

FID/CEDA Congress 2015 and E&D NRW Annual Meeting – Abstracts

ABSTRACT 1

Phenotype-directed personalisation of therapy in type 2 diabetes mellitus patients and criteria of prescription

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Background: Type 2 diabetes mellitus (T2DM) is a chronic and progressive disease that is rapidly increasing in prevalence over the world. Despite a number of guidelines and advices for physicians on how to start treatment, not all patients achieve target glycaemia and glycated hemoglobin (HbA_{1c}) level. The aim of this study is to research personalisation of therapy of T2DM patients and criteria of prescription.

Material and methods: We studied 228 patients with T2DM treated in Pauls Stradins Clinical University Hospital in Endocrinology department from Jan. 2013 to Sep. 2014. The greatest attention was paid to the patients' biochemical analyses, such as triglycerides (TG), C-peptide, HbA_{1c} and prescribed treatment, as well as BMI and the doctor who treated each patient.

Phenotypically patients were divided into 4 major groups. The first group included 73 patients with chronic kidney disease (GFR < 60 ml/min/1,73m²), the second 31 patients with obesity (BMI > 30 kg/m²), 66 persons represented older patients group (> 65 years old) and 58 with early diabetes (< 10 years).

Results: For patients with obesity, treatment was started with insulin and metformin combination; chronic kidney disease group with insulin monotherapy; in groups of old patients (> 65 years) and early diabetes patients with insulin monotherapy.

Insulin monotherapy was used for patients with C-peptide 0,1–0,9 ng/l; for

patients with C-peptide 1–2 ng/ml was used metformin combination with insulin, but patients with C-peptide more than 2 ng/ml were treated more often with DPP-4/metformin combination with insulin.

There was no correlation between HbA_{1c} level and prescribed therapy.

For patients with TG level from 1,7–5,6 mmol/l was used metformin together with insulin, with TG < 1,7 mmol/l only insulin, but with TG > 5,7 mmol/l DPP-4/metformin combination with insulin.

We found some correlation between prescribed therapy and treating physicians: 3 of 5 physicians treated their patients more often with insulin. One treated patients mostly with combination of insulin and metformin, while last with combination of DPP-4/metformin and insulin.

Conclusion: As T2DM is a special medical condition that requires an individualized approach to each patient to achieve adequate glycaemia and HbA_{1c} levels, most likely future T2DM treatment guidelines will be based on metabolic characteristics of each patient, although currently most physicians tend to prescribe therapy based on their experience.

Keywords: type 2 diabetes mellitus treatment, type 2 diabetes mellitus, endocrinology, diabetes

ABSTRACT 2

Why do the patients with diabetes believe about themselves that they have neuropathy? (QoL-DN Survey in Romania)

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Background and aims: Due to the number of the involved persons, the cross-sectional study on the quality of life in patients with diabetes mellitus and diabetic neuropathy done between June and December 2012 is the greatest of this kind in our country. We have used the linguistically validated Romania translation of the Norfolk QoL-DN questionnaire (Vinik EJ et al., 2005), distributed, after obtaining the informed consent, to 23 543 patients with diabetes. The questionnaire was conceived as a tool able to identify the type of the affected nerve fiber information ("fiber specific"), grouped in sub-domains and has proved a very good sensitivity and specificity in discriminating between patients with and without diabetic neuropathy and layering its severity.

Material and methods: In the current study we have analyzed from 19 892 validated questionnaires the demographic and clinical characteristics of the group of patients who answered "Yes" to the question "Do you have neuropathy (nerve affecting)?" and "No" to the question "Have you ever been told that you suffer from neuropathy?", group 1 (1 941 persons, 9.8 % of the total), compared with those who answered "No" to both questions, group 0 (6 069, 30.5 %) and those who answered "Yes" to both questions, group 2 (11 618, 58.4 %). We have tested the significance of the differences with the SPSS 15 program, by one-way analysis of variance, post-hoc test Scheffé and the Chi² test.

Results: The proportion of women in the groups 1 and 2 was significantly greater than in the group 0. The total score and the sub-domains scores were much higher in groups 1 and 2 compared to group 0. The percentage of pa-

tients who mentioned ulcerations, gangrene or amputations in the past (13.25, 3.7, 3.3 %) were significantly greater in group 1 compared with group 0 (3.67, 1.0, 1.0 %). The individual symptoms analyses revealed a significant frequency in group 1, higher than in group 0 but lower than in group 2 (except the location in the upper limbs).

Conclusion: The conclusions of our study are that a relatively important number of patients in our study have symptoms which they consider to be due to diabetic neuropathy, with an important impact on the quality of life, but which they either do not communicate to doctors or are not correctly interpreted.

Keywords: diabetes, neuropathy, quality of life

ABSTRACT 3

Kardiovaskuläre autonome Dysfunktion und Kleinfaserneuropathie in gut kontrollierten Typ-2-Diabetikern: Es besteht ein Zusammenhang

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Beschädigung der dünnen Nervenfasern ist die früheste Veränderung im Verlauf der Entwicklung der diabetischen Neuropathie. Kardiovaskuläre autonome Neuropathie ist bei diabetischen Patienten mit einer fünffach erhöhten Sterblichkeit gegenüber Diabetikern ohne kardiovaskuläre Neuropathie verbunden. Es wurde bewiesen, dass schmerzhafte Symptome mit einer größeren Abnahme der Herzfrequenzvariabilität (HRV) assoziiert sind. Allerdings hat man kaum Assoziation zwischen dem Ergebnis der traditionellen kardiovaskulären Reflextests und der Kleinfaserfunktion gefunden.

Ziel unserer Untersuchung war die detaillierte Charakterisierung des Zusammenhanges zwischen Temperaturempfindungsschwellen, die die Funktion der dünnen Fasern reflektieren, und den kardiovaskulären Reflextests bei Typ-2-Diabetikern.

Unsere Studie schloss 26 Typ-2-Diabetiker mit einem Durchschnittsalter von

64 ± 7 Jahren ein. Durchschnittliche Diabetesdauer und HbA_{1c}-Spiegel der Patienten waren 5 Jahre (Interquartilbereich: 8 Jahre) bzw. 6,9 % (Interquartilbereich (IQB): 6,2; 7,4). Die periphere Kleinfaserfunktion wurde durch Bestimmung des Temperatureizes mit Hilfe des Q-Sense-Gerätes (Medoc Ltd.) bewertet. Wärme- und Kälteempfindungsschwellen wurden für jede Hand und jeden Fußrücken bestimmt. Die kardiovaskuläre autonome Funktion wurde mit den standardisierten kardiovaskulären Reflextests beurteilt, die die Reaktion der Herzfrequenz auf Ein- und Ausatmung, Valsalva-Manöver, 30/15-Quotienten und die orthostatische Blutdruckabnahme (Cardiosys, MDE Heidelberg) umfassten.

