ABSTRACTS ADDRM 2020

1
Cytokine output of adipocyte-iNKT cell interplay is skewed by a lipid-rich microenvironment

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BACKGROUND

The complex direct and indirect interplay between adipocytes and various adipose tissue (AT)-resident immune cells plays an important role in maintaining local and whole-body insulin sensitivity. Adipocytes can directly interact with and activate AT-resident invariant natural killer T (iNKT) cells through CD1d-dependent presentation of lipid antigens, which is associated with anti-inflammatory cytokine production in lean AT (IL-4, IL-10). Whether alterations in the microenvironment, i.e. increased free fatty acids concentrations or altered cytokine/adipokine profiles as observed in obesity, directly affect adipocyte-iNKT cell communication and subsequent cytokine output is currently unknown.

METHODS

To study effects of a lipid-rich environment on the cross-talk between iNKT's and adipose we conditioned the adipose cell line 3T3-L1 with a commercially available lipid-mix. These cells were then co-cultures with a variety of iNKT cell lines as well as ex-vivo iNKT's. Cross-talk was assessed either by FACS (JE6-1$^{REP\text{-}iNKT\beta2M_KO}$) or IL4 and IFNγ elisa (DN32.D3 and ex-vivo iNKT's).

RESULTS

Here we show that cytokine output of adipocyte-iNKT cell interplay is skewed by a lipid-rich microenvironment. Incubation of mature 3T3-L1 adipocytes with a cocktail of saturated and unsaturated fatty acids specifically reduced phosphorylation of AKT to 31% of the control and increased lipolysis significantly, p = 0.0076. Reduced activation of the CD1d-invariant T-Cell Receptor (TCR) signaling axis was observed in Jurkat reporter cells expressing the invariant NKT TCR, p = 0.0044, while co-culture assays with an iNKT hybridoma cell line (DN32.D3) reduced IL-4 secretion and increased IFNγ secretion, p = 0.0319 and 0.0004 respectively. Importantly, co-culture with primary iNKT cells isolated from visceral AT showed a similar cytokine output, reduced IL-4, p = 0.0233 and increased IFNγ, p = 0.0086.

DISCUSSION/CONCLUSION

Here we show lipid-rich environments change adipocyte-iNKT cell communication, resulting in higher production of inflammatory factors by iNKT cells. Collectively, these data indicate that iNKT cells display considerable plasticity with respect to their cytokine output, which can be skewed toward a more pro-inflammatory profile in vitro by microenvironmental factors like fatty acids.

2
Evaluation of adherence to medication by LC-MS/MS urine testing and relation with clinical outcomes in type 2 diabetes: an analysis in the Diabetes and Lifestyle Cohort Twente

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BACKGROUND

To reach treatment targets in Type 2 Diabetes (T2D) and prevent long-term complications, medication adherence is essential, yet difficult to determine. A novel objective tool to assess medication adherence is biochemical urine testing of drug metabolites using liquid chromatography-tandem mass spectrometry (LC-MS/MS). We used this tool in a real-world setting to assess adherence to the main drug classes important for T2D and determined the association of non-adherence with clinical outcomes.

METHODS

Adherence to oral antidiabetics (OADs), antihypertensives, and statins was determined by LC-MS/MS in 457 patients included in the Diabetes and Lifestyle Cohort Twente. Non-adherence was defined as the absence of minimal one prescribed drug in the urine. Differences between groups were tested using the ANOVA, X_2 test, and the Kruskal-Wallis test.

RESULTS

Overall, 89.3% patients were adherent. Adherence to OADs, antihypertensives and statins was 95.7%, 92.0% and 95.5%, respectively. Prevalence of both microvascular and macrovascular complications was higher in non-adherent than adherent patients (81.6% vs 66.2%, p = 0.029 and 55.1% vs 37.0%, p = 0.014, respectively). Less non-adherent than adherent patients reached an LDL-cholesterol target of < 2.5 mmol/L (67.4% vs 81.1%, p = 0.029), and mean HbA1c was higher (62.9 ± 14.5 vs 57.4 ± 11.2 mmol/mol, p < 0.01). Among non-adherent patients were more smokers (28.6% vs 15.0%, p = 0.047) without other demographic differences. Logistic regression analysis demonstrated higher BMI, smoking, elevated LDL-cholesterol, high HbA1c, presence of diabetic kidney disease and macrovascular disease as significant predictors of non-adherence.

CONCLUSION

Overall medication adherence determined by LC-MS/MS in this real-world setting was relatively high. In non-adherent patients, overall prevalence of diabetic complications was higher and treatment targets were reached less frequently. This emphasizes the importance of objective detection and tailored interventions to optimize adherence.

3

Sexual dimorphism in body weight loss, improvements in cardiometabolic risk factors and maintenance of beneficial effects 6 months after a low-calorie diet: results from the DiOGenes trial

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BACKGROUND

Weight loss resulting from a low-calorie diet (LCD) is an effective strategy to improve risk factors for cardiometabolic diseases. However, sexual dimorphism may be present in LCD-induced body weight loss and improvements in cardiometabolic risk factors, and maintenance of changes during follow-up.

METHODS

782 overweight or obese participants (35% men) were included in the large-scale multicenter DiOGenes trial (ClinicalTrials.gov Identifier: NCT00390637). Participants followed an 8-week LCD (~800 kcal/day), with a 6-months follow-up weight maintenance period on *ad libitum* diets varying in protein content and glycemic index. Body weight and several cardiometabolic risk factors were determined. A mixed-model analyses was performed with adjustment for age, weight (loss and regain) and (change in) baseline value and diet.

RESULTS

Men lost more body weight during the LCD period (-12.8 ± 3.9 vs. -10.1 ± 2.8kg, respectively, p < 0.001), but regained more weight during the follow-up period than women (1.5 ± 5.4 vs. -0.5 ± 5.5kg, respectively, p < 0.001). Although beneficial LCD-induced changes in cardiometabolic risk factors were found for both sexes, improvements in HOMA-IR, muscle and hepatic insulin sensitivity, triacylglycerol, free fatty acids, cholesterol esters, sphingomyelins, total cholesterol, HDL-cholesterol, LDL-cholesterol and diastolic blood pressure were more pronounced in men than women (std. ß range: 0.073-0.144, all q < 0.05). During the weight maintenance period, women demonstrated a lower rebound in HDL-cholesterol, triacylglycerol and diacylglycerol (std. ß range: 0.097-0.164, all q < 0.05).

CONCLUSIONS

Men lose more weight and improve more in cardiometabolic risk factors following a LCD but are less able to maintain these improvements after 6 months of weight maintenance compared to women.

4

Moderate hypoglycaemia affects cognitive function in people with diabetes, irrespective of diabetes type, level of glucose control or hypoglycaemic awareness

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BACKGROUND

Hypoglycaemia is the most common adverse effect in people with type 1 or type 2 diabetes treated with insulin and creates an immediate threat for brain function. While it is evident that hypoglycaemia causes cognitive dysfunction, it is unclear how this is affected by factors like diabetes type, age, prior hypoglycaemic exposure or glycaemic control.

METHODS

Adults with type 1 diabetes, type 2 diabetes treated with insulin, and non-diabetic individuals (matched on age, sex and BMI) were recruited to undergo a hyperinsulinaemic-hypoglycaemic glucose clamp (nadir 2.8mmol/l). During baseline and hypoglycaemia, cognitive function was measured with the Paced Auditory Serial Addition Test (PASAT) and the Test of Attentional Performance (TAP; Alertness, Working memory). Paired t-tests were used to compare data obtained during baseline and hypoglycaemia.

RESULTS

An interim analysis was performed on the data of 29 participants with type 1 diabetes, 8 participants with type 2 diabetes and 23 controls (n = 60 in total). For the total sample, with PASAT, the proportion of correct answers was 66 ± 17% during baseline versus 62 ± 17% during hypoglycaemia (p = 0.001). On the TAP subtest Alertness, mean (± SD) reaction times increased from 291±77ms during baseline to 316 ± 84ms during hypoglycaemia (p = 0.010). On TAP working memory, a combination of the mean omissions and errors is increased from 4.9 ± 5.1 to 6.7 ± 7.7 (p = 0.002) baseline and hypoglycaemia, respectively. Hypoglycaemia-induced cognitive declines were seen in all subgroups and were not modified by the level of hypoglycaemic awareness or glucose control.

CONCLUSION

Based on these preliminary data, moderate hypoglycaemia results in a decline in auditory information processing speed, reaction time, and working memory that appears consistent in people with diabetes irrespective of diabetes type or glycaemic parameters, and people without diabetes.

5

Fully Closed Loop Glucose Control with a Bihormonal Artificial Pancreas in Adults with Type 1 Diabetes: an Outpatient, Randomized, Crossover Trial

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BACKGROUND

The bihormonal artificial pancreas can provide fully closed loop glucose control via automated delivery of both insulin and glucagon. The aim of this CE-mark trial was to demonstrate the performance and safety of this artificial pancreas.

METHODS

In this outpatient, randomized, crossover trial, two-week fully closed loop glucose control (artificial pancreas therapy) was compared to two-week open loop control (patient's normal insulin pump therapy with a glucose sensor if they had one). The primary outcome was the percentage of time in range (3.9-10 mmol/L).

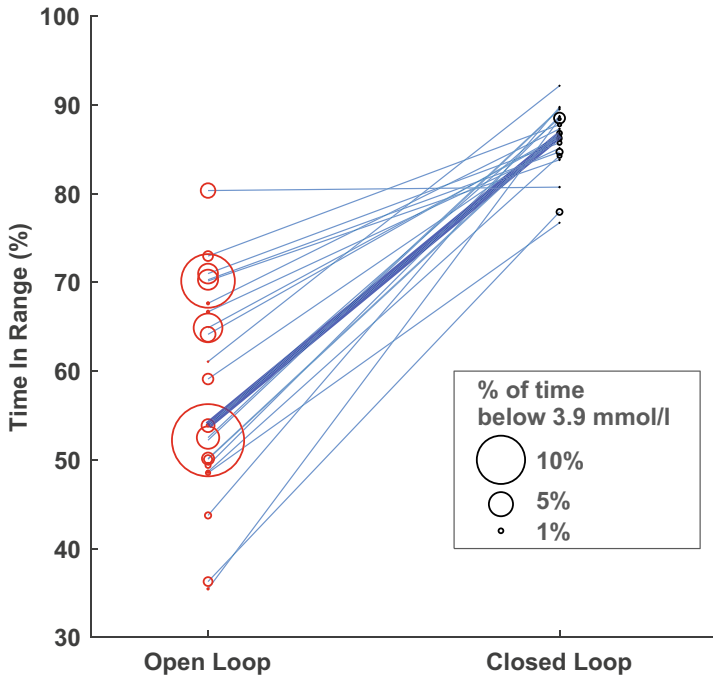

Figure 1. Time in range from open loop (left) to closed loop (right). The thin lines represent the individual patients and the bold line the median. The size of each circle is proportional to the time spent in hypoglycemia.

RESULTS

Twenty three patients were included in the analysis. Median (IQR) time in range was significantly higher during closed loop (86.6% (84.9-88.5)) compared with open loop (53.9% (49.7-67.2); $p < 0.0001$; Figure 1). Time in hypoglycemia was 0.4% (0.1-0.8) during closed loop and 2.0% (0.7-3.6) during open loop ($p < 0.0001$). Median glucose level was 7.2 mmol/L (7.0-7.4) during closed loop and 9.3 mmol/L (8.3-9.9) during open loop ($p < 0.0001$). No severe hypoglycemia or ketoacidosis occurred during the study.

CONCLUSION

Compared to insulin pump therapy, the bihormonal artificial pancreas provides superior glucose control, without meal or exercise announcements, and is safe in adults with type 1 diabetes. The available clinical evidence resulted in the first CE-marked bihormonal artificial pancreas.

6

Effects of diabetes mellitus on fibrin clot structure and mechanics in a model of acute neutrophil extracellular traps (NETs) formation

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BACKGROUND

Subjects with diabetes mellitus (DM) have an increased risk of arterial thrombosis, to which changes in clot structure and mechanics may contribute. Another contributing factor might be an increased formation of neutrophil extracellular traps (NETs) in DM. NETs are mainly formed during the acute phase of disease and form a network within the fibrin matrix, thereby influencing clot properties. Our aim was to study how DM affects clot properties in a model resembling an acute phase of disease with NETs formation.

METHODS

Clots were prepared from citrated plasma from subjects with and without DM with the addition of NETs, induced in neutrophils by S. aureus bacteria or phorbol myristate acetate (PMA). Structural parameters were measured using scanning electron microscopy, mechanical properties using rheology, and sensitivity to lysis using a fluorescence-based fibrinolysis assay.

RESULTS

Plasma clots from subjects with DM compared to clots from subjects without DM had significantly thicker fibers (195.0 ± 3.9 nm vs 186.6 ± 3.9 nm, $p < 0.01$) and fewer pores (1616.7 ± 120.0 vs 1907.1 ± 169.0, $p < 0.01$) and branchpoints (2.95 ± 0.21 vs 3.44 ± 0.29, $p < 0.01$). In addition, fibrinolysis was significantly slower (14873 ± 1321 AU vs 15995 ± 1337 AU, $p < 0.05$), while mechanical properties were similar between both groups (storage modulus DM 62.1 [29.7-188.9] Pa vs no DM 36.4 [23.0-80.0] Pa, $p = 0.24$).

CONCLUSION

In a model of acute NETs formation, DM plasma shows prothrombotic effects on fibrin clots.

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Associations of ultra-processed food and its underlying consumption patterns with incident Type 2 Diabetes: the Lifelines cohort study

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BACKGROUND

Although consumption of ultra-processed food (UPF) has previously been associated with adverse health outcomes, it is unclear how the consumption of UPF and its underlying habitual consumption patterns are associated with incident type 2 diabetes.

METHODS

In 70421 participants (35-70 years, 58.6% women) from the Lifelines cohort study, dietary intake was assessed with a food frequency questionnaire. Principal component analysis (PCA) was performed to derive UPF consumption patterns. UPF was related to incident diabetes with adjustments for confounders, including overall diet quality.

RESULTS

During a median follow-up of 41 months, the intake of UPF was associated with higher risk of type 2 diabetes (1128 cases, OR for a 10% increment in UPF intake 1.33 [95% CI 1.26, 1.41]), and remained significant after adjustment for confounders (OR 1.25 [95% CI 1.16, 1.34]). PCA revealed four habitual UPF consumption patterns. A pattern high in cold savory snacks (OR 1.16 [95% CI 1.09, 1.22]) and a pattern high in warm savory snacks (OR 1.15 [95% CI 1.08, 1.21]) were associated with an increased diabetes risk. A pattern high in traditional Dutch cuisine was not associated with diabetes risk (OR 1.05 [95% CI 0.97, 1.14]), while a pattern high in sweet snacks and pastries was inversely associated with diabetes risk (OR 0.82 [95% CI 0.76, 0.89]). There was a clear inverse association between diabetes risk at baseline and the sweet snacks and pastries pattern ($\beta = -0.104$ [95% CI -0.113, -0.094]).

CONCLUSION

A higher consumption of UPF was associated with higher risk of type 2 diabetes. For consumption patterns, this association was most pronounced for the patterns that were high in savory snacks. Our findings emphasize that, in addition to promoting the consumption of healthy food products, discouraging the consumption of UPF, specifically savory snacks, should be considered as part of future diabetes prevention strategies.

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Glucose regulation beyond HbA1c in Type 2 Diabetes treated with insulin

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BACKGROUND

We investigated the glucose variations behind HbA1c in a real-world setting in insulin treated patients with type 2 diabetes, the differences in time in range (TIR), time below range (TBR) and time above range (TAR) between different HbA1c categories used in clinical practice and whether there are differences in glucose variability. Because hypo- and hyperglycemic episodes are of special interest, we evaluated the frequency, duration and start time of the TBR and TAR episodes in different HbA1c categories.

METHODS

Patients included in the Diabetes and Lifestyle Cohort Twente (DIALECT)-2 were categorized in three HbA1c categories: low, intermediate and high (≤ 53; 54-62 and ≥ 63 mmol/mol or 7, 7.1-7.8, 7.9%). Blood glucose TIR, TBR, TAR, glucose variability parameters and day and night duration and frequency of TBR and TAR episodes were determined by continuous glucose monitoring (CGM) and compared between HbA1c categories.

RESULTS

No differences were found between low and intermediate HbA1c categories for TIR (76.8% [68.3-88.2] vs 76.0% [72.5.0-80.1]), whereas in the low category TBR was higher and TAR was lower (7.7% [2.4-19.1] vs 0.7% [0.3-6.1], and 8.2% [5.7-17.6] vs 20.4% [11.6-27.0], respectively, $p < 0.05$). Patients in the highest HbA1c category have lower TIR (52.7% [40.9-67.3]) and higher TAR (44.1% [27.8-57.0]) compared to the other HbA1c categories ($p < 0.05$), but do not have less TBR during the night. All patients had more (0.06 ± 0.06/h vs 0.03 ± 0.03/h, $p = 0.002$) and longer (88.0 [45.0-195.5] vs 53.4 [34.4-82.8] minutes, $p < 0.001$) TBR episodes during the night than during the day.

CONCLUSION

The findings, that a high HbA1c does not protect against hypoglycemia and a low HbA1c does not provide the highest TIR, demonstrate that personalization of glycemic control requires new tools such as CGM-derived parameters.

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Higher daily glucose variability is associated with worse cognitive performance – The Maastricht Study

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BACKGROUND

Type 2 diabetes is associated with an increased risk of Alzheimer's disease and vascular dementia. A better understanding of the drivers of diabetes-associated cognitive decline may provide entry points for preventive measures; daily glucose variability (GV) may be such a modifiable risk factor. We, therefore, investigated whether daily GV, assessed by continuous glucose monitoring (CGM) or oral glucose tolerance test (OGTT), is associated with cognitive performance.

METHODS

We used cross-sectional data from The Maastricht Study, a population-based cohort enriched with type 2 diabetes. We calculated standard deviation, coefficient of variation, and time in range (TIR) in participants with > 48h of CGM data (n = 853), and calculated the recently validated incremental glucose peak (IGP; peak minus fasting glucose value) in participants with complete seven-point OGTT data (n = 3586). The associations of these GV indices with overall cognitive perfor-

mance and individual cognitive domains (i.e., memory function, information processing speed, and executive function and attention [EFA]) were examined by use of multiple linear regression.

RESULTS

Higher IGP was associated with worse overall cognitive performance, irrespective of demographics, cardiovascular risk factors and lifestyle factors. The effect size (regression coefficient per 1 mmol/L IGP [95% CI]: -0.017 [-0.028; -0.006], p = 0.002) corresponded with 4 months of normal ageing. This association remained statistically significant after further adjustment for HbA1c or fasting plasma glucose. Of the individual cognitive domains, IGP was most strongly associated with EFA. This was consistent with our findings on CGM-measured indices, although only TIR was independently associated with EFA (regression coefficient per 10% TIR: 0.053 [0.001; 0.105], p = 0.044).

CONCLUSION

Our results show that daily GV is an independent, clinically relevant determinant of worse cognitive performance. Future studies should explore whether therapeutic interventions that specifically target daily GV can delay or prevent cognitive decline.

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Sedentary time and physical activity are associated with endothelial dysfunction and low-grade inflammation – the Maastricht Study

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BACKGROUND

Endothelial dysfunction and low-grade inflammation play an important role in the pathogenesis of cardiovascular disease. Physical activity and sedentary behavior are potential modifiable risk factors in the development of cardiovascular diseases. We investigated the association between physical activity and sedentary behavior and biomarkers of endothelial dysfunction and low-grade inflammation.

METHODS

Data from The Maastricht Study (n = 2336) were used. We measured biomarkers of endothelial dysfunction (von Willebrand factor, soluble intercellular adhesion molecule (sICAM-1), soluble vascular cell adhesion molecule 1 and soluble endothelial selectin), and low-grade inflammation (C-reactive protein, serum amyloid A, interleukin 6, interleukin 8, tumor necrosis factor alpha, sICAM-1). Biomarkers were combined into overall Z-scores (higher score, more dysfunction/inflammation). Physical activity (total, light, moderate-to-vigorous and vigorous) and sedentary time were measured with the activPAL3®.

RESULTS

All intensities of physical activity were inversely associated with endothelial dysfunction and low-grade inflammation. (β [95% CI], endothelial dysfunction: total -0.15[-0.21; -0.09]; light -0.04 [-0.06; -0.01]; moderate-to-vigorous -0.20 [-0.29; -0.11]; vigorous -0.26 [-0.49; -0.03]; low-grade inflammation: total -0.16 [-0.22; -0.11]; light -0.05 [-0.07; -0.02]; moderate-to-vigorous -0.25 [-0.33; -0.16]; vigorous -0.40 [-0.62; -0.17]. Independently of physical activity, sedentary time was associated with endothelial dysfunction and low-grade inflammation (endothelial dysfunction: 0.06 [0.02; 0.10], low-grade inflammation: 0.05 [0.01; 0.09]). Associations between physical activity (all intensities) and sedentary time on the one hand and endothelial dysfunction on the other were consistently stronger in individuals with prediabetes and T2DM than in individuals with normal glucose metabolism.

CONCLUSION

Physical activity (all intensities) and sedentary time can influence endothelial dysfunction and low-grade inflammation. Especially in individuals with pre-diabetes or T2DM, increasing physical activity and reducing sedentary time may be an important strategy to prevent endothelial dysfunction and low-grade inflammation.

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Polymorphisms in glyoxalase I gene are not associated with glyoxalase I expression or markers of methylglyoxal stress: The CODAM study

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BACKGROUND

Glyoxalase I (GLO1) is the rate-limiting enzyme in the detoxification of the reactive methylglyoxal (MGO) into D-Lactate. MGO is a major precursor of advanced glycation endproducts (AGEs). MGO stress – the accumulation of MGO and AGEs – is associated with the progression of diabetes and diabetes complications. Because genetic variation in the GLO1 gene may alter the expression and/or the activity of GLO1, we examined the association of single nucleotide polymorphisms (SNPs) in the GLO1 gene with GLO1 expression and markers of MGO stress.

METHODS

We used data from individuals of the Cohort on Diabetes and Atherosclerosis Maastricht [CODAM Study, n = 546, 60 ± 7 years, 25% T2DM]). Independent variables were nine tag SNPs that cover the common GLO1 gene variation, genotyped using the ABI-PRISM-7900HT sequence detection system. Outcome variables were circulating GLO1 mRNA measured using qPCR, concentrations of MGO in fasting plasma and after an oral glucose tolerance test, concentrations of D-Lactate in fasting plasma and urine, and the MGO-derived AGEs Nε-(carboxyethyl)lysine (CEL) and Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) in fasting plasma and urine, all measured via UPLC-MS/MS. Outcome variables were standardized and compared across genotypes using linear regression, adjusted for age, sex, and glucose metabolism status.

