 1 diabetes patients

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Background and aims: The rising prevalence of childhood obesity has led to a parallel increase in the prevalence of overweight and obesity in patients with type 1 diabetes (T1D). The prevailing obesity-related insulin resistance leads to increased insulin requirements but poor glycemic control. There is no consensus on the best therapeutic regimen for the management of overweight or obese patients with T1D.
Objective: To determine the effect of treat-to-target insulin protocol on glycemic control, body mass index (BMI), waist circumference (WC), total daily dose (TDD) of insulin and hypoglycemia in overweight/obese T1D patients.
Materials and methods: A 3-month prospective trial of self directed treat-to-target insulin regimen in 21 overweight/obese subjects (10 males) with T1D of ages 10-20 years (mean age 15.25 ± 2.5). Inclusion criteria were T1D > 12 mo, hemoglobin A1c (HbA1c) >8% but <14%, and BMI >85%. Exclusion criteria were pregnancy, systemic illnesses, medications that alter blood glucose levels and recent history of blood transfusions. All patients were on multiple daily injections as follows: once daily injection of insulin detemir at bedtime, and multiple injections of aspart insulin before meals. Participants were asked to maintain their fasting blood glucose level between 90-120 mg/dL (5.0-6.7 mmol/L) using a titration algorithm of 2-0-2 units of insulin to adjust their detemir dose every 4th day to maintain glycemia as shown in Table 1, as follows: increase detemir dose by 2 units if the average of the 3 preceding days' pre-breakfast fasting plasma glucose (FPG) is >120 mg/dL (>6.7 mmol/L); reduce detemir dose by 2 units if the average of 3 preceding days' FPG is <90 mg/dL (5 mmol/L), and make no changes to the detemir dose if the above 3-day average is between 90-120 mg/dL (5.0-6.7 mmol/L). Insulin to carbohydrate ratios and correction factors were adjusted as in routine care. Anthropometric data were collected at enrollment and at 3 months, while TDD of insulin and hypoglycemia records were collected daily.
Results: There was no difference in mean HbA1c levels between the preceding 3mo and at baseline (9.21±1.71 vs. 9.82 ± 1.96, p=0.1). However, there was a significant reduction in HbA1c at the end of the study (9.82 ± 1.96 vs. 8.84 ± 1.15, p=0.005), and a significant increase in the TDD of insulin (71.52 ± 28.89 vs. 99.95 ± 44.49 IU/day, p<0.001), but no change in the mean BMI SDS (1.74 ± 0.43 vs. 1.66 ± 0.49, p = 0.19), weight SDS (1.66 ± 0.73 vs. 1.61. ± 0.77), and WC (93.44 ± 14.48 vs. 92.74 ± 15.0, p=0.38). No severe hypoglycemic events were reported.
Conclusion: Treat-to-target insulin regimen effectively reduced HbA1c level in overweight/obese patients with T1D. There was a significant increase in the TDD of insulin, but no change in BMI and WC. Optimal glycemic control in overweight/obese patients with T1D may require frequent structured insulin dose titrations.

Titration algorithm for long-acting insulin analog - detemir	
Average fasting plasma glucose of 3 consecutive days	Recommended long-acting insulin dose adjustments
<90 mg/dL (5.0 mmol/L)	subtract 2 units from the total dose of detemir
90-120 mg/dl (5.0-6.7 mmol/L)	no adjustments
> 120 mg/dL (>6.7 mmol/L)	add 2 units to the total dose of detemir

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Study of circulating microRNAs in prepubertal obesity

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Background and aims: Since the presence of risk factors for metabolic disease in prepubertal children predicts the development in adulthood, it has been postulated that the prevention of obesity in children may reduce obesity-related diseases such as insulin resistance and type 2 diabetes (T2D). Thus, it is likely that the efforts thus far invested in the context of biomarker discovery for the diagnosis and clinical monitoring of obesity in children will experience major acceleration in the upcoming years, leading to a completely novel paradigm in the screening of chronic metabolic diseases. Circulating microRNAs (miRNAs) are valuable biomarkers of metabolic diseases and potential therapeutic targets in this field. In this study, we sought to define the circulating pattern of miRNAs in prepubertal obesity, identifying the miRNAs with the highest prediction power of overweight and the risk of T2D.
Materials and methods: The genome wide circulating miRNA profile was assessed in 10 boys (5 lean and 5 obese children). The most relevant miRNAs were cross-sectionally validated in 85 lean *versus* 40 obese children (63 boys and 62 girls), and prospectively validated in 45 children (23 boys and 22 girls, between age ~6 and ~10).
Results: The cross-sectional validation study disclosed that 15 specific circulating miRNAs were significantly deregulated in prepubertal obesity, includ-

