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– Abstracts –

P11

Seasonal trends in HbA_{1c} level in adult patients with type 1 diabetes treated with personal insulin pumps

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Introduction/aim: The DCCT and other studies showed that the variability in HbA_{1c} added to mean HbA_{1c} increases the risk of the development of complications of diabetes. There were some earlier reports showing a seasonal variability in the HbA_{1c} level in a pediatric population.

Methods: We evaluated seasonal HbA_{1c} changes over a period of 9 years (2009–2017) in 453 adults with type 1 diabetes (T1DM, 61 % women) treated with personal insulin pumps. HbA_{1c} was measured at a tertiary care university hospital on the Bio-Rad D10 haemoglobin testing system. Differences between groups (12 groups for 12 months and 6 for every consecutive two months) were assessed using the Kruskal-Wallis and post hoc tests.

Results: Patients' median age was 24 years [range 18–80 years], median BMI 22.9 kg/m² [15.6–43.7 kg/m²], median diabetes duration 12 years [1–40 years] and median duration on personal insulin pump 6 years [0–18 years]. A total of 1438 HbA_{1c} measurements were analyzed. Median HbA_{1c} level for the whole study period was 7.25 % [55.7 mmol/mol] (range 4.8–12.8 % [29–116.4 mmol/mol]). There were seasonal differences in HbA_{1c} over 12 months ($p = 0.02$): The lowest HbA_{1c} was observed in summer (July, 6.8 % [50.8 mmol/mol]) and the highest in winter months (from 7.1 % [54.1 mmol/mol] in January to 7.3 % [56.3 mmol/mol] in February). HbA_{1c} was lower in July than in February ($p = 0.03$). After combining two consecutive months in one group seasonality of HbA_{1c} values was still observed ($p = 0.008$). Median HbA_{1c} in July/August (6.9 % [51.9 mmol/mol]) was lower than in January/February (7.2 % [55.2 mmol/mol], $p = 0.01$) and in November/December groups (7.3 % [56.3 mmol/mol], $p = 0.02$).

Conclusion: The phenomenon of the seasonal fluctuations in HbA_{1c} levels in T1DM has been described previously. However, to our knowledge this is the first report showing the presence of HbA_{1c} seasonal fluctuations in a homogenous, well controlled cohort of adult T1DM patients treated with personal insulin pumps. Seasonal changes of HbA_{1c} levels (peak in summer months, drop in winter months) in such a group of patients should be considered in patient education, diabetes management and epidemiological interpretation. Physical activity, physiological changes, level of psychological stress are potential reasons of seasonal fluctuations in HbA_{1c} levels.

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Differential roles of osteopontin in the pathophysiology of metabolic syndrome-derived hepatocellular carcinoma

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Background and aims: Osteopontin (OPN, gene Spp1), a multifunctional protein and inflammatory cytokine, has been proposed to play a pivotal role in many pathophysiological events related to metabolic syndrome and carcinogenesis. OPN is overexpressed in adipose tissue and liver during obesity and concurs in the induction of adipose tissue inflammation and non-alcoholic fatty liver (NAFL). Studies performed in both mice and humans demonstrated a putative role for OPN in malignant transformation and tumor growth, including hepatocellular carcinoma (HCC). Metabolic syndrome is nowadays recognized as an important risk factor for HCC. In order to fully understand the role of OPN on the development of HCC in NAFL, we reproduced in our laboratories a recently

published NASH-HCC mouse model called STAM on a both wild type (WT) and OPN deficient (Spp1-/-) background, and evaluated its properties in non-alcoholic steatohepatitis (NASH), fibrosis and HCC.

Methods: Two-days-old WT and Spp1-/-mice received a low-dose streptozotocin (STZ) injection in order to induce diabetes and were fed a high-fat diet (HFD) starting from week 4. Different cohorts of mice of both genotypes were sacrificed at 8, 12 and 19 weeks of age in order to evaluate the NASH, fibrosis and HCC phenotypes, respectively.

Results: Lack of osteopontin prevented HCC progression to less differentiated tumors and improved overall survival rate while enhancing the development of well-differentiated liver tumors. On the other hand, Spp1-/- mice developed a stronger fibrosis due to increased hepatocellular apoptosis. OPN-deficient mice also showed an aggravated NASH due to increased CD36-mediated lipid uptake. The worse steatotic and fibrotic phenotypes observed in Spp1-/- mice were probably a consequence of overall improved metabolic condition.

Conclusions: In a model of metabolic syndrome, the lack of OPN improved overall outcomes but worsened hepatic inflammation and fibrosis. OPN appears necessary for dedifferentiation of HCCs.

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P 13

Multiple associations between biomarkers of inflammation and incident distal sensorimotor polyneuropathy: KORA F4/FF4 study

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Introduction: Inflammation contributes to the development of distal sensorimotor polyneuropathy (DSPN), but prospective data on biomarkers of inflammation and DSPN are scarce. The aim of this study was to identify inflammation-related biomarkers and pathways for incident DSPN using a novel multimarker assay.

Methods: The study was based on 133 incident DSPN cases and 397 non-cases aged 62–81 years from the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4/FF4 cohort. The mean follow-up time was 6.5 years. Ninety-two biomarkers of inflammation were measured in serum samples using primer extension technology. Neurotoxic effects of biomarkers were tested using the human SH-SY5Y neuroblastoma cell line. DSPN-associated pathways were analysed using the Ingenuity Pathway Analysis software.

Results: After quality control, 71 biomarkers were included in the analysis. Twenty-four biomarkers were associated with incident DSPN after adjustment for a range of anthropometric, metabolic, clinical and lifestyle factors ($p < 0.05$). After correction for multiple testing, higher levels of 3 chemokines (MCP-3/CCL7, MIG/CXCL9, IP-10/CXCL10) and of 3 soluble forms of transmembrane proteins (DNER, CD40, TNFRSF9) were associated with a higher risk of DSPN at a false-discovery rate of $q < 0.05$. Addition of all 6 biomarkers to a clinical risk model improved C-statistics from 0.750 (95 % CI 0.699–0.801) to 0.782 (95 % CI 0.734–0.830), $p = 0.015$. The chemokines had neurotoxic effects on SH-SY5Y cells in vitro. Pathway analyses indicated that multiple cell types from both innate and adaptive immunity may be involved in the development of DSPN.

Conclusion: The study identified novel inflammation-related biomarkers that predict incident DSPN. These biomarkers reflect different functions within the immune system suggesting a cross-talk between innate and adaptive immunity in the development of DSPN.

P 14

Correlations of serum mid-osteocalcin with NT-proBNP, asymmetric dimethylarginine, placental growth factor 1 in type 2 diabetes patients on oral antidiabetic treatment

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Serum levels of undecarboxylated osteocalcin (OC) have been associated with the glycaemic control and insulin secretion. On the other hand, serum OC has also been used as a marker of vascular status. New serum markers such as N-terminal pro-B type natriuretic peptide (NT-proBNP), asymmetric dimethylarginine (ADMA) and placental growth factor 1 (PIGF-1) are expected to better quantify cardiovascular risk.

Objectives: To describe the correlations of serum OC (as independent variable) with the serum levels of glycated haemoglobin A_{1c}, NT-proBNP, ADMA and PIGF-1 in type 2 diabetes (DM) patients on oral antidiabetic treatment.

Material and methods: 97 type 2 DM patients participated – 60 women and 37 men; mean age was 63.2 ± 9.3 years and diabetes duration 9.0 ± 7.2 years. HbA_{1c} was measured on a Nycocard

analyzer. Serum levels of NT-proBNP and PIGF-1 as well as serum N-MID-OC were measured by electro-hemiluminescence (Elecsys 2010, Roche Diagnostics) and ADMA by an enzymatic immunoassay (BioVendor). Curve estimation (10 curves) and regression analysis were performed on an IBM SPSS 19.0 for Windows platform (SPSS Corp., Chicago, IL).
Results: Mean levels of NT-proBNP, ADMA and PIGF-1 were 32.85 ± 55.35 pmol/l, 0.62 ± 0.19 µmol/l, and 16.41 ± 5.06 pg/ml. The mean HbA_{1c} was 7.63 ± 1.59 %, the mean N-MID-OC was 18.6 ± 12.7 ng/dl. The latter was not correlated to the levels of ADMA (best model is quadratic, $R^2 = 0.052$, $p = 0.082$), NT-proBNP (best model is inverse, $R^2 = 0.035$, $p = 0.066$), or HbA_{1c}. It was however related to PIGF-1 in multiple models – linear, quadratic, cubic, compound and growth (the best was the quadratic, $R^2 = 0.128$, $p = 0.002$).
Conclusion: There is no significant relationship of serum N-MID-OC with glycaemic control or NT-proBNP and ADMA as markers of endothelial dysfunction and heart failure. However, PIGF-1 was related to serum N-MID-OC. This relationship needs further investigations and explanation.