RESULTS

Concentrations of MGO, D-Lactate, and AGEs in plasma and urine did not differ across genotypes of the nine SNPs. SNP4 (rs13199033) was associated with GLO1 expression (standardized beta AT versus AA = -0.29, p = 0.02 and TT versus AA = -0.39, p = 0.3). Similarly, SNP13 (rs3799703) was associated with GLO1 expression (standardized beta AG versus GG = 0.17, p = 0.14 and AA versus GG = 0.36, p = 0.005). However, these associations were no longer significant after correction for multiple testing with FDR.

CONCLUSION

After correction for multiple testing, polymorphisms of GLO1 were not associated with GLO1 expression or markers of MGO stress. This suggests that these SNPs are not functional, although activity/expression might be altered in other tissues.

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Circadian misalignment disturbs the lipidome in human skeletal muscle

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BACKGROUND

Circadian misalignment, e.g. shift work, is associated with an increased risk to develop obesity and type 2 diabetes. We recently showed that simulated shift work in a laboratory setting leads to skeletal muscle insulin resistance in young healthy volunteers after 3 consecutive nights. Based on previously observed changes in gene expression related to PPAR signalling and fat metabolism, we here aimed to test the hypothesis that a disturbed muscle lipid metabolism contributes to the development of muscle insulin resistance upon circadian misalignment.

METHODS

In a randomized cross-over design, 14 healthy, lean, male volunteers underwent one control (aligned) period and one circadian misalignment period both consisting of ~3.5 days spent in a respiration chamber. In the aligned condition, participants followed a normal diurnal lifestyle, including scheduled sleep from 11 PM to 7 AM and meals provided at 8 AM, 12:30 PM, 3 PM and 8 PM. In the misaligned condition, day and night were rapidly shifted by 12h on day 2. This regime was continued for 2 days before

measurements were performed. For each condition, two skeletal muscle biopsies were taken from the m. vastus lateralis at 8AM and 8PM and subjected to semi-targeted lipidomics using UPLC/HRMS.

RESULTS

Only 2% (19 of 1178) of detected lipids were different between morning and evening in the aligned condition, whereas 9% (102 lipids) displayed a morning-evening difference upon misalignment. The majority of lipids that changed upon misalignment were triacylglycerols, in particular species of a carbon length ≥ 55. Cardiolipins were generally decreased upon misalignment. Cholesterol esters adjusted to the shifted behavior and were hence increased in the fed state of both conditions.

CONCLUSION

Our findings show that the skeletal muscle lipidome is disturbed under conditions simulating shift work which may contribute to the muscle insulin resistance upon circadian misalignment.

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Relationship between de novo lipogenesis and serum sex hormone-binding globulin in humans

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BACKGROUND

Obesity, type 2 diabetes and liver fat are associated with decreased levels of serum sex hormone-binding globulin (SHBG). Laboratory studies suggest that hepatic de novo lipogenesis (DNL) is involved in the downregulation of SHBG synthesis. The aim of the present study was to assess the role of DNL on serum SHBG in humans.

METHODS

This study consisted of two substudies; 1) a cross-sectional study that examined the association between DNL, measured by stable isotopes, and serum SHBG in healthy individuals (n = 55), 2) a case-control study that compared serum SHBG in healthy individuals (n = 14) with monogenetic disorders affecting DNL (i.e. individuals with glucokinase-maturity onset diabetes of the young (GCK-MODY; model of decreased DNL; n = 11), and glycogen storage disease type 1a (GSD1a; model of increased DNL; n = 9)) or monogenetic disorders causing liver fat through other pathways (i.e. familial partial lipodystrophy (FPL, model of increased fatty acid flux; n = 13) and abetalipoproteinemia (ABL, model of impaired VLDL secretion; n = 2)).

RESULTS

DNL was inversely associated with serum SHBG in women (β: -0.015, 95% CI: -0.03; -0.00), but not in men (β: 0.007, 95% CI: -0.005; 0.019). This strength of association decreased after correction for insulin in women (β: -0.013, 95% CI: -0.028; 0.003). SHBG levels were significantly lower in GSD1a patients when compared to controls (median: 13.0 nmol/L versus 43.0 nmol/L, p = 0.003). SHBG levels in GCK-MODY, FPL and ABL were not different from controls, despite a higher liver fat content in the latter two groups.

CONCLUSION

An inverse association between DNL and serum SHBG levels may explain the decreased serum SHBG levels that are observed in obesity and type 2 diabetes, at least in women.

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A novel microscopy tool to study *in vitro* lipid droplet dynamics in human primary myotubes

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BACKGROUND

High levels of intramyocellular lipid droplets (LDs) are associated with insulin resistance. Insulin sensitive athletes, however, have similar intramyocellular lipid levels as type 2 diabetes (T2DM) patients. LDs are dynamic organelles which release and store fatty acids (FAs) depending on energy demand. LD dynamics may be an underlying factor explaining this athlete's paradox. We aimed to develop an in vitro fluorescent microscopy tool to study LD dynamics in human primary myotubes (HPM) from athletes and T2DM patients.

METHODS

HPM were incubated overnight with 50 µM oleate and trace amounts of Bodipy-FL (green)-labeled C12-FA, followed by a 6-hour 50 µM oleate incubation with trace amounts of Bodipy 558/568 (red)-labeled C12-FA. ImageJ scripts were developed to analyze the Bodipy labeled-FA incorporation into LDs. To examine if the combined use of both Bodipy-labeled FAs resulted in visualization of all LDs, we stained LDs with MDH.

RESULTS

All LDs were labeled as indicated by Manders coefficients M1 and M2 of MDH with any of the Bodipy's approaching 1.000 (0.998 and 0.979 for Bodipy 558/568; 0.976 and 1.000 for Bodipy-FL). Bodipy-FL to Bodipy 558/568 content permitted to make the distinction between three LD pools (pre-formed, incorporating and new). Live-cell experiments showed that LD formation in HPM from an athlete occurred more rapidly compared to HPM from a T2DM patient and plateaued after 10 hours.

CONCLUSION

The use of two fluorescently-labeled FAs in combination with our developed ImageJ scripts is a promising tool to study LD dynamics in HPM. This methodology permits the detection of differences in LD formation rate in HPM from an athlete vs. a T2DM patient. More studies are needed to confirm this observation and to examine if regional differences within the cell exist with respect to FA incorporation rate into LDs and if this is different in HPM from athletes vs. T2DM.

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Development of a prediction model for foot ulcer recurrence in people with diabetes using easy-to-obtain clinical variables

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BACKGROUND

People with diabetes stratified as highest risk for foot ulceration vary widely in disease severity. It is important here to differentiate for ulcer risk to provide appropriate and personalized preventative strategies. We aimed to develop a prediction model for foot ulcer recurrence in people with diabetes using easy-to-obtain clinical variables and to validate its predictive performance.

METHODS

We included 304 persons with diabetes at high-risk for foot ulceration with 18 months follow-up from the DIATEMP foot temperature monitoring trial. Two logistic regression models were created: one for recurrent foot ulcers (n = 126) and one for recurrent plantar foot ulcers (n = 70). Ten-fold cross validation, each including five multiple imputation sets, was used to internally validate the models; model performance was assessed in terms of discrimination and calibration using the area under the receiver operator curve (AUC; range: 0-1), Brier score (range: 1-0) and calibration graphs.

RESULTS

Predictors for recurrent foot ulceration were: younger age,

more severe peripheral sensory neuropathy, shorter time since healing of previous ulcer, presence of minor lesion, using a walking aid, and not monitoring foot temperatures; AUC: 0.69 (IQR: 0.61-0.74); Brier score: 0.22 (IQR: 0.21-0.24). Predictors for recurrent plantar foot ulceration were: the same, but not monitoring foot temperature, and in addition plantar location of previous ulceration, consumption of > 1 unit alcohol per week, and foot care received in a university medical center; AUC: 0.67 (IQR: 0.66-0.77) and Brier score: 0.16 (IQR: 0.13-0.19).

CONCLUSION

These well-designed and internally validated prediction models are built from a representative group of people at high risk of diabetes foot ulceration, using easy to obtain variables. The models predict with good calibration and fair discrimination who is at highest risk of ulcer recurrence. These people should be monitored more carefully and treated more intensively than others.

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Increased stress, weight gain and less exercise in relation to glycaemic control in people with type 1 and type 2 diabetes during the COVID-19 pandemic

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BACKGROUND

Lockdown measures have a profound effect on many aspects of daily life relevant for diabetes self-management. We assessed whether lockdown measures, in the context of the COVID-19 pandemic, differentially affect perceived stress, anxiety, body weight, exercise and related this to glycaemic control in people with type 1 and type 2 diabetes.

METHODS

We performed an observational cohort study at the Leiden University Medical Center. People with type 1 and type 2 diabetes ≥ 18 years were eligible to participate. Participants filled out online questionnaires, sent in blood for HbA1c analysis and shared data of their flash or continuous glucose sensors. HbA1c during the lockdown was compared to the last known HbA1c before the lockdown.

RESULTS

In total 435 people were included (type 1 diabetes n = 280, type 2 diabetes n = 155). An increase in perceived stress, anxiety, weight gain and less exercise was observed in both groups. There was improvement in glycaemic control in the group with the highest HbA1c tertile (type 1 diabetes: -0.39% (-4.3 mmol/mol) ($p < 0.0001$ and type 2 diabetes: -0.62% (-6.8 mmol/mol) ($p = 0.0036$). Increased perceived stress was related to difficulties in diabetes self-management ($p < 0.0001$).

CONCLUSION

An increase in perceived stress, anxiety, weight gain and less exercise but no deterioration of glycaemic control occurs in both people with type 1 and type 2 during lockdown measures. As perceived stress showed to be associated to difficulties in self-management, an increased emphasis on the psychological impact of the lockdown measures by health care providers may be needed.

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Feasibility and Reproducibility of the Muscle Sound® Technique in a Morbidly Obesity Population: MUST-MOP Study

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BACKGROUND

In obesity, low muscle mass has been associated with higher risks of type 2 diabetes mellitus and hypertension. It is important to preserve muscle mass during weight loss, which leads to a need for validated methods for esti-

mating muscle mass in obese populations. Recently, new software to quantify muscle with ultrasonography has been developed. This software has yet to be tested in an obese population. However, before validation, feasibility and reproducibility have to be determined. This study was to evaluate the feasibility and reproducibility of measuring muscle mass using ultrasonography with the MuscleSound® software in a population with morbid obesity.

METHODS

During this prospective observational study, patients scheduled for bariatric surgery underwent ultrasound measurements, in which seven-points were examined. The measurements are performed twice. To examine the feasibility, a small descriptive analysis is performed to assess this outcome. The reproducibility of the body composition measurement is examined by a Bland-Altman plot and the intraclass correlation coefficient (ICC).

RESULTS

The population consisted of 52 patients, 43 females (82.7%), with a mean age of 44 (± 12) and a median BMI of 40 (38-43). The success rate of the measurement is 94.2%. The Bland-Altman plots of both intra- and inter-observer measurements shows no apparent bias between the 2 measurements. The ICC of the intra-observer lean mass difference is 0.991 (0.983-0.995), and the ICC of the inter-observer lean mass difference is 0.977 (0.702-0.924), both demonstrating an excellent reliability.

CONCLUSION

The use of ultrasound with the MuscleSound® technique proves to be feasible in a population with class II and III obesity. Both the intra-observer and inter-observer reproducibility are excellent. A study validating the MuscleSound® technique with DXA-scanning in this obese population is underway.

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The effectiveness of at-home foot temperature monitoring in reducing the incidence of ulcer recurrence in people with diabetes: a multicentre randomized controlled trial (DIATEMP)

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BACKGROUND

The skin of people with diabetic foot disease is suggested to heat up before it breaks down into ulceration. This allows for monitoring and early intervention to prevent ulcers. We assessed whether at-home plantar foot skin temperature monitoring can help prevent ulcer recurrence in people with diabetes

METHODS

In this multicentre outcome-assessor blinded randomized controlled trial we assigned 304 people with diabetes, neuropathy and a healed foot ulcer (< 4 years) or Charcot's neuro-arthropathy to usual care (i.e. podiatric care, education, and therapeutic footwear) or usual care plus measuring temperatures at 6-8 predefined plantar foot locations each day (enhanced therapy). With $\Delta T > 2.2$ °C between left-to-right-foot corresponding regions for two consecutive days, participants were instructed to reduce ambulatory activity until this hotspot disappeared. Primary outcome was ulcer recurrence in 18 months on the plantar foot, interdigital, toe apical or medial/lateral forefoot surfaces (i.e. at or adjacent to the measurement sites). Secondary outcomes were ulcer recurrence in adherent participants and at any foot site.

RESULTS

On the basis of intention-to-treat, 44 of 151 (29.1%) participants in enhanced therapy and 57 of 153 (37.3%) in usual care had ulcer recurrence (RR 0.782 [95% CI 0.566-1.080], p = 0.133). Ulcer recurrence survival curves showed no significant group differences (p = 0.167). Participants measuring foot temperature and reducing activity when finding a hotspot had fewer recurrences than those in usual care (RR 0.336 [95% CI 0.114-0.986], p = 0.017). Enhanced therapy was effective over usual care for recurrence at any foot site (RR 0.760 [95% CI 0.579-0.997], p = 0.046).

CONCLUSION

At-home daily foot temperature monitoring does not significantly reduce incidence of diabetic foot ulcer recurrence at or adjacent to measurement sites over usual care, unless participants reduce their activity with hotspots found or when ulcers can occur at any foot site.

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Circadian control of brown adipose tissue activity by glucocorticoids

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BACKGROUND

Brown adipose tissue (BAT) displays a strong circadian rhythm in metabolic activity (van den Berg, Cell Rep 2018). On the other hand, circadian disturbances affect BAT activity and result in weight gain (Kooijman, PNAS 2015). The aim of the current study was to investigate the role of the superimposed rhythm in the glucocorticoid corticosterone in the metabolic activity of BAT.

METHODS

Wildtype and hyperlipidemic APOE*3-Leiden.CETP mice were subcutaneously implanted with pellets releasing a continuous low dose of corticosterone to flatten corticosterone rhythm. Alternatively, daily corticosterone injections were given to study the effect of hypercortisolism and adipose-specific GRKO mice were employed to investigate the underlying mechanism.

RESULTS

Implantation of corticosterone-containing pellets resulted in constant and flattened circulating corticosterone, with slight hypercortisolism. Strikingly, flattened corticosterone rhythm caused a complete loss of circadian rhythm in the uptake of triglyceride-derived fatty acids by BAT in both male and female mice. In line with these data, lipoprotein lipase mRNA and protein were highly rhythmic in BAT of vehicle-implanted mice, but were blunted in mice with flattened corticosterone rhythm. In APOE*3-Leiden.CETP mice, long-term experimental flattening of corticosterone – and thus BAT activity rhythm – resulted in increased lipid deposition in adipose tissue depots and as a consequence weight gain. All described effects were independent of glucocorticoid receptor expression in (brown) adipocytes and not caused by hypercortisolism, but rather mediated by reduced sympathetic outflow to BAT as evidence by a blunted rhythm in norepinephrine production and reduced adrenergic signaling.

CONCLUSION

A physiological glucocorticoid rhythm is essential for rhythmic BAT activity and metabolic health. We anticipate that disruption of glucocorticoid rhythm, and thereby BAT activity rhythm, could partially underlie the relationship between rhythm disturbances and metabolic disease in humans.

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Human IAPP drives painful diabetic peripheral neuropathy

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BACKGROUND

Peripheral neuropathy is a common complication in type 2 diabetes mellitus (T2DM). Insulin treatment reduces hyperglycaemia, but not neuropathy in T2DM, indicating that hyperglycaemia is not sufficient to cause neuropathy in T2DM. Human islet amyloid polypeptide (hIAPP) is overproduced with insulin by the pancreatic islet β-cells as a consequence of insulin resistance. Human IAPP forms pathogenic aggregates and amyloid leading to beta-cell death and possible damage to other tissues. Here, we investigated whether hIAPP contributes to neuropathy in transgenic mouse models of T2DM.

METHODS

Pain-like behaviours were assessed in hIAPP mice, obese hIAPP mice (hIAPP/ObOb) and wildtype (WT) mice. In addition, the ability of hIAPP to induce nerve damage was assessed in vivo by assessing intraepidermal nerve fiber (IENF) density.

RESULTS

hIAPP Ob/Ob mice had hyperglycaemia and elevated blood IAPP levels as compared to WT mice (glucose: 29 vs 10 pM; $p < 0.0001$; IAPP 729 vs 4.28 pM; $p < 0.0001$). hIAPP Ob/Ob mice developed signs of neuropathy because they had reduced skin IENF density (16.3 vs 47.2 IENF/mm; $p < 0.0001$), mechanical hypersensitivity (50% threshold of 0.0875 vs 0.4398 g; $p < 0.0001$) and thermal hyposensitivity (thermal withdrawal latency time of 10.02 vs 7.112 sec; $p < 0.0001$). hIAPP transgenic mice had elevated hIAPP levels (35 vs 4.2 pM; $p < 0.05$) but normal glycaemia. Intriguingly, hIAPP mice developed mechanical hypersensitivity (threshold 0.155 vs 0.438 g; $p < 0.0001$) and had reduced IENF density compared to WT mice (25.5 vs 47.2 IENF /mm; $p < 0.0001$). Moreover, hIAPP injection, into the paw or intravenously in WT mice dose-dependently induced long lasting (1-2 weeks) mechanical hypersensitivity and reduced skin IENF numbers at 1 week after injection (24.1 vs 35.6 IENF /mm; $p < 0.01$), whilst non-amyloidogenic mouse IAPP did not.

CONCLUSION

Human IAPP, contrary to non-aggregating mIAPP, induces signs of peripheral neuropathy in mice. Therefore, human IAPP is a potential driver of peripheral neuropathy in T2DM patients.

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111In-exendin SPECT imaging suggests presence of residual beta cells in patients with longstanding type 1 diabetes

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BACKGROUND

There is increasing evidence for the presence of residual, dysfunctional beta cells in patients with type 1 diabetes (T1D), but research is hampered by the lack of methods to quantify beta cell mass (BCM) in vivo in humans. Image-based quantification of pancreatic BCM using radiolabeled exendin-4 might provide such a method. We hypothesized that T1D patients have considerable remaining BCM and therefore should have detectable 111In-exendin-4 uptake in the pancreas.

METHODS

Ten T1D patients and ten matched healthy controls underwent quantitative SPECT following injection of 150 MBq 111In-exendin-4 after which pancreatic tracer uptake was determined. In addition, immunohistochemical analysis of human pancreatic sections from organ donors with longstanding T1D (C-peptide negative) was performed to assess GLP-1R expression, insulin, glucagon and somatostatin. See figure 2.

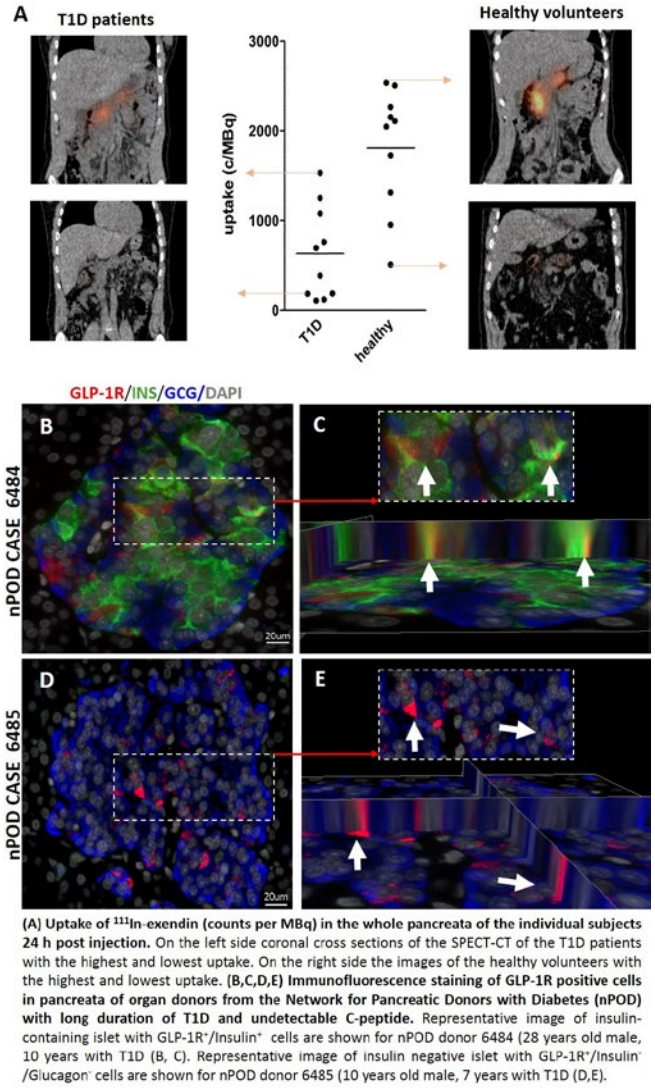
RESULTS

Uptake of 111In-exendin-4 was above background levels in 6/10 individuals with T1D and even comparable to levels in healthy controls in 5/10 patients. In all remaining patients, only background uptake (~30% of the mean uptake in T1D patients) was observed. Uptake was independent of stimulated C-peptide levels (< 0.03 nmol/L in 8/10 patients).

Immunohistochemistry demonstrated the presence of insulin/GLP-1R positive cells in 12/19 cases, explaining the high radiotracer uptake found in a subgroup of T1D patients. Furthermore, insulin-negative/GLP-1R positive cells were found, which proved to be somatostatin-positive, showing GLP-1R expression on delta cells and explaining the background tracer uptake in patients without remaining beta cells.

CONCLUSION

Quantitative exendin imaging was able to show differences in beta cell mass between patients and uncover the presence of residual beta cells in a subgroup of patients with T1D with low and stable background uptake levels. This demonstrates the value of this technique for in vivo determination of human pancreatic BCM and its potential use as a tool to further elucidate the complex pathophysiology of diabetes or study the effect of various interventions on BCM.



(A) Uptake of ^{111}In-exendin (counts per MBq) in the whole pancreata of the individual subjects 24 h post injection. On the left side coronal cross sections of the SPECT-CT of the T1D patients with the highest and lowest uptake. On the right side the images of the healthy volunteers with the highest and lowest uptake. **(B,C,D,E) Immunofluorescence staining of GLP-1R positive cells in pancreata of organ donors from the Network for Pancreatic Donors with Diabetes (nPOD) with long duration of T1D and undetectable C-peptide.** Representative image of insulin-containing islet with GLP-1R$^+$/Insulin$^+$ cells are shown for nPOD donor 6484 (28 years old male, 10 years with T1D (B, C). Representative image of insulin negative islet with GLP-1R$^+$/Insulin$^-$ /Glucagon$^-$ cells are shown for nPOD donor 6485 (10 years old male, 7 years with T1D (D,E).