Wärmeempfindungstemperaturen der oberen und unteren Extremitäten korrelierten mit den Veränderungen der Herzfrequenz auf Ein- und Ausatmung ($r = -0,413$ und $r = -0,436$, $p < 0,05$). Wärmeempfindungsschwellen der oberen Extremitäten zeigten eine signifikante Assoziation mit dem Valsalva-Quotienten ($r = -0,419$, $p < 0,05$). Darüber hinaus ergab sich eine signifikante Differenz zwischen den kleinfaserneuropathiepositiven und -negativen Patienten bezüglich der orthostatischen Hypotonie ($-9,2$ (IQB: 1; 14) mmHg versus 0 (IQB: 0) mmHg, $p = 0,004$). Diese zwei Gruppen unterschieden sich in Bezug auf Alter ($p = 0,737$), glykämische Kontrolle ($p = 0,238$), Diabetesdauer ($p = 0,167$), BMI ($p = 0,784$), Rauchen ($p = 0,677$), systolische und diastolische Blutdruckwerte ($p = 0,670$ und $p = 0,285$) nicht.

Unsere Ergebnisse weisen darauf hin, dass die Beschädigung der Kleinfasern mit Beeinträchtigung der kardialen autonomen Funktion assoziiert ist und mit Resultaten der Reflextests bei gut kontrollierten Diabetikern korreliert. Orthostatische Hypotonie ist in erster Linie bei Diabetikern zu erwarten, die abnorme Temperaturempfindungsschwellen haben. Die Kleinfaserfunktion sollte bei Patienten mit autonomer Dysfunktion bewertet werden, während kardiovaskuläre Reflextests bei Patienten mit Temperaturempfindungsstörungen durchzuführen sind.

Keywords: cardiovascular autonomic neuropathy, small-fibre neuropathy

ABSTRACT 4

Serum levels of IL-6 and sICAM-1 are associated with painful polyneuropathy and pain intensity in the elderly: KORA F4 Study

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Background and aims: Distal sensorimotor polyneuropathy (DSPN) represents the most common diabetic microvascular complication affecting at least one third of patients with type 2 diabetes. Painful DSPN is encountered in a considerable proportion of patients with DSPN and leads to substantial impairment in quality of life due to limited treatment options. Factors contributing to painful DSPN are incompletely understood. The aim of our study was to investigate whether circulating levels of biomarkers of subclinical inflammation were associated with painful DSPN in old age.

Methods: The study population consisted of individuals with painless ($n = 337$) and painful DSPN ($n = 54$) from a source population of men and women aged 61–82 years who participated in the population-based KORA F4 survey (2006–2008). We measured circulating levels of seven immune mediators and assessed their associations with the presence of painful DSPN and pain intensity using multiple regression models.

Results: After adjustment for age and sex (model 1), we found positive associations between serum concentrations of interleukin (IL)-6 and the soluble intercellular adhesion molecule (sICAM)-1 and painful DSPN ($P = 0.005$ and $P = 0.004$, respectively), whereas no associations were observed for C-reactive protein (CRP), IL-18, tumour necrosis factor- α (TNF- α), adiponectin and IL-1 receptor antagonist (IL-1RA, P between 0.07 and 0.38). Associations between IL-6, sICAM-1 and painful DSPN re-

mained significant in model 2 after additional adjustment for waist circumference, height, hypertension, cholesterol, smoking, alcohol intake, physical activity, history of myocardial infarction and/or stroke, presence of other neurological conditions and use of non-steroidal anti-inflammatory drugs ($P=0.016$ and $P=0.005$, respectively). High serum levels of IL-6 and sICAM-1 were also positively associated with pain intensity ($P=0.003$ and $P=0.041$, respectively), whereas no significant associations were found for the other biomarkers (P between 0.06 and 0.91).

Conclusion: Our data indicate a differential association of biomarkers of subclinical and vascular inflammation with the presence of painful DSPN and pain intensity in older individuals from the general population. Mechanistic studies exploring the relationship between inflammation, peripheral and central sensitisation appear relevant to improve our limited understanding of the underlying pathophysiology and to identify potential novel therapeutic targets.

Keywords: inflammation, cytokines, polyneuropathy, epidemiological study

ABSTRACT 5

Dietary modulators of insulin sensitivity and insulin secretion during the first two years in patients with newly diagnosed type 1 and type 2 diabetes

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Background and aims: Dietary habits relate to the prevention and the manifestation of diabetes. However, their impact on the early development of insulin sensitivity and insulin secretion in

type 1 (T1D) and type 2 diabetes mellitus (T2D) remain to be elucidated. Thus, we analyzed the impact of the consumption frequencies of selected food groups on insulin sensitivity and secretion in patients with diabetes during the first two years after diagnosis.

Material and methods: In a prospective observational study, glycemic control, insulin sensitivity and secretion from intravenous glucose tolerance and glucagon stimulation testing, and food consumption frequencies derived from a food propensity questionnaire were measured in 127 (31 % T1D) patients with diabetes. Multivariable regression models were used to assess the prospective associations of food consumption frequencies at baseline with changes in glycemic control, insulin sensitivity and secretion between baseline and two-year follow-up.

Results: Patients with T1D and T2D exhibited good glycemic control (A_{1c} in T1D: 7.1 ± 1.6 % and T2D: 6.4 ± 1.0 %) at diagnosis and two years later (A_{1c} in T1D: 7.0 ± 1.2 % and T2D: 6.7 ± 1.2 %). In T1D, increased consumption frequency of refined grains by one time/day at baseline associated with an increase in A_{1c} by 0.60 % (95 % CI: 0.04; 1.16, $P=0.035$) within the first two years after diabetes diagnosis when adjusted for age, sex, body mass index, and type of glucose-lowering medication. In T2D, increased consumption frequency of meat and meat products by one time/day at baseline related to a lower beta cell adaptation index by 7.25 % (95 % CI: -13.16; -0.93, $P=0.026$) after two years.

Conclusion: During the initial course of the disease, intake of refined grains may negatively affect glycemic control in T1D, whereas intake of meat and meat products may contribute to impaired beta cell function in T2D.

Keywords: insulin resistance, beta-cell function, HbA_{1c} , lifestyle

ABSTRACT 6

Proteolytic cleavage of osteopontin in adipose tissue enhances its inflammatory effects on human adipocytes which can be blocked with a monoclonal antibody

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Background and aims: Obesity-driven adipose tissue (AT) inflammation results in adipocyte dysfunction and insulin resistance. Osteopontin (OPN) is an inflammatory cytokine, which is highly upregulated in AT in obesity where it contributes to chronic subclinical inflammation and has been shown to directly affect adipocyte function by inducing inflammatory signaling and decreased insulin sensitivity. Based on the fact that OPN's activity could be amplified by matrix-metalloprotease (MMP) cleavage, this study aimed at investigating the presence of OPN cleavage products in human adipose tissue in obesity and its impact on adipocyte function.

Material and methods: AT from severely obese and non-obese donors was analyzed for gene expression of OPN and MMPs. OPN fragments in AT were detected via Western blot and immunohistochemistry. Primary human in-vitro differentiated adipocytes were stimulated with human recombinant OPN or MMP-7 cleaved OPN (MMP-cOPN). Inflammatory signaling, gene expression, insulin-stimulated signaling respectively glucose uptake and integrin receptor binding were analyzed including their blockade by a monoclonal antibody directed against the free MMP-cleavage site of OPN (CMIP 9-3).