ing the decreased miR-221 and miR-28-3p, and increased concentrations in plasma of miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, and miR-423-5p (all p<0.0001). Indeed, the circulating concentration of these miRNAs was significantly associated with body mass index and other measures of obesity such as percent fat mass, waist circumference, regional fat distribution, and with laboratory parameters such as HOMA-IR, high molecular weight adiponectin, C-reactive protein, and circulating lipids in concordance with anthropometrical associations. Plasma concentrations of 10 of these circulating miRNAs changed significantly and differently during the 3-year follow-up in children who increased or decreased their normalized weight.
Conclusion: This study provides the first evidence that circulating miRNAs are deregulated in prepubertal obese children. Thus, the very early detection of metabolic abnormalities in obese children, as inferred by their circulating miRNA profile, may be a promising strategy in predicting metabolic disturbances such as T2D.

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OGTT-modelled beta cell function and incretins in obese youth from normal to pre diabetes to overt type 2 diabetes

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Background and aims: We previously demonstrated, using the hyperglycemic clamp together with the hyperinsulinemic-euglycemic clamp, diminished β-cell function (BCF) in obese youth with impaired glucose tolerance (IGT) and type 2 diabetes (T2DM) with ~40% and 80% impairment respectively, compared with their normally glucose tolerant (NGT) peers. The aim of the present study was a) to examine whether parameters of βCF, modeled from a simple 2-hr glucose tolerance test (OGTT), reflect similar impairments of BCF in obese adolescents across the spectrum of glucose tolerance; and b) to examine incretin hormones and their relationship to BCF.
Materials and methods: In 255 obese adolescents with NGT (173), IGT (48) and T2DM (34) we employed mathematical modeling of C-peptide during a 75g 2-hr OGTT to assess β-cell glucose sensitivity (βCGS), rate sensitivity and oral glucose insulin sensitivity (OGIS). Body composition was assessed by DEXA, subcutaneous (SAT) and visceral adiposity (VAT) by computed tomography or magnetic resonance imaging at L_{4-5} intervertebral space. Incretin hormones [total glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP)] and glucagon concentrations were measured during the OGTT.
Results: Data are presented in the Table. Compared with NGT, βCGS was ~ 30% and 65% lower in IGT and T2DM respectively. Rate sensitivity was not different in IGT but was lower (~ 40%) in T2DM. OGIS was lower in IGT and T2DM and the significance remained after adjusting for BMI $\%^{tile}$ or VAT. Total insulin secretion was higher in IGT compared with NGT and T2DM. GLP-1 area under the curve (AUC) was higher in IGT compared with NGT with no differences in GIP AUC. Among the BCF parameters, βCGS correlated with GLP-1 AUC (r=-0.14, p=0.02) but not with GIP AUC. Glucagon AUC relative to glucose AUC increased significantly across the groups from NGT to IGT to T2DM. In a multiple regression analysis, age, race, sex, VAT, OGIS, βCGS and rate sensitivity explained 40% of the variance (R^2=0.40, p<0.001) in 2-hr glucose with βCGS (R^2=0.17, p<0.001), OGIS (R^2=0.15, p<0.001), rate sensitivity (R^2=0.07, p<0.001) and VAT (R^2=0.01, p<0.001) being significant independent contributors.
Conclusion: OGTT-modeled βCGS is a key determinant of glucose tolerance in obese youth and declines progressively across the spectrum of glucose tolerance from NGT to IGT to T2DM accompanied with escalating hyperglucagonemia. Obese youth with IGT who have normal rate sensitivity demonstrate an increase in total insulin output together with higher incretin response; however, against the backdrop of impaired βCGS, insulin secretion remains insufficient to compensate for their IGT. The inverse relationship between βCGS and GLP-1 may suggest an incretin-driven compensatory mechanism to improve insulin secretion.