Figure 2. Ten T1D patients and ten matched healthy controls underwent quantitative SPECT.

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Social determinants of health and complications among young adults with type 2 diabetes in an urban population

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BACKGROUND

Type 2 diabetes mellitus (T2DM) in young adults (YA) (18-45 years) is associated with more complications than when the disease develops in later life. Stratification of this population is needed to match care to risk on the development of diabetes complications. We aim to identify determinants that are associated with the development of diabetes complications in YA with T2DM in the urban region of The Hague.

METHODS

An observational retrospective cohort study using routine primary care linked to the Social Statistical Datasets hosted by Statistics Netherlands. Multivariate Cox regression was used to examine the association between the development of diabetes complications within 8 years after diagnose and medical (comorbidity, medication, body measurements) and social determinants (welfare, migration background).

RESULTS

In The Hague a prevalence of 8/1000 YA with T2DM was estimated. Out of 253 YA with T2DM, 35 developed at least one complication. People treated with medication in the year of diagnosis had a higher non-significant hazard on developing complications than peers with lifestyle advice only; blood-glucose-lowering medication (HR: 2.61, 95% CI: 0.61-11.11); insulins (HR: 3.44, 95% CI: 0.68-17.35). Furthermore, YA with low welfare compared to moderate or high welfare and YA with a Surinamese migration background compared to western or other non-western peers had a higher hazard; moderate (HR: 0.50, 95% CI: 0.22-1.18), high (HR: 0.58, 95% CI: 0.21-1.58); Surinamese (HR: 1.28, 95% CI: 0.58-2.79), other non-western (HR: 0.78, 95% CI: 0.32-1.88). These effects were not significant.

CONCLUSION

Due to the limited size of our dataset, the study has not enough power to draw conclusions about risk factors on the development of complications. Recently our database has been updated, giving access to approximately 4 times more inclusions. Currently we are working on an update of this research, aiming to improve the population description and prediction model.

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Effects of macronutrient intake in obesity: a meta-analysis of low-carbohydrate and low-fat diets on markers of the metabolic syndrome

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BACKGROUND

The metabolic syndrome (MetS) comprises cardiometabolic risk factors frequently found in individuals with obesity. Guidelines to prevent or reverse MetS suggest limiting fat intake. However, lowering carbohydrate intake has gained attention too. The aim for this review was to determine to what extent weight loss, reduction in caloric intake, or changes in macronutrient intake contribute to improvement in markers of MetS in persons with obesity without cardiometabolic disease.

METHODS

PubMed searches (search terms: low carbohydrate diet, low fat diet, Atkins, insulin, cardiovascular, human) yielded 17 articles describing 12 studies assessing changes in MetS markers of persons with obesity assigned to LC (< 40% energy from carbohydrates) or LF (< 30% energy from fat) diets. Meta-analysis and meta-regression analysis were performed.

RESULTS

Participants lost 6.8 kg on the LC diet and 5.3 kg on the LF diet. Both diets improved markers of MetS. Weight loss reduced fasting glucose levels (B = 0.065 mmol/kg) at the end of the study. Actual carbohydrate intake and actual fat intake at the end of the study, improved diastolic blood pressure (Carbohydrates: B = 0.093 mmHg/en%, Fat: B = -0.135 mmHg/en%) and circulating triglyceride levels (Carbohydrates: B = 0.012 mmHg/en%, Fat: B = -0.018 mmHg/en%), without an effect of weight loss. Remarkably, changes in caloric intake did not play a primary role in altering MetS markers.

CONCLUSION

Beyond the general effects of the LC and LF diet categories to improve MetS markers, there are also specific roles for weight loss, LC and HF intake, but not reduced caloric intake, that improve markers of MetS irrespective of diet categorization. Therefore, guidelines to prevent MetS may need to be re-evaluated.

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Dietary protein composition and renal outcome: a prospective study in the Diabetes and Lifestyle Cohort Twente (DIALECT)

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BACKGROUND

Chronic kidney disease is a common complication of diabetes mellitus type 2 (T2DM), accompanied with increased mortality. We investigated the effects of nutrition on renal endpoints in DIALECT, an observational study in T2DM patients treated in secondary care.

METHODS

Nutritional intake was assessed at baseline using a validated Food Frequency Questionnaire. Univariate and multivariate Cox Proportional Hazards models were used to calculate Hazard Ratios, time to event for competing risk analysis was calculated by the number of days to renal endpoint, death, or follow-up end date. The composite renal endpoint was defined as a 50% increase in serum creatinine from baseline visit, initiation of dialysis, or kidney transplantation.

RESULTS

There were 40 renal endpoints in 410 patients with mean follow-up duration of 6.0 ± 2.5 years. An inverse trend towards progression of renal function decline was found in the highest quartile of total protein intake compared to the lowest quartile (HR 0.37, 95% CI 0.11-1.22, p = 0.10). Patients in the highest quartile of red meat intake have a higher HR of 3.68 (95% CI 1.41-9.58, p = 0.008) compared to patients in the lowest quartile of red meat intake. On the other hand, patients in the highest quartile of oily fish intake have a lower HR of 0.29 (95% CI 0.09-0.93, p = 0.038) compared to patients in the lowest quartile of oily fish intake. Theoretical replacement models for substituting red meat by oily fish (HR 0.76 95% CI: 0.62-0.94, p = 0.011) and dairy (HR 0.88 95% CI: 0.82-0.95, p = 0.001) intake showed significantly lower hazard ratios of progression of renal function decline.

CONCLUSION

The findings suggest that dietary protein from red meat could have an adverse effect on the kidneys in T2DM, while oily fish and dairy-derived protein is renoprotective. Protein sources are important for renal outcome in T2DM in addition to overall protein intake.

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Dairy product consumption and incident prediabetes in Dutch middle-aged adults: The Hoorn Studies

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BACKGROUND

Evidence from cross-sectional studies suggests that higher dairy product consumption is associated with lower or no risk of prediabetes, an early phase in type 2 diabetes development. We investigated prospective associations of consumption of total dairy and dairy types with incident prediabetes in a Dutch population-based study.

METHODS

We calculated relative risks (RRs) between dairy, fermented dairy, milk, yogurt (total, high and low fat), cream and ice cream and prediabetes in 2262 participants without (pre)diabetes at enrolment (mean age 56 ± 7.3 years; 50% male) from the Hoorn Studies. Additionally, the substitution of one serving/day of dairy types associated with prediabetes with alternative dairy types was examined.

RESULTS

During a mean 6.4 (± 0.7) years of follow-up, 810 participants (35.8%) developed prediabetes. High fat fermented dairy, cheese and high fat cheese were associated with a 17% (RR 0.83, 95% CI 0.69-0.99, p^{trend} = 0.04), 14% (RR 0.86, 95% CI 0.73-1.02, p_{trend} = 0.04) and 21% (RR 0.79, 95% CI 0.66-0.94, p_{trend} = 0.01) lower risk of incident prediabetes, respectively, in higher compared to lower quartiles,

after adjustment for risk factors (demographic, lifestyle and dietary intake). High fat cheese consumption was linearly associated with a lower risk of prediabetes ($RR_{servings/day}$ 0.94, 95% CI 0.88-1.00, p = 0.04). Total dairy and other dairy types were not associated with prediabetes risk in multivariate adjusted models, irrespective of fat content (RR~1). None of the substitutions for high fat cheese were associated with prediabetes risk.

CONCLUSION

In this Dutch population-based cohort, a high intake of high-fat fermented dairy, cheese and high fat cheese were associated with a lower risk of prediabetes, whereas other dairy types were not associated. Cheese seems to be beneficial in diabetes prevention, despite high levels of saturated fatty acids and sodium.

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Complement factor D (adipsin) is positively associated with cfPWV in individuals with T2D: The Maastricht study

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BACKGROUND

Factor D (FD, also known as adipsin) is the rate-limiting protease of the alternative pathway of complement activation. FD was reported to be associated with a higher risk of stroke, while its relation with coronary heart disease is less clear. Carotid-femoral pulse wave velocity (cfPWV), an indicator for aortic stiffness, is independently associated with coronary heart disease and cerebrovascular disease. We herein investigated the association between FD and cfPWV.

METHODS

FD plasma concentrations and cfPWV were measured in 2270 participants (51% men, 60 ± 8.0 years, 26% type 2 diabetes [T2D]; oversampled) of The Maastricht study, a large population-based observational cohort. We conducted multiple linear regression analyses to investigate the association between FD (main independent variable) and cfPWV (outcome) with adjustments for confounders.

RESULTS

Per SD higher FD, cfPWV was 0.415 m/s greater (95% confidence interval (CI) [0.329; 0.501], p < 0.001). After adjustment for age, sex, mean arterial pressure and heart rate, this was attenuated (0.089 m/s, 95% CI, [0.014; 0.164], p = 0.021), and after additional adjustment for prediabetes and T2D no longer significant (0.065 m/s, 95% CI [-0.010; 0.139], p = 0.088). This remained so in the fully adjusted model. Since we observed interaction with T2D (Pinteraction FD*prediabetes = 0.107, FD*T2D = 0.002), we stratified on glucose metabolism status. In those with normal glucose metabolism, FD was positively associated with cfPWV, but only in the crude model (0.296 m/s, 95% CI, [0.186; 0.406], p < 0.001). In prediabetes, the association of FD with cfPWV was not significant in any model. In T2D patients, the crude association of FD with cfPWV was 0.404 m/s (95% CI [0.244; 0.563], p < 0.001. When adjusted for all confounders, this association was attenuated, but remained significant (0.177 m/s, 95% CI [0.023; 0.331], p = 0.025).

CONCLUSION

The independent positive association between FD concentrations and aortic stiffening was statistically significant only in individuals with T2D.

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Carnitine supplementation improves insulin sensitivity and skeletal muscle acetylcarnitine formation in type 2 diabetes patients

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BACKGROUND

We have previously shown that increasing free carnitine availability (via oral carnitine supplementation) resulted in elevated skeletal muscle acetylcarnitine concentrations and restored metabolic flexibility in impaired glucose tolerant (IGT) individuals. Therefore, we here investigated if carnitine supplementation elevates skeletal muscle acetylcarnitine formation and thereby improves insulin sensitivity and glucose homeostasis in type 2 diabetes patients.

METHODS

32 type 2 diabetes patients followed a 12-week L-carnitine treatment (2970 mg/day). A two-step hyperinsulinemic-euglycemic clamp was performed to assess hepatic and peripheral insulin sensitivity. In vivo skeletal muscle acetylcarnitine concentrations at rest and post exercise (30 minutes 70% Wmax), as well as intrahepatic lipid content (IHL) was determined by proton magnetic resonance spectroscopy (1H-MRS). All measurements were performed before and after carnitine supplementation.

RESULTS

Carnitine supplementation increased plasma free carnitine levels (from 35.6 ± 1.3 to 54.7±1.7 µmol/L, $p < 0.01$) indicating good compliance. Furthermore, we reported improvements in insulin-induced suppression of endogenous glucose (31.9 ± 2.9 vs. 39.9 ± 3.2 %, $p = 0.020$) and peripheral insulin sensitivity (Δ rate of disappearance, ΔRd: 10.53 ± 1.85 vs 13.83 ± 2.02 µmol/kg/min, $p = 0.005$). Resting and post-exercise skeletal muscle acetylcarnitine concentrations were both elevated after carnitine supplementation (1.18 ± 0.13 vs 1.54 ± 0.17 mmol/kgww, $p = 0.008$ and 3.70 ± 0.22 vs 4.53 ± 0.30 mmol/kgww, $p < 0.001$, respectively). A trend towards reduced plasma glucose levels (from 8.1 ± 0.3 to 7.7 ± 0.3 mmol/L, $p = 0.083$) and IHL (from 14.7 ± 2.6 to 12.8 ± 2.2%, $p = 0.098$) was found after carnitine supplementation.

CONCLUSION

The current study revealed very pronounced positive effects of carnitine supplementation on insulin sensitivity and a trend for an effect on intrahepatic lipid content and fasting plasma glucose levels in type 2 diabetes patients. Furthermore, we demonstrated that carnitine supplementation increases acetylcarnitine concentration in muscle, which may be underlying the beneficial effect on insulin sensitivity.

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Sansevieria trifasciata leaf extract protects pancreatic beta-cells against streptozotocin-induced cell death via the Nf-κB pathway

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BACKGROUND

An improved understanding of the mechanisms linking inflammation to type 2 diabetes (T2DM) has stimulated interest in targeting inflammatory pathways as part of the strategy to prevent or control T2DM. To this end, traditional medicines from medicinal plants offer great potential for the discovery of new anti-diabetic drugs, due to the versatile bioactive molecules with different biological activities. Therefore, the anti-diabetic activities of the leaves of the Sansevieria trifasciata (ST) plant were investigated, by assessing the effect of ST-extract on the inflammatory response and against streptozotocin (STZ)-induced beta-cell death in vitro.

METHODS

Cell viability was studied after STZ-induced cell death in the mouse insulinoma (MIN6) cells in an MTT-assay. Treatment with ST-extract (2.5-15 mg/mL) was combined with either TNF-α, to mimic a pro-inflammatory environment, or with the inflammatory Nf-κB pathway blocker MG132.

RESULTS

ST-extract protected MIN6 cells against STZ-induced cell death (LD50 = 10 mM) in a prophylactic and therapeutic manner (Dose 5-15 mg/mL; ~15% versus ~50% cell death). This protective effect of ST-extract disappeared when the Nf-κB pathway was blocked with MG132 (> 5 mg/mL ST; ~50% cell death). Remarkably, when ST-extract was combined with TNF-α administration the protective effect of ST extract also disappeared (> 5 mg/mL ST; ~50% cell death)

CONCLUSION

Our results show that ST-extract protects against STZ-induced cell death in MIN6 cells, which is dependable on the Nf-κB pathway. However, when ST treatment is combined with TNF-α, the protective effect of ST disappears. This effect could be induced by TNF-α pathways that are independent of Nf-κB activation, like the caspase apoptotic pathway resulting in cell death.

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Effects of 12 different heel rocker designs, configurated with different rocker radii, apex positions and apex angles, on plantar pressure

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BACKGROUND

Rocker shoes are used to offload high-risk areas of the foot to prevent foot ulcers in diabetic patients with loss of protective sensation. These high-risk areas are the hallux, metatarsal heads (MTH) and heel region [1]. Forefoot rocker shoes can reduce the peak plantar pressure of the hallux and MTH regions, however, pressure in the heel is often elevated by this type of footwear [2,3,4]. No studies have analyzed the effect of different heel rocker designs on the heel plantar pressure.

METHODS

Shoes with 12 different heel rocker configurations were designed with different heel rocker radii, apex positions and apex angles (figure 3). The relative peak plantar pressure (RPP) of each configuration in 7 heel masks figure 3) was studied in 10 healthy participants.

RESULTS

There is a significant main effect of the rocker radius on the RPP for the different heel masks. A larger radius (LR), compared to a smaller radius (SR), causes significantly lower RPP in mask 1, 2 and 3, whereas the same radius causes a significant increase in RPP in mask 5 and 7. Moreover, a significant interaction effect between rocker radius and apex position for mask 1 and 3 was found. A LR, compared to a SR, with a proximal apex position causes a significantly lower RPP in mask 1 and 3. The same is seen in mask 1 with the mid apex position.

CONCLUSION

The radius of the heel rocker affects heel pressure distribution. A steep curve (= SR) resulted in an increased RPP in the proximal heel compared to a LR, probably due to a shortened rear curve rolling time. Patients with high risk areas in the proximal heel region benefit more from a rocker shoe with larger heel radii, whereas smaller heel radii are beneficial for patients with high risk areas in the distal heel region.

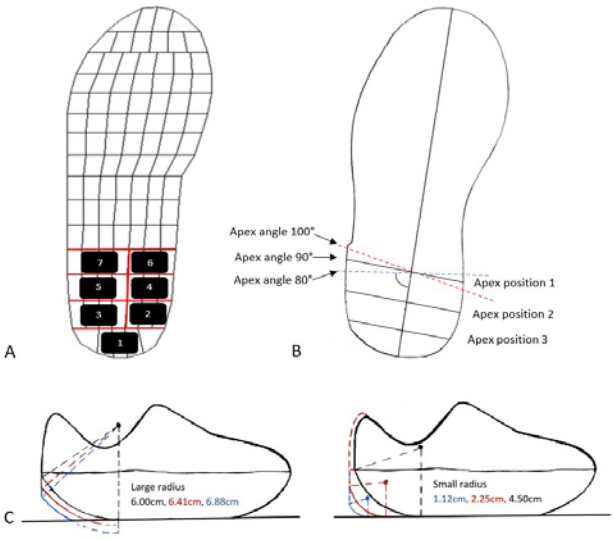

Figure 3. Heel masks of the Pedar insole and design parameters for the heel rocker shoes. (A) The numbers represent the following masks, 1: proximal heel region, 2: medial side of proximal heel region, 3: lateral side of proximal heel region, 4: medial side of midheel region, 5: lateral side of midheel region, 6: medial side of distal heel region, 7: lateral side of distal heel region. (B) The different apex angles are represented by the red dotted line: apex angle of 100°, the black line: apex angle of 90° and blue dotted line: apex angle of 80°. The distal, mid and proximal apex position are also shown in the same image. (C) The left image illustrates the heel rocker shoe with the large radius (range: 6.00 - 6.88cm) and the right image shows the heel rocker shoe with the small radius (range: 1.12 - 4.50cm).

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Timing of physical activity in relation to body weight and metabolic health in sedentary older people: a cross-sectional and prospective analysis

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BACKGROUND

Although increased physical activity is a known contributor to metabolic health, less is known about the impact of timing of physical activity. We assessed the associations between accelerometery-based daily timing of physical activity and measures of metabolic health in sedentary older people using cross-sectional and prospective analyses.

METHODS

Objectively measured physical activity was obtained through accelerometery in participants from the prospective Active and Healthy Ageing study. Raw acceleration was transformed to an hourly mean physical activity over a 6-day period. This was repeated after 3 months. For the cross-sectional analyses, we only used data collected at baseline. For the longitudinal analysis, we calculated the hourly difference in objective physical activity between baseline and 3-months follow-up. We calculated clusters resembling periods with similar physical activity patterns through a principal component analysis. We used a linear regression analysis to associate the principal components with body mass index (BMI), fasting glucose and insulin, HbA1c and the homeostatic model assessment for insulin resistance (HOMA-IR).

RESULTS

207 individuals (61.4% male, mean age: 64.8 [SD, 2.9], mean BMI: 28.9 [4.7]) were included in the analysis. We found 5 baseline physical activity clusters and 8 clusters for change in physical activity periods. At baseline, higher daytime physical activity was associated with lower BMI. In addition, a higher increase in physical activity between 4 and 7 AM and between 6 and 9 PM during follow-up was associated with decreased BMI (-0.4% [95% confidence interval, CI: -0.8, 0.0] and -0.5 [95% CI: -0.9, -0.1], respectively). Furthermore, higher increase in physical activity between 8 AM and 12 PM was associated with decreased HOMA-IR (-7.7% [95% CI: -14.2, -1.6]), independent of change in BMI.

CONCLUSION

Specific timing of physical activity was associated with several measures of metabolic health, suggesting time-dependent physical activity interventions are required to reach maximum health benefits.

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Liraglutide treatment improves immune gene expression in blood of overweight type 2 diabetes patients of South Asian descent

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BACKGROUND

Several recent meta-analyses have demonstrated beneficial effects of glucose lowering glucagon-like peptide-1 receptor (GLP1R) agonists on cardiovascular outcomes. Preclinical studies suggest that these effects are exerted by inducing anti-inflammatory effects rather than by improving dyslipidemia. However, in humans the effects of GLP1R agonists on the immune system remain to be determined. A population typically at risk to develop cardiovascular diseases and T2DM are the South Asians. In this study, we aimed to investigate the effect of treatment with the GLP1R agonist liraglutide on immune gene expression in blood in overweight T2DM patients from South Asian and Europid descent.

METHODS

Fasted blood samples were obtained before and after 26 weeks liraglutide treatment (1.8 mg/day SC), from overweight T2DM patients from South Asian (n = 7; age 58 ± 7.9 years) and Europid (n = 11; age 62 ± 3.7 years)

descent. mRNA expression of 144 immune genes in blood was measured using a dual-color reverse transcriptase multiplex ligation-dependent probe amplification (dcRT-MLPA) assay.
Results At baseline, 11/144 genes were significantly differently expressed in South Asians compared to Europids. Liraglutide treatment modified expression of these genes in South Asians to similar level as in Europids. In South Asians only, expression of 51/144 genes was modulated after treatment. More specifically, liraglutide downregulated several markers of pattern recognition receptors (TRL2-5, TLR8, NOD1, NOD2; -25-57%, $p < 0.05$) and T-cells (TBX21, CCR7, IL9, IL13, IL2RA, LAG3, FOXP3, TNFRSF18; -43-90%, $p < 0.05$).

CONCLUSION

South Asian patients with overweight and T2DM exhibit a different expression of several immune related genes compared to Europids. Liraglutide treatment downregulates expression of several markers of pattern recognition receptors and T-cells in South Asians only.

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Genetically-influenced lower TG levels on top of genetically-influenced lower LDL-C levels are associated with an improved lipoprotein profile

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BACKGROUND

Lipoprotein lipase (LPL) is a key player in lipoprotein remnant clearance, and therefore a druggable target. Recent Mendelian randomization studies have provided evidence that TG-lowering alleles in the LPL pathway are associated with lower risk of coronary disease independently of LDL-C-lowering genetic mechanisms (Lotta et al. JAMA Cardiology. 2018). Here, we aimed to provide insight into the casual mechanisms behind these effects by assessing through Mendelian randomization the effect of genetically-influenced lower TG levels via LPL alleles on the nuclear magnetic resonance (NMR) determined metabolomic profile on top of genetically-influenced lower LDL-C levels.

METHODS

We quantified over 100 lipoprotein (sub)components in 4838 participants of the NEO study. The TG genetic score was based on five TG-lowering LPL alleles and the LDL-C score on 19 LDL-C–lowering alleles. These genetic scores were dichotomized at their corresponding median value to "naturally randomize" the participants into 4 groups comprising high/low TG levels and high/low LDL-C levels and were analysed in a 2 × 2 factorial design. Replication of these analyses was performed in an independent cohort of Oxford Biobank (OBB) (n = 6999).

RESULTS

In the discovery cohort, 44 % were male and the mean (SD) age was 55.9 (6.01) years. Naturally randomizing people to both lower TG and lower LDL-C levels resulted in the highest number (102) of significant NMR-metabolite associations and the largest effect sizes of these associations. The effects of the LPL and LDL-C genetic scores were additive, but independent from each other (p-interaction<0.05). Our findings were confirmed in the replication cohort.