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P 17

Basal insulin dose in adults with type 1 diabetes mellitus on insulin pumps in real-life clinical practice: a single-center experience

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Introduction: Basal insulin (BI) infusion in pump therapy of type 1 diabetes (T1DM) mimics physiological secretion during the night and between meals. The recommended percentage of the total BI to daily insulin dose (termed the %BI) ranges between 30 and 50 %. We analyzed whether this recommendation was followed in adults with T1DM from a university center, and whether BI doses were linked with glycaemic control.
Materials and methods: We included 260 consecutive patients with T1DM (159 women and 101 men) treated with continuous subcutaneous insulin infusion at the Department of Metabolic Diseases, Krakow, Poland. Data were downloaded from patients' pumps and collected from medical records. We analyzed the settings of BI and the association of %BI with HbA_{1c} level. Linear regression was performed.
Results: The mean age of T1DM individuals was 26.6 ± 8.2 years, BMI 23.1 ± 3.0 kg/m², T1DM duration 13.3 ± 6.4 years, and HbA_{1c} level 7.4 %. There were 69.6 % (n = 181) of T1DM patients with %BI in the recommended range. The T1DM duration and HbA_{1c} level of patients with a %BI < 30 % (n = 23) were 9.5 years and 6.4 %, respectively; for a %BI of 30 – 50 %, it were 13.2 years and 7.4 %; and for a %BI > 50 % (n = 56), it were 15.8 years and 7.8 % ($p < 0.001$ for both three-group comparisons). Multiple regression identified %BI among independent predictors of the HbA_{1c} level.
Conclusion: In this real-life analysis, the recommendations concerning %BI dosing were not followed by almost one third of adult T1DM patients. Low %BI was associated with better glycaemic control; however, this requires further confirmation.

P 18

Correlation between the frequency of consumption of selected food groups and the lipid profile in adult patients treated with a personal insulin pump

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Objective: Lipid control is one of the key elements of metabolic control of diabetes. The patient's lipid profile is largely determined by his eating habits. It is worth emphasizing that the impact of individual elements of the diet on the lipidogram largely depends on the clinical characteristics of the studied population, if such relationships have been well documented for obese people or those with type 2 diabetes, there are no analogous, on large populations, analyzes for patients with type 1 diabetes. The current cross-sectional study aimed to identify clinical and nutritional factors correlated with individual lipid profile fractions in adult patients with T1DM treated with a personal insulin pump.
Research design and methods: The study group consisted of 89 patients suffering from T1DM (55 % women) with an average age of 25.8 years, with an average BMI of 23.1 kg/m², and an average duration of diabetes of 12.8 years. Data from routine annual patient tests were used. The mean of HbA_{1c} in the study group was 7.3 % (56 mmol/mol). The mean total cholesterol level was 4.4 mmol/l, HDL cholesterol 1.7 mmol/l, LDL cholesterol 2.3 mmol/l and triglycerides (TG) 0.9 mmol/l. The validated KomPAN questionnaire was used to assess the frequency of consumption of individual food ingredients. To assess the correlation between the frequency of consumption of selected dietary components and clinical parameters (independent variables: sex, BMI, age, duration of diabetes, HbA_{1c}) with individual lipid fractions, single and multi-factor linear regressions were used.
Results: In multivariate regression with total cholesterol, sex was significantly correlated; $p = 0.000$, HbA_{1c} percentage; $p = 0.004$ and the frequency of vegetable consumption (daily vs. less than once a week; $p = 0.025$). The lower level of LDL cholesterol was correlated with high intake of vegetables (daily vs. less than once a week; $p = 0.025$) and milk (less than once a week vs. no intake; $p = 0.015$). Higher levels of triglycerides were correlated with higher intake of white bread and rolls (daily vs. no intake; $p = 0.049$). The level of HDL cholesterol was influenced by gender; $p = 0.000$, consumption of oil, margarine or spreads (less than once a week vs. no intake; $p = 0.019$ and less than once a month vs. no consumption; $p = 0.029$) and butter intake (less than once a week vs. no intake; $p = 0.006$ and daily vs. no

consumption; p = 0.023) and consumption of buckwheat, oatmeal or other whole grains (less than once a month vs. no intake; p = 0.034).
Conclusions: The results of our analysis indicate the necessity of more effective implementation of elements of a healthy diet in patients with type 1 diabetes, treated with a personal insulin pump. They also point to some statistical relationships between the lipid profile and individual elements of the diet, although inference about cause-and-effect relationships must be very careful and requires further research.

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Should we avoid the handgrip test in the assessment of cardiovascular autonomic neuropathy in diabetic patients? – exploratory factor analysis

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Introduction: Originally, a set of five cardiovascular autonomic reflex tests (CARTs) was considered to be the gold standard of cardiovascular autonomic neuropathy (CAN) assessment. Current guidelines however suggest only the use of four with the omission of the diastolic blood pressure response to sustained handgrip. Therefore we aimed to assess the association between the handgrip and the other tests.
Methods: We recruited 353 diabetes patients (age: 60.2 ± 7.4 years; female: 57.2 %; BMI: 29.3 ± 2.1 kg/m²; diabetes duration: 15.6 ± 9.9 years; HbA_{1c}: 8.2 ± 1.9 %; type 1 diabetes: 18.1 %). We measured the following CARTs: deep breathing test, Valsalva ratio, handgrip test, and orthostatic hypotension test. Definite CAN was defined as ≥ 2 abnormal CARTs excluding the handgrip test.
Results: The handgrip test had a sensitivity of 24.6 % (95 % CI 17.7 – 33.1 %) and a specificity of 79.4 % (95 % CI 73.3 – 84.4 %) for the diagnosis of definite CAN. According to exploratory factor analysis, the four examined CARTs showed a 2-factor structure with the handgrip test loading to one factor (factor loading: 0.98) and the deep-breathing test, Valsalva ratio and orthostatic hypotension test clustered on another component with factor loadings 0.68, 0.77 and 0.66, respectively. Handgrip test abnormality showed an independent association with higher initial diastolic blood pressure values (OR: 1.05, p = 0.0009) and an independent inverse association with the presence of hypertension (OR = 0.42, p = 0.006).
Conclusions: According to the exploratory factor analysis, there is an independent factor underlying the results of the handgrip test differing from that underlying the results of the other cardiovascular reflex tests. Potential factors influencing handgrip test results could be the presence of hypertension and baseline diastolic blood pressure.

P 20

Evaluation of the gut microbiota in patients with type 1 and 2 diabetes using next generation sequencing (NGS)

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Introduction: The recent scientific reports concerning the incidence of diabetes indicate the possible influence of the gut microbiota on the development of type 1 and 2 diabetes (T1DM, T2DM). The objective of the study is to evaluate the qualitative and quantitative composition of the colon microbiota in patients with T1DM, T2DM, hospitalized at the Department of Metabolic Diseases, University Hospital, Kraków, and to establish a link between data obtained from microbiological testing and the patients' clinical data.
Methods: The material examined was DNA isolated from fecal samples of 68 adults: patients with T1DM (n = 22), T2DM (n = 23) and people from the control group (C, n = 23) using one of the NGS methods – sequencing by synthesis, which was performed according to Illumina 16S protocol and MiSeq machine (Illumina).
Results: At the phylum level, the dominant bacteria in all groups were Firmicutes (> 77 %), whereas only the percentage of bacteria belonging to Bacteroidetes statistically significantly differed in T2DM as compared to C and to T1DM (p = 0.006). At the taxonomic levels L2 (phylum) and L6 (genus), statistically significant differences were demonstrated in bacterial profiles, particularly in T2DM group. A correlation has been established between: 1) several genera of bacteria and the percentage of haemoglobin A_{1c} in T2DM group (negative correlation), and 2) bacteria belonging to the genus Bifidobacterium and high density lipoprotein cholesterol level in both T1DM and T2DM groups (positive correlation).
Conclusion: The results of this study are a good reason to expand research in this field in order to develop individualized therapy modifying the composition of the intestinal microbiota. It would constitute a new method for preventing the complications of diabetes or even curing it.

