Session 11. EMBO and Poland: Metabolic Disorders: External and Internal Signaling

Lectures

L11.1

How to optimize pancreatic beta-cell function — is the regulation of lipid metabolism a golden tool?

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Pancreatic beta cells exhibit a remarkable ability to tune their secretory functions in response to altered tissue insulin demands. Glucose, nutrients (amino acids and lipids), neurohormonal signals and lipid mediators (i.e. free fatty acids, diacylglycerol, phosphatidic acid) all efficaciously modulate insulin secretion. Recently, the presence of a functional endocannabinoid (eCB) system (that is, the enzymatic machinery to biosynthesize and degrade 2-arachidonoylglycerol (2-AG) and anandamide (AEA), as well as cannabinoid receptors) was identified in the endocrine pancreas. eCB signaling has been implicated in modulating insulin release from beta cells of the endocrine pancreas. However, the molecular cascade coupling cannabinoid receptor (CB R) activation to insulin release remains unknown. By combining molecular pharmacology and genetic tools, we recently showed that eCBs (AEA and 2-AG) activate CB_1R to induce insulin hypersecretion. In doing so, CB_1Rs recruit Akt/PKB and extracellular signal-regulated kinases 1/2 (ERK1/2) to phosphorylate focal adhesion kinase (FAK). FAK activation induces the formation of focal adhesion plaques, multimolecular platforms for second-phase insulin release. Inhibition of eCB synthesis or FAK activity precluded insulin release. Cytoskeletal remodeling induced by agonist-activation of CB_1R was independent of extracellular calcium pool. Furthermore, eCBs-induced insulin secretion might also be associated with changes in lipid metabolism and activation of mitochondrial CB_1Rs. We concluded that FAK downstream from CB_1Rs mediates eCBs-induced insulin release by allowing cytoskeletal reorganization that is required for the exocytosis of secretory vesicles. The mechanism we identified suggests that acute CB_1R activation may be critical for the adaptation of pancreatic beta cells to insulin resistance. Thus these findings show a mechanistic link between increased circulating and tissue eCB levels and hyperinsulinemia in type 2 diabetes.

L11.2

Immune mechanisms of hypertension and vascular dysfunction

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Inflammation significantly increases cardiovascular risk. This is manifested clinically by increased atherosclerosis in rheumatoid arthritis or systemic lupus erythematous or by increased risk of myocardial infarction following infections. Importantly, recent evidence suggests that inflammation is very important in the pathogenesis of hypertension and associated organ damage. We have recently shown that $RAG1^{-/-}$ mice, which lack mature T and B lymphocytes, are protected from severe angiotensin II induced or DOCA-salt hypertension and vascular damage. Similar findings were confirmed in mice with severe combined immunodeficiency (SCID), and in RAG1-/- Dahl salt-sensitive rats. These hypertensive phenotypes are dependent on T cell activation. Interestingly, lack of monocytes also protected form severe hypertension. Blockade of TNF-alpha can lower blood pressure. Similarly, Myclophenolate Mofetil (MMF), which primarily targets T and B cells, prevents the development of hypertension and urinary excretion of TNF-α in patients with psoriasis or rheumatoid arthritis and in animal models of hypertension. More recently, we found that the IL-17 contributes to hypertension. This cytokine is produced by TH17 cells or by gamma-delta T cells. Il-17 KO mice are protected from hypertension and vascular dysfunction. T regulatory cells play an important modulating role in hypertension, as their transfer confers protective phenotype in animal models. Moreover both monocyte and lymphocyte characteriscs are correlated to high blood pressure in primary hypertension as well as immune diseases such as periodontitis, indicating the key role of preactivation of the immune system. Understanding of these novel, immune mechanisms of hypertension will create the possibility of immunomodulatory approaches to treat hypertension.

Oral presentations

O11.1

Variability in adipose tissue expansion genes may determine phenotype and insulin resistance in high-fat fed mice