CONCLUSION

Our study provides evidence of an additional beneficial effect of pharmacologically enhanced LPL activity on top of cholesterol lowering, which may consequently further improve cardiovascular outcomes.

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Association between dietary carbohydrates and liver fat content: the NEO study

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BACKGROUND

Liver fat is a major cause of chronic liver disease and associated with type 2 diabetes. Current treatments to reduce liver fat are mainly aimed at weight loss by caloric restriction. However, changing the composition of the diet may be a more feasible option. Because dietary carbohydrates may increase liver fat content through de novo lipogenesis, we aimed to investigate the association between isocaloric replacement of dietary carbohydrates with fat or protein and liver fat.

METHODS

In this cross-sectional analysis of the Netherlands Epidemiology of Obesity study, liver fat was assessed by proton-MR spectroscopy and intake of macronutrients was estimated with a food frequency questionnaire. Macronutrients were converted to percent of total energy intake (En%). Dietary fat and protein were further subdivided into sources (plant-, dairy- and meat-based). We used linear regression analysis to model isocaloric replacement of 5 En% carbohydrates with 5 En% fat or protein in relation to liver fat.

RESULTS

In total, 1814 participants (44% men) were analysed, with a mean (SD) age of 55 (6) years, BMI of 26 (4) kg/m^2 and liver fat content of 5.5% (7.7). Isocaloric replacement of 5 En% carbohydrates with 5 En% of protein was associated with less liver fat (0.82 times; 95% CI 0.72, 0.94), in particular dairy (0.78 times; 0.63, 0.96) and plant-based protein (0.74 times; 0.51, 1.06). We observed no association between replacement of dietary carbohydrates with dietary fat and liver fat content. However, the associations with fat from meat (1.10 times; 0.95, 1.28) and fat from dairy (0.96 times; 0.88, 1.03) had opposite directions.

CONCLUSION

These results indicate that, in order to reduce liver fat content, it appears best to replace dietary carbohydrates with dietary protein, possibly due to the inhibited fat oxidation and increased satiety induced by dietary protein. However, the source of these proteins appears important, which is in line with the current food group-based dietary guidelines.

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Growth in children prior to diagnosis of juvenile type 1 diabetes: A systematic review

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BACKGROUND

Juvenile onset type 1 diabetes (T1D) is one of the most common chronic diseases in childhood and shows a rising incidence over the past decades. The exact pathogenesis is still not completely understood, especially regarding possible environmental factors triggering disease onset. We aimed to systematically review literature on growth in children prior to diagnosis of juvenile type 1 diabetes and to ascertain whether specific patterns of growth prior to diabetes onset, are a consistent phenomenon.

METHODS

This systematic review was fulfilled according to the PRISMA Guidelines. In April 2020, three online databases were consulted (PubMed, Embase and Cochrane Library). Studies describing growth in children prior to juvenile type 1 diabetes onset and covering patient populations from birth till age at onset and not older than 20 years were assessed.

RESULTS

37 studies were included, involving 156609 T1D cases. Of these, 5065 cases were matched with 462772 healthy individuals and 1230 non-diabetic siblings. An increased weight gain, expressed as weight SDS from birth till diagnosis, in early childhood was found to be positively associated with the risk of T1D development. Moreover, we were able to ascertain a higher weight SDS in children at diagnosis. High BMI SDS at birth did not show any significant outcome.

CONCLUSION

There appears to be a clear association between the early environmental factor of accelerated weight gain in the first years of life, higher weight at diagnosis on one hand, and the risk for (later) juvenile onset type 1 diabetes, on the other.

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The Associations of Leptin and Adiponectin with the Metabolic Syndrome in an Indonesian and a Dutch Population

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BACKGROUND

Asian populations develop cardiometabolic complications at a lower BMI than Western populations. In part, this may be the result of a different body fat distribution and consequent excretion of the adipocyte-derived hormones leptin and adiponectin. We aimed to investigate the associations of leptin and adiponectin with the metabolic syndrome (MetS) in an Asian-Indonesian and a Caucasian-Dutch population.

METHODS

We performed cross-sectional analyses investigating baseline measurements of the Netherlands Epidemiology of Obesity Study (n = 6671; 56% women) and the Indonesian SUGARSPIN Study (n = 1669; 61% women). We performed sex-stratified logistic regressions to examine the associations of leptin and adiponectin with MetS, adjusted for age, education, alcohol, smoking, diabetes, and further adjusted for total body fat.

RESULTS

The mean (SD) leptin (mcg/L) were 4.7 (6.0) in Indonesian men, 18.6 (12.0) in Indonesian women, 9.1 (7.7) in Dutch men, and 23.4 (17.4) in Dutch women. The mean (SD) adiponectin (mg/L) were 5.7 (5.4), 7.5 (7.1), 6.6 (3.3), and 11.3 (4.9), respectively. Within the same BMI category, leptin concentrations were similar in both populations, whereas adiponectin was lower in the Indonesian than the Dutch population. Per SD of leptin, adjusted prevalence odds ratios (ORs, 95% CI) of MetS were 2.5 (1.9-3.1) in Indonesian men, 2.6 (2.2-3.1) in Indonesian women, 4.6 (3.6-5.7) in Dutch men, and 3.6 (3.1-4.2) in Dutch women. Per SD of adiponectin, the ORs were 0.8 (0.6-1.0), 0.7 (0.6-0.8), 0.6 (0.5-0.7), and 0.4 (0.3-0.5), respectively. After further adjustment for total body fat, leptin and adiponectin were not associated with MetS in the Indonesian population.

CONCLUSION

Adiponectin concentration was lower in the Indonesian than the Dutch population. In both populations, total body fat strongly influenced the associations of leptin and adiponectin with MetS. Despite lower levels of adiponectin, adiponectin was not related to the risk of MetS and does not explain the increased cardiometabolic risk in the Asian-Indonesian population.

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Pancreatic uptake of radiolabeled exendin as a measure of beta cell mass in T2DM before and after bariatric surgery

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BACKGROUND

An approach for in vivo beta cell imaging is targeting the glucagon-like peptide-1 (GLP-1) receptor by radiolabelled-exendin-4. Currently, the role of beta cell mass (BCM) and function (BCF) in type-2 diabetes mellitus (T2DM) is not clear, and might be related to T2DM remission after gastric bypass surgery (RYGB). This study aims to examine BCF and BCM as pancreatic uptake of 68Ga-exendin-4 in patients with T2DM before and after RYGB.

METHODS

Oral glucose tolerance test (OGTT) and 68Ga-exendin-4-PET/CT were performed pre- and one year post-RYGB. Total pancreatic uptake of 68Ga-exendin-4 per injected activity (kBq/MBq) was measured as marker for BCM.

RESULTS

Thirteen patients were included, analysis in nine patients with complete follow-up was performed and shown in

Table 1. Pre- and postoperative outcomes as average and [range] per group.

	Insulin group	Metformin group	P-value
N (female)	6 (3)	3 (0)	
Age	54 [45-65]	54 [50-57]	0.96
T2DM duration			0.31
Pre-RYGB			
Insulin dose (IU/day)	104 [52-198]	0	na
Metformin dose (g/day)	1.4 [0-2.5]	1.5 [1.0-2.0]	na
BMI (kg/m2)	39 [34-41]	40 [35-49]	0.82
HbA1c (mmol/mol)	68 [56-82]	55 [50-60]	0.089
c-peptide response during OGTT (nmol/l)	1.1 [0.7-1.9]	2.9 [1.8-4.3]	0.015
Total pancreatic uptake of 68Ga-exendin-4 (kBq/MBq)	1.6 [0.53-2.7]	3.0 [2.5-3.2]	0.017
Post-RYGB			
Insulin dose (IU/day)	1.7 [0-10]	0	na
Metformin dose (range, g/day)	0.4 [0-1500]	0	na
BMI (kg/m2)	28 [22-31]	26 [22-30]	0.34
HbA1c (mmol/mol)	54 [37-77]	31 [25-35]	0.045
c-peptide response during OGTT (nmol/l)	1.7 [0.4-2.8]*	2.5 [1.4-5.0]	0.55
Total pancreatic uptake of 68Ga-exendin-4 (kBq/MBq)	2.3 [1.9-3.1]	2.5 [1.7-3.6]	0.66

0* missing in 2 patients

table 1. Six patients had insulin therapy and three only metformin. BMI and HbA1c decreased significantly after RYGB ($p < 0.01$). Preoperatively, pancreatic uptake and c-peptide response were lower in insulin compared to metformin group ($p = 0.017$ and $p = 0.015$). In the insulin group, one patient had complete remission (no antidiabetics, normal HbA1c), two patients had little improvement (insulin or sulfonylurea and unchanged HbA1c) and three patients had improvements in between, one year after RYGB. Pancreatic uptake increased in this group ($p = 0.23$), which seems related to the degree of improvement. Relative increase of 56-340% vs 8-30% in three patients with most and least improvement. All patients on metformin had complete remission. Pancreatic uptake decreased; relative change of -47% to +13%.

CONCLUSION

As could be expected, patients with insulin dependent T2DM have lower BCM and BCF compared to patients without insulin therapy. In the metformin group average pancreatic 68Ga-exendin-4 uptake decreased, probably reflecting reduction of beta cell hyperplasia. Insulin-dependent patients with large improvement, showed increased pancreatic uptake. This may indicate towards recovered beta cell mass and has not been observed in patients before. Mechanisms behind BCM recovering and 68Ga-exendin-4 uptake need further investigation.

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Predicting liver fat content using routinely measured predictors: a development study

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BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a strong prognostic factor for type 2 diabetes. In clinical practice, estimations of liver fat content could be useful for encouraging lifestyle change or to improve cardiometabolic risk stratification. We developed a model to predict liver fat content with routinely measured predictors and evaluated whether this model could be improved by including liver enzymes and metabolomics.

METHODS

We performed cross-sectional analyses on data of 2053 middle-aged individuals from the Netherlands Epidemiology of Obesity study with proton magnetic resonance

measurements of hepatic triglyceride content (HTGC). We used linear regression to estimate a core model including age, sex, BMI and fasting glucose and triglyceride concentrations with HTGC as outcome and evaluated the added value of several predictors including liver enzymes, insulin and uric acid concentrations, waist and hip circumference and Nightingale metabolomics measurements. All models were internally validated through bootstrapping.

RESULTS

The included participants (52.1% men) had a mean BMI of 29.4 (SD: 4.2) and a median HTGC of 5.0% (interquartile range: 2.2-12.1%). The core model had an optimism-corrected explained variance (R2) of 0.22, a root-mean-squared error (RMSE) of 8.8% and a c-statistic of 0.80 for a HTGC cut-off of 5.56%, which improved to a R2 of 0.37, RMSE of 7.9% and a c-statistic of 0.83 after including aspartate and alanine transaminase concentrations. Further adding insulin and uric acid concentrations, and waist and hip circumference, resulted in small improvements (R2 of 0.39, RMSE of 7.7% and a c-statistic of 0.84), while measurements from the Nightingale metabolomics platform did not further improve model performance.

CONCLUSION

Liver fat content can be predicted using relatively routine clinical measurements such as age, sex, BMI and liver enzyme concentrations and does not require the additional measurement of expensive or time-consuming predictors such as waist circumference or metabolomics.

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12-week combined polyphenol supplementation: indications for sex-specific differences in gut microbiome-host metabolism interaction in individuals with overweight and obesity

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BACKGROUND

Alterations in gut microbiota composition and functionality are related to obesity-related metabolic diseases. We previously showed that polyphenol supplementation exerts beneficial effects on host metabolism, which may be mediated through changes in the gut microbiota. However, it is not fully clear whether and how polyphenols impact gut microbiota composition, and whether this is sex-specific. Here, we investigated the interactions between combined polyphenol supplementation, fecal microbiota profile, and associations with tissue-specific insulin sensitivity, substrate oxidation and skeletal muscle mitochondrial function in men and women.

METHODS

In a double-blind, randomized, placebo-controlled study, 18 men and 19 women with overweight/obesity received either epigallocatechin-3-gallate and resveratrol (EGCG+RES, 282 and 80 mg/day) or placebo for 12 weeks. Before and after the intervention, fecal samples were collected, tissue-specific insulin resistance was determined by a two-step hyperinsulinemic-euglycemic clamp with [6,6-2H2]-glucose infusion, fasting/postprandial substrate oxidation by indirect calorimetry, and skeletal muscle mitochondrial oxidative capacity by ex vivo respirometry.

RESULTS

Baseline microbiota composition of specific genera ($q < 0.2$) and phyla (Verrucomicrobia, $q = 0.02$) were significantly different between men and women. Overall, 12-week EGCG+RES supplementation did not induce significant changes in fecal microbiota composition ($q > 0.05$) and α- and β-diversity ($p = 0.69$ and $p = 0.95$ respectively). However, baseline abundance of specific microbial genera highly correlated with polyphenol-induced changes in skeletal muscle oxidative capacity in men ($p < 0.05$), but not in women.

CONCLUSION

Our findings suggest that combined polyphenol supplementation has no effect on fecal microbiota composition in individuals with overweight/obesity. However, baseline microbiota composition may be more predictive for changes in metabolic outcomes in men compared to women. Future studies investigating gut microbiome-host metabolism interactions in humans should therefore consider employing a sex-specific approach.

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Type 2 diabetes in South Asians compared to white Caucasians: higher risk and earlier development of major cardiovascular events irrespective of the presence and degree of retinopathy. Results from The HinDu The Hague Diabetes Study

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BACKGROUND

To study the relationship between diabetic retinopathy (DR) and a first non-fatal Major Adverse Cardiovascular Event (MACE) in South Asians and white Caucasians, including the effect of age of diabetes diagnosis.

METHODS

A total of 3831 adults with type 2 diabetes, consisting of 1358 South Asians and 2473 white Caucasians, treated in our diabetes clinic between 2006-2017 were included in the study. Data on risk factors, diabetes duration, age of diagnosis and diabetes complications were collected and analyzed using descriptive statistics and Cox regression. DR was graded in 3 categories and MACE was pre-specified.

RESULTS

Prevalence of MACE increased with increasing severity of DR in both ethnic groups, but MACE was more frequent in South Asians with DR compared to white Caucasians with DR (mild 42 vs. 50% and severe 46 vs. 62%). Furthermore, time until first (TUF) MACE was significantly shorter in South Asians, an effect also seen in the no-DR group (4 years; HR 1.5 and 7 years earlier; HR 2.0 for no-DR and severe DR, respectively) after correction for both classic risk factors and age at diagnosis. (table 2)

CONCLUSION

TUF MACE in South Asians is significantly shorter compared with white Caucasians, even in the no-DR group. Our findings corroborate data on the relationship between the severity of DR and MACE in both ethnic groups.

Table 2.

Ethnic group	WC‖	SA ¶	p-value	WC	SA	p-value	WC	SA	p-value
All Patients n(%)	1675(67.5)	709(52.2)	0.001	494(20)	288(21)	ns	303(12)	359(26)	0.001
Diabetes duration yrs/m±sd.	12.6±8.9	13.3±9.8	ns	15.6±8.9	14.8±8.5	ns	18.2±9.9	18.7±9.7	ns
Never Smoked n(%)	926(55.3)	515(72.6)	0.001	293(59.3)	215(74.7)	0.001	173(57.1)	266(73.9)	0.001
Hypertension n(%)	980(58.8)	358(50.5)	0.001	324(65.6)	180(62.5)	ns	202(66.7)	245(68.1)	ns
Dyslipidemia n(%)	656(39.2)	288(40.6)	ns	232(47)	134(46.5)	ns	122(40.3)	174(48.3)	0.037
MACE n(%)	589(35.2)	242(34.1)	ns	208(42)	145(50)	0.001	139(46)	224(62)	0.001
Crude model* TUF§ MACE(yrs)	-1.4		0.079/ns		-2.1	0.054/ns		-2.1	0.054/ns
HR TUF MACE (CI 95%)	0.87(0.8-1.0)		0.079		1.2(0.97-1.5)			1.2(0.99-1.5)	
Model 2 † TUF MACE(yrs)	-0.9		ns		-2.8	0.012		-1.9	ns
HR TUF MACE (CI 95%)	0.9(0.8-1.1)				1.3(1.1-1.6)			1.2(0.97-1.5)	
Model 3 † TUF MACE in yrs		-4.1	0.001		-7.3	0.001		-7.4	0.001
HR TUF MACE (CI 95%)		1.5(1.3-1.8)			2.1(1.6-2.6)			2.0(1.6-2.6)	

* Crude model only ethnicity included
† Model 2 additionally including the classic risk factors (hypertension, dyslipidemia, smoking)
‡ Model 3 = after adjustment for classic risk factors and age at diabetes diagnosis
§ TUF= Time until first MAC
‖ WC = White Caucasians
¶ SA = South Asians

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Microbiological safety in islet transplantation: a 13-year retrospective analysis

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BACKGROUND

The transplantation of islets of Langerhans is an effective therapy for the treatment of patients with type 1 diabetes. For a successful transplantation the final product must not only meet criteria for islet quality and quantity but must also be free of pathogens and have a low endotoxin concentration. Standardized protocols define the manufacturing processes from a contaminated donor organ towards a clean product. In this study, the prevalence of microbiological contamination at different steps in the isolation process was established.

METHODS

Samples were taken routinely at 3 timepoints during the islet isolation procedure (before start (organ preservation solution (OPS), during the isolation (in-process), final product (islets considered for transplantation)). Aerobic and anaerobic BACTEC cultures were performed for all timepoints. Gram staining was performed in-process and for the final product. Endotoxin was determined for the final product.

RESULTS

For this analysis a total of 410 islet isolation procedures (400 allogenic, 10 autologous) were included. No difference in contamination was detected between donation after brain death (74%) and donation after circulatory death (76%) procedures. In allogenic procedures the prevalence of contamination measured by BACTEC cultures was 77% for OPS, 2% during the isolation, and 3% in the final product. These contaminations were not detected by gram staining. Nevertheless gram detected contamination in two BACTEC negative products. Furthermore, three final products exceeded the threshold for endotoxin concentrations but were not contaminated in the other assays.
In the context of islet autotransplantation 88% of OPS and 75% of the final products contained microorganisms. Gram staining was positive in one product which was BACTEC negative. Two autologous products had elevated endotoxin concentration.

CONCLUSION

These results demonstrate that contaminations are only reliably detected by a combination of several microbiological assays. The established protocols ensure a safe product for allogenic procedures in contrast to autotransplantations. Here adjustments are required to reduce the contamination rates and the potential risks for transplant recipients.

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The gut microbiome in long-standing type 1 diabetes

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BACKGROUND

The presence of Type 1 diabetes (T1D) leads to various complications that are associated with altered immune responses including an increased risk for infections and vascular disease. Although changes in the microbiome during the development of T1D have been linked to alterations in the immune response, not much is known about the role of the gut microbiome in long-standing T1D. We therefore set out to determine differences in the gut microbiome of T1D patients compared to healthy controls and to associate the microbiome with diabetes-related complications.

METHODS

239 T1D patients were included with an average disease duration of 28,4 years. Clinical characteristics and faecal samples were collected. Metagenomic shotgun sequencing was performed and the results were associated to T1D-related characteristics and complications including HbA1c, and macrovascular and microvascular complications. Microbiome data were compared to a healthy cohort, consisting of 2937 age and sex matched individuals.

RESULTS

No significant difference in diversity of the gut microbi-

ome was found between T1D patients and healthy controls. However, the proportions of several bacterial taxa were altered in T1D. 20 bacterial taxa were significantly depleted in T1D, for examples S. Alistipes Putredinis (FDR = 1.6 x 10-12). Furthermore, 76 bacterial taxa were significantly enriched in T1D, such as G. Clostridium (FDR = 4.0 x 10-15). Glycaemic control, measured by HbA1c (ranging from 34 to 136 mmol/mol), explained a significant part of variation in gut microbiome (R2 > 0.010, FDR < 0.05). Furthermore, variation in gut microbiome was also explained significantly by the absence or presences of vascular complications (R2 > 0.0075, FDR < 0.05).

CONCLUSION

Although the diversity is not affected, the composition of the gut microbiome T1D patients is significantly different compared to healthy controls. T1D related characteristics and vascular complications are associated with changes in the gut microbiome. These data suggest that the gut microbiome is not only important in the context of disease development, yet may also contribute to the development of diabetes-associated complications.

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PPARdelta in hypothalamic microglial controls glucose metabolism and insulin sensitivity

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BACKGROUND

Microglia are the brain innate immune cells essential for maintaining a local micro-environment optimal for neuronal function. Our previous study showed that lack of lipoprotein lipase (a key enzyme that gates lipid uptake) in microglia worsened glucose metabolism in high fat diet induced-obese (DIO) and insulin resistant mice. These effects are largely mediated by neurons located in the mediobasal hypothalamus (MBH) that control glucose metabolism and energy homeostasis. In the current study, we explored whether enhancing microglial fatty acid oxidation in the MBH by a peroxisome proliferator-activated receptor (PPAR)-δ agonist would exert beneficial effects on glucose metabolism in DIO rats.

METHODS

To deliver the PPAR-δ agonist GW0742 specifically into microglia in the MBH, we developed polymer hybridized PLGA-PEG nanoparticles that can pack the GW0742 (NP-GW0742). Nanoparticles packed with vehicle were used as control (NP-Veh). We tested the efficacy of the NP-GW0742 in stimulating lipid utilization in microglial cells in vitro, by measuring the oxygen consumption rate (OCR). Subsequently, we infused the NPs into the MBH of DIO rats, and tested their effects on insulin sensitivity.

RESULTS

We found that in a concentration of 5 μM NP-GW0742 increased the OCR by 52% in cultured microglial cells. In the in vivo study, after 12 days of NP-GW0742 infusions in the MBH, basal blood glucose levels in DIO rats were not different from the NP-Veh group. However, endogenous glucose production (EGP) was significantly higher in the NP-GW0742 infused rats as compared to the NP-Veh infused rats, indicating that rats received the NP-GW0742 also had a higher glucose uptake. Moreover, the NP-GW0742 induced increased EGP did not correlate with plasma insulin levels. In the insulin tolerance test, we found a significantly higher insulin sensitivity in the rats that had received NP-GW0742 as compared to those that had received NP-Veh.

CONCLUSION

Thus, administration of NP-GW0742 in the MBH resulted in an improvement in insulin sensitivity.

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A Complex System Approach to the Assessment of Homeostasis Loss in Type 2 Diabetes

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BACKGROUND

The role of individual circulating biomarkers in the development of type 2 diabetes (T2D) has been broadly studied, but interactions of such biomarkers as proxy of homeostasis dysregulation remain underexplored. The aim of the present study was to analyze biomarkers in the context of T2D development using Mahalanobis Distances (MDs), which are unitless measures of the dispersion of reduced dimensionality features (i.e. Principal Components).