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Association of nuclear-mitochondrial epistasis with BMI in T1DM patients

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Introduction: Obesity results from an imbalance between energy intake and its expenditure. Genome-wide association study (GWAS) analyses have led to

discovery of only about 100 variants influencing body mass index (BMI), which explain only a small portion of genetic variability. Analysis of gene epistasis gives a chance to discover another part. Since it was shown that interaction and communication between nuclear and mitochondrial genome are indispensable for normal cell function, we have looked for epistatic interactions between the two genomes to find their correlation with BMI.

Methods: The analysis was performed on 366 T1DM patients using Illumina Infinium OmniExpressExome-8 chip and followed by imputation. Only genes which influence mitochondrial functioning (listed in Human MitoCarta2.0) were included in the analysis – variants of nuclear origin (MAF > 5 %) in 1 140 genes and 42 mitochondrial variants (MAF > 1 %). Gene expression analysis was performed on GTex data. Association analysis between genetic variants and BMI was performed with the use of Linear Mixed Models as implemented in the package 'GENESIS' in R. Analysis of association between mRNA expression and BMI was performed with the use of linear models and standard significance tests in R.

Results: Among genes involved in epistasis between mitochondria and nucleus we have identified mitochondrial transcription factor TFB2M. It interacted with few mitochondrial variants localized to MT-RNR1 ($p = 0.0004$, MAF = 15 %), MT-ND2 ($p = 0.07$, MAF = 5 %) and MT-ND4 ($p = 0.01$, MAF = 1.1 %). Analysis of the interaction between nuclear variant rs6701836 localized to TFB2M and rs3021088 localized to MT-ND2 mitochondrial gene has shown that the combination of the two led to BMI decrease ($p = 0.02411025$). Each of the variants on its own does not correlate with higher BMI ($p(nuc) = 0.8566547$, $p(mito) = 0.1160552$). Although rs6701836 is intronic it influences gene expression in thyroid ($p = 0.000037$). rs3021088 is a missense variant that leads to alanine to threonine substitution in the MT-ND2 gene which belongs to complex I of the electron transport chain. The analysis of the influence of genetic variant on gene expression has confirmed the trend explained above – each of the mRNAs on its own is associated with higher BMI ($p(mito) = 0.0244$ and $p(nuc) = 0.0269$), however, their interaction leads to BMI decrease ($p = 0.0308$).

Conclusions: Our results show that nuclear-mitochondrial epistasis can influence BMI in T1DM patients. The correlation between transcription factor expression and existence of genetic variants will be subject of further analysis.

P 2 2

Levels of adipokines – adiponectin and leptin, and adipocytokines – interleukin-1, interleukin-6, tumor necrosis factor-α and C-reactive protein in patients with metabolic syndrome

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It has been recently proven that adipose tissue is an active endocrine organ. Adipokines, adipocytokines, hormones and growth factors are released on the surface of adipocytes.

The aim of the present study was to examine the serum levels of adipokines – adiponectin and leptin, adipocytokines – interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) in normoglycaemic patients with metabolic syndrome (MS).

Methods: A prospective comparative observational study was performed. The serum levels of adipokines (adiponectin and leptin), adipocytokines (IL-1, IL-6, TNF-α) and CRP were measured and compared among 35 subjects with MS ($n_1 = 35$) and 35 clinical healthy subjects ($n_2 = 35$). Two homoeostasis models assessment of insulin resistance (HOMA-IR) and of β-cell function (HOMA-%B) were calculated.

Results: Statistically significant differences in body mass index (BMI), waist circumference, systolic and diastolic blood pressure, HDL cholesterol, basal insulin and HOMA-IR were observed among the patients with MS in comparison with the controls. A significantly higher level of leptin ($n_1 = 33.58 \pm 14.6$ vs. $n_2 = 21.55 \pm 16.1$ ng/ml; $p < 0.05$), IL-1 ($n_1 = 16.80 \pm 11.4$ vs. $n_2 = 6.4 \pm 3.7$ pg/ml; $p < 0.05$) and CRP ($n_1 = 8240.81 \pm 4763.36$ vs. $n_2 = 4232.33 \pm 433.22$ mg/dl; $p < 0.05$) were found in patients with MS in comparison with the control group. Patients with MS had significantly lower levels of TNF-α compared to the healthy subjects ($n_1 = 2.49 \pm 1.17$ vs. $n_2 = 11.49 \pm 8.57$ pg/ml; $p < 0.05$). Positive correlation between leptin and BMI was found in MS subjects. In this group adiponectin showed negative correlation with BMI and serum level of triglycerides. There was positive correlation between IL-1 and HOMA-%B, and between IL-6 and CRP in patients with MS.

Conclusion: Patients with MS exhibited significant changes in levels of adiponectin, leptin, IL-1 and TNF-α. Indicated adipokines and adipocytokines may have predictive value of progression from normal to pathological carbohydrate metabolism.

P 2 3

Defining glycaemic control in type 2 diabetic patients based on continuous glucose monitoring (CGM) data

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Introduction (background/aims): Glycaemic control is usually defined based on level of glycated haemoglobin (HbA_{1c}) and self-monitoring of blood glucose (SMBG), but these methods are insufficient to present overall control in some cases. CGM is used to adjust therapy mainly in insulin treated patients but CGM data could be helpful in defining glucose control. Our aim is to assess whether CGM data could be useful for defining control in type 2 diabetic patients.

Methods: We studied 85 type 2 diabetic patients (35 women, 50 men; mean age 43.93 ± 10.87 years, mean disease duration 21.91 ± 6.07 years), receiving different therapies – 31 on oral therapy, 33 on

insulin mixtures, 21 on multiple daily insulin injections (MII). Patients were followed up for three months and were asked to perform multiple daily blood glucose measurements of pre- and postprandial blood glucose for this period. CGM by using iPro™ Professional was performed for seven days and HbA_{1c} was measured at the end of this period.

Results: Based on HbA_{1c} assessment 54.84 % of patients on oral therapy, 36.36 % of those on premixed insulin and 19.05 % on MII were with good control. After estimation of results from SMBG these percentages were respectively 34.4 %, 32.1 %, 17.8 % according to fasting glucose level, while according to postprandial glucose level, fewer patients reach therapeutic goals. CGM defined 54.04 % of patients on oral medications, 27.27 % of those on premixed insulin and 23.80 % on MII as well controlled. In addition CGM gave information about insulin excursions – they were significantly lower in tablet treated group (11.71 ± 6.15, $p < 0.05$). CGM was more informative than SMBG in presenting hypoglycaemic episode ($p = 0.01$) including night hypoglycaemia and their duration ($p = 0.001$).

Conclusion: Nevertheless of short period reflected by CGM, derived data could give additional information about overall glucose control in patients with type 2 diabetes. CGM shows some advantages when compared to other methods used for glucose control assessment, especially in estimating hypoglycaemic episodes and glucose variability. Performing of CGM should be included in recommendations for assessment of glucose control in type 2 diabetic patients.

P 2 4

The comparison of the occurrence of beta cell autoantibodies and regulatory T cells (CD4+CD25+FOXP3+) in patients with type 1 diabetes, their siblings and control group

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Background: Regulatory T cells (Treg) of phenotype CD4+CD25+FoxP3+ involve active suppression of excessive immune response. The population of Treg cells from patients with type 1 diabetes (DM1) has numeric and functional abnormalities. Although there are many reports of investigations on human and animal populations, the role of regulatory T cells in the development of type 1 diabetes is still unclear.

Objective and hypotheses: The aim of the study is to compare the population of regulatory T cells and the correlation between Treg cells and beta cell autoantibodies in healthy siblings of children with DM1 to healthy children from non-diabetic families and to children with DM1.

Method: Peripheral blood mononuclear blood cells were obtained from 76 children with DM1, their siblings – 101, and 30 healthy children. Treg cells were characterized by flow cytometry FACSCalibur (Becton Dickinson, USA). The autoantibodies were determined by ELISA. The results were analyzed with STATISTICA 10 PL.

Results: The number of regulatory T cells from diabetic patients was higher (average percentage 0.23 ± 0.20) than that in the siblings (0.15 ± 0.14) ($p = 0.004$). There was no significant difference in the number of Treg cells between children with DM1 and the control group (0.19 ± 0.15; $p = 0.11$) and between siblings and the control group ($p = 0.09$).