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Obesity is a common and serious metabolic disorder in the developed world. There is a strong correlation between the hypertrophy of adipose tissue and the development of insulin resistance and tissue inflammation. By regulating adipocyte hypertrophy, the initial phase of adipose tissue expansion at the onset of a positive energy balance may prevent the development of insulin resistance. Mesoderm specific transcript (*Mest*) gene expression is a biomarker linked to the capacity for adipocyte hypertrophy. Caveolins (*Cav1*, *Cav2*), components of caveolae structure, were also indicated as adipose tissue expansion genes (ATE). Certain white adipose tissue depots present different dynamics and growth regarding the accumulation of new fat cells, throughout the process of adipogenesis. The objective of this study is to evaluate the effect of long-term diet-induced obesity in ATE genomic profile in different fat depots across C57BL6/J (B6) and AXB8 (A8) strains of mice, which differ in susceptibility to obesity. We wished to determine whether *Mest* and the other biomarkers play a role in the development of insulin resistance. Adult mice at 56 days of age were introduced to high-fat diet for 12, 16 or 20 weeks. Nuclear magnetic resonance was used to characterize changes in fat and lean mass and adiposity index. Quantification of mRNA level was performed by real time PCR, in inguinal, epididymal and retroperitoneal fat pads. As a results, A8 mice presented lower body weight, fat mass and adiposity already at the 16 weeks on HFD ($p<0.05$) than B6 mice. Additionally, A8 mice presented smaller insulin resistance at each time point (p0.05). Regarding the gene expression profile between strains throughout the time: (i) in inguinal fat, *Mest* and *Cav2* showed increased mRNA at 20 weeks for B6 (p0.05), while A8 did not change; (ii) in epididymal fat, besides the increased *Mest* mRNA for 12 weeks and 16 weeks in B6 (p0.05), no changes were observed in A8 mce; and (iii) in retroperitoneal fat transcription of studied genes not only showed the highest expression at 16 weeks for B6, but also an increased mRNA level for B6 at 20 weeks comparing to A8, which evidenced no changes. Also, retroperitoneal fat phenotypic data present the highest correlation to ATE genes for both strains of mice. In conclusion, phenotypic differences in adiposity between strains may be determined by differential genomic profile of *Mest*, *Cav1* and *Cav2* among the 3 fat pads. Adiposity index reaching plateau may indicate some of fat depots stop expanding, characterizing the phenotype susceptible to insulin resistance.

O11.2

Activation of podocyte autophagy by insulin-dependent ROS production

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Autophagy constitutes an important adaptive and defense mechanism which serves to maintain intracellular homeostasis. Autophagy plays a key role in non-proliferating cells, eg. podocytes. Altered morphology and diminished podocyte number are the hallmarks of pathological changes in diabetic nephropathy. Early stages of type 2 diabetes are characterized by progressive insulin resistance with concomitant increases in blood insulin and glucose concentrations and enhanced production of reactive oxygen species (ROS). To shed light on the autophagy role in this complication, we investigate how autophagic pathways are regulated by a (patho)physiological factor, such as insulin.
All experiments were conducted on primary culture of rat podocytes. Insulin effects on autophagy were investigated in cells cultured for 60 minutes, 3 or 5 days with 300 nM insulin. In order to determine the role of ROS in insulin-dependent autophagy regulation, podocytes were incubated with both insulin (300 nM) and apocynin (100 μM) — inhibitor of NAD(P)H oxidase. The expression of proteins involved in autophagy (LC3, beclin1, Atg5-Atg12, ULK1, mTOR, AMPK, class III phosphatidylinositol 3-kinase (PI3KC3)) was analysed by immunodetection. Changes in AMPK and mTOR phosphorylation were determined by immunoblotting against AMPKα P-Thr^{172} and mTOR P-Ser^{2448}. PI3KC3 activity was analysed by PI(3)P quantification (ELISA).
The increase of LC3-II expression was observed in podocytes after 60 minutes and after 3 days incubation with insulin (by 55% and 24%, respectively, p0.05). The amount of Atg5-Atg12 complexes was also augmented after 3 days (117% of control, p0.05). Insulin effects on LC3 and Atg5-Atg12 were abrogted by apocynin. The expression of belin1 in podocytes showed slightly upward tendency dependent on time of incubation, though there was no effect of insulin on PI3KC3 expression, nor its activity. Insulin increased P-AMPK/actin ratio after 3 days, while mTOR expression was decreased by about 47%. Despite the decline in mTOR expression, there were no changes in its phosphorylation after insulin incubation. The effects of insulin on AMPK and mTOR were abrogated by apocynin.
It seems plausible, that insulin stimulates the processes of autophagosome maturation and closure in podocytes independently of PI3KC3-beclin1 complex, and this effect is mediated by ROS.