Results: OPN expression in human obesity highly correlates with MMP-9 expression, known to be capable of OPN cleavage. Moreover, we detected increased levels of OPN cleavage products including free MMP cleavage sites in AT of obese compared to lean individuals. Stimulating adipocytes with OPN, most likely via binding of αv -integrin chains, resulted in p38 and ERK phosphorylation and increased expression of MMP-9 and CCL-5 while adiponectin, GLUT-4 and PPAR γ expression was decreased, in parallel with decreased insulin-stimulated glucose uptake. All these effects were enhanced by MMP-cleavage of OPN. CMIP

9-3 significantly blocked inflammatory effects of MMP-cOPN on adipocytes.

Conclusions: Our findings show that MMP cleavage takes place in obese adipose tissue thereby enhancing OPN's inflammatory and pro-diabetic activity in adipocytes. Specifically targeting MMP-cleaved OPN opens avenues for prevention and treatment of obesity-induced insulin resistance and type 2 diabetes. This work is supported by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (to T.M.S.).

Keywords: osteopontin, matrix metalloproteinase, adipocyte, inflammation, diabetes

ABSTRACT 7

Adiponectin may mediate the association between omentin, circulating lipids and insulin sensitivity: KORA F4 Study

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Background and aims: Reduced circulating omentin levels have been reported in obesity and type 2 diabetes, but data were mostly derived from univariate analyses in small study samples. This study aimed to investigate the relationship between omentin, abnormal glucose tolerance and related metabolic factors in a large population-based cross-sectional study.

Methods: Serum omentin was measured by ELISA in 1 092 participants of the German KORA F4 survey (2006–2008). Associations between omentin serum levels, glucose tolerance (assessed with an oral glucose tolerance test) and diabetes-related factors were assessed using logistic and linear regression models, respectively.

Results: Serum levels of omentin were not related to categories of glucose tolerance. However, serum omentin was

positively associated with whole-body insulin sensitivity index (ISI(composite)) and HDL cholesterol and showed inverse associations with two-hour post-load glucose, fasting insulin, HOMA-IR, body mass index (BMI) and triglycerides (all $P \leq 0.03$ after adjustment for age, sex and lifestyle factors). Further adjustment for BMI and/or serum lipids attenuated the associations with parameters of glucose metabolism. Adjustment for waist-hip ratio or waist-height ratio as alternative indices of obesity or adjustment for kidney function assessed by estimated glomerular filtration rate (CKD-EPI) had no impact on our results. Finally, adding serum adiponectin to regression models virtually abolished all aforementioned associations. In contrast, adjustment for omentin did not alter the positive association between adiponectin levels and ISI(composite).

Conclusion: The data from this large population-based cohort show that circulating omentin levels are associated with insulin sensitivity. Our observations further suggest that omentin acts via upregulation of adiponectin, which in turn affects lipid metabolism and thereby also indirectly enhances insulin sensitivity, but mechanistic studies are required to corroborate this hypothesis.

Keywords: omentin, adiponectin, insulin resistance, diabetes, lipids

ABSTRACT 8

Non-obese diabetic mice lacking the innate immune receptor TLR4 exhibit hyperlipidemia and altered gut morphology

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Background and aims: Our previous studies in non-obese diabetic (NOD) mice lacking the innate immune receptor toll-like receptor (TLR) 4 showed an accelerated diabetes development and point to a role of the receptor in the in-

duction of diabetes-promoting autoimmunity as well as metabolic disorders such as hyperlipidemia. Peripheral levels of fatty acids (FA) and triglycerides (TG) are largely dependent on the efficiency of lipid resorption via the intestinal barrier which is supposed to be affected by TLR4. We therefore tested the hypothesis that TLR4 deficient mice exhibit gastrointestinal abnormalities driving diabetes development. In a comparative approach we investigated the impact of the TLR4 expression status on gut morphology in NOD mice and in mice of a control strain without risk to develop diabetes.

Methods: Female TLR4 expressing (TLR4+/+) and TLR4 deficient (TLR4-/-) C57BL10 and prediabetic (normoglycemic) NOD mice were monitored for diabetes development and peripheral lipid levels. In parallel, morphometric analyses of frozen sections from the intestine were performed.

Results: Female TLR4-/-NOD mice developed diabetes earlier (mean age of diabetes manifestation 152 ± 28 days) and exhibited higher peripheral levels of triglycerides (TG) (78.4 ± 3.6 mg/dl) and free fatty acids (FFA) (0.75 ± 0.04 mmol/l) than their TLR4 expressing littermates (mean age of diabetes manifestation 208 ± 40 days).

Conclusion: Our results demonstrate that hyperlipidemia in TLR4 deficient NOD mice is associated with atrophy of the ileal tunica muscularis, the intestinal muscle layer regulating gut motility. Our findings point to the involvement of the TLR4 in the development of gastrointestinal disorders and accelerated diabetes in this model of T1D.

Keywords: non-obese diabetic mouse, toll-like receptor 4, animal model of type 1 diabetes, gut morphology

ABSTRACT 9

Intranasal insulin lowers liver fat content and stimulates hepatic energy metabolism in healthy humans, but not in type 2 diabetes patients

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Background and aims: Studies in rodents suggest that insulin controls hepatic glucose metabolism through brain-liver crosstalk. Human studies using intranasal insulin to mimic central insulin delivery provided conflicting results, by reporting either reduction of endogenous glucose production (EGP) or no acute changes in glucose metabolism.

Material and methods: In this randomized controlled cross-over trial we investigated effects of intranasal insulin on hepatic insulin sensitivity and energy metabolism in 10 patients with type 2 diabetes (T2D) and 10 lean healthy humans (CON). All participants were studied on two study days according to identical protocols, except for the intranasal administration of 160 IU human insulin (Actrapid, Novo Nordisk, Denmark) or placebo. On a third day, a subgroup of 8 CON received 0.1 IU of human insulin intravenously in order to study the metabolic effects of a transient increase in serum insulin, observed upon intranasal insulin application. Rates of EGP were monitored using [6,6- $^2\text{H}_2$]glucose. Hepatocellular lipids (HCL) and hepatic concentrations of adenosine triphosphate (ATP) and inorganic phosphate were assessed with $^{31}\text{P}/^1\text{H}$ magnetic resonance (MR) spectroscopy on a clinical MR-scanner (Philips Healthcare, Best, The Netherlands).

Results: Intranasal insulin transiently increased serum insulin, followed by gradual lowering of blood glucose in CON only. Fasting hepatic insulin sensitivity index (HIS) was not affected by intranasal insulin in CON and patients with T2D. Only in CON, HCL decreased by 35 %, whereas absolute hepatic ATP concentrations increased by 18 % at three hours after intranasal insulin. In the subgroup of CON, who received intravenous insulin, similar changes in serum insulin, blood glucose and plasma free fatty acids were observed compared to intranasal insulin condition. Intravenous insulin administration resulted in a 34 % increase in HCL, without altering hepatic ATP concentrations.

Conclusion: Intranasal insulin does not affect HIS but rapidly reduces HCL and stimulates hepatic energy metabolism in healthy humans, which is independent of peripheral insulinemia. These effects are blunted in patients with T2D, which may result from the impairment of the indirect effects of insulin on peripheral metabolism.

Keywords: intranasal insulin, hepatic insulin sensitivity, liver fat, hepatic energy metabolism

ABSTRACT 10

Serum progranulin concentrations decrease after a 6-month exercise program in overweight or obese sedentary subjects

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Background and aims: Progranulin, a multifunctional protein with crucial role in multiple physiologic and pathologic processes, was recently suggested to link obesity with inflammation, insulin resistance and atherosclerosis. Exercise reduces circulating progranulin in type 2 diabetes. We examined the effect of exercise on circulating progranulin in non-diabetic subjects, and the relation of progranulin with cardiometabolic parameters.