	NGT (173)	IGT (48)	T2DM (34)	ANOVA, P
Age (yrs) Sex (male/female)	14.7±0.1 68/105	15.2±0.3 19/29	15.1±0.3 16/18	NS NS
Race (AA/CA/Bi) BMI (kg/m^2)	89/78/6 34.2±0.5	16/31/1 36.5±0.9	18/16/0 36.6±1.0	NS 0.01
BMI percentile % BF	97.4±0.2 42.2±0.5	98.4±0.4 44.8±1.0	99.0±0.5 42.4±1.3	0.003 NS
VAT (cm^2) Fasting glucose (mmol/L)	61.5±2.4 4.9±0.1	75.2±4.7 5.2±0.1	86.0±5.5 6.4±0.1	<0.001 <0.001
2-hour glucose (mmol/L) OGIS (ml·min^{-1}·m^{-2})	6.5±0.1 357.0±5.2	8.6±0.2 295.5±9.7	11.0±0.2 288.9±11.8	<0.001 <0.001
βCGS (pmol·min^{-1}·m^{-2} · mM^{-1})	177.8±7.7	125.0±14.7	64.3±17.4	<0.001
Rate sensitivity (pmol·m^{-2}·mM^{-1})	1788.2±100.2	1607.8±190.2	1094.5±225.9	0.02
Total insulin output (nmol·m^{-2})	58.1±1.7	71.5±3.3	57.0±3.9	0.001
GLP-1 AUC (pmol/L·min)	1609.1±78.9	1868.4±149.9	1793.3±178.1	0.03
GIP AUC (pg/ml·min) Glucagon AUC relative to glucose AUC (µg/ml·min)	20284.8±657.1 9850.8±414.1	20698.7±1247.5 12061.3±781.6	19274.6±1482.2 17934.5±972.6	NS <0.001

Mean±SEM. AA=African American; CA=Caucasian; Bi=biracial; % BF=percent body fat; NS=not significant

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The AGE methylglyoxal-derived hydroimidazolone and early signs of atherosclerosis in childhood diabetes

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Background and aims: Advanced protein glycation is an important mechanism for the development of late diabetic complications and atherosclerosis. Hydroimidazolones are the most abundant advanced glycation end products (AGEs) in human plasma, and methylglyoxal-derived hydroimidazolone (MG-H1) is a major contributor. Our aim was to investigate the relationship between MG-H1 and early signs of atherosclerosis in children and adolescents with type 1 diabetes compared to healthy controls.
Materials and methods: 314 diabetic patients aged 8-18 years, were compared to 118 healthy controls of similar age and gender. Serum MG-H1 was measured by immunoassay. The development of atherosclerosis was evaluated by measuring carotid intima-media thickness by ultrasound (cIMT), arterial stiffness using Young's modulus and inflammation assessed by C-reactive protein (CRP).
Results: Mean age of the diabetic patients was 13.8 (SD = 2.8) years, diabetes duration 5.5 (SD = 3.4) years and HbA1c 8.4 (SD = 1.2) %. MG-H1 was significantly increased in the diabetic group compared to controls, 155.3 (SD = 41.0) vs. 142.7 (SD = 35.1) U/ml, p = .002. The CRP levels were also significantly higher in the diabetic group 2.0 (SD = 3.9) vs. 0.9 (SD = 2.3) mg/l, p < .001. Diabetic patients had more frequently elevated cIMT than healthy control subjects, as 19.5% were above the 90th percentile of healthy control subjects, and 13.1% were above the 95th percentile (P < 0.001). Mean cIMT was significantly higher in diabetic boys compared to controls 0.46 (SD = 0.06) vs. 0.44 (SD = 0.05) mm, p = .04, but not in girls. There was no significant difference between the groups regarding Young's modulus. However, by multiple regression analysis we found a significant positive association between MG-H1 and both Young's modulus and CRP in the diabetes group.
Conclusion: Serum levels of methylglyoxal-derived hydroimidazolone are increased, and despite short duration of disease, MG-H1 is an independent risk factor for both arterial stiffness and low grade inflammation indicative of an accelerated early atherosclerotic process in diabetic children and adolescents.