The levels of anti IA2 and anti ZnT8 antibodies were statistically significant higher in siblings in comparison to the control group (anti IA2 Ab $p = 0.0000001$; anti ZnT8 Ab $p = 0.00001$). The level of anti-GAD in siblings was similar to that in the control group. There was no correlation between the number of Treg cells and the co-occurrence of beta cell autoantibodies.

Conclusion: The results suggest that regulatory T cells probably provide protection from development of disease and the dysfunction of Treg cells contributes to the autoimmune pathogenesis of type 1 diabetes.

P 2 5

Treatment with sulfonylurea and insulin during the long-term follow-up including three pregnancies in Slovak patients with permanent neonatal diabetes mellitus

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Background and aims: Majority of the patients with permanent neonatal diabetes mellitus (PNDM) carrying activating mutation in the KCNJ11 and ABCC8 genes could be successfully treated with a sulfonylurea derivate (SU). We aimed to report on up to 12-year follow-up of Slovak PNDM patients treated with SU.

Methods: Four patients carrying a mutation in KCNJ11 (p.R201H in SK1 and SK6 patients, p.H46Y in SK4) or ABCC8 (p.V86A in SK8 patient) genes switched in 2005/2006 from insulin to SU, were regularly checked during up to 12-year follow-up.

Results: Data of the first five years of the follow-up showed better diabetes control and increased quality of life in all four patients. During the next years, two patients (SK1 and SK6) failed to retain good diabetes control. Nevertheless, we showed that switch to insulin monotherapy or combination of SU and insulin treatment in SK1 and SK6 did not help to achieve better HbA_{1c} levels. Moreover, we report the outcome of three pregnancies – one extrauterine and two successful pregnancies treated with SU and insulin in SK1 (added in the third trimester) and insulin alone in SK4. Both children inherited the KCNJ11 mutations from their mothers, and were successfully treated with SU since diabetes onset.

Conclusions: We have shown in the long-term follow-up that SU therapy is preferable to insulin in PNDM patients sensitive to SU even in the case of poor glycaemic control.

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P27

Association between dietary factors, clinical parameters and neuropeptide Y in type 1 diabetes in Latvia

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Background: Diet plays an important role in the management of type 1 diabetes. However, the association between dietary intake and health has not been extensively studied in this population. Neuropeptide Y (NPY) is a major integrator of appetite control in the hypothalamus. NPY is an interesting candidate gene for obesity.

Aims: We studied the cross-sectional association between dietary factors, clinical parameters, neuropeptide Y and different complications status in patients with type 1 diabetes.

Methods: Samples and data of 267 patients with type 1 diabetes duration more than 1 year were analyzed. Dietary intake was assessed using a self-reported questionnaire and a diet score, expressing the extent to which individuals adhered to standard dietary recommendations. NPY in serum was measured by ELISA.

Results: We found significant correlations between diet score and BMI, waist circumference, metabolic syndrome, levels of blood pressure, age, diabetic retinopathy ($p < 0.005$), duration of diabetes, cardiovascular disease (CVD), diabetic polyneuropathy, smoking, TG level and NPY ($p < 0.05$). Level of NPY was higher in patients with advanced diabetic nephropathy ($p = 0.03$).

Conclusions: Dietary habits are associated with prevalence of diabetic complication, CVD and cardiovascular risk factors. Data on association between these markers provide new knowledge about links between dietary factors, clinical parameters and neuropeptide Y in type 1 diabetes. More attention should be paid to dietary counselling of patients with type 1 diabetes.

P28

Impaired cardiovascular autonomic function and peripheral sensory nerve function are present among subjects with high risk for the development of type 2 diabetes mellitus screened by the FINDRISC questionnaire

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Introduction: Diabetes mellitus and even impaired glucose tolerance are associated with autonomic and sensory nerve dysfunction. The Finnish Diabetes Risk Score (FINDRISC) is a validated and one of the most widely used T2DM risk score questionnaires that comprises questions on age, body mass index (BMI), waist circumference, physical activity, diet, history of antihypertensive medication, high blood glucose and family history of diabetes. The aim of our study was to compare autonomic and sensory nerve function, as well as anthropometric data among patients with higher future T2DM risk (minimum 12 points in the FINDRISC questionnaire) with healthy control subjects.

Patients and methods: Our study involved 30 patients with higher future T2DM risk (mean age: 58.3 ± 13, female 12, fasting glucose 5.7 ± 0.4 mmol/l, mean FINDRISC score 18 [15; 19]) and 18 healthy control subjects (mean age: 52.8 ± 13, female 6, fasting glucose 5.03 ± 0.5 mmol/l, mean FINDRISC score 8 [7; 10]). Sensory function was evaluated by Neurometer (Neurotron Inc., Baltimore, USA) device, 128 Hz calibrated tuning fork, Semmes-Weinstein monofilament and Q-sense (Medoc Ltd., Yamat Rishai, Israel) device. Neuropathic symptoms were measured by NTSS-6 (Neuropathy Total Symptom Score) questionnaire.

Results: Patients with higher future T2DM risk had significantly higher vibration perception thresholds both on the upper extremities (6.6 vs. 7.6, $p = 0.037$) and both on the lower extremities (5.8 vs. 7.4, $p = 0.004$) than healthy control subjects. In case of stimulating the median nerve at 2 000 Hz (2.99 mA vs. 2.65 mA, $p = 0.014$) and at 250 Hz (1.36 mA vs. 0.86 mA, $p = 0.0008$) the current perception thresholds were significantly higher among patients with higher future T2DM risk compared to controls. Moreover, patients with higher future T2DM risk had significantly higher heat perception thresholds assessed by the Q-Sense device, both on the upper extremities (35.5 °C vs. 34 °C, $p = 0.02$) and both on the lower extremities (41.5 °C vs. 38 °C, $p = 0.02$) than healthy control subjects. During the determination of cold perception patients with higher future T2DM risk had significantly lower cold perception thresholds in case of the upper (29.3 °C vs. 30.5 °C, $p = 0.042$) and the lower extremities (26.9 °C vs. 29.5 °C, $p = 0.019$). Using the 10-g monofilament diminished protective sensory function was detected among patients with higher future T2DM risk compared to healthy controls (3.8 vs. 4.8, $p = 0.073$). None of the subjects examined had any symptoms of neuropathy. Assessing autonomic function, we detected attenuation of respiratory arrhythmia in the high risk group compared with the healthy group (11 vs. 18.4, $p = 0.001$). The total autonomic impairment score (2.67 vs. 1, $p = 0.007$) was higher among patients with higher future T2DM risk compared to controls.

Conclusion: Impaired cardiovascular autonomic function and peripheral sensory nerve function might be present among subjects with high risk for the development of type 2 diabetes mellitus screened by the FINDRISC questionnaire, compared to healthy controls. Our results highlight the importance of early neuropathy assessment, as well as for the development of effective risk reduction strategies among these patients.

P 29

Non-alcoholic fatty liver disease is associated with cardiorespiratory fitness in patients with type 2 diabetes

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Introduction: Studies have shown that physical activity effectively reduces liver fat in patients with type 2 diabetes mellitus (T2DM). There is a question whether level of cardiorespiratory fitness (CF) is associated with the risk of liver steatosis. Fatty liver index (FLI), hepatic steatosis index (HSI), non-alcoholic fatty liver disease liver fat score (NAFLD-LFS) have been validated for evaluation of risk of hepatic steatosis in healthy subjects and patients with T2DM. The aim was to study hepatic steatosis markers in T2DM patients with different levels of CF.

Methods: 63 previously untrained patients with T2DM aged 35–75 have been enrolled and divided in groups according to relative VO_2max (low CF group: VO_2max males < 29 ml/min/kg, females < 25 ml/min/kg; high CF group: VO_2max males ≥ 29 ml/kg/min, females ≥ 25 ml/min/kg). FLI, HSI, NAFLD-LFS were calculated based on clinical features and blood test. Leisure time physical activity was evaluated via Minnesota leisure time activity questionnaire and expressed in metabolic equivalent (MET).