Material and methods: Serum progranulin was measured in 79 overweight or obese, sedentary non-diabetic individuals who participated in a 6-month exercise program (exercise group = EG, n = 51) or served as controls (n = 28). The relation of progranulin with various cardiometabolic risk factors was examined in a non-diabetic population (n = 104) with a wide range of age and body mass index (BMI).

Results: After the exercise program circulating progranulin decreased in the EG ($P < 0.001$) and controls ($P = 0.002$), the progranulin decrease in the EG being significantly greater ($P = 0.023$) vs. controls. The change (Δ) in progranulin correlated with ΔBMI , Δwaist , $\Delta\text{C-reactive protein (CRP)}$, Δleptin and $\Delta\text{maximal aerobic capacity (inversely)}$. In

multivariable linear regression analyses ΔCRP was an independent determinant of $\Delta\text{progranulin}$ after the exercise program. Serum progranulin correlated with age, BMI, waist, fat mass, total cholesterol, LDL cholesterol, insulin, HOMA-insulin resistance, leucocytes count and CRP.

Conclusions: A 6-month exercise program reduces serum progranulin in overweight or obese, sedentary non-diabetic subjects and these changes are independently associated with reduced low-grade inflammation. Serum progranulin correlates with various cardiometabolic risk factors, in particular with subclinical inflammation. These data further suggest a role of progranulin as proinflammatory adipokine linking chronic inflammation to cardiometabolic diseases.

Keywords: progranulin, adipokines, exercise, obesity, chronic inflammation, atherosclerosis

ABSTRACT 11

Eicosapentaenoic acid but not docosahexaenoic acid promotes a brite phenotype in primary human adipocytes

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Background and aims: Both, increasing brown adipose tissue (BAT) mass/activity and inducing UCP1-expressing brown-like (brite/beige) adipocytes within white adipose tissue (WAT) represent strategies to counteract obesity. Studies in rodents have shown that supplementation with the n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) reduces obesity. Furthermore, adaptive thermogenesis in BAT and oxidative metabolism in WAT are increased after n-3 PUFA intake. Therefore, we aimed to assess the direct effect of the n-3 PUFAs EPA (20:5n-3) and DHA (22:6n-3) in comparison to arachidonic acid (ARA, 20:4n-6) and oleic

acid (OA, 18:1n-9) on WAT browning in humans.

Material and methods: Primary human adipose-derived stem cells (hASCs) isolated from the subcutaneous depot were challenged with EPA, DHA, ARA or OA at 20 μ M during adipocyte differentiation (12 days). Lipid accumulation was measured by Oil Red O staining, adipogenic and brown marker gene expression were assessed by qRT-PCR as well as Western Blot, and mitochondrial function was measured using the XFe96 Flux Analyzer.

Results: Treatment of hASCs with EPA, DHA, and ARA increased lipid accumulation and HSL protein levels, indicating enhanced adipogenesis. As expected, adiponectin protein levels were only higher in hASCs exposed to EPA and DHA. Interestingly, hASCs treated with ARA displayed larger lipid droplets. Importantly, UCP1 and CPT1B mRNA levels were solely upregulated by EPA, while ARA significantly enhanced mRNA expression of the WAT-specific marker TCF21. The beneficial effects of EPA on UCP1 and CPT1B expression were abrogated when EPA was combined with ARA. Protein levels of OXPHOS complexes in total, an indicator of mitochondrial density, were not altered by any of the fatty acids. However, citrate synthase activity was increased in EPA treated hASCs. Moreover, maximum respiratory capacity was reduced in hASCs challenged with ARA compared control.

Conclusion: In summary, solely the n-3 PUFA EPA but not DHA promotes a brite gene expression pattern and improves mitochondrial function, providing a potential mechanism for the beneficial anti-obesity effects of dietary n-3 PUFAs. Moreover, these results may help to improve nutritional recommendations for a healthy ratio of EPA/ARA intake.

Keywords: primary human adipocytes, polyunsaturated fatty acids, brite adipocytes, mitochondrial function

ABSTRACT 12

Decline of extracellular superoxide dismutase (SOD3) concentrations in recently diagnosed diabetic subjects over 2 years

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Introduction: Oxidative stress is thought to play a key role in the pathogenesis of diabetic neuropathy. We determined the course of extracellular superoxide dismutase (SOD3) concentrations in relation to nerve conduction in recently diagnosed diabetic subjects over 2 years. **Material and methods:** We prospectively assessed serum concentrations of SOD3 and nerve conduction attributes over 2 years in 40 type 1 diabetes (T1D) and 88 type 2 diabetes (T2D) subjects of the German Diabetes Study (GDS). Baseline characteristic [T1D/T2D] – age: 35.0 \pm 12.0/54.2 \pm 9.7 [SD] years; male: 55/73 %; BMI: 24.8 \pm 4.0/31.3 \pm 5.8 kg/m², diabetes duration: 5.4 \pm 3.7/5.0 \pm 3.7 months; HbA_{1c}: 7.0 \pm 1.6/6.5 \pm 1.0 % (53.5 \pm 17.5/47.0 \pm 10.8 mmol/mol). Two-year follow-up – BMI: 26.2 \pm 3.8/31.5 \pm 5.5 kg/m², HbA_{1c}: 7.0 \pm 1.2/6.6 \pm 1.1 % (53.0 \pm 13.4/46.6 \pm 12.0 mmol/mol).

Results: SOD3 concentrations at baseline (T1D: 51.5 \pm 30.7 ng/ml; T2D: 54.8 \pm 32.5 ng/ml) and two years follow-up (T1D: 35.5 \pm 16.1 ng/ml; T2D: 33.1 \pm 15.4 ng/ml) were similar between the groups. However, compared to baseline, SOD3 levels declined in both groups ($P < 0.0001$). Linear regression analyses (adjusted for sex, age, and BMI) revealed that at baseline in patients with T2D low SOD3 concentrations were associated with reduced peroneal ($\beta = 0.412/P < 0.0001$), median ($\beta = 0.257/P < 0.05$), and ulnar ($\beta = 0.407/P < 0.0001$) motor nerve conduction velocity (NCV) and sural ($\beta = 0.407/P = 0.001$) sensory NCV. In addition, reduced median ($\beta = 0.326/P = 0.008$), ulnar ($\beta = 0.364/P = 0.001$), and sural ($\beta = 0.373/P = 0.001$) sensory nerve amplitude were associated with low baseline SOD3 concentrations. Low baseline SOD3 concentrations were associated with reduced ulnar motor NCV over 2 years ($\beta = 0.372/P = 0.001$). No associations between the changes in SOD3 and changes in NCV were found.

Conclusions: In conclusion, in patients with recently diagnosed T2D, low SOD3 serum concentrations were associated with nerve conduction slowing, declined

over 2 years despite good glycemic control, and predicted the progression of ulnar motor NCV deficits.