METHODS

We calculated the MDs of the Principal Components (PCs) containing information of 27 plasma biomarkers (comprehending glycemic, lipid, microbiome and one-carbon metabolism) measured in 4446 participants from the PREVEND study. Cox regression analyses were performed using the MDs as predictors of T2D.

RESULTS

After a median follow-up of 8.6 years, incident T2D was ascertained in 227 subjects. PCs number 1, 2, 4, 7, 8, 10, which incorporate the variability of iron metabolism and Branched Chain Amino Acids were associated with a reduced risk of T2D; and PCs number 6, 9, 11, 12, 13, 17, 22, accounting for hepatic, lipids and glucose metabolism were associated with an increased risk of T2D. The hazard ratio of the MDs calculated from the 27 PCs was 1.87 (95% CI, 1.53-2.29; $p < 0.001$). The highest hazard ratio was obtained using the MDs calculated from the first 13 PCs (2.14 (95% CI, 1.77-2.59; $p < 0.001$)). Such associations remained after the adjustment for age, being 1.91 (95% CI, 1.58-2.30; $p < 0.001$) and 2.10 (95% CI, 1.75-2.53; $p < 0.001$), respectively. Interestingly, the association of MDs calculated from all different subsets of PCs were stronger in women than in men ($p < 0.01$).

CONCLUSION

This study suggests that MDs contain information about the homeostasis loss that precede the unset of T2D, and it is significantly associated with the risk of T2D independent of age and clinical risk factors.

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Impact of differences in white blood cell composition in insulin resistant individuals on whole blood transcriptome analysis and interpretation – The CODAM study

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BACKGROUND

Worldwide, the prevalence of obesity and insulin resistance has grown dramatically. Gene expression profiling in peripheral blood represents a powerful means to explore disease pathogenesis, but often the potential impact of inter-individual differences in cell-type composition is not taken into account. As such, the objective of this project was to investigate the peripheral blood transcriptome profile of insulin resistant as compared to insulin sensitive individuals independent of inter-individual differences in white blood cell (WBC) composition.

METHODS

We used the Bioconductor package EpiDISH to infer the WBC subtypes from genome-wide DNA methylation data for 157 individuals that were categorized as insulin resistant or insulin sensitive. We performed differential gene expression and Gene Ontology analysis in peripheral

blood, implementing a linear regression model adjusting for sex, body mass index, and WBC composition.

RESULTS

We report a 3% higher relative monocyte amount in the insulin resistant individuals. Furthermore, independent of WBC composition our analysis revealed: 1) a significant upregulation of interferon-stimulated genes (ISGs) by interferon, and 2) a significant downregulation of genes involved in cellular differentiation and remodelling of the actin cytoskeleton in the insulin resistant individuals.

CONCLUSION

A specific ISGs signature characterizes the peripheral blood transcriptome profile of the insulin resistant individuals, independent of WBC composition, suggesting a role in the etiology of insulin resistance. The upregulation of the ISGs indicates increased inflammation due to an innate immune response. Altered gene expression in specific organs may be reflected in peripheral blood cells, hence our results may reflect obesity-associated adipose tissue inflammation.

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Monitoring liver glucose metabolism with deuterium metabolic imaging

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BACKGROUND

Deuterium metabolic imaging (DMI) is a new technique to study metabolism in vivo, which relies on magnetic resonance spectroscopic imaging combined with the administration of deuterated compounds, such as deuterated glucose.[1] In type 2 diabetes, hepatic glucose metabolism is dysregulated, but our understanding of the disturbances is far from complete. In this study, DMI was performed to show that metabolic imaging of the liver is feasible using deuterated glucose.

METHODS

Healthy volunteers were scanned in a 7T MRI scanner, equipped with a body array coil for DMI. To assess the repeatability of the DMI measurements, 5 scans (nominal resolution 30 x 30 x 30 mm^3, 5 min per scan) were acquired consecutively at natural abundance and the coefficient of variation (CoV) of the deuterated water peak intensity was calculated for each voxel. Finally, DMI scans with oral intake of [6,6'-2H2]-glucose (0.75 g per kg body weight) were performed after an overnight fast with a temporal resolution of 5 min, starting at baseline and continuing up to 2 hours after intake of deuterated glucose.

RESULTS

The mean CoV of the natural abundance deuterated water signal intensity in the liver over time was 7%. Deuterated glucose signal in the liver progressively increased after deuterated glucose intake (figure 4). Immediately after intake, a very strong signal from deuterated glucose in the stomach and portal vein was observed (figure 4). These signals rapidly decayed, but at 2 hours the signal from the

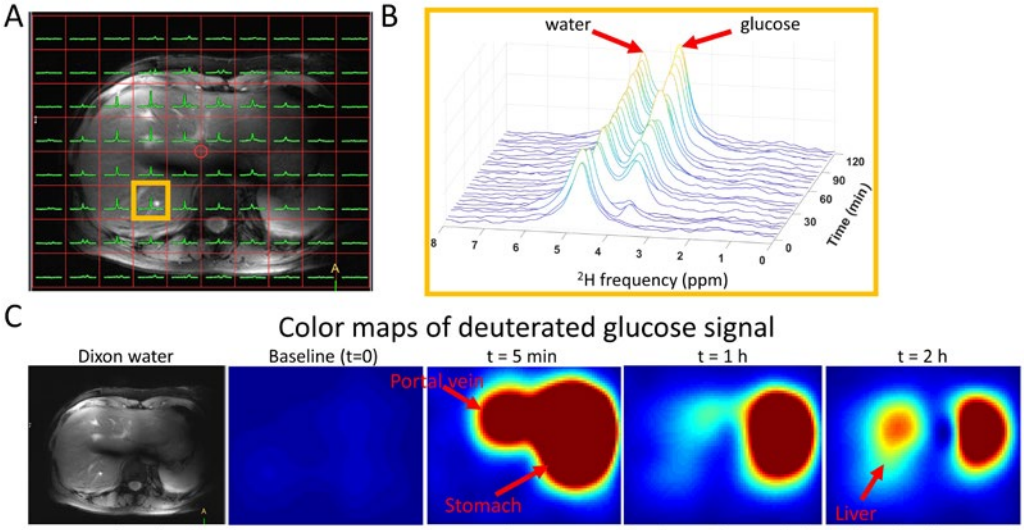

Figure 1: A) Overlay of baseline DMI scan on Dixon water image. B) DMI spectra at baseline and up to 2 h after oral intake of deuterated glucose (5 min per scan), from the voxel indicated with the orange square in the posterior section of the liver. C) Color maps of deuterated glucose signal intensity.

Figure 4. Monitoring liver glucose metabolism with deuterium metabolic imaging.

stomach was still high compared to the liver.

CONCLUSION

Our results show that glucose uptake in the liver can be monitored with DMI.

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Integrative bioinformatic and laboratory approaches shed new light on the molecular mechanisms underlying beta cell identity loss in T1D

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BACKGROUND

Type 1 diabetes (T1D) is an inflammatory disease that is characterized by the destruction of beta-cells in pancreatic islets due to autoreactive actions from immune cells. The goal of this study is to characterize the inflammatory physiopathology that takes place in T1D, and explore the molecular mechanisms driving loss of beta-cell identity and function.

METHODS

EndoC-βH1 cells and primary human islets were treated with various metabolic and inflammatory stressors mimicking the pathophysiological conditions in T1D. Gene expression and beta-cell function were assessed by RT-PCR and glucose-stimulated insulin secretion (GSIS), respectively. Single-cell RNA sequencing was carried out on primary human islets. Data processing, cell clustering and gene expression analysis was performed using Seurat packages from R.

RESULTS

The combination of IL1β and IFNγ repressed gene expression of beta-cell maturity markers such as NKX6.1 (0.71-fold change) and MAFA (0.39-fold change), and reduced the expression of duct/differentiation marker HES1 (0.67-fold change) compared to untreated islets (n = 3). Furthermore, treatment with IL1β/IFNγ impaired insulin secretion by 62%. Bioinformatic analysis revealed that exclusive heavy metal-related biological pathways were altered in specific endocrine pancreatic cells under cytokine treatment: IL1β/IFNγ promoted the expression of the metallothionein gene family and zinc transporter ZnT8 exclusively in beta-cells, while the expression of distinct genes involved in iron homeostasis was found to be altered in beta- (ferritin subunits) and alpha-cells (ceruloplasmin).

CONCLUSION

IL1β/IFNγ treatment induces loss of beta-cell maturity and impairment of insulin secretion in human pancreatic islets. Further pathway analysis revealed that alpha- and beta-cells respond and adapt differently to inflammatory triggers. We hypothesize that a difference in protective molecular mechanisms between beta-cells and alpha-cells could account for the loss of beta-cells and survival of alpha-cells despite exposure to the same inflammatory stress during the onset of T1D.

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The assessment of intrahepatic islet transplantation using exendin PET imaging

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BACKGROUND

Intrahepatic transplantation of islets is performed in patients with complicated type 1 diabetes (T1D) and unstable glycemic control. This procedure leads to an improved glycemic control and quality of life. Graft function can however deteriorate over time and a tool to assess transplantation success and monitor islet survival and function-

ality would be of great clinical value. We used dynamic PET imaging with the beta cell specific tracer 68Ga-exendin to study the presence of intrahepatic islet grafts in T1D patients.

METHODS

Dynamic exendin PET scans of 8 T1D patients with functional intrahepatic islet grafts (Tx-group) and 3 controls with T1D awaiting islet transplantation, were acquired and hepatic tracer uptake was measured by kinetic modeling. Islet function was measured through a mixed-meal tolerance test (MMTT) and expressed as AUC for C-peptide, to determine its relation with the PET signal.

RESULTS

The control and Tx-group did not differ in age (58.7 ± 5.5 vs 57.6 ± 9.1 years, p = 1.00), BMI (24.5 ± 4.5 vs 24.2 ± 3.8 kg/m^2, p = 0.92) and HbA1c (62.3 ± 6.1 vs 46.3 ± 10.0 mmol/mol, p = 0.052), though AUC for C-peptide (22.6 vs 145.2 nmol.min/L, p = 0.01)) significantly differed. The average number of transplanted islet equivalents (IEQ) was 9.4 * 105 ± 2.9 * 105. The distribution volume (Vt) of the PET tracer was significantly higher in the Tx-group, indicating an increased retention of radiolabeled exendin in the liver, i.e. the presence of islets (0.43 ± 0.02 vs 0.57 ± 0.08, p = 0.01). There was no significant correlation found in the Tx-group between Vt and IEQ, nor between Vt and function.

CONCLUSION

The data of this explorative study indicate that PET imaging using radiolabeled exendin is a promising tool to monitor pancreatic islet grafts in patients. Our observation that no correlation was found between the PET signal and C-peptide production suggests the potential of exendin PET to detect viable beta cell mass that has lost its functional capacity.

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Monogenic Diabetes: the Impact of Making the Right Diagnosis

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BACKGROUND

Maturity Onset Diabetes of the Young (MODY) is the most common type of monogenetic diabetes in Europe, with an estimated total of 20.000 patients in the Netherlands. However, the large majority of patients are still genetically unaccounted for. While diagnosing monogenic diabetes often has clinical consequences for the index patient and importance for family members.

METHODS

We studied 1951 index patients with diabetes referred for genetic testing between 2015-2020 in the Netherlands. Genetic testing was performed using a custom gene-panel including 24 genes associated with monogenic diabetes.

RESULTS

Pathogenic germline variants were identified in 287 of 1951 (15%) referred diabetic patients. The median age at diagnosis was 27 years (range, 0 to 80 years) in mutation carriers compared with 34 years (range, 0 to 83 years) in nonmutation carriers (p = 0.000). Pathogenic germline variants were found in 17% of females compared to 11% of males (p = 0.001). GCK-MODY and HNF1A-MODY were the largest MODY-subgroup with respectively 37% and 34% of all mutation carriers. Furthermore in 8% a variant of unknown significance was found in one of the MODY-associated genes.

CONCLUSION

This nationwide study showed a mutation detection yield of 15% with our MODY-gene-panel, associated with younger age at diagnosis and female gender. In general, comprehensive testing increases efficiency both in terms of time and costs if more than one gene is related to a certain disease. The drawback of testing many genes is the complex interpretation of the results. Therefore, close collaboration between treating physicians and clinical geneticists is of utmost importance, including the interpretation of variants of unknown significance. Based on our data and literature; genetic analysis is recommended in individuals with diagnosis diabetes below age 35 years, in absence of clinical characteristics of diabetes type 1 (e.g. auto-antibodies) or diabetes type 2 (e.g. lifestyle risk factors) and/or positive family history of diabetes. These criteria would increase MODY detection, enabling optimal clinical management as well as genetic counseling of family members.

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The glycolytic by-product methylglyoxal is present in immune cells and may affect their recruitment

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BACKGROUND

The reactive dicarbonyl compound methylglyoxal (MGO) is mainly formed as a byproduct of glycolysis. Immune cell activation leads to a switch to glycolysis for their energy demand. We investigated whether MGO is formed in immune cells and whether MGO affects immune cell function.

METHODS

MGO was measured in human blood fractions and circulating immune cell fractions using ultra-performance liquid chromatography-tandem mass spectrometry. Oral glucose tolerance tests (OGTT) were performed in 20 abdominal obese individuals, and a fluorescent probe was used to track MGO in immune cells using flow cytometry. In mice, we injected an iv bolus of 25 µg highly purified MGO in 2 hours, 24 hours, and 72 hours prior to sacrifice. Immune cell numbers were studied in blood and tissues using flow cytometry and immunohistochemistry, respectively.

RESULTS

In human, about 50% of whole blood MGO content (3.61 ± 1.04 µM) was confined to leukocytes (2.02 ± 0.7 µM), whereas MGO levels in plasma (0.12 ± 0.03 µM) and platelets (0.007 ± 0.004 µM) were very low. Purified leukocyte subsets showed a very high cellular MGO concentrations in lymphocytes (4409 ± 1095 µM) followed by monocytes (2804 ± 2455 µM) and granulocytes (1683 ± 437 µM). An increased glucose load during OGTT resulted in increased MGO levels in these immune cell fractions. In mice, injection of highly purified MGO resulted in a significant increase of circulating Ly6Chi monocytes (+207%), T cells (+67%), and neutrophils (+61%) after 2 hours compared to PBS injection, while these immune cell numbers were decreased after 72 hours. We observed similar results in the liver 2 hours post injection, there was an increasing tendency of hepatic Ly6Chi monocytes (+83%) and neutrophils (+36%). However, hepatic macrophage numbers did not change after 2 hours, but increased (+48%) after 72 hours.

CONCLUSION

MGO is present in a high concentration in lymphocytes, granulocytes, and monocytes, is further increased during an OGTT and may affect immune cell recruitment.

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PCr/ATP ratio's measured with 31P-MRS do not correlate with atrial cardiac mitochondrial function

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BACKGROUND

It has been suggested that cardiac PCr/ATP-ratio, measured with ^{31}P-Magentic Resonance Spectroscopy (^{31}P-MRS) non-invasively in vivo, reflects cardiac mitochondrial function, however this has never been validated. Therefore, we aimed to examine if the PCr/ATP-ratio correlates with human cardiac mitochondrial function.

METHODS

We enrolled 10 lean, 15 obese and 13 type 2 diabetes patients (T2DM) who were scheduled for open-heart surgery. The day before surgery, a ^{31}P-MRS scan was performed. During surgery, tissue specimens from the right atrial appendage were obtained for measuring mitochondrial respiration ex vivo (Oroboros).

RESULTS

In contrast to our expectations, we did not find any differences in the MRS-based PCr/ATP-ratio between lean (0.98 ± 0.29), obese (1.11 ± 0.33), and T2DM patients (0.99 ± 0.31, p = 0.76). ADP-stimulated and maximal uncoupled mitochondrial respiration, were similar across groups on a lipid and a glucose derived substrate. Linear regression

analysis revealed no relationship between PCr/ATP-ratio's and ADP-stimulated respiration (pyruvate R2 < 0.001, p = 0.95, octanoyl R2 < 0.03, p = 0.33) nor between PCr/ATP-ratio's and maximally uncoupled respiration (pyruvate R2 < 0.05, p = 0.19, octanoyl R2 < 0.05, p = 0.60). Nonetheless, left ventricular end systolic and end diastolic mass tended to correlate with the PCr/ATP ratio (p = 0.04 and p = 0.08). In contrast, correlation analysis showed no relation between state 3 or uncoupled mitochondrial respiration with any of the parameters of cardiac function.

CONCLUSION

These results show that in vivo cardiac energy status does not reflect ex vivo mitochondrial respiratory capacity as measured in the right atrial appendage tissue. This suggests that PCr/ATP is influenced by other factors than merely cardiac mitochondrial function. Nonetheless, the PCr/ATP ratio does relate to cardiac function parameters and hence does seem important in cardiac metabolism. However, the exact underlying physiology remains unclear.

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Is the diurnal variation of plasminogen activator inhibitor-1 disturbed in prediabetes?

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BACKGROUND

In the general population, the incidence of myocardial infarction (MI) peaks in the morning. This high point is preceded by the diurnal peak in the plasma level of the MI risk factor plasminogen activator inhibitor-1 (PAI-1). Contrastingly, type 2 diabetes (T2D) patients exhibit higher rates and an altered temporal distribution in MI onset. Previous research has demonstrated both morning and evening MI peaks, as well as no maximum at all. Therefore, we hypothesize that individuals with prediabetes have an altered diurnal variation in the level of PAI-1 compared to controls.

METHODS

Twelve young, healthy, lean (YHL) male volunteers (age ± SD: 22.2 ± 2.3 y, BMI: 22.4 ± 2.0 kg/m^2) and twelve older and overweight men with prediabetes (65 ± 9 years, 30.3 ± 2.7 kg/m^2) stayed at our metabolic research unit for 1.5 days. During their stay, we provided them with standard meals and a set activity routine. At 10.00, 14.00, 22.00 and 04.00, we obtained blood samples to quantify PAI-1 around the clock using ELISA.

RESULTS

The average daily PAI-1 level was significantly higher in prediabetic than YHL men (mean ± SEM: 1105.04 ± 127.92 pg/mL vs. 557.48 ± 120.21 pg/mL, p < 0.0001). In YHL, the PAI-1 peak occurred at 10.00 (824.79 ± 138.89 pg/mL) with a trough at 22.00 (332.77 ± 40.06 pg/mL). In older and overweight men with prediabetes, PAI-1 peaked earlier at 04.00 (1376.06 ± 130.81 pg/mL) and dropped earlier at 14.00 (941.46 ± 127.47 pg/mL).

CONCLUSION

Our data demonstrate that the PAI-1 levels are higher and phase-advanced in older, overweight men with prediabetes compared to YHL. This disturbed diurnal PAI-1 pattern could contribute to higher MI rates and the altered MI onset timing in T2D. More broadly, our findings highlight the crucial role the timing of sample acquisition plays in clinical practice and health research.

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Timing of physical activity may impact insulin resistance

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BACKGROUND

Physical activity (PA) is one of the Zeitgebers to maintain a normal circadian rhythm, which is important to prevent type 2 diabetes. It is unclear if timing of PA is important to prevent type 2 diabetes, therefore our aim was to investi-

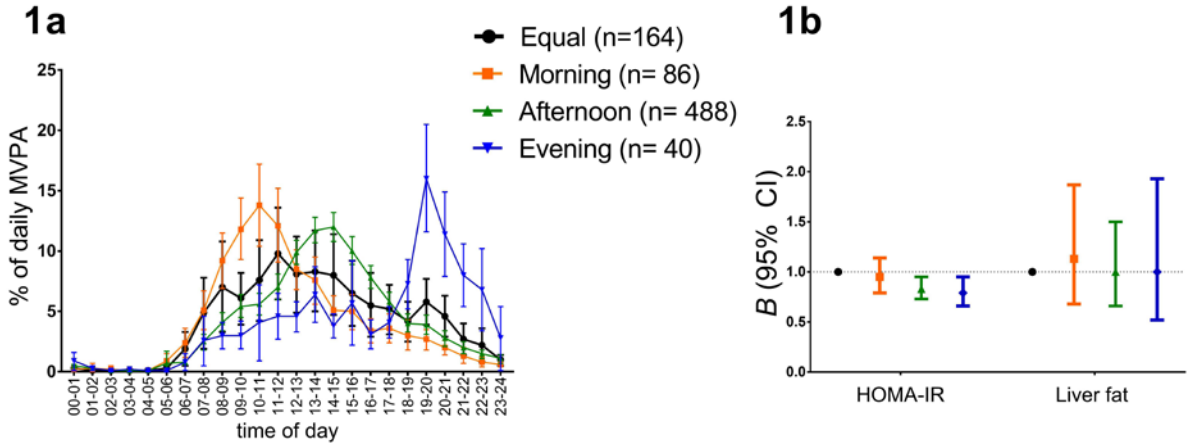

Figure 5. 1a. Distribution of moderate-vigorous physical activity (MVPA) per hour for each subgroup. **1b**. Regression coefficients (B) with 95% confidence intervals that were back-transformed from the natural log scale (1b) representing proportional differences in HOMA insulin resistance (IR) and liver fat content for each subgroup (with equal distribution as the reference group).

gate timing of PA in relation to insulin resistance and liver fat content.

METHODS

In the Netherlands Epidemiology of Obesity (NEO)-study, physical activity was assessed using activity sensors and participants were categorised as being most active in the morning (06-12 hours), afternoon (12-18 hours), evening (18-24 hours), or as having an equal distribution of activity throughout the day (Figure 5). We examined differences in HOMA insulin resistance and the amount of liver fat, as assessed by MR spectroscopy, between these subgroups using linear regression analyses. Results were adjusted for demographic and lifestyle variables and total physical activity.

RESULTS

We analysed data of 778 participants (43% men with a mean (SD) BMI of 26.2 (4.1) kg/m^2), aged 56 (4) years. Compared with participants with an equal distribution of activity, insulin resistance was reduced in participants who were most active in afternoon (-20% [95% CI: -37; -5%]) or evening (-27% [-52; -5%]), whereas it was similar (-5% [-27; 12%]) in those most active in morning. Timing of physical activity was not associated with liver fat content (Figure 5).

CONCLUSION

Physical activity in the afternoon or evening was associated with up to 27% reduced insulin resistance compared with equal distribution of activity, but not with liver fat. Prospective studies are warranted to examine the causality of physical activity timing in relation to risk of type 2 diabetes.

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Therapeutic efficiency of lowering branched-chain amino acid levels in patients with type 2 diabetes using sodium-phenylbutyrate: a randomized placebo-controlled clinical intervention study

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BACKGROUND

Branched-chain amino acid (BCAA) levels are elevated in patients with type 2 diabetes (T2DM) and associate with insulin resistance. An impaired BCAA catabolism may possibly lead to increased BCAA levels and affect metabolic health. Sodium-phenylbutyrate (NaPB), a drug known to boost BCAA oxidation, may therefore lower BCAA levels and improve metabolic health in T2DM. This was investigated in the present study.