Acknowledgements

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O11.3

Cystathionine-β synthase deficiency changes expression of major urinary proteins in mice

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Major urinary proteins (MUP), present at high concentrations in mice urine are members of the lipocalin family, coded by highly polymorphic and polygenic MUP complex located on chromosome 4. By binding pheromones in scent marks, and thus extending the time of their release to the environment, they play an important role in sexual signaling. Alone, they may also act as pheromones. It has been recently shown, that in senesced mice the amount of expressed MUP is significantly reduced. Hereby we report, that cystathionine ß-synthase (CBS) deficiency resulting in homocystinuria, changes the profile of expression of MUPs and influences sexual signaling in mice. In this study we examined 24 hour urine collections from homocystinuric C57BL/6J Tg-I278T $Cbs^{-/-}$ mice (Gupta S *et al.*, 2009, *FASEB J* **23**: 883–893) and $Cbs^{+/+}$ littermates. MUPs concentrations and urinary creatinine in $Cbs^{-/-}$ mice and $Cbs^{+/+}$ littermates were determined using turbidymetric protein quantification method (utilizing tannin) and picric acid, respectively. Our results show, that $Cbs^{-/-}$ male mice (n=6) had significantly (P0.005) lower urinary protein:creatinine ratios: 6.21±3.14 versus 12.03±0.9 present in wild type males (n=4), while the differences in females were insignificant. To evaluate qualitative change in MUP profiles, urine samples of $Cbs^{-/-}$ male and female as well as wild type mice urine were subjected to ESI-MS analysis. We found that only one major MUP (molecular weight 18,891 Da) was present in wild type $Cbs^{+/+}$ urine, while four major MUPs (molecular weights 18,643 Da, 18,692 Da, 18,706 Da, 18,891 Da) were present in wild type $Cbs^{+/+}$ males. In wild type female $Cbs^{+/+}$ urine we found one major MUP (18,706 Da). However, the 18,706 Da MUP was absent in $Cbs^{-/-}$ female urine, in which a male-specific MUP of higher molecular weight (18,891 Da) was present. Analysis of tryptic digests by LC-MS confirmed that the 18,891 Da MUP, identical in both $Cbs^{-/-}$ female and male urines corresponds to MUP24, previously described as male specific MUP and also known as darcin. Other MUPs in both $Cbs^{-/-}$ male and female urine were identified as MUP11/8 and MUP26. We also used behavioral tests to examine responses of wild type males to $Cbs^{-/-}$ *vs.* $Cbs^{+/+}$ urine from males or females. We found that WT males (n=10) similarly countermarked urine spots from unfamiliar $Cbs^{-/-}$ and $Cbs^{+/+}$ males. Responses of WT males to urine from unfamiliar $Cbs^{-/-}$ and $Cbs^{+/+}$ females were also similar. In conclusion, our results suggest that Cbs deficiency changes patterns of MUP expression in male mice but appears not to affect murine sexual signaling.

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Posters

P11.1

Free amino acids spectrum of the thymus after administration of cyclophosphamide and oregonin

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Oregonin (1,7-bis(3,4-dihydroxyphenyl)-heptane-3-one-5-O-b-D-xylopyranoside,) one is of the well-known diarylheptanoid obtained from the bark of various species of alder and used as a food additive in the EU. We have developed basic indications for the use of the treatment, however taking into accounts its high biological activity it is recommended continue research work. Administration of oregonin to animals increases the rate of activation and cytotoxic activity of NK cells, which in it turn increases an antitumor effect. In particular, the use of oregonin in treatment of mice with B16-F10 melanoma significantly reduces the number of metastases.

Animals (rats, female, 160–200 g) were injected intraperitoneally with cyclophosphamide in the dose of 160 mg/kg, and followed with further oregonin (5 mg/kg 17 days). After 18 day of the last oregonin administration the animals were decapitated.

After 8 days of cyclophosphamide administration in thymus tissue total content of free amino acids and their nitrogen-containing derivatives of proteinogenic amino acids as the nitrogen-containing amino acid metabolites decreasing. Concentration of aspartate, glutamate, asparagine, glutamine, alanine, and essential - threonine, tyrosine, tryptophan and phenylalanine significantly reduces. The lost of the total concentration free amino acids and their nitrogen-containing derivatives in thymus tissue has been reveled in animals additional administrated with oregonin. The spectrum of the indication under the study has changed simultaneously. The decrease of concentration of glycine, phosphoethanolamine, arginine, α-aminoadipic acid, citrulline, β-alanine and taurine especially significant. The increased concentration of ethanolamine indicates the change in the synthesis of membrane phospholipids.