Keywords: diabetic neuropathy, oxidative stress, SOD3

ABSTRACT 13

Soluble dipeptidyl peptidase-4 promotes endothelial dysfunction through the release of vasoconstrictor prostanoids: protective effect of dipeptidyl peptidase inhibitors

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Background and aims: Soluble dipeptidyl peptidase (sDPP)-4 is a novel adipokine whose release is increased in patients with the metabolic syndrome. The potential cardioprotective effects of DPP-4 inhibitors are currently under debate, little is known on the direct effects of DPP-4 on the vascular wall. We aimed to explore if sDPP-4 can directly impair vascular reactivity, as an early hallmark of endothelial dysfunction.

Material and methods: Vascular reactivity was explored in mesenteric microvessels from 3 months old female mice with a small vessel myograph. TXA₂ release was monitored in human coronary endothelial (HCAEC) cells by EIA. COX-2 expression was assessed in HCAEC by qRT-PCR.

Results: sDPP-4 (20–500 ng/ml) did not affect the contractility to noradrenaline (3–30 μ M) neither the endothelium independent relaxations induced by sodium nitroprusside (1–100 μ M) in microvessels. However, sDPP-4 impaired in a concentration-dependent manner the endothelium-dependent relaxation elicited by acetylcholine (ACh; 1 nM–10 μ M) with pD₂ values of 6.88 \pm 0.17, 6.86 \pm 0.25, 6.24 \pm 0.18, 5.54 \pm 0.31 for 0, 20, 100 and 200 ng/ml sDPP-4, respectively. At 500 ng/ml, sDPP-4 reduced the maximal relaxation induced by ACh from 78.20 \pm 8.83 % to 20.29 \pm 3.94 %. The inhibition of cyclooxygenase and the blockade of thromboxane TP receptors with indomethacin (10 μ M), celecoxib (3 μ M)

and SQ29548 (100 nM), respectively, prevented the impaired relaxation to ACh evoked by a submaximal concentration of sDPP-4 (200 ng/ml). Accordingly, sDPP-4 (500 ng/ml) stimulated the release of thromboxane A2 (TXA2) in cultured human coronary artery endothelial cells (HCAEC), although sDPP-4 did not induce the expression of COX-2 in the cells. Both DPP-4 inhibitors K579 (100 nM) and linagliptin (10 nM) prevented both TXA2 release and the impaired relaxation caused by sDPP-4. Importantly, GB83 an antagonist of the protease activated receptor 2 (PAR2; 10 nM), prevented the deleterious effect of DPP-4 on endothelium-dependent relaxation.

Conclusion: sDPP-4 arises as a prominent player in endothelial dysfunction by inducing the release of COX-derived vasoconstrictor prostanoids. Moreover we propose that this deleterious effect is mediated through the activation of PAR2 and can be prevented by DPP-4 inhibitors. Therefore, DPP-4 inhibitors and PAR2 antagonists might prevent the deleterious actions of sDPP-4 on endothelial function in clinical conditions where sDPP-4 is upregulated such as metabolic alterations such as obesity and/or type 2 diabetes.

Keywords: DPP-4, adipokines, obesity, endothelial dysfunction, thromboxane A2, cyclooxygenases

ABSTRACT 14

Glucose metabolism after renal denervation

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Objective: Renal denervation has been established in the treatment of resistant hypertension. Reduction in blood pressure is seen first after months up to one year. In addition single findings suggest a positive influence on glucose metabolism.

However, effects of renal denervation on glucose metabolism are unclear.

Design and method: In this prospective cohort study with 47 patients suffering from resistant hypertension, 20 women and 27 men, 62 years old (SEM \pm 1.4), first diagnosed 13.8 years ago

(SEM \pm 1.7), receiving 5.3 (SEM \pm 0.2) hypertension lowering agents, HbA_{1c} concentration was measured and a oral glucose tolerance test was carried out at standardised conditions before intervention and after 6 and 12 months. Data analysis were performed with Graphpad Prism Software using the Student's t-test.

Results: In patients with type 2 diabetes we found a significant reduction in systolic blood pressure values at six and twelve months after renal denervation (baseline 178 \pm 8 mmHg to 152 \pm 21 mmHg after twelve months), no differences in HbA_{1c} concentration in patients with or without diabetes and a significant reduction of postprandial blood glucose levels (oral glucose tolerance test) in patients with impaired glucose tolerance (76 \pm 20 mg/dl to 28 \pm 26 mg/dl).

Conclusions: We performed catheter-based renal denervation in 47 patients without any complication. The procedure is effective and safe in patients either with or without type 2 diabetes mellitus. In patients with impaired glucose tolerance a significant reduction of postprandial blood glucose levels is observed.

Keywords: resistant hypertension, renal denervation, blood pressure, HbA_{1c}

ABSTRACT 15

¹³C-MRS natural abundance liver glycogen measurements at 3.0 T

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Introduction: The assessment of hepatic glycogen fluxes is of great interest regarding the pathophysiology of metabolic diseases like type 2 diabetes [1, 2]. ¹³C MRS is the only method to non-invasively measure changes in net hepatic concentrations, with experiments traditionally conducted on MR-systems designed for spectroscopy. Results with clinical scanners prove more challenging due to limited ranges of sequence parameters and RF pulses. However, the expansion to FDA approved scan-

ners could play a vital role in clinical research. Therefore, the goal of this study was to develop a robust ¹³C MRS method to detect net hepatic glycogen breakdown on a 3.0 T clinical scanner. **Subjects:** Healthy, lean volunteers (n=7, age: 26 \pm 1 yr, BMI: 21.9 \pm 0.8 kg/m²), maintained a diet of 60 % carbohydrates for three days prior to the study. On the night before the 19 hr fast, a 800 kcal meal was consumed.

Material and methods: ¹³C liver glycogen MRS measurements were made the next morning at 13 hr, 16.5 hr, and 19 hr into the fast. A 7 cm ¹³C coil with ¹H decoupling (PulseTeq, UK) was positioned over the liver and verified with scout images. Pulse acquire measurements (TR: 230 ms, BW: 8 kHz, NSA: 4 000, decoupling: CW, scan time: 15 min) with a COV = 12 % [3] were acquired on a 3.0 T Achieva MRI (Philips Healthcare, The Netherlands). Coil loading was corrected via integration of the right most peak of a ¹³C enriched sample of formic acid placed in the coil housing. Glycogen concentration was determined from the integration of the C1-glycogen resonance after the addition of two scans (2 x 4 000) (NUTS, Acorn NMR Inc, USA). The glycogen signal was corrected for distance, and quantified via aqueous glycogen phantom measurements of 70 and 140 mM measured at a distances of 15–37 mm. Corrections for liver volume were made with a high resolution T2 weighted turbo spin echo sequence.

Results: A ¹³C MRS spectrum with the proton decoupled C1-glycogen peak is shown along with the inverted methyl and methylene peaks from adipose tissue. Glycogen concentrations were detected with SNRs ranging from 32:1 at 514 mM, to 5:1 at 106 mM, with an average linewidth of 58.77 \pm 6.98 Hz. Liver volume corrected rates of net hepatic glycogen breakdown were 5.53 \pm 0.22 μ mol/kg BW/min (mean \pm SEM), with a range of 3.16–7.44 μ mol/kg BW/min.