METHODS

Sixteen men and women (3 females and 13 males) with

T2DM underwent a 2-week NaPB (4.8 mg/kg/day) treatment in a randomized, placebo-controlled, double-blind cross-over design with a wash-out of 6-8 weeks. The primary outcome was whole-body insulin sensitivity, measured with 2-step hyperinsulinemic-euglycemic clamps expressed as insulin-stimulated glucose disposal rate minus baseline (ΔRd). Secondary outcomes were ex-vivo mitochondrial oxidative capacity measured with high-resolution respirometry expressed as O2-flux and metabolic flexibility using indirect calorimetry expressed as the insulin-stimulated respiratory exchange ratio minus baseline (ΔRER).

RESULTS

End-of-treatment fasting BCAA levels significantly decreased after NaPB vs. placebo (479 ± 12 vs 501 ± 16 µmol/l, p = 0.05) and tended to decrease for glucose levels (7.8 ± 0.4 vs 8.2 ± 0.5 mmol/L, p = 0.06). Furthermore, whole-body insulin sensitivity was 27% higher (ΔRd: 13.2 ± 1.84 vs 9.7 ± 1.8 µmol/kg/min, p = 0.02) and ex-vivo mitochondrial oxidative capacity on glycolytic substrate was 10% higher after NaPB compared to placebo (O_2-flux: 74.0 ± 4.1 vs 67.1 ± 4.3 pmol/(s*mg), p = 0.05). In addition, metabolic flexibility tended to be higher after NaPB treatment compared to placebo (ΔRER: 0.09 ± 0.01 vs 0.08 ± 0.01, p = 0.09).

CONCLUSION

NaPB-reduced BCAA plasma levels in patients with T2DM seem to improve glucose metabolism. This data strengthens future research to investigate the metabolic effects of long-term NaPB administration in T2DM.

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The impact of Personalized Lifestyle Advice on type 2 diabetes remission as compared to usual care in newly diagnosed type 2 diabetics in a primary care setting

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BACKGROUND

The core pathophysiological defects of type 2 diabetes (T2D) are insulin resistance (IR) in the organs (e.g. muscle and liver) and reduced β-cell function (BCF). As a result of differences in pathophysiology between patients, specific phenotypes exist within the (pre)-T2D population. These specific phenotypes may require a more tailored treatment instead of a "one-size-fits all" solution. We describe an innovative personalized lifestyle approach and tested its effectiveness, in comparison to usual care, in a primary care setting.

METHODS

Subjects underwent an oral glucose tolerance test (OGTT) to assess subtypes of 82 (pre)T2D subjects according to BCF and hepatic and muscle IR, and were then allocated to one of seven personalized lifestyle treatments. Fasting plasma glucose (FPG), HbA1c and body weight (BW) were measured at baseline, after 13 weeks and at 6, 12 and 24 months.

RESULTS

After 13 weeks of intervention there was a significant reduction in BW (-8.2 kg; p < 0.0001), HbA1c (-4.0 mmol/mol; p < 0.0001), and FPG (-0.8 mmol/l; p = 0.0004) as compared to baseline, while there were no significant changes in the control group. Additionally, 32% of the subjects obtained a healthy subtype (no reduced BCF and no IR) after 13 weeks of intervention, as compared to 0% at the start of the study. After two years of follow-up, BW (-7.4kg; p < 0.0001), HbA1c (-1.2 mmol/mol; p = 0.0098) and FPG (0.5 mmol/l; p = 0.0044) remained significantly lower as compared to baseline for the intervention group. In the control group, a modest reduction in body weight (-2.6 kg; p = 0.0067) and a reduction in FPG (-0.9 mmol/l; p = 0.0395) were observed.

CONCLUSION

The personalized lifestyle treatment more effectively addressed the core defects of T2D and thereby significantly improved the health status of T2D patients, in comparison to usual care, even after two years follow-up.

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Serum magnesium is an independent predictor of macrovascular and microvascular complications in type 2 diabetes

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BACKGROUND

Vascular complications are the primary cause of morbidity and mortality in type 2 diabetes mellitus (T2DM). Magnesium (Mg2+) deficiency is highly prevalent in T2DM and is associated with poor glycaemic control. Therefore, hypomagnesemia has been suggested to contribute to vascular complications. We aim to evaluate if serum Mg2+ is associated with macro- or microvascular events in the Diabetes Care System cohort.

METHODS

In total 4400 serum samples obtained between 2008-2013 were measured for Mg2+. Endpoints were incident macrovascular (Acute Myocardial Infarction, Coronary Heart Disease, Heart Failure, Cerebrovascular Accident, Peripheral Arterial Disease) and microvascular (nephropathy, foot complications, and retinopathy) between the data of blood sampling and 2018. We analysed the association of serum Mg2+ (per 0.1 mmol/l) and HbA1c (per 5 mmol/l) with endpoints using Cox regression, adjusted for confounders.

RESULTS

Total of 9.1% of people are determined hypomagnesemic. During 10 years of follow-up, 334 of 3428 macrovascular and 1107 of 2550 microvascular incidents were identified. Serum Mg2+ was associated with major macrovascular incidents with a hazard ratio of 0.86 (p = 0.035, 95% CI: 0.75; 0.99), and specifically heart failure with 0.75 (p = 0.005; 95% CI: 0.62; 0.92), while other macrovascular endpoints were not associated. Microvascular incidents were associated with serum Mg2+ with a hazard ratio of 0.82 (p < 0.001; Cl: 0.76; 0.89), nephropathy 0.75 (p < 0.001; CI: 0.68; 0.84), retinopathy 0.76 (p < 0.001; CI: 0.60; 0.95), and foot complications 0.85 (p < 0.001; CI: 0.7; 0.91). Mediation is only shown in the association of Mg2+ with retinopathy adjusted for glycaemic control resulting in a higher hazard ratio from 0.76 (95% CI: 0.60; 0.95) to 0.90 (95% CI: 0.70; 1.16).

CONCLUSION

Mg2+ is independently associated with a decreased risk of heart failure and microvascular morbidity and mortality (diabetic nephropathy, foot complications, and retinopathy). The serum Mg2+ association on the risk of retinopathy is partially mediated by glycaemic control.

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Dapagliflozin is cost-effective compared to DPP-4 inhibitors in the treatment of type 2 diabetes mellitus in the Netherlands

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BACKGROUND

In the Netherlands, dapagliflozin is currently reimbursed as dual or triple therapy in combination with metformin with or without sulfonylurea for patients with type 2 diabetes. While the use of SGLT2 inhibitors such as dapagliflozin is until now limited in the Netherlands, the use of DPP-4 inhibitors is more common and rising. This study compares the cost-effectiveness of dapagliflozin versus DPP-4 inhibitors when added to metformin and sulfonylurea in the treatment of type 2 diabetes.

METHODS

A cost-utility analysis is performed with the Cardiff diabetes model based on 'UK Prospective Diabetes Study 68' risk equations. In line with Dutch pharmacoeconomic guidelines, a societal perspective, discounting 4% for costs, 1.5% for effects, and a 40-year time horizon are used.

RESULTS

Using dapagliflozin results in a € 990 cost saving and a 0.28 quality-adjusted life year gain over 40 years compared with DPP-4 inhibitors. This is mainly due to a lower incidence of micro- and macrovascular complications, delayed insulin treatment, and improved quality of life, partially due to lower BMI. Results are robust to changes in input parameters, including a potential price decrease of DPP-4 inhibitors due to expected generic entry. When the willingness-to-pay is € 20,000 per quality-adjusted life year, the probability of dapagliflozin being cost-effective compared with DPP4-inhibitors is 99.9%.

CONCLUSION

Adding dapagliflozin instead of a DPP-4 inhibitor to metformin and sulfonylurea saves costs while improving outcomes. Sensitivity analyses show uncertainty around this outcome is low. Recent studies with dapagliflozin show that apart from the glucose lowering effect it also has a clinically significant effect on cardio-renal outcomes that starts soon after treatment initiation. This drives the position of SGLT2 inhibitors more and more towards the start of the treatment pathway, as mono or add on to any other glucose lowering drug. The corresponding economic value needs to be studied in a separate economic evaluation based on these cardio-renal outcome studies.

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Body fat at adolescence and early changes in atherogenic metabolomic measures during young adulthood

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BACKGROUND

Obesity in adulthood is a strong risk factor for cardiovascular disease. It is unknown whether total fat mass at adolescence is associated with early changes in atherogenic metabolomic measures during adolescence and young adulthood.

METHODS

In the first-generation offspring of the Avon Longitudinal Study of Parents and Children (ALSPAC), total fat mass was measured using dual-energy X-ray absorptiometry at age 15, and 146 nuclear magnetic resonance (NMR)-based metabolomics were repeatedly measured at age 15, 18 and 24 years. Using multilevel models with two splines we examined changes in metabolomic measures between age 15 to 18, and age 18 to 24 years in relation to baseline fat mass, adjusted for sex, ethnicity, age at peak height velocity, and educational level of the mother.

RESULTS

We included 3851 participants in the analyses, 51% men, with a mean (SD) age of 15.5 (0.4) years, mean BMI of 21.5 (3.6) kg/m^2 and total fat mass of 15.4 (9.2) kg. 1 SD higher log fat mass at age 15 was associated with increasing levels of several atherogenic metabolomic measures between age 15 to 18 (e.g. triglycerides in large VLDL 0.003 mmol/l [95% 0.002; 0.004], ApoB 0.005 g/l [0.003; 0.007]) and a decrease in extra-large to large HDL particles. In contrast, between age 18 to 24 baseline fat mass was associated with a decrease in VLDL and LDL particles, small HDL (e.g. cholesterol in small HDL -0.002 mmol/l [-0.003; -0.002]) and glycoproteins (-0.002 mmol/l [-0.003; -0.0008]), whereas levels of extra-large to medium HDL particles increased.

CONCLUSION

Body fat mass at age 15 years was associated with changes in metabolomic measures towards an atherogenic metabolomic profile between age 15 and age 18, but with a subsequent decreased atherogenic metabolomic profile between age 18 and 24. Our results suggest that adolescence is a critical period with regard to adiposity-related changes in atherogenic risk factors.

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Kidney hemodynamic profile and systemic vascular function in adults with type 2 diabetes: analysis of four clinical trials

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BACKGROUND

Glomerular hyperfiltration is indicated to play a key role in the pathophysiology of diabetic kidney disease (DKD). Mechanisms underlying this adverse hemodynamic profile are incompletely understood. We hypothesized that systemic vascular pathology, a common observation in type 2 diabetes (T2D), relates to glomerular hyperfiltration.

METHODS

We used baseline data of three randomized trials in adults with T2D (Cohort A, n = 111) which assessed kidney hemodynamics including glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and their quotient filtration fraction (FF), with gold-standard measurements of inulin and para-aminohippurate clearance. Systemic vascular resistance (SVR) and pulse pressure (PP) were derived from continuous beat-to-beat monitoring. Additionally, we examined the effects of the nitric oxide synthase inhibitor L-NG-monomethyl Arginine (L-NMMA) on these parameters in healthy overweight males (Cohort B; n = 10).

RESULTS

In cohort A, SVR negatively related to GFR (β: -0.382, $p < 0.001$) and ERPF (β: -0.475, $p < 0.001$), and positively related to FF (β: 0.369 $p < 0.001$). Associations between SVR, ERPF and FF persisted after multivariable adjustments. PP negatively associated to ERPF (β: -0.190, $p = 0.048$), and positively related to FF (β: 0.232, $p = 0.030$), of which the latter remained significant in multivariable regression. In cohort B, L-NMMA increased SVR (median difference (Δ) 138.9 dyn·s/cm-5, $p = 0.022$), in parallel with decreased ERPF (median Δ -108.0 ml/min $p = 0.011$) and increased FF (median Δ 5%, $p = 0.007$), while GFR remained unchanged. SVR and FF were significantly related during L-NMMA infusion (r0.638, $p = 0.047$).

CONCLUSION

Parameters of systemic vascular function including SVR (suggestive of endothelial dysfunction), and PP (common marker for extent of arterial stiffness), are positively related to FF (indicative for glomerular hyperfiltration). Based on these findings, systemic vascular dysfunction could contribute to the adverse kidney hemodynamic profile, promoting hyperfiltration and predisposing to the development of DKD.

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Different insulin sensitivity of plasma metabolites in a two-step hyperinsulinemic-euglycemic clamp study

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BACKGROUND

Numerous studies have investigated the association of plasma metabolites with insulin resistance and type 2 diabetes, but few studies have assessed the direct effects of hyperinsulinemia on plasma metabolite levels. The aim of this study was to explore the insulin sensitivity of metabolites based on the data acquired from a two-step hyperinsulinemic-euglycemic clamp study in healthy middle-aged individuals.

METHODS

In this study, data was acquired from a two-step hyperinsulinemic-euglycemic clamp study on 24 healthy individuals. Concentrations of 159 metabolites were obtained using an NMR-based metabolomics platform (Nightingale Inc, Finland) at baseline glucose infusion, 10 mU/m^2/min (low dose) insulin infusion, and 40 mU/m^2/min (high dose) insulin infusion. To correct for dilution of the blood during the experiment, metabolite concentrations were normal-

ized to albumin and data were analyzed by using linear mixed effects models after exclusion of outliers (beyond ± 4 SD).

RESULTS

After low dose insulin, a total of 120 metabolites changed significantly (Bonferroni adjusted p < 1.34e-3) and after high dose insulin, 150 metabolites changed significantly as compared to baseline. Interestingly, some of the insulin sensitive metabolites showed maximal effect sizes already at the low dose insulin with no further increase at high dose insulin. These metabolites included beta-hydroxybutyrate and virtually all lipids in all sizes of low-density lipoprotein (LDL) particles. In contrast, some metabolites showed significant further decrease at high dose insulin. For example, the levels of the branched-chain amino acids valine, leucine and isoleucine showed an approximately fourfold additional decrease after high dose insulin.

CONCLUSION

In conclusion, the majority of plasma metabolites measured by an NMR metabolomics platform are sensitive to insulin levels and some of these responses are insulin dose-dependent. Since low and high dose insulin levels are assumed to target, respectively, the liver and the liver plus peripheral organs (i.e. muscle and fat), our data provide insight into the role of insulin in specific tissues in determining plasma metabolite levels.

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Microglial insulin signalling plays a role in the progression of dietary-induced obesity

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BACKGROUND

Obesity and type 2 diabetes mellitus (T2DM) are highly prevalent metabolic disorders which are among the leading causes of death worldwide. One of the hallmarks of obesity and T2DM is insulin resistance. Microglia – the resident immune cells in the brain responsible for keeping a healthy microenvironment for neurons to survive and function, have been shown to get activated by an obesogenic diet. Microglia express insulin receptors, however, we have very limited understanding of the involvement of microglial insulin signaling in the pathogenesis of obesity and T2DM. We hypothesize that reduced insulin signaling affects microglial immune function, which could ultimately result in dysfunction of neighboring neurons and impaired CNS control energy homeostasis.

METHODS

We induced microglia-specific knock-down of the insulin receptor gene in vivo in InsRfl/fl-Cx3Cr1CreERT2 mice (InsR-KD). Male and female InsR-KD mice and InsRwt/wt-Cx3Cr1CreERT2 controls (InsR-WT) were fed with high-fat diet (HFD) or Chow diet for 10 weeks. Animals were perfused and fixed in paraformaldehyde. Coronal slices were used to evaluate microglial cell number and primary branching in the arcuate nucleus of the hypothalamus. Statistical analysis was performed with one- or two-way ANOVA. Data are presented as mean ± SEM.

RESULTS

Following 10 weeks of HFD, both male and female mice showed higher body weight (BW) gain in InsR-WT and InsR-KD animals, compared to the respective control group (p < 0,0001). We observed no difference between in InsR-WT and InsR-KD animals in males and females fed Chow or HFD. We found no difference in number of microglial soma in any of the groups, however we found a reduced number of primary projections in InsR-KD animals, compared to InsR-WT, irrespective of the sex or diet.

CONCLUSION

We observed a decrease in microglial primary projections in InsR-KD, compared to InsR-WT animals, which could be indicative of higher microglial activation in the absence of microglial insulin signaling.

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Towards a GMP-Compliant Protocol for the Differentiation of Human Pluripotent Stem Cells to β-like Cells for the Treatment of Type 1 Diabetes

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BACKGROUND

β-cell replacement therapy by allogeneic pancreas or islet transplantation is a promising approach for patients with type 1 diabetes. However, there is a scarcity of organ donors. The generation of insulin-producing pancreatic β-cells from human pluripotent stem cells (hPSC) in vitro would provide an unlimited cell source for drug discovery and cell replacement therapy in diabetes.

METHODS

We applied a modified seven-stage differentiation protocol derived from Cosentino et al 2018 to generate hPSC-derived insulin-producing β-cells in a 3D microwell culture system. Next, we adapted the scalable production of hPSC-derived β-cells to spinner flask culture. Importantly, our protocol was developed to be compliant with Good Manufacturing Practices (GMP) regulations. Differentiation was assessed by qPCR, immunocytochemistry and flowcytometry for stage-specific markers. Immunodeficient mice were transplanted with ~2x106 stage-7 cell clusters per mouse. Intraperitoneal glucose tolerance tests were performed on day 14, 28 and 60 post-transplantation and human C-peptide secretion in plasma was measured by ELISA.

RESULTS

After stage 1 (day 3), cells acquired a definitive endoderm phenotype expressing SOX17 (87.2% ± 4.6; n = 5), FOXA2 (82.8% ± 4.6; n = 3) and co-expressing c-KIT and CXCR4 (93.3% ± 5.6; n = 4), and a silenced pluripotency marker OCT4 (2.8% ± 6; n = 5). At the end of stage 4 (day 12), pancreatic progenitor populations were identified by the co-expression of PDX1/NKX6.1 (46.7% ± 3.5; n = 3). Finally, at the end of stage 7 (day 30), C-peptide-positive β-like cells (49.5% ± 10.5; n = 3) and glucagon-positive α-like cells (18.4% ± 6.1; n = 3) were produced. Following transplantation of day-30 clusters into mice, human C-peptide levels reached 29.9 pmol/L ± 14.3; n = 4 and 141.1 pmol/L ± 24.8; n = 4 at day 14 and 28 respectively.

CONCLUSION

hPSCs-derived β-like cells, with the capacity of glucose-responsive insulin secretion, are a promising future alternative to donor islets for the treatment of type 1 diabetes.

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Towards a comparative assessment score for β-cell replacement therapy and automated insulin delivery: updating the Igls Criteria

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BACKGROUND

β-cell replacement therapy is a treatment option for patients with diabetes and severe β-cell failure. In 2017, the Igls criteria were developed as a standardised functional score for β-cell replacement therapy. With automated insulin delivery (AID) becoming increasingly important in diabetes care of this patient group, comparing these two treatment strategies is useful. Working towards a comparative scoring tool, we aimed to evaluate the usefulness of current Igls criteria in β-cell replacement therapy and propose improvements, including providing opportunity for future comparison to AID.

METHODS

Current Igls criteria (table 3A) were evaluated by assessing outcomes of pancreas (PancreasTx) and islet transplantation (IsletTx) at 1, 2 and 4 years post-transplantation for patients with at least one year of follow-up in 2019. Limitations were identified and applied in the proposition of updated Igls criteria.

RESULTS

One year post-transplantation, successful treatment outcome was reached in 25/36 (69.5%) of IsletTx, 18/29 (62.3%) PancreasTx and 20/22 (90.9%) simultaneous-pan-

Table 3. Igls criteria for functional and clinical outcomes for β-cell replacement therapy and automated insulin delivery systems.

A. Igls criteria 1.0

β-cell graft functional status	HbA1c, % (mmol/mol)[a]	Severe hypoglycemia, events per yr	Insulin requirements, U•kg^{-1}•d^{-1}	C-peptide	Treatment success
Optimal	≤ 6.5 (48)	None	None	> Baseline[b]	Yes
Good	< 7.0 (53)	None	< 50% baseline[c]	> Baseline[b]	Yes
Marginal	Baseline	< Baseline[d]	≥ 50% baseline	> Baseline[b]	No[e]
Failure	Baseline	Baseline[f]	Baseline	Baseline[g]	No

B. Proposed Igls criteria 2.0

Treatment outcome	Glycemic control		Hypoglycemia		Treatment success
	HbA1c, % (mmol/mol)[a]	CGM, % time-in-range	Severe hypoglycemia, events per yr	CGM, % time < 54 mg/dl (3.0 mmol/l)	
Optimal	≤ 6.5 (48)	≥ 80	None	0	Yes
Good	< 7.0 (53)	≥ 70	None	< 1	Yes
Marginal	≤ Baseline	≤ Baseline	< Baseline[d]	< Baseline	No[e]
Failure	~ Baseline	~ Baseline	~ Baseline[f]	~ Baseline	No

β-cell graft functionh	C-peptide, ng/ml (nmol/l)	Insulin requirements, U•kg-1•d-1	β-cell graft success[i]
Optimal	Any	None	Yes
Good	> 0.5 (0.17) stimulated ≥ 0.3 (0.10) fasting	Any	Yes
Marginal	≥ 0.3 (0.10) stimulated ≥ 0.1 (0.04) fasting	Any	No[e]
Failure	< 0.3 (0.10) stimulated < 0.1 (0.04) fasting	Any	No

Baseline, pre-transplant assessment (not applicable to total pancreatectomy with islet autotransplantation patients).

[a]Mean glucose should be used to provide an estimate of the HbA1c, termed the glucose management indicator (GMI), in the setting of disordered red blood cell life span.

[b]Should also be > 0.5 ng/ml (> 0.17 nmol/l) fasting or stimulated.

[c]Should also be < 0.5 U•kg-1•d-1; might include the use of non-insulin antihyperglycemic agents.

[d]Should severe hypoglycemia occur following treatment, then continued benefit may require assessment of hypoglycemia awareness, exposure to serious hypoglycemia (< 54 mg/dl [3.0 mmol/l]), and/or glycemic variability/lability with demonstration of improvement from baseline.

[e]Clinically, benefits of maintaining and monitoring β-cell graft function may outweigh risks of maintaining immunosuppression.

creas-kidney (SPK) recipients. Four years post-transplantation, 10/23 (43.5%) IsletTx, 12/19 (63.2%) PancreasTx and 17/20 (85.0%) SPK recipients scored treatment success. For IsletTx, scores were equally distributed between Failure, Marginal, Good and Optimal, whereas PancreasTx and SPK showed marked dichotomy between either Optimal or Failure.