Thus, the restoration of thymus tissue (the 8th day after the administration of cytostatic) causes the amino acid pool in thymus cells, which may be associated with activation of biosynthetic processes and accelerated maturation of immune cells. Administration of oregonin together with cyclophosphamide activates these processes, which manifests more profound drop of the amino acids supply of the thymus cells, as well as common changes in homeostatic processes in the thymus tissue.

P11.2

Hypertension-induced heart steatosis is not associated with increased cardiac lipogenesis

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The amount and composition of lipids in the myocardium contributes to the development of cardiac dysfunction through so called "lipotoxic" effects. Spontaneously hypertensive rat (SHR), an animal model of hypertension and cardiovascular disease, is characterized by increased cardiac triglyceride (TG) level, however the mechanism responsible for the development of heart steatosis in this model is unknown. Therefore, we investigated the expression of lipogenic factors in the heart of SHR and normotensive Wistar-Kyoto rats (WKY). Plasma and cardiac TG levels were significantly higher in SHR rats at 6 and 18 weeks of age compared with age matched WKY rats. Interestingly, accumulation of TG in the heart of SHR rats increased gradually with age. The protein levels of sterol regulatory element-binding protein 1 (SREBP1), fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1) and acetyl-co carboxylase (ACC) were decreased in 6 weeks old SHR compared with littermate WKY rats. At 18 weeks of age protein levels of SREBP1 and FAS were not different whereas levels of SCD1 and ACC were decreased in SHR when compared with WKY rats. Obtained results showed that lipogenesis is not upregulated in the myocardium of SHR rats suggesting that accumulation of TG in this animals is not associated with increased cardiac lipid synthesis. Future studies involving fatty acid uptake and degradation of lipids are needed to clarify causative mechanism behind lipid stores in the heart of SHR rats.

Acknowledgements

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P11.3

Time-dependent inflammatory response in adipose tissue during obesity progression

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Obesity is associated with a low-grade systemic inflammatory state. Different dietary factors may activate inflammation in adipose tissue, which presents specific receptors able to sense saturated fatty acids, glucose and/or changes in gut microbiota. Nonetheless, the pathways triggering and promoting inflammation in adipose tissue during obesity are yet to be fully understood. In the present report we describe the effect of high-fat diet (coconut oil 58% calories saturated fat) on Toll-like receptor (TLR)s and inflammatory cytokines interleukin (IL)β and Tumor necrosis factor-α (TNF) transcription level. An *in vivo* study was conducted on C57Bl/6J female mice (n=8/group) fed a chow-diet versus high-fat diet during four- (C-4w; HFD-4w) or sixteen weeks (C-16w; HFD-16w). Further on, gonadal fat-depot was used for quantification of *TLR1*, *TLR2*, *TLR4* and *TLR6*, as well as *IL1β* and *TNF mRNA* transcription by Real-Time PCR. As a result, in HFD-4w treatment the *mRNA* was decreased for *TLR1* (p0.1), *TLR4* (p0.5) and both *IL1β* (p0.01 and TNF (p0.05). Conversly, HFD-16w showed increased mRNA for *TLR1* (p0.00), *TLR2* (p0.01, *TLR6* (p0.05 and cytokines *IL1β* (p0.01 and *TNF* (p0.05. Analysis of present data suggests an opposing response from members of innate immune system and inflammatory cytokines in adipose gonadal tissue to HFD in the course of obesity. Apparently, soon after the HFD introduction, TLRs and inflammatory cytokines transcription in gonadal fat is downregulated. After 16w the inflammatory state was confirmed in HFD group. Further studies are needed to better understand the biological significance of TLRs and inflammation downregulation in the initial phase of adipose tissue expansion and its correlation with insulin resistance.

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P11.4

Argininosuccinic acid analysis in dry blood samples by Tandem Mass Spectrometry in the newborn screening