Results: Characteristics of the group: mean age 58.6 ± 9.5 years, mean duration of diabetes 6.9 ± 5.1 years, mean BMI 33.5 ± 5.5 kg/m², mean HbA_{1c} 6.9 ± 1.3 %, mean FLI 79.8 ± 25.9, mean HSI 37.7 ± 5.6, mean NAFLD-LFS 1.73 ± 1.8, mean VO_2max 24.0 ± 95.1 ml/kg/min, mean MET 33.7 ± 25.2. HSI, FLI and NAFLD-LFS were higher in patients with low CF (FLI: low CF group 82.0 ± 21.3 versus high CF group 66.5 ± 31.1, p = 0.023; HSI: low CF group 39.1 ± 5.1 versus high CF group 34.9 ± 5.4, p = 0.004; NAFLD-LFS: low CF group 2.2 ± 1.7 versus high CF group 0.9 ± 1.8, p = 0.009). Serum insulin concentration was higher in low CF group compared to high CF group (11.7 ± 8.5 µV/ml versus 6.3 ± 8.6 µV/ml (p = 0.045). We did not observe differences between VO_2max groups in HbA_{1c}, duration of diabetes, MET as well as cytokines associated with risk of diabetic complications (VEGF-A, angiopoietin-2, MMP7, MMP2). There were significant correlations between VO_2max and indices (VO_2max and FLI p = 0.029, VO_2max and HSI p = 0.000, VO_2max and NAFLD-LFS p = 0.005) as well as insulin (p = 0.012) and waist (p = 0.004).

Conclusions: Markers of hepatic steatosis HSI, FLI and NAFLD-LFS are associated with CF in untrained subjects with T2DM. These results provide new data on association between hepatic steatosis and cardiac complications of diabetes.

P 30

Non-alcoholic fatty liver disease is associated with diabetic cardiovascular autonomic neuropathy

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Introduction: Cardiac autonomic neuropathy (CAN) is a serious complication of longstanding diabetes. For patients with diabetes hepatic steatosis is an independent risk factor for cardiovascular diseases. Fatty liver index (FLI), hepatic steatosis index (HSI), non-alcoholic fatty liver disease liver fat score (NAFLD-LFS) have been validated for evaluation of risk of hepatic steatosis in healthy subjects and patients with type 2 diabetes. We offer hepatic steatosis markers as a useful tool for detecting CAN as well.

Methods: The study examined 64 type 2 diabetes patients aged 35–75. Cardiovascular autonomic function tests were performed on tilt table: heart-rate (HR) response to Valsalva manoeuvre (VM), HR variation during deep breathing, blood-pressure (BP) response to sustained handgrip, immediate HR and BP response to standing. Ewing et al. (1985) classification was used for staging of CAN. FLI, HSI, NAFLD-LFS indices were calculated by formulas.

Results: Characteristics of the group: mean age 58.6 ± 9.5 years, mean duration of diabetes 6.9 ± 5.1 years, mean BMI 33.5 ± 5.5 kg/m², mean HbA_{1c} 6.9 ± 1.3 %, mean FLI 79.8 ± 25.9, mean HSI 37.7 ± 5.6, mean NAFLD-LFS 1.73 ± 1.8. The prevalence of possible CAN was detected in 40 (62.5 %), definite CAN in 15 (23.4 %) and severe CAN in 4 (6.3 %) patients, for 5 patients (7.8 %) all tests were normal. HSI, FLI and NAFLD-LFS were higher in patients with definite CAN (FLI: CAN patients 89.7 ± 15.4 versus patients without CAN 71.5 ± 27.6, p = 0.009; HSI: CAN patients 39.6 ± 4.0 versus patients without CAN 36.9 ± 6.0, p = 0.037; NAFLD-LFS: CAN patients 2.7 ± 1.7 versus patients without CAN 1.3 ± 1.7, p = 0.002). HR variation during deep breathing correlates with NAFLD-LFS (p = 0.035); BP response to standing correlates with HSI (p = 0.000) and FLI (p = 0.007); HR response to VM correlates with FLI (p = 0.022); BP response to sustained handgrip correlates with FLI (p = 0.002), NAFLD-LFS (p = 0.001) and HSI (p = 0.047).

Conclusions: In our study we found an association between hepatic steatosis markers and CAN. This can help distinguish patients who should undergo tilt table test for detecting possible CAN, as Ewing's battery autonomic function tests used for detecting CAN are time-consuming and special equipment is needed. Liver steatosis might be associated with increased cardiovascular morbidity through CAN.

P 31

Assessment of glomerular filtration rate in type 1 diabetic children

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Introduction: Macro-, and microvascular complications in the long-term diabetes significantly impair quality of life and survival. A typical manifestation of microvascular events is the decreased glomerular filtration capacity. The original method of determination of glomerular filtration rate (GFR) was creatinine clearance – based on serum and 24 h or overnight urine creatinine assay. But proper urine collection is in

general practice not an easy task, so the gold standard remained the inulin clearance. However, this is quite difficult in the routine clinical practise. The use of estimated GFR (eGFR) methods is gaining increasing popularity recently. Significant improvement of eGFR calculations has been observed in recent years. At present, the MDRD-EPI calculation formula is used by Hungarian laboratories. However, the application of MDRD-EPI has a number of limiting factors. Under the age of 18, the most widely used eGFR determination method was the Schwartz formula, which has been recently revised and used as Bedside-Schwartz formula. In the past two decades, the cystatin C (CysC) based assessment of GFR has been included in the renal function test methods. Our objective is the comparison of the utility of creatinine and cystatin C in renal function tests in type 1 DM cases.

Patients and methods: In the study, 89 DM1 (ages 5–20) and 31 non-diabetic controls (6–17 years) participated. Serum creatinine concentration was measured by routine enzymatic clinical chemistry tests and cystatin C was determined using an immunochemical (Siemens) reagent in ADVIA 2400 analyser. The creatinine filtration rate was calculated according to followed: GFR = 0.43 * (L/CR), where L is the height of the children and CR is the creatinine concentration. Filtration from cystatin calculated by this equation: GFR = K * 84.69 * (CY - 1.68) ("K" = 1.384 if the age of the child is less than 15 years [otherwise 1]) and CY is the serum concentration of cystatin C.

Results: There was no significant difference between the DM1 and the control group either in serum creatinine or cystatin C concentrations. There was a weak, but significant, correlation between the concentration of creatinine and cystatin C ($r = 0.39$, $p < 0.05$). GFR values calculated from cystatin concentrations were on average 33.7 % higher than calculated from creatinine, but there was no significant difference between DM1 group and control group data. In the DM1 group correlation there wasn't detectable any correlation between eGFR and other glycaemic parameters (serum glucose and HbA_{1c}). According to the creatinine-based GFR 3 DM1 children had slightly reduced (< 90 ml/min) filtration capacity. All other parameters were in the normal range.

Conclusion: Although only 3 diabetic children (from 89) were classified differently with CR and CY based assessment of GFR, the detailed analysis showed a considerable degree of discrepancy between them. GFR assessment equations are validated against standard methods of clearance but their validity in a different setting is questionable. These differences should be taken into account in children with diseases affecting their renal function.

P 32

Changes in gene expression of selected genes in patients with type 1 diabetes in peripheral blood

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Background: Insulin is a main anabolic peptide hormone, that regulates metabolism of carbohydrates, proteins, lipids and affects DNA synthesis or apoptosis. Lack of insulin secretion by destroyed beta cells, in type 1 diabetic patients, not only leads to disorders in regulation of blood glucose levels, but also has direct impact on proper functioning of many key biological processes, like immune response or suppression of carcinogenic processes. Analysis of gene expression profiles allows to search for new pathogenic biomarkers and helps to understand the mode of action of pathological factors involved in the development of diabetes. The scope of this project is to identify and further validate biomarkers in diabetes.

Method: For the preliminary study, we analyzed changes of transcript levels between three study groups: three healthy, three diabetic and two individuals after pancreas transplant. RNA was extracted from peripheral blood of each individual and reverse transcribed to cDNA. Changes in gene expression were quantified by Real-Time Polymerase Chain Reaction. Significantly changed genes were analyzed by ANOVA study.

Results: We observed a decrease in expression of genes NKX6-1, CDH1, PFKFB2, TP53, and GCG in type 1 diabetic patients. This was accompanied by increase in expression of HHEX, DPP4 and ABCC8. Interestingly, while the expression of TP53 is lower in patients with type 1 diabetes, after pancreas transplant the level of the transcript is comparable to level in healthy individuals. In this study we also identified a group of transcripts that while upregulated in diabetic patients were downregulated after pancreas transplant (compared to healthy individuals): ABCC8 and PFKFB2.