CONCLUSION

Igls criteria provide meaningful clinical assessment on an individual and treatment group level, allowing comparison both within and between β-cell-replacement modalities. Important limitations for future comparison with AID include the C-peptide criterium, prominent role of insulin requirements and absence of CGM-metrics. Proposed Igls criteria 2.0 (table 3b) separate graft outcome from treatment outcome and consider CGM-metrics on par with HbA1c and hypoglycaemia, providing for direct comparison of β-cell replacement therapy with AID.

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Modest increase in hepatic lipid content in early postnatal female offspring of non-obese mice with gestational diabetes mellitus

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BACKGROUND

Clinical studies indicate that gestational diabetes mellitus (GDM) increases the risk for mother and child to develop non-alcoholic fatty liver disease (NAFLD) in later life. In order to define preventive or curative therapies, we need to better understand the course and mechanisms underlying NAFLD development in GDM mothers and offspring.

METHODS

In this preclinical study we therefore quantified hepatic lipid content in non-obese GDM mouse dams and their offspring. Female mice challenged with a high fat diet (HF) and low-dose streptozotocin (STZ, 60 mg/kg) injections were mated to induce GDM. Livers from GDM dams were collected at gestational day 17.5 (GD17.5) or at the end of lactation, postnatal day 15 (PN15). We additionally collected livers from male and female offspring at PN15. Separate control groups to establish the independent effects of HF and STZ on maternal and offspring lipid contents were included.

RESULTS

Total hepatic lipid content were comparable between GDM and controls at GD17.5. At PN15, hepatic triglyceride (TG) contents were higher in GDM dams compared to controls (TG 108 vs 76 nmol/mg, $p < 0.05$). TG and cholesteryl-ester (CE) contents were higher in female offspring from GDM dams at PN15 as compared to non-GDM offspring (TG 15 vs 8 nmol/mg, $p = 0.058$; CE 5.2 vs 1.3 nmol/mg, $p < 0.001$), while no changes were observed in male offspring. Increased hepatic lipid content in PN15 female GDM offspring was paralleled by higher TG/phospholipid ratios, suggestive of increased lipid droplet size.

CONCLUSION

Non-obese GDM does not affect maternal liver lipid content during gestation, while hepatic TG content is elevated at the end of lactation. Hepatic TG and CE levels are modestly increased in female GDM offspring by the end of lactation. Follow up research will establish whether these early changes may predispose towards NALFD development in later life, and potentially provide insight into the underlying mechanisms.

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Long-RNA sequencing and ribosome profiling reveal novel candidate autoantigens in type 1 diabetes

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BACKGROUND

Type 1 diabetes is an autoimmune disease characterized by autoreactive T-cell mediated destruction of the pancreatic beta-cells. Increasing evidence suggest that beta-cells contribute to their own destruction by generating neo-antigens through the production of aberrant or modified proteins that escape central tolerance. We have recently demonstrated that ribosomal infidelity amplified by stress could lead to the generation of neoantigens in human beta-cells, emphasizing the participation of nonconventional translation events to autoimmunity, as occurring in cancer or virus-infected tissues.

METHODS

Human beta cells were cultured in the presence or absence of proinflammatory cytokines for 24 hours. Following stimulation, protein synthesis initiation was blocked by harringtonine/cycloheximide combined treatment and a ribosome profiling library was generated and processed on next generation sequencing. In parallel beta cell transcriptome was deciphered by long-RNA sequencing. Resulting databases were integrated for identification of inflammatory specific RNA isoforms and translation start sites (TIS).

RESULTS

We show that nearly 40% of the overall TIS derived from events of non-canonical translation and could potentially generate neo-polypeptides. A fraction (~3%) of TIS occurs within lncRNA, even in resting conditions. Moreover, inflammation leads to a significant increase in the number of ORFs per transcript and in particular an increased ribosome density within 5'-UTR regions. Finally, we describe the presence of potential neoantigens in T1D associated genes, showing alternative splicing in combination with non-canonical translation initiation.

CONCLUSION

Our data underline the extreme diversity of the beta-cell translatome and the profound changes induced by T1D pathophysiological environment. Our database, may reveal new functional biomarkers for beta-cell distress, disease prediction and progression and therapeutic intervention in type 1 diabetes.

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The use of GLP-1 agonists and SGLT2 inhibitors during pregnancy and lactation: a systematic review of the evidence

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BACKGROUND

Glucagon-like peptide 1 receptor agonists (GLP-1RA) and sodium-glucose co-transporter-2 inhibitors (SGLT2i) are novel drugs for the treatment of type 2 diabetes mellitus. Their use during pregnancy and lactation is discouraged, however few data are available. The aim of the present study is to systematically review all available data on the safety of GLP-1RA and SGLT2i during this period.

METHODS

PubMed, clinicaltrials.gov and FDA as well as EMA product information were searched up to June 2020 using terms for current GLP-1RA and SGLT2i combined with terms for pregnancy, lactation and diabetes. 14 animal and 8 human studies on GLP1-RA and 9 animal and 5 human studies on SGLT2i were included.

RESULTS

In animal studies, use of all GLP-1RA caused reduced fetal weight and/or growth, delayed ossification and skeletal variants usually accompanied by a reduction in maternal weight. Visceral abnormalities and skeletal malformations were seen with liraglutide as well as semaglutide. In animal studies exendin-4 was shown to not diffuse through the maternal-fetal interface in the absence of systemic inflammation. Exenatide showed a fetal-to-maternal peptide concentration ratio of ≤ 0.017 in ex vivo placental perfusion. In animal studies SGLT2i were generally safe during the first trimester but exposure during the period coinciding with the late second and third trimester of human renal development, caused dilatation of the renal pelvis and tubules. In animal studies GLP-1RA and SGLT2i are excreted in breast milk, human data are not available.

CONCLUSION

Exendin-based GLP-1RA and albiglutide do not cross the placenta. Harmful fetal effects seen while using these drugs are therefore likely caused by caloric restriction induced in the mother. SGLT2i show adverse effects on the developing kidney in animal studies, confirming the advice to discontinue these during pregnancy and lactation since human kidney maturation continues during the first 2 years of life.

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Effects of gestational hyperglycemia on placental function in a mouse model for lean gestational diabetes mellitus

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BACKGROUND

The placenta is the interface between the maternal and fetal circulation and is essential for proper fetal development. In gestational diabetes mellitus (GDM), placental function is disturbed, affecting fetal development. Increased weight, higher nutrient transporter expression and signs of oxidative stress and inflammation have been reported in human GDM placenta. However, preclinical studies are needed to establish to what extent such changes affect offspring development.

METHODS

Female mice were challenged with a high fat diet (HF) and low dose streptozotocin (STZ, 60 mg/kg) injections to induce hyperglycemia and glucose intolerance during pregnancy. On gestational day 17.5 (GD 17.5) placentas were collected to evaluate lipid and metabolite levels and to perform histological analysis. Different control groups were included to establish the independent effects of HF and STZ.

RESULTS

Placental glucose content was significantly increased in the HF/STZ group compared to HF animals (0.20 vs 0.14 AU, $p < 0.05$), with several glucose intermediates such as pyruvate trending in the same direction. Placental and fetal weight remained unaffected while placental lipid accumulation was increased by HF/STZ treatment compared to low fat controls (triglycerides 5.0 vs 2.7 nmol/mg, $p < 0.01$; phospholipids 22 vs 16 nmol/mg, $p < 0.01$). Interestingly, placental triglyceride content correlated with blood glucose levels ($R2 = 0.67$, $p < 0.001$). Moreover, histological assessment revealed an increase in necrotic foci in the labyrinth and giant trophoblast layers, indicating placenta deterioration.

CONCLUSION

HF/STZ-induced GDM in mice increases placental glucose and lipid contents and accelerates placental ageing. These changes resemble what has previously been observed in human GDM. This novel preclinical model therefore allows investigation of the relationship between placental function and offspring development in GDM. It can furthermore to be employed to establish the potential of therapeutic interventions through improved placental function.

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Evaluating glycaemic control of patients with type 1 diabetes mellitus in a Dutch hospital: a cross-sectional real-world database study using electronic medical records

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BACKGROUND

Acquiring a snapshot of the profile of patients with type 1 diabetes mellitus (T1DM) in a hospital can give valuable insights in helping to achieve glycaemic control and therefore reducing the risk of micro- and macrovascular complications. The objective was to select T1DM patients within the Northwest clinics to evaluate glycaemic control, stratified by use of device features.

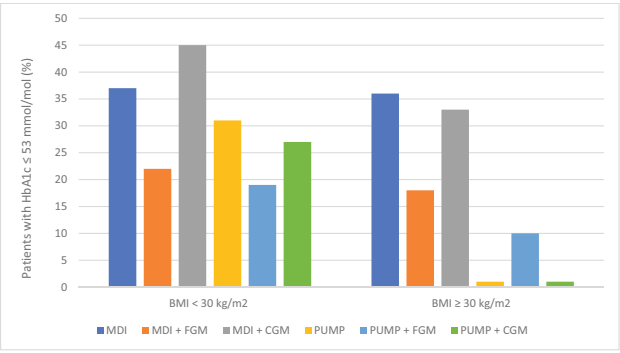

Figure 6. Glycaemic control of T1DM patients stratified by device.

METHODS

Data on diabetes management and outcomes from Northwest clinic electronic medical records were collected between July 2019 and July 2020. T1DM patients were selected using an artificial intelligence algorithm developed by CTcue and validated afterwards.

RESULTS

1505 patients with T1DM were identified, in the age range 18-90 years, of whom 53% were male and 15.7% had a body mass index (BMI) ≥ 30 kg/m^2. The majority of the patients used flash glucose monitoring (FGM) with multiple daily injections (MDI; 46.8%). Only 15% used continuous glucose monitoring (CGM). Overall more than a quarter of the patients had an HbA1c ≤ 53 mmol/mol. Variations were observed between devices used (figure 6). Patients with a BMI < 30 kg/m^2 were more likely to reach HbA1c ≤ 53 mmol/mol, compared to BMI ≥ 30 kg/m^2 (28.8% vs

19.3% respectively).

CONCLUSION

This is a preliminary overview of the T1DM population in the Northwest Clinics. Despite intensified glucose monitoring and insulin delivery, almost 75% of the patients did not reach the target of HbA1c ≤ 53 mmol/mol. Further analysis of the data needs to be done to draw valid conclusions about the added value of an insulin pump versus MDI and the advantage of continuous or flash glucose monitoring.

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Diazoxide pre-treatment prevents glucotoxicity-induced beta-cell dysfunction and death in isolated human islets

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BACKGROUND

Islet transplantation is a promising approach for the treatment of type 1 diabetes patients. However, the procedure is not as effective as expected due to the early exposure of the islets to a high glucose environment, inflammation, ischemia, and ultimately cell death. Diazoxide not only inhibits insulin secretion, leading to "beta-cell rest" but has also anti-apoptotic and anti-ischemic properties. Here, we hypothesise that diazoxide is able to prevent beta-cell dysfunction and death in human islets exposed to the glucotoxic environment present in type 1 diabetes patients after islet transplantation.

METHODS

Human pancreatic islets were incubated with or without 325 µmol/l diazoxide for 24 hours, washed for 24 hours without drug, and later exposed to high glucose (20 mM) for 96 hours and 24 hours. Islets were analysed for cell viability (Annexin staining as an apoptotic marker), insulin function (glucose-stimulated insulin secretion) and gene expression (qPCR).

RESULTS

Preliminary data suggests that diazoxide pre-treatment improves insulin secretion of 96 hours glucotoxicity-exposed human islets (stimulation index treated vs control, 0.9X ± 0.5 vs 0.4x ± 0.1, n = 3). In addition, islets pre-treated with diazoxide showed 8% reduction in the percentage of Annexin+ cells as compared to islets exposed to glucotoxicity alone (24.4% vs 32.4%, n = 2). Diazoxide pre-treatment prevented the increased expression of the oxidative stress marker TXNIP both in 24 hours and 96 hours of high glucose (fold expression relative to control from treated vs glucotoxicity, 1.3X vs 5X and 3.9X vs 8X, respectively, n = 2). Moreover, diazoxide pre-treatment also prevented the increased expression of ER-stress markers ATF3 (5.4X vs 7.6X to control, n = 1), and XBP1s/XBP1u (0,6X vs 2.2X, to control n = 1) in islets exposed to high glucose for 96 hours.

CONCLUSION

We propose that diazoxide preincubation prevents beta-cell secretory dysfunction and death in glucotoxicity by preventing the increased expression of ER-stress related markers.

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Accelerometer-Measured Sedentary Time and Physical Activity and Incident Cardiovascular Disease: The Maastricht Study

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BACKGROUND

The association of objectively-measured estimates of sedentary behavior and physical activity with incident cardiovascular disease (CVD) has not been well elucidated. We aimed to examine the association between the time spent and the pattern of sedentary behavior and physical activity with incident CVD.

METHODS

Among 4706 participants free of CVD at baseline from The Maastricht Study (aged 59.3 ± 8.6 years, 52.2% women), we assessed sedentary time and pattern variables (number of sedentary breaks, number of prolonged sedentary bouts (≥ 30 minutes), average sedentary bout duration), light-intensity physical activity (LIPA), and moderate-to-vigorous-intensity physical activity (MVPA) with the activPAL3 activity monitor. We used an annual questionnaire to assess incident CVD.

RESULTS

Over a median follow-up of 5.1 years (23303 person-years), 336 participants developed incident CVD. We found a significant sex difference in associations of the time spent of sedentary behavior (p for interaction = 0.066), LIPA (p for interaction = 0.002), and MVPA (p for interaction = 0.093) with incident CVD. The association of sedentary time with incident CVD was non-linear in women (p for non-linearity = 0.032); more sedentary time increased CVD risk only in women who had ≥ 9 hours/day of sedentary time (hazard ratio [HR] peFr hour/day = 1.40, 95% CI [1.09, 1.80]), but not in women with sedentary time < 9 hours/day (HR = 0.94 [0.74, 1.19]) after adjustment for age, sex, education level, accelerometer wake time, history of diabetes, smoking status, and Dutch Healthy Diet index. More prolonged sedentary bouts (HR per bout/day = 1.13 [1.00, 1.28]) and less MVPA (HR per 30 minutes/day = 0.74 [0.57, 0.95]) were linearly associated with a higher CVD risk in women. More LIPA was significantly associated with a higher CVD risk only in men with < 5 hours/day of LIPA (HR per hour/day = 1.42 [1.05, 1.92], p for non-linearity = 0.020).

CONCLUSION

The present study found that more sedentary time and prolonged sedentary bouts and less MVPA are associated with a higher CVD risk in women. More LIPA is associated with a higher CVD risk in men, independently of potential confounders.

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Large-scale electron microscopy database for human type 1 diabetes

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BACKGROUND

The underlying mechanism(s) initiating beta cell destruction resulting in type 1 diabetes (T1D) are still poorly understood. T1D etiology demands full knowledge of cellular composition and microenvironment of the islets of Langerhans. Electron microscopy (EM) allows to study ultrastructure, but typically only reveals high resolution of limited subcellular areas.

METHODS

We routinely perform large-scale EM for unbiased analysis of complete islet cross-sections at nanometer-resolution, which we call 'nanotomy' for nano-anatomy and thereby transform biobanked material from the Network for Pancreatic Organ donors with Diabetes (nPOD) into an open access EM database.[1] The repository currently contains over 50 datasets from 45 donors (asymptomatic autoantibody-positive (n = 13), T1D (n = 16), and control (n = 16)). Analysis of these gigabytes grey-scaled data was aided by label-free elemental fingerprinting of secretory granule content.

RESULTS

Our analysis of the database revealed significant increased presence of specific mast cell subtypes in both autoantibody-positive (p = 0.02) and T1D (p = 0.005) compared to control donors. Moreover, we found that endocrine cells co-appearing with exocrine granules were present in a greater extent among autoantibody-positive (23%) and T1D donors (38%) compared to control donors (13%).

Furthermore, these 'intermediate' cells in both autoantibody-positive and T1D donors display a stressed morphology.

CONCLUSION

The first datamining showed innate immune cell alterations as well as aberrant exocrine and endocrine interactions that fit with the growing notion that T1D is a pancreas-wide disease. We are currently addressing a possible cause-consequence relationship between exocrine alterations and T1D pathology in a model for dynamic in vivo imaging experiments. In conclusion, we present the largest repository for human EM data. The information-dense character of these zoomable EM maps is excellent for data reuse and can now be accessed by researchers worldwide to address their own questions on T1D pathology at the nanometer scale.

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The relation between insulin resistance and risk of cardiovascular events, vascular interventions and all-cause mortality in people with type 1 diabetes: results from the UCC-SMART cohort study

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BACKGROUND

The presence of the metabolic syndrome and insulin resistance (IR) is increasing among people with type 1 diabetes (T1D). The estimated glucose disposal rate (eGDR) is a validated measure of IR in patients with T1D with lower eGDR levels indicating higher IR. The aim of this study was to identify determinants associated with a lower eGDR and to assess the association between eGDR and cardiovascular events, vascular interventions and mortality.

METHODS

195 people with T1D from the Utrecht Cardiovascular Cohort - Secondary Manifestations of ARTerial disease (UCC-SMART) study were included. The effect of eGDR on cardiovascular events, cardiovascular events or vascular interventions (combined endpoint) and on all-cause mortality was analysed using Cox proportional hazard models adjusted for confounders.

RESULTS

A total of 25 cardiovascular events, 26 vascular interven-

Table 4. Relation between eGDR and cardiovascular events, vascular interventions and all-cause mortality

	Model		High risk n = 48	Low risk n = 147	Total n = 195
			HR (95% CI)	HR (95% CI)	HR (95% CI)
Cardiovascular events	# events		12	13	25
		I	0.81 (0.54-1.21)	0.71 (0.55-0.91)	0.72 (0.58-0.88)
		II	0.71 (0.44-1.17)	0.73 (0.57-0.93)	0.74 (0.61-0.91)
Cardiovascular events and vascular interventions	# events		22	18	40
		I	0.82 (0.59-1.14)	0.71 (0.58-0.88)	0.71 (0.60-0.83)
		II	0.86 (0.59-1.26)	0.74 (0.60-0.91)	0.74 (0.63-0.87)
All-cause mortality	# events		11	16	27
		I	0.59 (0.38-0.92)	0.85 (0.69-1.06)	0.79 (0.65-0.96)
		II	0.51 (0.29-0.88)	0.86 (0.70-1.08)	0.81 (0.67-0.98)

Model I: adjusted for age and sex. Model II: adjusted for age, sex, current smoking, non-HDL and eGFR.
High risk is defined as a history of CVD or eGFR < 60 ml/min/1.73 m^2. Low risk is defined as no history of CVD or eGFR ≥ 60 ml/min/1.73 m^2. CVD is defined as coronary artery disease, cerebrovascular disease, peripheral artery disease, and/or aneurysm of the abdominal aorta.
Cardiovascular events is defined as myocardial infarction, stroke, subarachnoid hemorrhage or vascular mortality. Vascular interventions is defined as revascularisation, vascular surgical intervention or amputation.

tions and 27 deaths were observed during a median follow-up of 12.1 years (interquartile range 5.2-16.9). A lower eGDR was associated with a higher risk of cardiovascular events (HR 0.74; 95% CI 0.61-0.91), a higher risk of cardiovascular events or vascular interventions (HR 0.74; 95% CI 0.63-0.87) and a higher risk of all-cause mortality (HR 0.81; 95% CI 0.67-0.98). (Table 4)

CONCLUSION

A lower eGDR as a measure of IR in individuals with T1D is associated with a higher risk of cardiovascular events, cardiovascular events and vascular interventions and all-cause mortality. Therefore, individuals with T1D and insulin resistance should be identified early and treated appropriately to prevent cardiovascular disease.

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Monoclonal alpha-cells display a heterogenous calcium response when subjected to glucose stimulation

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BACKGROUND

In both health and disease, little is known about the mechanisms behind glucagon secretion, whilst there are indications for alpha-cell dysfunction in type 1 and 2 diabetes, contributing to inappropriate glucagon response in patients. For beta-cells, pacemaker-like behavior in calcium metabolism has been shown to be necessary for insulin secretion, but it is largely unclear how calcium fluxes in alpha-cells contribute to glucagon secretion. In this study, we monitor calcium activity of individual alpha-cells, in an effort to describe the general cell response to increased glucose levels.

METHODS

The murine alpha-TC6 cell line was seeded as a monolayer at different densities, and incubated with 5uM of calcium indicator Cal-520, followed by imaging on an Andor Dragonfly spinning disk microscope in HBSS with 10mM HEPES containing low (1.67 mM) or normal (5.5 mM) glucose. Recordings were analyzed with Fiji software for mean intensity for Regions of Interest (ROIs) containing one cell per ROI and normalized to the first recorded fluorescence (F0).

RESULTS

Individual alpha-cell calcium responses found, were dividable in 3 categories: low (A, continuous activity, with peaks < 0.05), high (B, continuous activity, with peaks > 0.05), and pacemaker-like (C, non-continuous activity defined by irregular but repetitive periods of on/off). Quantification of individual calcium responses are described as AUC.

Preliminary data suggests that in low to normal glucose stimulation, the high-activity cells (19 cells analyzed)

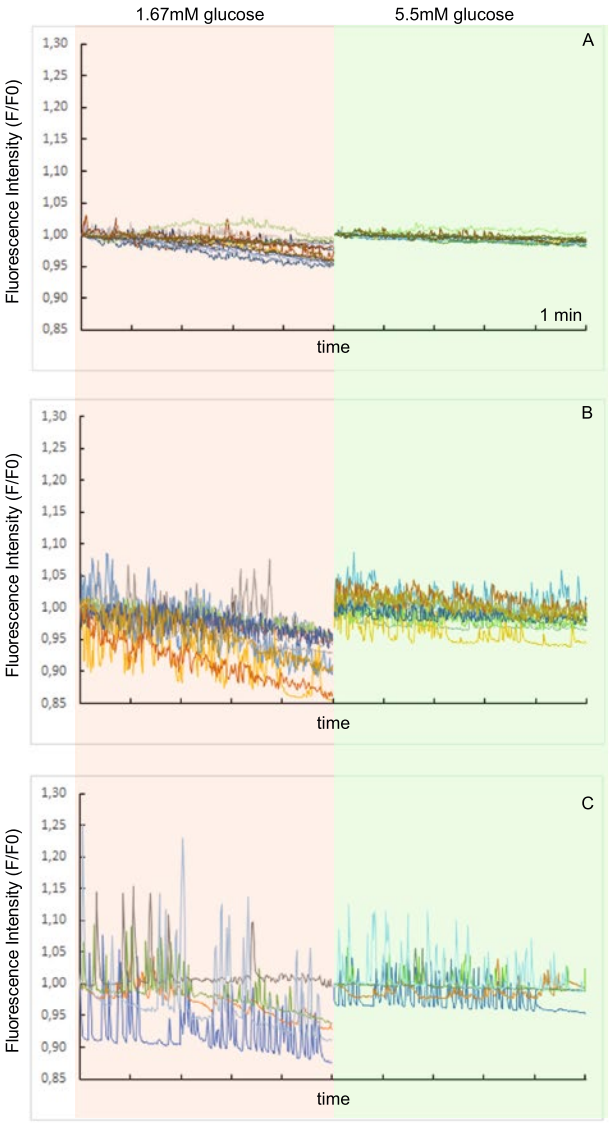

Figure 7. Monoclonal alpha-cells display a heterogenous calcium response when subjected to glucose stimulation.