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Background: Argininosuccinic acid (ASA) is a by-product of urea cycle formed from citrulline and aspartic acid and in the next step is converted into arginine and fumarate by argininosuccinate lyase (ASL). ASL deficiency causes the accumulation of argininosuccinate in blood. The lack of the enzyme activity can lead to severe clinical symptoms of argininosuccinic aciduria: hiperammonemia, vomiting, lethargy, seizures, loss of appetite, hepatomegaly.
Materials and Methods: Tandem Mass Spectrometry (MS/MS) is used in the newborn screening for amino acids and acylcarnitines profiles analysis in the dry blood spot. Argininosuccinate is additional parameter for diagnosis of argininosuccinic aciduria, which can be measured together with amino acids profile after butylation of samples with 3N-HCl in n-butanol. Argininosuccinate content in blood is estimated as a ratio of Asa70 (m/z=70) and Asa144 (m/z=144) fragments to internal isotopic standard of palmitoylcarnitine (m/z=459) which is used in acylcarnitines profile measurement, therefore internal isotopic standard of ASA is not necessary.
Results: 258,175 blood samples from newborns were tested in the period of 10 years. The cut-off ratio of Asa70 and Asa144 to C16 in newborns was set 0.1; reference range for citrulline was 3–57 μmol/L. There were 4 positive cases of ASL deficiency (Asa70 range 1.8 to 12.6; Asa144 range 034 to 0.83; citrulline range 67 to 347 μmol/L). All cases were confirmed as argininosuccinic lyase deficiency by plasma amino acids HPLC profile. The frequency of ASA is estimated to 1:300 000.
Conclusions: Introduction of ASA as new metabolite analysed in the newborn screening test allows to detect wider spectrum disorders in urea cycle and it is helpful in their differentiation. Tandem Mass Spectrometry is useful, simple and fast method enabling to detect inborn errors of metabolism before clinical symptoms appear.

P11.5

The problem of determining the prescription of death coming by indicators of energy and amino acid metabolism in tissues

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As is known, the enzymatic activity in some tissues and organs continues a certain time after the death of the body. We can assume that between the level of enzyme activity and the time elapsed after death, there must be a clear correlation.
Before us, in the liver of rats and humans have already studied the levels of activity SDH, ADH, MDH, α-GPDH, GDH, G-6-PDH, NADH-DH and NAD-FN-DH in terms 0, 6, 12, 18, 24, 36 and 48 hours after the time of death.
In the myocardium of rats and humans — SDH, MDH, LDH, G-6-PDH, α-GPDH, NADH-DH (Zarov V *et al.*, 1975, *Works II MOLGMI* **3**: 23–24), in skeletal muscle also — GDH, ADH, NADPH-DH and ATP (Zarov V, 1966, *Forensic Medical Examination* **2**: 24–26). LDH and acid phosphatase in the spleen, activity of SDH, α-GPDH and NADH-DH was investigated in brain (Lisjanskij B, 1975, *Works II MOLGMI* **3**: 48–50). At histochemical study of the kidney was determined activity of LDH, SDH, ADH (Pashinjan G, 1976, *1st All-Union Congress of Forensic*: 231–232).
From the data we can conclude that in the liver, myocardium, kidney and skeletal muscle activity of studied dehydrogenases naturally decreases and disappears completely for 48 hours. Myocardium feature is the "mosaic" in the distribution of dehydrogenases, disappearing to 36–48 h postmortem period.
In quantitative histochemical study found that the most indicative is dynamic of LDH and SDH activity in the kidney and myocardium, because after 6 and 24 hours after death marked the wave rises in their activity. In the spleen and brain cortex in all periods shown the gradual decrease of activity of dehydrogenases, except cortex SDG (the rise of activity through 12 hours).
To date, no comprehensive methods of determining the limitation of death with the exact hour. Therefore, we offer a definition of activity of some dehydrogenases, macroergic compounds, structure of the biogenic amines and amino acids pool and, as the indicators of energy metabolism efficiency in more narrow period postmortem changes (0.5–6 h) to determine the exact correlation with prescription of death.
Definition of these indicators can be performed in skeletal and cardiac muscle, as in tissues exposed to the most local changes in different pathologies, and also, if possible, in the liquor as a connective fluid of the body, moreover, should be undertaken histological analysis.

P11.6

Dipeptidyl peptidase-IV: a new biomarker for diagnosing of mucopolysaccharidoses — preliminary results