Conclusion: Reduced expression of CDH1 (coding cadherin, protein involved in cell-cell interaction) in patients with type 1 diabetes could be responsible for escalating of perturbation of islet cells integrity or interactions. Furthermore, the decrease of expression of NKX6-1 (involved in transcriptional regulation of insulin) in diabetes may be connected with diminished number of pancreatic beta cells. We have also observed increased expression of DPP4, responsible for immunological responses and apoptosis, two main processes having influence on pathogenesis of type 1 diabetes. Interestingly, after pancreas transplant expression of DPP4 is decreased, compared to diabetic individuals. In addition, in patients with type 1 diabetes, we observed lower expression of gene TP53, a well-known suppressor of carcinogenesis, which could elucidate their increased risk of developing cancer.

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P 33

Insulin requirement for pure protein meal in type 1 diabetic children treated with insulin pumps

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Fat and protein meals need square-wave insulin boluses because of higher late postprandial glycaemia. Protein and fat content independently increase the

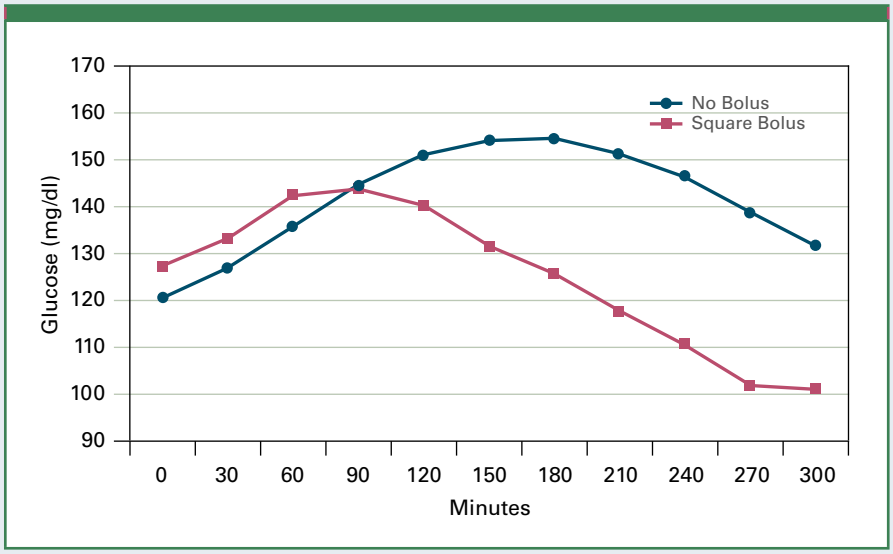

Fig. P33: Postprandial glycaemia in each time interval.

postprandial glucose level and together have an additive effect. Recent studies in adults demonstrated that delivery of insulin for pure protein meals may not be obligatory.

The objective of this study was to assess the insulin requirement and to compare the postprandial glycaemia after 200 kcal of pure protein meal in adolescents with type 1 diabetes (T1D) treated with insulin pumps.

We conducted a randomized, double-blind, cross-over study, including 48 children with T1D (mean age 14.4 ± 1.8 years, mean HbA_{1c} 8.7 ± 1.5 %, insulin requirement 0.88 U/kg, mean basal insulin of 37 %) with diabetes duration longer than one year (mean 6.2 ± 3.9). Participants were randomly assigned into two treatment orders: NO BOLUS or SQUARE BOLUS. They consumed standardized meal: 200 kcal of protein shake. The primary outcome was postprandial glycaemia (PPG) based on capillary measurements (SMBG), checked every 30 minutes, and CGM during 5-h follow-up. The secondary outcomes were the frequency of hypoglycaemia (< 70 mg/dl), mean amplitude of glycaemic excursion (MAGE) and glycaemic rise.

Significantly lower PPGs were noticed since 2.5 hours of the test after insulin bolus delivery: NO BOLUS vs. SQUARE (150 min) 146 vs. 124.5 mg/dl, (180) 143 vs. 124.5, (210) 138.5 vs. 113, (240) 133 vs. 108.5, (270) 121.5 vs. 98, (300) 115 vs. 93.5. In addition, more overall hypoglycaemic episodes were observed in SQUARE BOLUS group (6.04 % vs. 2.29 %). There were no statistical differences in the number of hypoglycaemia in each time frame.

This preliminary result showed that applying a dose of insulin in square-wave bolus for 200 kcal of pure-protein meal improved PPG in type 1 diabetic children. A smaller dose of insulin should be considered individually due to higher risk of hypoglycaemia associated with this intervention.

P 34

Haptoglobin (2-2) polymorphism together with elevated homocysteine levels enhance the risk for subclinical atherosclerosis in type 2 diabetic men

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Patients with type 2 diabetes mellitus are predisposed to accelerated atherosclerosis. Subclinical atherosclerosis can be measured by carotid artery intima-media thickness (cIMT), which predicts cardiovascular and all-cause mortality independently. cIMT is determined by several traditional risk factors such as diabetes mellitus, hypercholesterinaemia, hypertension, smoking, etc. Lately the role of non-traditional risk factors such as haptoglobin polymorphism, elevated homocysteine levels and inflammatory factors has been investigated in the pathogenesis of atherosclerosis and diabetic vascular complications.

Haptoglobin (Hp) plays an important role in iron metabolism and additionally modulates atherosclerosis given its antioxidant effect. Hp (1-1) provides a stronger antioxidant effect as compared to Hp (1-2) and Hp (2-2) polymorphisms. A strong association between haptoglobin polymorphism and coronary sclerosis, as well as carotid artery intima-media thickness has been reported previously. Elevated homocysteine level was also found to be an important factor in atherosclerosis. Atherosclerosis and all-cause mortality are independently associated with elevated plasma homocysteine levels.

Rundek et al. reported that traditional vascular risk factors explain only a part of the variance in cIMT. Lioupis et al. showed that the iron content of carotid artery plaques was significantly elevated in type 2 diabetic men with Hp (2-2) polymorphism and elevated homocysteine levels.

The aim of our work was to study the effect of Hp polymorphism and homocysteine levels on cIMT in type 2 diabetes.

Patients and methods: Out of 212 examined patients altogether 86 patients' results were evaluated on the basis of sex, Hp polymorphism and homocysteine levels, in 8 age and sex-matched groups. cIMT, homocysteine levels, haptoglobin polymorphism and traditional risk factors of atherosclerosis were determined.

Results: The best results were detected in females with Hp (1-1, 1-2) polymorphism and homocysteine levels lower than 12.5 µmol/l, while the worst ones were found in men with Hp (2-2) polymorphism and homocysteine levels above 12.5 µmol/l. The difference between the worst and the best values was significant ($p = 0.04$) and the results of other groups depending on the sex, homocysteine levels and Hp polymorphism ranged between these results showing continuously growing tendency. There was no significant difference in HbA_{1c} and lipid parameters between each group.

Conclusion: As a novel finding, our study reports that male sex, Hp (2-2) polymorphism and homocysteine levels above 12.5 µmol/l in type 2 diabetic patients confer a significantly higher risk for subclinical atherosclerosis.

P 35

Whole exome sequencing for the identification of rare monogenic forms of diabetes

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Diagnosis of monogenic as well as atypical syndromic forms of diabetes mellitus has important clinical implications for their specific diagnostics, prognosis and targeted treatment. However, phenotypic heterogeneity can limit the diagnostics of monogenic diabetes. Next generation sequencing technologies provide an excellent opportunity to screen large number of genes for causal mutations in affected individuals. The aim of our study was to identify genetic causes of rare syndromic forms of monogenic diabetes using methods of whole exome sequencing (WES) and, subsequently, to perform the functional analysis of selected genes variants realized by DNA recombination techniques in cell culture experiments.

Results: We identified the genetic cause of rare forms of monogenic diabetes in: a) probands with MEHMO syndrome where diabetes was associated with microcephaly, epilepsy, hypogonadism, mental retardation and obesity, and we connected this syndrome with mutations in the EIF2S3 gene. Moreover, functional analyzes confirmed the effect of a frameshift mutation found in probands on the phenotype; b) in a proband with glycogenosis-like phenotype, where we surprisingly identified a new heterozygous variant in the HNF4A gene, causing an incorrect splicing resulting in deletion of aminoacids from the ligand binding domain; c) in a proband with diabetes and gastrointestinal malformations, we found two new mutations in the RFX6 gene responsible for Mitchell-Riley syndrome with atypical later diabetes onset; d) in a proband with atypical phenotype of DIDMOAD syndrome (Diabetes Insipidus, Diabetes Mellitus, Optical Atrophy, Deafness) we identified a new heterozygous dominant in-frame deletion in the WFS1 gene; and e) in a proband with insulin resistance syndrome with unclear etiology, we found new pathogenic homozygous variant in the TRIM37 gene causing Mulibrey syndrome.