Discussion: A method for accessing net hepatic glycogen breakdown through ¹³C MRS on a 3.0 T clinical scanner is shown. In vivo hepatic glycogen peaks were measured at SNRs that allowed for reproducible and accurate absolute quantification. Results of net he-

patic glycogen breakdown, corrected for liver volume and body weight, were also consistent with previous studies [2]. These findings prove that methods outlined here can be implemented for use in clinical studies, to detect changes in the rate of net hepatic glycogen breakdown caused by intervention in healthy lean subjects.

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Keywords: liver, glycogen, glucose production

ABSTRACT 16

Associations between the anti-inflammatory adipokine Sfrp5 and chemotactic proteins in humans

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Background and aims: Secreted frizzled related-protein 5 (Sfrp5) has been identified as adipokine with anti-inflammatory properties in mice. Accordingly, Sfrp5 lowers the secretion of interleukin (IL)-6 after stimulation with the proinflammatory cytokine tumor necrosis factor (TNF)- α in primary human adipocytes. However, it is unclear whether Sfrp5 also regulates other inflammatory proteins that have been implicated in the development of obesity, type 2 diabetes (T2D) and its complications in humans. This study aimed to assess (i) whether serum Sfrp5 levels are associated with obesity and T2D and (ii) whether serum Sfrp5 is correlated with a range of circulating cytokines and chemokines in our study population.

Material and methods: Serum concentrations of Sfrp5, cytokines and

chemokines were determined by ELISA or magnetic-bead based assays in morbidly obese men without T2D (n=28, age (mean \pm SEM) 41.3 \pm 2.3 years, BMI 41.1 \pm 1.5 kg/m²) and with T2D (n=45, age 51.9 \pm 1.6 years, BMI 43.5 \pm 0.9 kg/m²) and in 26 normal-weight men (age 52.3 \pm 3.1 years, BMI 23.4 \pm 0.6 kg/m²). Pearson correlation analyses were used to investigate whether Sfrp5 was associated with circulating levels of 39 cytokines and chemokines in three models (unadjusted, age-adjusted as well as age- and BMI-adjusted).

Results: Morbidly obese men with T2D had the lowest serum levels of Sfrp5 (174.9 \pm 33.3 ng/ml) compared to those without T2D (236.5 \pm 56.2 ng/ml) and normal-weight men who had the highest levels of Sfrp5 (262.7 \pm 42.4 ng/ml). Serum Sfrp5 correlated positively with both total (r=0.24, p<0.05) and high-molecular weight adiponectin (r=0.32, p<0.05). Furthermore, serum Sfrp5 showed positive associations with eotaxin/CCL11 (r=0.42, p<0.01), MCP-1/CCL2 (r=0.31, p<0.05), MIP-1 β /CCL4 (r=0.32, p<0.05) and RANTES/CCL5 (r=0.38, p<0.05). Adjustment for age and BMI had almost no impact on all these associations.

Conclusion: Serum levels of Sfrp5 are decreased in obesity and T2D. Serum Sfrp5 is positively correlated with the anti-inflammatory adipokine adiponectin, but also with pro-inflammatory chemokines that are involved in insulin resistance and atherosclerosis in humans. Further studies are required to investigate the potential dual role of Sfrp5 in the regulation of these chemokines and its relevance in particular for cardiovascular phenotypes.

Keywords: Sfrp5, obesity, inflammation

ABSTRACT 17

Reproducibility of ATP and Pi concentrations in the liver of overweight humans using ³¹P MRS

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Background and aims: Hepatic energy metabolism plays an important role in insulin resistance and liver diseases. ³¹P MR spectroscopy is able to monitor the energy metabolism non-invasively by measuring ATP and Pi in-vivo.

In order to observe the success of medical treatment or lifestyle changes, high reproducibility of the measurements needs to be achieved. Previous studies only measured the intra- (1) and inter-day (2, 3, 4) reproducibility in normal weight volunteers. This study aimed to investigate the reproducibility of ³¹P measurements in the human liver in obese patients.

Material and methods: Nine healthy (26 \pm 4 years; BMI: 22.2 \pm 1.3 kg/m²) and ten obese volunteers (61 \pm 3 years; BMI: 28.1 \pm 1.5 kg/m²) consented to the approved protocol were examined in a 3-Tesla MRI scanner (Philips Achieva 3.0T X-series, The Netherlands). ³¹P spectra were acquired using a 14-cm diameter ³¹P surface coil, with the built-in ¹H body coil used for NOE enhancement and decoupling (TR: 4 sec; acquisition time: 13 min). Localization in the liver was achieved using ISIS. The volunteers were measured two times within a period of less than one month.

Results: The reproducibility after 18 \pm 9 days was 6.3 \pm 5.7 % for γ -ATP and 10.2 \pm 10.5 % for Pi in the lean subjects. In the obese volunteers the reproducibility after 15 \pm 7 days was 17.2 \pm 13.3 % for γ -ATP and 14.1 \pm 9.2 % for Pi. The stability after 350 \pm 107 days in the lean subjects was 12.9 \pm 9.6 % for ATP and 16.9 \pm 14.9 % for Pi.

Conclusion: Our values for obese volunteers are comparable to results from previous studies in normal weight volunteers (4). Nevertheless, the interday variability for obese patients is higher compared to lean volunteers. Based on this finding, it is more difficult to detect small changes in ATP and Pi reliably in the liver of overweight subjects.

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Keywords: ³¹P MRS, liver, energy metabolism, adenosine triphosphate

ABSTRACT 18

Family with mutation in NEUROD1 identified through targeted next-generation sequencing in the MODY cohort from Poland

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Background and aims: Monogenic diabetes constitutes a heterozygous group of single gene disorders. A genetic diagnosis often leads to significant alterations in treatment, allows for better prediction of disease prognosis and progression, and has implications for family members. Genetic testing usually has been restricted to small fragments of genes suggested by patients phenotype. Nowadays, next generation sequencing-based exome sequencing (NGS) seems to provide additional diagnostic potential as it enables simultaneous analysis of multiple genes in a single test. Our aim was to search for monogenic background of diabetes in patients from the MODY registry cohort of Poland in whom results of standard Sanger sequencing in HNF1A or GCK genes were negative.

Material and methods: We designed a custom Agilent Sure Select exon-capture assay with baits for 13 known MODY genes (GCK, HNF1A, HNF4A, HNF1B, NEUROD1, INS, CEL, PDX1, PAX4, BLK, KLF11, KCNJ11, ABCC8), two gene mutations which cause lipodystrophy, mitochondrial genome and 20 neonatal diabetes genes. A total of 96 patient samples were analyzed: 10 with known mutations and 86 with a clinically suggestive phenotype but lacking a positive genetic diagnosis. All previously identified mutations were detected, validating our assay.

Results: High or medium impact mutations were identified in 71 samples (74 % of the studied patient cohort). Among them the mutation in NEUROD1 was detected in one patient. It was a SNP (cGc/cCc) in the second exon causing a substitution of arginine to proline in position 103. Sanger sequencing confirmed the existence of the mutation.

We collected blood from 10 members of proband's family. Four diabetic family members carried the mutation, while 6

not suffering from diabetes carried wild type variant. Among those with mutated variant was a 3 year old girl who had an episode of hypoglycemia when she was born. Since the time of analysis DNA from another 11 family members was collected and is being analyzed now.