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Microstructural abnormalities in white and gray matter in obese adolescents with and without type 2 diabetes

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Background and aims: Compared to the general population, people with type 2 diabetes (T2DM) are at increased risk of cognitive decline. Recent studies have shown brain atrophy in T2DM in both white and gray matter of the brain, even in the absence of dementia or macro-vascular complications. Gray matter abnormalities have been associated with obesity but changes in white matter were associated with duration of diabetes. Worryingly, these brain abnormalities may already occur in adolescents with T2DM. To date, reports have focused on specific anatomical regions and have not considered more diffuse cortical losses. Given the association of T2DM and obesity, we compared white and grey matter properties in adolescents with T2DM, obesity and normal weight.
Materials and methods: 15 adolescents with T2DM (> 6 months), 21 obese (age-specific BMI > 30) and 22 normal weight adolescents underwent MRI scanning on a 3T Philips Achieva MRI system with 8-channel phased array SENSE head coil. A sagittal T1-weighted sequence and diffusion MRI sequence employing echo planar imaging (64 slices with isotropic 2x2x2 mm3 voxel) were acquired in 61 gradient directions with a b value of 1500s/mm2. Structural T1 images were processed using SPM8 with VBM8 (Statistical Parametric Mapping, Welcome Department of Cognitive Neurology, London UK). Age and gender specific templates were generated using The Template-O-Matic toolbox. DTI images were analysed using FSL DTIFIT (FMRIB, Oxford UK) to create Fractional Ansiotrophy (FA) maps. Blood glucose was measured prior to scanning. Participants also completed the Child version of the Dutch Eating Behaviour Questionnaire (DEBQ-C).
Results: Normal weight adolescents showed greater gray matter volume in the caudate and putamen bilaterally compared to those with T2DM and greater gray matter volume in the right hippocampus, left putamen, left caudate and amygdala bilaterally than obese participants ($p<0.001$, extent>200voxels; FEW cluster level corrected). Scores on DEBQ-C emotional subscale were negatively associated with gray matter density in the left hippocampus (r=-0.33), left caudate (r=-0.32), left amygdala (r=-0.34) and right putamen (r=-0.34). There was also decreased FA between controls and T2DM participants ($p<0.05$, FSL Randomise non-parametric statistical tool). Adolescents with T2DM showed reduced FA in left corticospinal tract, left thalamic radiation, body and genu of corpus callosum, left fornix, left retrolenticular internal capsule, right anterior corona radiata, left uncinate, left IFOF and left anterior external capsule.
Conclusion: T2DM is linked to significant loss in both gray matter volume and integrity of several white matter tracts. Gray matter losses were in areas previously found to be involved in emotion and memory. Participant's structural losses were also linked to higher emotional eating, possibly reflecting early loss of function linked to these areas. Widespread white matter changes could indicate vulnerability to hastening cognitive decline in adolescent with T2DM.
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Brain responses to food cues in adolescents with and without type 2 diabetes

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Background and aims: Good diet management is integral to the successful management of type 2 diabetes (T2DM). Yet, adolescents with type 2 diabetes (T2DM) have difficulty following dietary recommendations and succumb to frequent overeating. Restrictive feeding practices, often imposed by concerned parents, may have a deleterious effect on children's abilities to self regulate their own food intake. In adults with T2DM, a restrictive diet may alter brain responses to food stimuli and this is related to dietary self-care. However, little is known about the psychological effects of diets imposed for medical reasons in young people. Using combined behavioural, questionnaire and fMRI methods, we investigated i) differences in reactivity to food cues between adolescents with T2DM, obese and normal weight adolescents and ii) the effect of restrictive diets and parental feeding practices on adolescent's food responsiveness and fMRI response.
Materials and methods: Food images and visually matched non-food images were presented to 15 adolescents with T2DM, 20 with obesity and 20 normal weight adolescents. We recorded brain activity (fMRI) whilst participants imagined eating the presented food items. Questionnaire data regarding their feeding/eating behaviours were collected from both the parent and adolescent. We also measured the macro-nutrients of food consumed by both the parent and adolescent during a prepared lunch offered during the study visit.
Results: All fMRI activation was significant at $p<0.05$, $z>2.3$, cluster corrected for multiple comparisons. All groups showed higher activation for food than non-food images but activation was significantly higher in the T2DM group, illustrating the increased salience for food in this group. Participants with T2DM also had greater activation to food in brain regions associated with attention and control (medial frontal gyrus, cingulate, precuneus), if they experienced high parental restriction (as measured by the questionnaire data). This was not shown in the Obesity or Control groups. Restriction by parents however did not predict lower food consumption (if anything consumption was higher with more restriction).
Conclusion: For young people with T2DM their brain activation to food suggests parental restriction may be aiding ability to learn to control their responses to food. However the failure of cognitive control to predict a reduction in food consumption suggests at this stage it is not sufficient for effective behavioural change. As adolescents mature into adults it is not known how these increases in activation of control areas to food will interact with the known losses of cognitive function found in older adults with T2DM. It is possible that increased cognitive load may lead to erosion of the cognitive resources and a subsequent decline in control related activation. On the other hand, parental restriction encouraging the practice of cognitive control at a time of increased cognitive flexibility could improve the likelihood of better behavioural outcomes in the future.
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Corneal confocal microscopy detects neuropathy before retinopathy and nephropathy in children with type 1 diabetes: a preliminary study