Conclusion: Using the whole exome sequencing approach we identified disease-relevant mutations in individuals with unclear phenotype. Functional studies are an essential part of the process of detecting the etiology of rare diseases, such as syndromic forms of monogenic diabetes.

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P 36

Thyroid cancer and other malignant disorders in patients with type 2 diabetes

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Background and aims: It is generally agreed that type 2 diabetes and obesity are associated with thyroid nodular pathology both malignant and nonmalignant. Increased incidence of thyroid nodules of different origin in exposed population in Belarus in Post-Chernobyl period is still under investigation. The aim of the study was to find out the place of thyroid cancer in patients with diabetes mellitus in the structure of all malignancies in Belarus.

Materials and methods: Cases of diabetes mellitus (DM) with malignant disorders were analyzed in two registries, the database was fulfilled with verified information. Only cases with verified diagnosis of DM and malignant disease were included. The duration of diabetes and dates of malignant disorder diagnosis were rechecked. In those with thyroid cancer and DM metabolic and anthropometric parameters, insulin and metformin therapy were analyzed.

Results: The prevalence of all malignant diseases in type 2 DM patients is about 9.4 %. According to the Belarusian Cancer Registry the prevalence of thyroid cancer in women with type 2 DM is 7.3 % and 4.1 % – in women without diabetes and much lower in male patients – 2.3 % (with DM) and 1.0 % (without DM). Thyroid cancer in DM female patients is on the third position after the breast cancer (23.4 %) and uterus cancer (19.2 %). In female patients without diabetes the prevalence of the breast cancer is lower – 17.9 % and uterus cancer is twice lower (8.4 %). In male patients, prostate cancer is leading malignancy both in those with DM (22.15 %) and without (17 %). The structure of malignancies in men with and without DM is little different – the renal cancer is on 2nd position in DM men (10.1 %) and lung cancer in male patients without DM 15.2 % (9.1 % in DM men). The prevalence of intestine cancer is in DM 8.4 %, without DM 5.5 %.

Conclusion: In the structure of all the malignancies in Belarus thyroid cancer is on 3rd position in women with DM and the prevalence is higher than in those without DM. The possibility of DM to increase the risk of thyroid cancer is discussed.

P 37

The long-term outcome in children with type 1 diabetes treated with continuous subcutaneous insulin infusion

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Objectives: The growing popularity of type 1 diabetes (DM1) treatment based on continuous subcutaneous insulin infusion (CSII) raises a question of the group of patients that benefit most from the treatment.

Patients and methods: Clinical observation was carried out during 10 years in 285 1- to 18-year-old patients diagnosed with DM1 treated with CSII. Every 3 months, HbA_{1c} was determined by an agglutination inhibition immunoassay.

Results: The greatest benefits from the treatment with CSII using an insulin pump were noted in type 1 diabetes children aged 1 – 5: the mean HbA_{1c} decreased in these patients from 7.98 % to 6.75 % ($p < 0.01$) over 6 years. Slight-

ly lesser outcomes were noted in the group of 6- to 10-year olds: the mean HbA_{1c} value increased slightly from 7.6 % before the CSII to 7.89 % after 6 years of treatment ($p > 0.01$). Somewhat worse outcomes were reported in the group of 11- to 15-year-old children: HbA_{1c} increased from 8.05 % to 8.72 % ($p > 0.01$). The lowest outcomes were found in the group of the 16- to 19-year-old patients, as HbA_{1c} rose from 7.8 % to 8.82 % ($p < 0.01$) over 6 years. The children receiving the CSII treatment as early as in the first year of treatment exhibited better diabetes control (HbA_{1c} 8.1 % declined after 6 years to 7.1 %, $p < 0.01$) than patients who received CSII at an older age (HbA_{1c} increased from 7.92 % to 8.2 %, $p < 0.01$).

Conclusions: The CSII offers the greatest benefits for patients aged 1–5 and those with the treatment commenced in the first year after diagnosis of type 1 diabetes.

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Difficulties in the treatment of metabolic disturbances in patient with Seip-Berardinelli syndrome

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Objectives: Seip-Berardinelli syndrome is a rare form of congenital generalized lipodystrophy with secondary muscle hypertrophy from early childhood, hypertriglyceridaemia and hepatomegaly, accompanied by acromegaloid features, later in time also insulin resistance leading to the disclosure of glucose intolerance or diabetes. Patients with this syndrome have shown mutations in four genes: AGPAT2, seipin, CAV1 and PTRF/Cavin.

Case report: At 2 years of age Seip-Berardinelli syndrome was suspected on the base of the acromegaloid features, generalized lipoatrophy and hypertriglyceridaemia in the additional testing. On admission to our clinic diabetic history was negative, in physical examination acromegaloid features, generalized lipoatrophy with muscle hypertrophy. Laboratory tests: in OGTT abnormal levels of glucose (0 min 94 mg/dl, 60 min 238 mg/dl, 120 min 157 mg/dl) and insulin (0 min 3.8 mU/l, 60 min 211.4 mU/l, 120 min 142.9 mU/l) were found; HbA_{1c} 8.81 %. Because of Seip syndrome lipid homeostasis (TG 180.5 mg/dl) was performed, in ultrasound liver steatosis was found. She was trained in the field of fat and sugar-restricted diet. Hospitalized in December of 2013, glucose levels at home were often > 300 mg/dl, HbA_{1c} 11.9 %, TG > 1000 mg/dl, total cholesterol 253 mg/dl, no typical symptoms of diabetes, it was decided to implement metformin, insulin and fenofibrate. In 2014 intolerance to fenofibrate (muscle pain, allergic reaction), changing to atorvastatin. The patient was under the control of our clinic up to 18 years of age: HbA_{1c} 8–9.5 %, multiple attempts to increase insulin doses were ineffective (currently approx. 60–70 U/day), metformin 1000 mg/day, TG 300–800 mg/dl and total cholesterol approx. 240 mg/dl – atorvastatin 20 mg, very low physical activity.

Conclusions: The available methods of treatment of metabolic disorders in the course of Seip syndrome are ineffective. There is a need to search for other experimental methods (currently leptin is under investigation).

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Glucagon-like peptide-1 receptor agonists modulate metabolism of human adipocytes by up-regulation of irisin

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Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are used to normalize glucose levels in type 2 diabetes and to reduce body weight in non-diabetic patients. However, the mechanisms of extra-pancreatic action of GLP-1RAs are still not fully understood [1, 2]. The study was aimed to investigate the action of GLP-1RAs in human adipocytes.

For this purpose, human cell line CHUB-S7 was differentiated to mature adipocytes and than exposed for 24 hrs to GLP-1 [100 nM] or GLP-1R agonist, exendin-4 [100 nM]. Mitochondrial respiration was measured by high resolution respirometry, mitochondrial membrane potential (MMP) was analyzed by flow cytometry using JC-1, irisin and NAD+ levels in culture medium were measured by ELISA, gene expression was determined by RT-PCR.

GLP-1 and exendin-4 led to mild uncoupling of mitochondrial electron transport from ATP synthesis, slightly decreasing MMP. Basal respiration rate and the relative contribution of uncoupled respiration were also significantly higher compared to control cells. The influence of GLP-1RAs on the NAD+ level was comparable to the effect of irisin – adipokine known to induce browning of adipocytes. Increased secretion of irisin by GLP-1RAs treated adipocytes was observed. The FNDC5 (encoding irisin) gene expression was also up-regulated by GLP-1. Enhanced gene expression of PGC1alpha and UCP2 suggests switch of cells phenotype towards beige adipocytes.

In conclusion, the stimulatory effects of GLP-1 or exendin-4 on mitochondrial bioenergetics in mature CHUB-S7 adipocytes were observed. Increase in energy expenditure by GLP-1 may be associated with the up-regulation of irisin synthesis, secretion and autocrine action in adipocytes.

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References:

[1] Góralska J et al., Acta Biochim Pol 2017; 64: 423–429

[2] Drucker DJ, Cell Metab 2018; 27: 740–756

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Relationship between glycaemic changes and physical activity parameters during semi-competitive football matches in adolescents with type 1 diabetes

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Background and aims: Maintaining normoglycaemia throughout football matches is difficult for young players with type 1 diabetes mellitus (T1DM). Moreover, it is not clear whether detailed parametrization of player's performance may help predict and react to glycaemic excursions. The aim of this study was to parametrize physical activity and glycaemic variability of adolescents with T1DM during two football matches.