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change their behavior after 5 minutes (p = 0.0174, n = 1). (Figure 7.)

CONCLUSION

We identified three distinct types of calcium flux exhibited by alpha-cells. Only the high activity-cells seem to show a difference after changing from low to normal glucose, suggesting that the other cells may not be inherently responding to their glucose surroundings.

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Long term follow up of low dose metformin in prediabetic individuals: an interim analysis

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BACKGROUND

Metformin therapy has been proven to delay the onset of diabetes in high risk individuals and to have a beneficial effect on the cardiovascular risk profile.[1] Despite recommendations to treat prediabetic individuals with metformin just a small minority of these patients actually adheres to such a regime.[2] The main reason for non-adherence with the metformin therapy has been attributed to intolerance of high dose metformin in prediabetic individuals.

METHODS

To evaluate the long term effect of low dose metformin on the development of diabetes and on cardiovascular risk factors in prediabetic individuals. Patients diagnosed with prediabetes (fasting glucose levels 6.1-6.9 mmol/L) were invited to participate in a long term observational follow-up study with an annual visit of the outpatient department. Weight and systolic blood pressure was measured and adverse events ascribed to the use of metformin were recorded. Blood samples for the determination of the HbA1c percentage, levels of total cholesterol, HDL, LDL, triglycerides and fasting glucose were drawn after 1 and 2 years of follow up.

RESULTS

A total of 36 patients were included in the study, 27 men and 9 women, mean age at entrance 58 years (+/- ? years). After 1 and two years of follow-up we noted a gradual decrease in mean weight (100,3 vs 98,5 and 91.6 kg), systolic (129,6 vs 126,3 and 124,6 mmHg) and diastolic blood pressure (81,2 vs 80,6 and 79,7) ($p < 0.05$, table 5). The same gradual decline was noted for HbA1c (41,1 vs 40,6 and 40,4 mmol/mol), for fasting glucose (6,4 vs 5,9 and 6,0 mmol/l) ($p < 0.05$) for total cholesterol (5,2 vs 4,9 and 5,0 mmol/l) and for LDL (3,1 vs 2,8 and 2,8 mmol/l). The concentrations of total triglycerides and HDL remained stable over time. During the study period of two years one major cardiovascular event was reported and there was no progression to diabetes recorded so far. The metformin medication was well tolerated and continued for the time of the study by all participating patients

CONCLUSION

The preliminary results of this observational study of the

Table 5.

Years	0	n	1	n	2	n
Age (years)	58	34				
Male/female	25/9	34				
Weight (kg)	100,3	34	98,5	25	91,6	20
Systolic bp (mmHg)	129,6	33	126,3*	27	124,6	20
Diastolic bp (mmHg)	81,2	33	80,6	27	79,7	20
Fasting glucose (mmol/L)	6,4	35	5,9*	29	6,0	23
HbA1c	41,1	34	40,6	29	40,4	23
Total cholesterol (mmol/L)	5,2	33	4,9	28	5,0	23
HDL (mmol/L)	1,2	31	1,1	26	1,2	23
LDL (mmol/L)	3,1	31	2,8	26	2,8	23
Triglycerides (mmol/L)	2,2	31	2,1	26	2,3	23
Hb	9,2	26	9,5	15	9,6	13

*$p < 0.05$
Paired sample T test Y = 0 vs Y = 1 or 2

effect of a low dose metformin regimen in patients with pre-diabetes are in line with the results of previous studies.[2,3] The decrease in weight, blood pressure and LDL concentration however are more pronounced than previously reported. This may be explained by the substantial loss in mean weight which may be partially responsible for the improvement in the above mentioned cardiac risk factors. This mechanism has been suggested before as an explanation for the beneficial effects of metformin therapy.[2] Though lifestyle intervention was not initiated in this study, the awareness of the risks involved with the diagnosis prediabetes and the start of metformin therapy may have motivated life style changes in individual patients.

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Degree of sensory loss predicts the risk of foot ulceration in patients with diabetes mellitus

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BACKGROUND

The aim of this study was to assess the relationship between the degree of loss of foot sensation at baseline and incident foot ulceration (DFU) in a cohort of patients with diabetes, using a valid instrument for the assessment of sensory loss.

METHODS

(Non)-neuropathic subjects (n = 416) participating in the observational Rotterdam Diabetic Foot (RDF) Study were followed prospectively (median 955.5 days (IQR, 841.5-1121)). Subjects underwent sensory testing of the feet (39-item RDF Study Test Battery) at baseline and were assessed regarding incident DFU. Seven groups of incremental degree of sensory loss were distinguished, according to the RDF-39 sum score. Kaplan-Meier and Cox's regression analyses were used to determine the independent hazard of baseline variables for new DFU.

RESULTS

40 participants developed DFUs. The mean incident rate of new-onset ulceration from study start was 4.5 (95% CI: 3.3-6.1) per 100 person-years, which increased significantly from 0 to 67.70 in the seven groups (X2 (6) = 129.704, $p < 0.0005$). Predictors for DFUs were higher RDF-39 score (aHR: 1.173, $p < 0.0005$) and kidney function (aHR: 1.022, $p = 0.016$). Disease-free survival in patients without prior DFU was predicted by the RDF-39 with a positive likelihood ratio of 3.77. Prior DFU suggests increased mortality risk.

CONCLUSION

The degree of sensory loss at baseline was associated with progression to DFU during follow-up. Grading the loss of sensation using the RDF Study Test Battery may result in a more precise risk stratification compared to the use of the 10 g monofilament according to current guidelines.

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Hair glucocorticoids, obesity and fasting blood glucose levels: results from a 3 year longitudinal study

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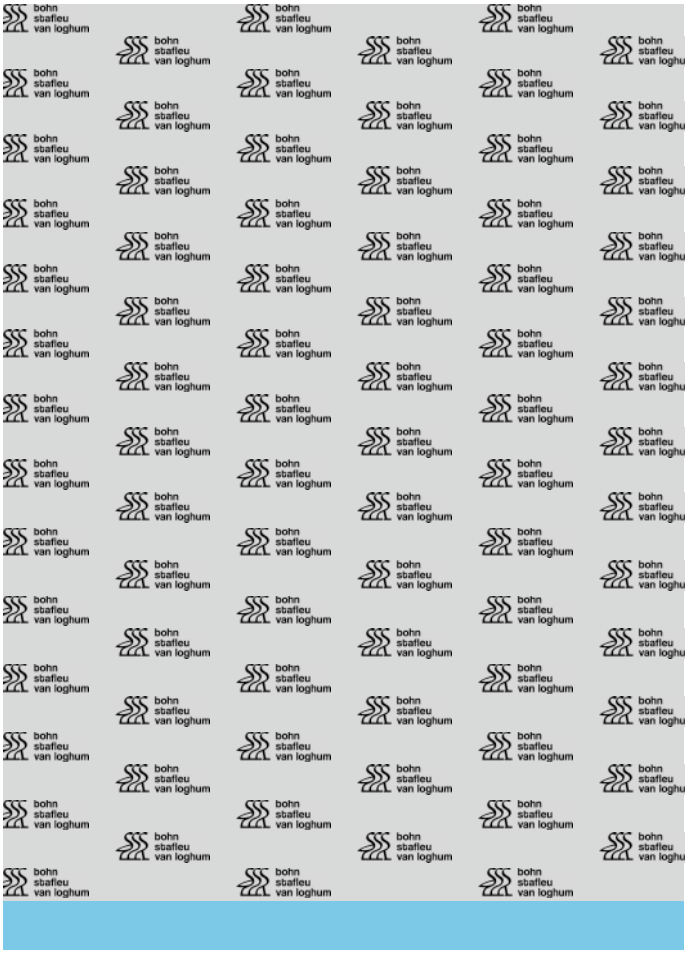
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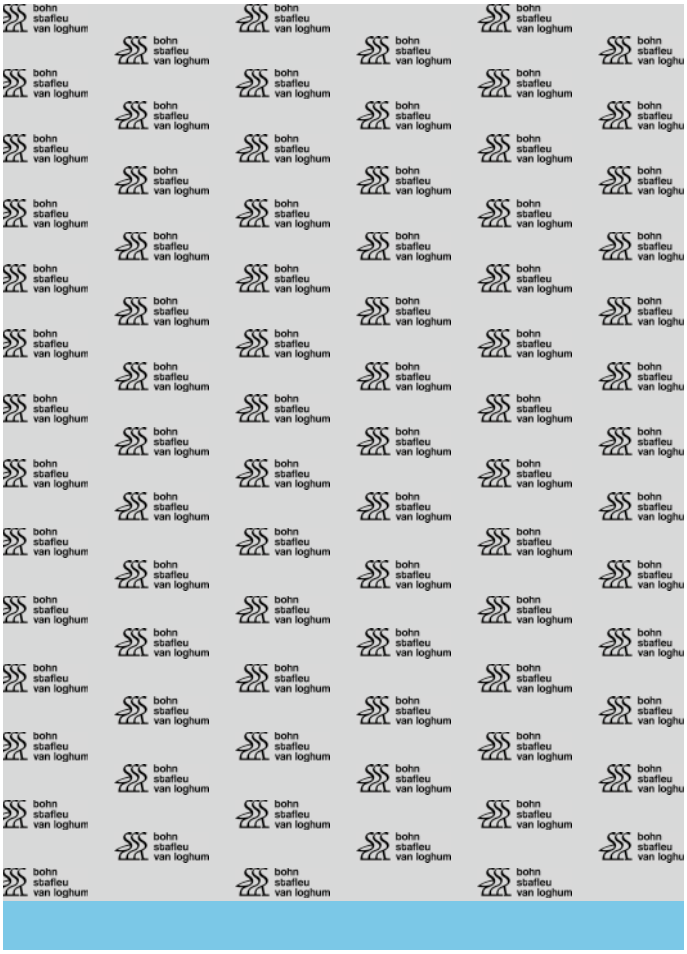
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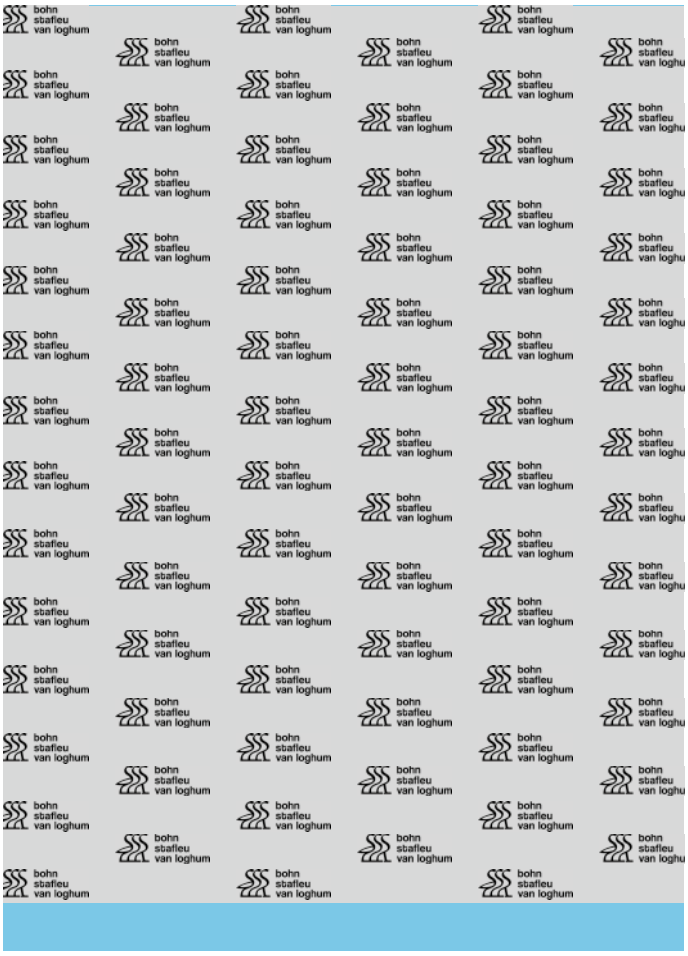
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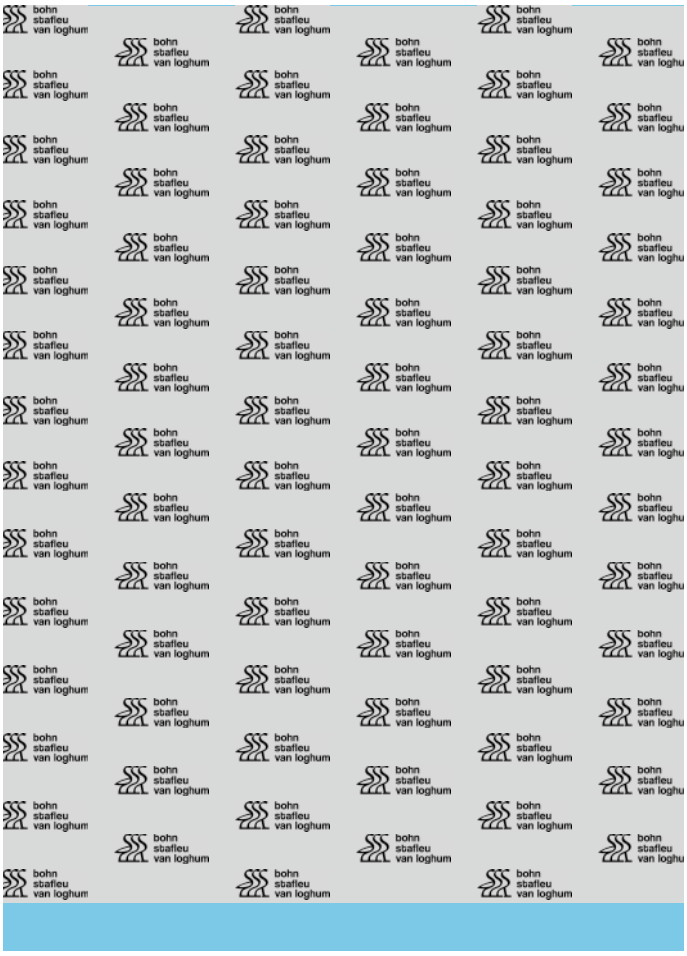
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BACKGROUND

Long-term glucocorticoids measured in scalp hair are correlated to obesity, components of the metabolic syndrome and depressive symptoms. It is however unknown whether hair cortisol and hair cortisone also predict changes in body mass index (BMI), waist circumference (WC), and glucose levels over time.

METHODS

We measured hair glucocorticoids in 1604 participants of the Netherlands Study of Depression and Anxiety (NESDA), including healthy controls and participants with a past or current diagnosis of depressive or anxiety disorder. Hair glucocorticoids were related to BMI, WC and fasting blood glucose levels at the moment of hair sampling, but also to changes of these parameters at the next study visit.

RESULTS

In cross-sectional analysis, hair cortisol and hair cortisone are correlated to an increased BMI (β = 2.06 and 2.84 respectively), WC (β = 5.36 and 8.54 respectively), and higher fasting glucose (β = 0.39 and 0.44 respectively, all $p < 0.001$). The highest quartile of baseline hair cortisol was related to an increase of BMI over time (adjusted β = 0.57, p = 0.003 compared to lowest quartile), whereas the highest quartile of hair cortisone was related to an increase in WC (β = 0.73, p = 0.009), after adjustment for confounders. Changes in glucose levels were not associated to baseline hair glucocorticoids.

CONCLUSION

Long-term exposure to endogenous glucocorticoid levels in the high-physiologic range can be linked to adverse anthropometric changes over time, but not to glucose levels.

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Long-term glucocorticoids are associated with increased odds of metabolic syndrome after a combined lifestyle intervention

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BACKGROUND

Long-term glucocorticoids, as measured in scalp hair, have been associated with obesity and the metabolic syndrome. Factors that are known to increase daily cortisol production are psychological stress, sleep deprivation and intake of food with high glycemic index. This study examines hair cortisol and cortisone concentrations in participants of a long combined lifestyle intervention (CLI), hypothesizing that increased long-term glucocorticoid exposure is associated with metabolic syndrome.

METHODS

Adults at the CGG outpatient clinic with a body-mass index (BMI) of > 30 kg/m2 were enrolled in the CLI, consisting of guided exercising, dietetics, and cognitive behavioural therapy. Antropometric measures, blood testing and hair corticosteroids were assessed at baseline, after 10 weeks and at the end of the program (75 weeks). A hair of 3 cm closest to the scalp was cut and analyzed for glucocorticoid concentrations using liquid chromatography-mass spectrometry (LC/MS).

RESULTS

163 participants (mean age 42 years, 75% female) were included. Mean weight, BMI and waist circumference decreased from 116 kg, 39.5 kg/m2, and 113 cm at baseline to 111 kg, 37.7 kg/m2, and 106 cm respectively after 75 weeks (p = 0.023, p = 0.018, and 0.000 respectively). Mean (log) hair cortisol and hair cortisone decreased from 0.61 and 1.05 pg/mg at baseline to 0.37 and 1.00 pg/mg (p = 0.000 and p = 0.305 respectively). HbA1c, insulin levels, HOMA-IR significantly decreased after 75 weeks (p = 0.001, p = 0.002, and p = 0.002 respectively). Hair cortisol concentrations were not correlated with weight and BMI, but there was a trend towards correlation with waist circumference (Pearson's r = 0.183, p = 0.082). Hair cortisone concentrations were correlated in trend with weight and were significantly correlated with waist circumference (Pearson's r = 0.245, p = 0.018). Adjusted OR for the metabolic syndrome at 75 weeks per quartile increase in baseline hair cortisol and hair cortisone was 1.74 (95% CI: 0.96-3.32) and 2.21 (95% CI: 1.16-4.19). Adjusted OR for the metabolic syndrome at 75 weeks for the highest quartile of baseline hair cortisol was 12.34 (95% CI: 1.76-86.36).

CONCLUSION

High baseline hair cortisol and cortisone concentrations are associated with higher odds of having the metabolic syndrome after a CLI.

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Improvements in Appetite-Regulating Hormones and Eating Behaviour in Response to a Combined Lifestyle Intervention for Obesity

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BACKGROUND

Obesity (BMI ≥ 30.0 kg/m2) is associated with dysregulations of appetite-regulating hormones such as insulin, leptin, adiponectin and cortisol. These dysregulations promote adverse clinical outcomes such as type 2 diabetes (T2D), but also pose a hormonal disposition for uncontrolled overeating. Improvements in hormonal appetite regulation and eating behavior are therefore beneficial, not only for glucose homeostasis, but also for successful weight loss and long-term weight loss maintenance.

METHODS

To investigate changes in appetite-regulating hormones (serum leptin, insulin, adiponectin and long-term hair cortisol) as well as eating behaviour (emotional, external and restrained eating) in patients with obesity who completed a 75-week combined lifestyle intervention (CLI) which aims to promote a sustainable healthier lifestyle and improvements in long-term weight management.

RESULTS

N = 76 patients were included of whom n = 13 were diagnosed with T2D). Levels of serum insulin (n = 76), leptin (n = 45) and adiponectin (n = 45) and hair cortisol (n = 51) as well as eating behaviour (n = 73) after 75 weeks of intervention were compared to baseline.

RESULTS

After 75 weeks of CLI, there were significant decreases in BMI (40 kg/m2 ± 5.5 vs 38.1 kg/m2 ± 6.1, p < .001), serum insulin (170 pmol/l ± 45 vs 136 pmol/l ± 83, p < .001), leptin (44.9 ng/ml ± 16 vs 38.5 ng/ml ± 15.9, p < .01), hair cortisol (5 pg/mg ± 4.9 vs 3.7 pg/mg ± 4,0 p < .01) as well as emotional eating (2.90 ± .84 vs 2.61 ± .83, p < .01) and external eating (3.00 ± .60 vs 2.65 ± .58, p < .001). Meanwhile, there were significant increases in restrained eating (2.83 ± .57 vs 3.16 ± .49, p < .001) as well as adiponectin (3.4 ug/ml ± 2.1 vs 3.8 ug/ml ± 2.7, p < .05).

CONCLUSION

A CLI promoted significant improvements in BMI, appetite-regulating hormones and eating behavior. The changes are assumed to support metabolic outcomes such as glucose homeostasis as well as long-term weight loss and weight management.

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Effects of liraglutide treatment in genetic obesity

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BACKGROUND

Obesity is associated with numerous comorbidities, like metabolic syndrome and type 2 diabetes (T2D). In rare cases, obesity is caused by disruptions in the leptin-melanocortin pathway, e.g. melanocortin 4 receptor (MC4R). In these patients, little effect of lifestyle treatment is seen. Moreover, bariatric surgery seems less successful. Liraglutide is a Glucagon-Like-Peptide-1 agonist, primarily developed for the treatment of T2D, showing positive effects on metabolic parameters and weight loss in lifestyle-induced obesity. We present a case report of the results of liraglutide treatment in a patient with a MC4R mutation.

METHODS

A 29 year old female patient developed hyperphagia and progressive obesity at the age of 5. At the age of 13 a heterozygous pathogenic variant in MC4R was identified. Intensive supportive lifestyle treatment had little effect. At the age of 29, liraglutide treatment was initiated because of her therapy-resistant obesity. She had a weight of 188,7 kg,

BMI of 57.09 kg/m2 and weight circumference of 129 cm. Her resting energy expenditure (REE) was 34% lower than expected (1738 kcal/day). Laboratory tests showed increased fasting glucose (6.4 mmol/l), dyslipidaemia, and leptin resistance.

RESULTS

Alongside intensive supportive lifestyle treatment, liraglutide was initiated and dosing could be titrated towards 3.0 mg. After 16 weeks, her weight, BMI and weight circumference respectively decreased to 179,2 kg, 54.22 kg/m2 and 128 cm. Her REE was 27% higher than expected (3453 kcal/day). Laboratory testing showed a normalised fasting glucose (5.4 mmol/l), improved dyslipidaemia, and decreased leptin levels. She reported improved satiety feelings and no serious side effects.

CONCLUSION

We show beneficial effects of liraglutide on metabolic parameters, weight, and satiety in a patient with a pathogenic MC4R mutation. Even in a period with limited intensive physical activities during corona lockdown, this patient achieved a clinically relevant 5% weight loss. Our findings suggests that liraglutide might be an effective treatment option, as an adjunct to a healthy lifestyle, for patients with monogenic obesity.