Conclusions: The study shows that identification by NGS a rare mutation will allow a presymptomatic diagnosis in the younger generation and will improve medical follow up of the predisposed individuals.

Keywords: NEUROD1, NGS, MODY

ABSTRACT 19

Offspring of parents with obesity. Complex investigations. Risk of carbohydrate disturbances and diabetes

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Aim of the study: To examine offspring of patients with simple obesity. To ascertain, if there are some disturbances in the carbohydrate or lipid metabolism or unknown type 2 diabetes in these subjects.

Method and subjects: Examined were 132 families, 108 families with obesity, and 24 families without obesity, the control group. 14 additional were excluded because of ascertained at the time of examination unknown type 2 diabetes in the parents. In all of the offsprings and their parents performed were: weight, height, BMI, WHR, HDL, TGD, LDL, glycaemia, HbA_{1c}, in the offsprings additionally HOMA. The control group included 30 healthy subjects with a negative anamnesis of obesity and/or diabetes in the family.

Results: Observed was overweight and obesity in a high percentage, increased BMI, WHR, significant differences in the level of HDL, TGD, LDL and HOMA between the examined and control group. Additionally introduced was HHR < HWRWMI < HJMI < Zot Zot4, ZL. In 7 of the examined offsprings ascertained was unknown type 2 diabetes, in 8 morning hyperglycaemia, in 5 glucose intolerance.

Conclusion: 1) In offsprings of obese parents observed are obesity and disturbances in the carbohydrate, lipid metab-

olism and unknown diabetes. 2) In offsprings of obese patients very important and necessary are repeated prophylactic investigations. 3) Useful will be an education about the prevention of obesity and diabetes, or a better analysis of the obesity is in our opinion important the examination of HHR (height to hip ratio) and HWR (height to waist ratio).

Keywords: obesity, diabetes, family examinations, diagnostic factor

ABSTRACT 20

Microarray analysis of liver gene expression in severely obese patients treated with long chain n-3 PUFA

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Background and aims: Long chain n-3 polyunsaturated fatty acids (n-3 PUFA) have beneficial metabolic and cardiovascular effects, due to their triglyceride lowering and anti-inflammatory properties. Severely obese patients are at an increased risk of premature death caused by metabolic and cardio-vascular diseases. Our aim was to assess the effects of an 8 week treatment with n-3 PUFA on liver gene expression in severely obese patients, with a focus on genes related to cardio-metabolic disorders.

Material and methods: Twenty nine severely obese patients (6 m/23 f) were treated with either 3.6 g/d n-3 PUFA (n=14) or the same amount of butterfat as a control (n=15) for 8 weeks in a randomized manner. All patients underwent elective bariatric surgery after the treatment, during which liver biopsy specimens were collected. Whole genome gene expression was analyzed by microarray analysis (Affimetrix Gene Chip Prime View Human Gene Expression Array®). Data were first normalized then analyzed by gene set enrichment analysis (GSEA).

Results: Pathways involved in blood pressure regulation, fatty acid oxida-

tion, triglyceride synthesis, glucagon receptor signaling and Hedgehog signaling were downregulated after n-3 PUFA treatment. Gene sets related to carbohydrate metabolism, immune regulation, inflammatory response and of note, apoptosis showed a marked upregulation. Specifically, n-3 PUFA treatment decreased expression of fat mass and obesity associated (FTO), glucagon receptor (GCR) and thromboxane A2 receptor (TBXA2R), and increased gene expression of CCAAT/enhancer binding protein beta (CEBPB), lipopolysaccharide-binding protein (LBP) and perilipin 2 (PLIN2), all $P < 0,05$.

Conclusion: This microarray analysis was the first to investigate effects of n-3 PUFA treatment on human liver gene expression. The data points to the conclusion that n-3 PUFA have pleiotropic effects with regards to metabolic, immune and endocrine pathways.

This work was supported by the Austrian National Bank (P12735) and by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (all to T.M.S.).

Keywords: obesity, n-3 PUFA, liver, gene expression, microarray

ABSTRACT 21

Secreted frizzled-related protein 4 impairs insulin action in skeletal muscle

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Background and aims: Secreted frizzled-related protein 4 (sFRP4) is a regulator of the activity of the Wnt signaling pathway. The expression of sFRP4 is increased in adipose tissue from patients with type 2 diabetes. The current study examined whether sFRP4 interferes with insulin action and the expression of genes involved in glucose and lipid metabolism in primary human skeletal muscle cells (hSkMC).

Methods: The effects of recombinant sFRP4 on insulin action were examined by Western blot analysis using hSkMC from four different donors. Expression of genes regulating glucose and lipid me-

tabolism were examined by real-time PCR.

Results: Exposing hSkMC to sFRP4 impaired insulin-stimulated Akt-phosphorylation by 50 % ($P < 0,05$). Furthermore, sFRP4 exposure increases the levels of CPT1B (1.8-fold), pyruvate dehydrogenase kinase 3 (PDK3; 2.5-fold), phosphorylase kinase gamma 1 (PHKG1; 2.7-fold), AMPK α 2 (1.7-fold), and phosphoribosyl pyrophosphate synthetase 1 (PRPS1; 1.7-fold), and lowered the levels of HSL (1.3-fold), AMPK β (1.9-fold), and the fatty acid transporter solute carrier family 27 member 6 (SLC27A6; 2.3-fold) ($n = 4$; all $P < 0,05$).

Conclusion: Exposing hSkMC to sFRP4 induces insulin resistance. Furthermore, the alterations in gene expression suggest that this Wnt regulator impacts on energy substrate metabolism in skeletal muscle.

Keywords: insulin resistance, skeletal muscle, Sfrp4

ABSTRACT 22

Secretory products from epicardial adipose tissue impair mitochondrial respiration in cardiomyocytes via upregulation of miR-208a

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Background and aims: Alterations in cardiac energy substrate metabolism contribute to the development of diabetic cardiomyopathy. We have recently found that secretory products from epicardial adipose tissue (EAT) from patients with type 2 diabetes impair cardiomyocyte function as illustrated by the induction of insulin resistance, contractile dysfunction, and changes in microRNA expression. The present study examines whether EAT affects mitochondrial respiration in cardiomyocytes and whether this can be ascribed to changes in cardiac microRNA expression.

Material and methods: Biopsies from EAT from patients with or without type 2 diabetes were collected from males of Caucasian origin undergoing open-heart surgery, and used to generate conditioned media (CM). Mitochondrial respiration was measured in primary adult rat cardiomyocytes exposed to CM using a Seahorse analyzer. The car-

diac mouse cell line HL-1 was used for transfection with precursor-miRNAs to investigate the impact of miRNAs on mitochondrial respiration and fatty acid oxidation. MicroRNA, mtDNA copy number, and gene expression was measured by qRT-PCR.

Results: Exposing primary adult rat cardiomyocytes to CM from patients with type 2 diabetes reduced maximal mitochondrial respiration (20 %), and spare respiratory capacity (40 %), and increased the levels of miR-208a (1.8-fold) as compared to cells exposed to CM from patients without type 2 diabetes. Expression of the precursor for miR-208a in HL-1 cardiomyocytes reduced maximal mitochondrial respiration (19 %), and spare respiratory capacity (25 %). Furthermore, miR-208a lowered palmitate-induced maximal respiration by 22 %. This decrease in mitochondrial function associated with changes in the expression of several subunits of AMP-activated protein kinase, a central regulator of cell energy metabolism, and reduced of carnitine palmitoyltransferase 1, a regulator of mitochondrial β -oxidation. Furthermore, miR-208a reduces the mtDNA copy number and decreases the expression of mitochondrial transcription factor TFAM.