Materials and methods: During summer camp for adolescents with T1DM, two football matches (each lasting 80 minutes + 10-minute break) were organized for two 9-players adolescent teams (convenient sample, mean age 14.9 ± 1.4 years, diabetes duration 7.2 ± 3.9 years, HbA_{1c} 7.1 ± 0.6 % [54 ± 0.32 mmol/mol]). The meetings were separated by a 4-day rest period. During the matches, players wore chest straps heart rate (HR) monitors coupled with GPS, which allowed for continuous tracking of their position and movement. To assess glycaemic and metabolic response to exercise blood glucose (BG) was measured in capillary blood at rest, after the first half and at the end of each match. Relative changes in glycaemia during the game [%BG = (BG_final – BG_start)/BG_start * 100 %] and amounts of consumed carbohydrates (CARBS) were recorded and compared against parameters of exercise (HR, covered distances, velocities, sprints and accelerations).

Results: Mean BG before and after each match was as follows – match: no. 1: 139 ± 61 mg/dl and 151 ± 95 mg/dl; no. 2: 164 ± 80 mg/dl and 131 ± 78 mg/dl. No significant difference in BG between the matches (p = 0.32) or during each match (p = 0.5) was noted, changes between matches were also similar (p = 0.9). However, during the second game players consumed significantly less carbohydrates (9.6 ± 12 g vs. 29.6 ± 25.8 g). Recorded HRs (match no. 1: 77 ± 7 % of maximum HR for a given age; match no. 2: 76 ± 8 %) revealed a mixed aerobic-anaerobic character of exercise and were similar between the meetings (p = 0.58). Mean distance covered by the players was comparable in both matches (match no. 1: 6.1 ± 1 km, no. 2: 6.2 ± 1.4 km, p = 0.89), as were velocities and accelerations.

During match no. 1 %BG and CARBS were not significantly correlated with any exercise-related parameters (p > 0.05), however, a moderate positive correlation of borderline significance was found between CARBS and mean HR during the game (r = 0.45, p = 0.077). During the second game, %BG was significantly correlated with minimal recorded HR (r = 0.61, p = 0.016), total covered distance (r = 0.66, p = 0.007), mean velocity (r = 0.65, p = 0.009), number of events with acceleration reaching 1 – 1,99 m/s² (r = 0.66, p = 0.007). During the same game, CARBS were significantly correlated with minimal recorded HR (r = 0.56, p = 0.16), mean HR (r = 0.63, p = 0.012), maximum HR (r = 0.73, p = 0.002), mean velocity (r = 0.73, p = 0.002), total number of sprints (r = 0.52, p = 0.047) as well as number of accelerations in range of 2 – 2.99 m/s² (r = 0.55, p = 0.032) and 1 – 1,99 m/s² (r = 0.69, p = 0.05). After excluding two players with extreme glycaemic changes (+111 and 134 %), only correlation between CARBS and maximum HR remained significant (r = 0.73, p = 0.005). Notably, excluded players displayed the best overall performance in terms of physical capacity, one was a competitive teenage player.

Conclusion: HR monitoring coupled with GPS-based tracking can effectively parametrize physical activity during a football match. Under similar exercise workload particular participants displayed comparable changes in glycaemia but consumed different amounts of carbohydrates. The amount of needed carbohydrates was related to exercise burden measured by mean HR. The relationship between glycaemic excursions may differ according to players performance and overall fitness.

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Osteocalcin decrease in response to oral glucose overload is suppressed in subjects with glucose tolerance disturbances

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Introduction: Disturbances in glucose metabolism affect bone remodeling, which explains increased fracture risk in subjects with T2DM. Carboxylated osteocalcin (Gla-OC) participates in bone formation whereas its undercarboxylated form (Glu-OC) acts as hormone in glucose metabolism. Food intake modulates bone turnover. The aim of the study was to compare the effect of mixed meal to glucose on Gla-OC, Glu-OC and total osteocalcin in subjects with different oral glucose insulin sensitivity index (OGIS) value within 2 hours of nutrients oral intake.

Methods: Subjects (BMI: 25 – 40 kg/m², n = 80) underwent an oral glucose tolerance test (OGTT) and mixed meal tolerance test (MMTT, 1018 kcal). Blood samples (0, 1 and 2 hours) were drawn and analyzed for concentrations of glucose and insulin to calculate post oral glucose load insulin sensitivity which was determined using an OGIS. Fasting and postprandial blood samples were collected and determined for Gla-OC and Glu-OC concentration.

Results: Subjects were divided into 2 groups depending on calculated median value of OGIS. After 2 hours of both tests suppression of Glu-OC level was observed independently on OGIS value. In turn Gla-OC was lower after 2 hours of glucose intake versus baseline value in subjects with higher than median value of OGIS (P < 0.01). Subjects with higher OGIS level presented suppression of Gla-OC also after 1 hour of OGTT (P < 0.01). Postprandial suppression of total osteocalcin concentration calculated as the sum of Gla-OC and Glu-OC was also influenced by the type of nutrient and OGIS value (P = 0.030) and suppressed after 2 hours of OGTT in subjects with higher OGIS value (P < 0.01).

Conclusion: Subjects with defects in glucose tolerance seem to present dis-

turbances in bone turnover, determined by marker of bone formation – Gla-OC, within 2 hours of glucose overload but not after 2 hours of mixed meal intake.

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Utility of paired 1,5-anhydroglucitol and hsCRP analysis in MODY patient prescreening selection algorithm

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The differential diagnosis of maturity onset diabetes of the young (MODY) is of great clinical importance due to its potential for treatment optimization, improved glycaemic control, and quality of life enhancement. The definitive diagnosis of MODY is confirmed by molecular analysis, which is both laborious and costly. Biomarker utility has formed the focus of recent efforts to improve the current imperfect MODY selection criteria in qualifying patients for genetic screening. Several biomarkers for MODY selection such as 1,5-anhydroglucitol (1,5-AG) and C-reactive protein (hsCRP) have been examined so far; however, the sensitivity and specificity of each individual factor has proven inadequate as a MODY biomarker. The aim of our study was to examine the utility of selected biomarkers for the differential diagnosis of GCK-MODY, HNF1A-MODY, T2DM, and T1DM.
Methods: Clinical characteristics and markers such as age of diabetes diagnosis, BMI, serum C-peptide, hsCRP, and 1,5-AG were used as potential biomarkers differentiating the study groups. Data were summarized as means and standard deviations of counts where applicable. Sequential ROC analyses were used to identify the single marker or set of markers which would best differentially characterize each study group from the rest. The procedure was repeated until all study groups were characterized. C-statistic was used as a discrimination measure. The procedure resulted in a sequential diagnostic algorithm – a clinical decision tree. Statistical analysis was performed in R 3.4.4, with a p-value < 0.05 set as significant.
Results: In the first step of the ROC analysis, serum C-peptide was found to be the best differentiating marker among the study groups. The marker identified the T1DM group, and was characterized by a high C-statistic value of 0.98. In the second step, the T2DM group was uniquely characterized by a combination of C-peptide and BMI with C-statistic 0.92. The final step of the algorithm distinguished between HNF1A and GCK-MODY using a combination of 1,5-AG and hsCRP. The C-statistic in the last step was 0.87. Subsequently we used the algorithm to identify the MODY groups among the 355 study participants. The cutoffs used and associated identified group in the algorithm were: C-peptide < 0.61 (T1DM), C-peptide + BMI > 8.58 (T2DM), and hsCRP + 1,5-AG < 3.53 (HNF1A MODY), while GCK-MODY was identified as the patients remaining above the last cutoff. We were able to correctly identify 51 (66.2%) of the HNF1A-MODY patients and 46 (52.3 %) of the GCK patients. The positive predictive values were 63.0 % and 71.9 %, respectively for HNF1A- and GCK-MODY.
Conclusions: Currently known biomarkers are insufficiently reliable for the diagnosis of monogenic diabetes, and hence cannot replace genetic testing. Utilizing inexpensive and readily available biomarkers, the proposed algorithm in this study is easy to perform. It can be used to prioritize genetic testing in large cohorts of patients based on clinical criteria such as those described by Shields et al. (2012).