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Background and aims: Early detection and prevention of long-term complications by maintaining good metabolic control is the key goal of paediatric diabetes management. Whilst we have readily available diagnostic techniques for retinopathy (fundus photography) and nephropathy (UAER), there is no equally sensitive measure for diabetic neuropathy. The aim of the present study was to assess the utility of in vivo corneal confocal microscopy (IVCCM) in identifying early nerve damage in children with T1DM.
Materials and methods: 25 children with type 1 diabetes mellitus (average age: 13±1 yrs; average duration of diabetes 8 years) with no evidence of retinopathy or microalbuminuria and 10 aged matched control subjects underwent assessment with IVCCM (HRT III) to quantify corneal nerve fibre density (NFD), branch density (NBD) and length (NFL). Each examination took ~ 6 minutes and no child reported that CCM was uncomfortable.
Results: There was a significant reduction in NFD (no/mm^2) (32.5 ± 1.9 v 41.1±1.8, P=0.007), NBD (no/mm^2) (50.6 ± 4.5 v 72.5 ± 6.5, P=0.008) and NFL (mm/mm^2) (20.6 ± 0.9 v 29.4 ±1.3, P<0.0001) in children with T1DM versus control subjects. Patients were stratified into better (HbA1c <8%, n=15) and poorer (HbA1c >8%, n= 10) glycaemic control. Even in patients with better (HbA1c-7.9±0.14) glycaemic control, NFD (34.2 ± 2.4, P=0.07), NBD (51.9 ± 4.4, P=0.04), and NFL (21.3 ± 1.2, P<0.0001) were reduced compared to controls. Those with poorer (HbA1c=10.08±0.04) glycaemic control had a further non-significant reduction in NFD (27.3 ± 1.5, P=0.2), NBD (46.7 ± 13.0, P=0.8), and NFL (18.2 ± 1.4, P=0.3) compared to those with better glycaemic control.
Conclusion: Corneal confocal microscopy detects early corneal nerve damage in children with Type 1 diabetes, which worsens with poorer glycaemic control. CCM provides a fast, non-invasive and well tolerated technique to detect early nerve damage which precedes retinopathy and microalbuminuria. Longitudinal studies are required to predict those who develop overt neuropathy.
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Prevalence of asthma in children and adolescents with type 1 diabetes in Germany and Austria: Is there an impact on metabolic control?

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Background and aims: Recent data suggest an increased prevalence of asthma in diabetic youth in contrast to previous reports, and the concomitant diagnosis was associated with poor metabolic control. The aim of the study was to estimate the prevalence of asthma in a large cohort of children and adolescents with Type 1 diabetes mellitus (DMT1) in Germany and Austria and to analyse a possible impact on metabolic control.
Materials and methods: Observational cohort study based on the DPV-database (German/Austrian DPV initiative) data from 51926 patients with DMT1 (<20 years (Tab 1). All clinical data were documented prospectively in the DPV-data base. The data base was searched for the concomitant diagnosis asthma and for asthma medication (inhaled corticosteroids, leukotriene modifiers, sympathomimetic drugs and others). Data were analysed using the SAS software. For group comparisons, non-parametric statistical tests and a linear regression model were used, with adjustment for multiple comparisons.
Results: 1755 (3.4 %) of the whole cohort had the diagnosis asthma or received medical treatment for asthma. Patients with asthma were more often males, used higher insulin doses, had decreased height SDS, an increased BMI-SDS and experienced more often severe hypoglycaemias. No significant difference was found for HbA_{1c} between patients with and without asthma. 495 (28%) patients with asthma were treated with inhaled corticosteroids, 425 (24%) with sympathomimetic drugs, 104 (6 %) used leukotriene receptor antagonists, 72 (4%) various other medications, while 659 (37%) were without pharmacotherapy. No relevant influence of the type of asthma medication on metabolic control or BMI -SDS could be found.
Conclusion: Prevalence of asthma in children and adolescents with DMT1 in Germany and Austria seems not to be elevated compared to the background population. The diagnosis asthma and pharmacological treatment have no relevant influence on metabolic control in our cohort.

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European Association for the Study of Diabetes

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