Conclusion: Secretory products from epicardial adipose tissue impair mitochondrial respiration in cardiomyocytes via upregulation of miR-208a. This miRNA inhibits mitochondrial energy metabolism via the down-regulation of fatty acid oxidation.

Keywords: diabetic cardiomyopathy, mitochondrial function, adipokines, miRNA

ABSTRACT 23

Effects of chronic variable stress and dietary fat on insulin sensitivity

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Background and aims: Stress is a state of threat to homeostasis that may contri-

bute to metabolic disorders such as visceral obesity and type 2 diabetes. However, there is a lack of studies analyzing the long-term consequences of chronic stress on insulin sensitivity and its relation to fat consumption. Our aim was to characterize how chronic variable stress (CVS) in relation to dietary fat affects insulin sensitivity in mice.

Material and methods: Three months old, body weight (BW)-matched male C57BL/6 mice were exposed to a random series of stressors for 15 days (CVS), the unstressed control mice were housed separately (Ctrl). Body composition was analyzed with NMR. After CVS, blood was collected (8:00–12:00 am) for corticosterone and insulin analysis. Subsequently, mice were consuming a low- (Chow) or a high-fat diet (HFD) for three months and insulin sensitivity was measured in vivo with hyperinsulinemic-euglycemic clamps. Plasma hormones were analyzed with multiplex immunoassay. Statistical differences were considered significant at $p < 0.05$ (two-tailed unpaired t-test).

Results: CVS mice ($n = 24$) had lower BW (25.49 ± 0.33 g, $p < 0.001$) and lean mass (23.08 ± 0.37 g, $p < 0.001$) with no changes in fat mass (2.23 ± 0.08 g) compared to the Ctrl group ($n = 24$), (28.32 ± 0.36 g, 25.37 ± 0.39 g, 2.25 ± 0.09 g, respectively). Plasma levels of corticosterone were higher in the CVS group ($n = 22–24$) (109.9 ± 13.71 vs. 60.37 ± 7.55 ng/ml, $p < 0.01$) as well as insulin levels (0.65 ± 0.06 vs. 0.47 ± 0.03 ng/ml, $p < 0.01$) compared to the Ctrl group ($n = 19–24$). When fed Chow, the CVS group ($n = 10$) showed a trend to lower basal endogenous glucose production (EGP) (20.01 ± 1.12 vs. 23.86 ± 1.48 mg/kg/min, $p = 0.055$), while insulin-stimulated glucose disposal (Rd) in peripheral tissues was increased in CVS compared to Ctrl (363 ± 29 vs. 275 ± 17 % of basal, $p < 0.05$). On HFD, basal EGP was unchanged, while Rd (135 ± 12 vs. 192 ± 14 % of basal, $p < 0.01$) was decreased with CVS. Suppression of EGP by insulin was unaffected by CVS. CVS-Chow mice showed lower plasma adiponectin (5.87 ± 0.43 vs. 11.29 ± 1.34 μ g/ml, $p < 0.01$) and a trend towards lower resistin (0.13 ± 0.005 vs. 0.14 ± 0.005 μ g/ml, $p = 0.06$) compared to Ctrl. CVS-HFD mice showed lower plasma adi-

ponectin (7.99 ± 0.89 vs. 15.74 ± 3.27 μ g/ml, $p < 0.05$) and a trend towards higher resistin (0.16 ± 0.02 vs. 0.11 ± 0.02 μ g/ml, $p = 0.06$) compared to Ctrl.

Conclusion: When fat consumption is low, chronic stress improves insulin sensitivity through higher peripheral glucose disposal. On the contrary, HFD consumption exacerbates insulin resistance under these conditions. Changes in adipokine profiles could be a possible mechanism underlying the effects of CVS and dietary fat on peripheral insulin sensitivity.

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Keywords: chronic variable stress, insulin sensitivity, dietary fat, adipokines

ABSTRACT 24

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Reduced oxidative respiration of peripheral blood mononuclear cells associates with insulin resistance

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Background and aim: Impaired oxidative metabolism and subclinical inflammation contribute to the development of insulin resistance. In adipose tissue, pro-inflammatory polarization of lymphocytes and macrophages has been linked to insulin resistance. According to the Warburg effect, pro-inflammatory TH1 lymphocytes and M1 macrophages present with higher non-oxidative respiration, even during oxygen excess. We hypothesized that whole-body insulin resistance may associate with lower oxidative respiration may associate with higher pro-inflammatory polarization in circulating lymphocytes and macrophages.

Methods: Patients with recently diagnosed type 2 diabetes (T2D), recruited from the German Diabetes Center Study (GDS), and 10 age-matched healthy volunteers (CON) underwent hyperinsulinemic-euglycemic clamps to assess whole-body insulin sensitivity (M-value). Leukocytes were isolated from fasting blood

samples using density gradient centrifugation. Oxidative capacity was measured with high-resolution respirometry in isolated leukocytes, using multiple substrate protocols. Respiration in the absence of exogenous substrates represents basal respiration. Tricarboxylic acid cycle activity was assessed by using saturating concentrations of malate, glutamate, succinate and adenosine diphosphate. Beta-oxidation, the first step of fatty acid catabolism was measured in response to octanoyl carnitine. Finally maximal respiration capacity in the uncoupled state u was investigated using the uncoupler carbonyl cyanide-4-trifluoromethoxy phenylhydrazone (FCCP). Mitochondrial content was assessed by citrate synthase activity (CSA) in the leukocytes. Heparinized blood was used for the quantification of the ratio of Th1:Th2 lymphocyte and the ratio of M1:M2 macrophage subsets.

Results: Rates of oxygen consumption by leukocytes was markedly lower in patients with T2D than in CON (State u: 4.0 ± 1 vs. 8.3 ± 1.3 pmol/s/mg protein, $P < 0.05$). Beta-oxidation was also lower in leukocytes of T2D (3.9 ± 0.7 vs. 6.2 ± 0.6 pmol/s/mg protein, $P < 0.05$). CSA did not differ between groups. The ratios of Th1:Th2 and M1:M2 expression were higher in leukocytes of T2D (1.4 ± 0.3 vs. 0.7 ± 0.1 and 0.2 ± 0.01 vs. 0.1 ± 0.01 , $P < 0.05$) in line with a pro-inflammatory phenotype. Leukocyte state u respiration related positively to whole-body insulin sensitivity (M-value: $R = 0.45$, $P < 0.05$). Beta-oxidation was also related positively to M-value ($R = 0.57$, $P < 0.05$) and negatively with total blood cholesterol ($R = -0.47$, $P < 0.05$).

Conclusion: In conclusion, lower oxidative respiration in circulating leukocytes may reflect both the pro-inflammatory polarization of leukocytes and the impaired oxidative capacity in other tissues, which have been related insulin resistance. Leukocyte respiration may serve as a surrogate of whole-body metabolism and represent a novel target for addressing insulin resistance in T2D.

Keywords: inflammation, metabolism, polarization, insulin resistance