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The mucopolysaccharidoses (MPS) are a group of rare inherited metabolic disorders which result from the lack of one of the lysosomal enzymes responsible for the glycosaminoglycans degradation. Clinically, MPS patients present mainly with coarse faces, visceromegaly, joint stiffness, skeletal deformities, and in some cases neurodegeneration, corneal clouding, and hearing loss. Early recognition of MPS is important for these patients in whom the enzyme replacement therapy (ERT) can be implemented.
Dipeptidyl peptidase-IV (DPP-IV) is a ubiquitous ectopeptidase, which cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position. DPP-IV activity has been associated to a cell surface protein CD26.
Aims of this study were to investigate the level of plasma DPP-IV activity in patients with MPS type I and II in comparison to control individuals and to evaluate changes of DPP-IV during ERT.
One MPSI and three MPSII patients were treated with ERT for up to 19 months. DPP-IV activity was measured in serum/plasma with a colorimetric method using Gly-Pro-p-nitroanilide as a substrate. The reference ranges were estimated in 17 healthy donors and in 9 MPSII patients before ERT implementation.
DPP-IV activity ranged from 557 to 1959 nmol/ml/hr (mean ±SD: 1298±429, n = 17) in serum/plasma of healthy reference samples. In 9 MPSII patients, DPP-IV activity was higher and ranged from 2565 to 5968 nmol/ml/hr (mean ±SD: 4526±1023). In 4 MPS patients on ERT, DPP-IV activity ranged from 3323 to 8628 nmol/ml/hr. In untreated patients, DPP-IV activity did not depend on the patient's age and was not a measure of clinical status, type or progress of the disease. No declining tendency was observed in the treated patients.
We conclude that DPP-IV activity is a good new biomarker distinguishing between MPS and healthy individuals. However, it cannot be considered as a marker of treatment effectiveness and it is unsuitable for the monitoring.

P11.7

Major lipid peroxidation product 4-hydroksynonenal affects dna damage response and modulates gene expression in HeLa cells

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Lipid peroxidation (LPO) is involved in the pathogenesis of several human diseases, including cancer. LPO products react with cellular proteins changing their properties, and with DNA bases to form mutagenic exocyclic-DNA adducts, DNA-DNA and DNA-protein crosslinks. Here we showed that 4-hydroxynonenal (HNE) induces oxidative stress and generates single- (SSB) and double-strand DNA breaks (DSB) in HeLa cells. DNA damage response to HNE was delayed up to 6 h after treatment. When HeLa cells were pre-treated with HNE and subsequently exposed to another DNA damaging agent, camptothecin (CPT) and hydroxyurea (HU) the level of phosphorylation of DDR proteins, Chk1, Chk2, RPA32, H2AX was partially decreased or totally inhibited. We then studied the consequences of DDR inhibition by HNE, namely the presence of damage which could inhibit DNA replication and the kinetics of transcription. Using GFP reporter gene in pEPI-EGFP plasmid transformed into HeLa cells we observed that 2 h treatment of cells with HNE generated DNA damage that inhibited DNA synthesis performed by T4 DNA polymerase *in vitro* on re-isolated plasmid template. To our surprise higher HNE concentrations, 75 and 100 μM, but not 50 μM caused a complete loss of GFP fluorescence, which could suggest loss of the plasmid or inhibition of transcription by higher HNE concentrations. However, neither PCR nor Western analysis confirmed loss of GFP protein from HNE treated cells. Moreover, after cell treatment with 75 μM HNE the amount of GFP protein doubled within 24 h following treatment. This may suggest that HNE persists in cells, and modifies GFP structure, as well as its transcription or/and degradation. Thus, genotoxic activity of HNE is complex and involves DNA damage, modulation of DNA damage response and modulation of gene expression.

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P11.8

Plasma GSH in animal model of antiretroviral therapy

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The HIV pandemic has placed a great demand upon the scientific community to develop effective prevention and treatment methods. Highly active antiretroviral therapy (ART) often leads to dramatic improvement in clinical, viral, and immunologic parameters in HIV-infected patients. However, a favorable response is not achieved in all patients, and the emergence of drug-resistant strains and serious long-term side effects of ART have increasingly been reported. Among the approaches that have been considered to control HIV *in vitro*, attempts to discover biological processes that were important in perpetuating the virus's activation and replication were given a high priority. Nevirapine (Viramune) is a non-nucleoside reverse transcriptase inhibitor that is widely used for the treatment of HIV infections in the developing world. Nevirapine is associated with two serious clinically restrictive side effects: skin reactions and hepatotoxicity.

The objective of the study was to determine whether ART by nevirapine is associated with poor reduced glutathione status. Adult male rats were used. Animal received nevirapine 30 mg/kg daily. Plasma samples were taken after 2 weeks of ART. Concentration of GSH levels were determined by HPLC.

Plasma glutathione concentrations was significantly (Students' *t*-test) lower in ART-animals than in control subjects. The mean for ART — 8.24±1.002 mmol/ml (n=6) was two times lower than intact group — 4.37±0.89 mmol/ml (n=7, p = 0.016).

The current analysis indicates that antioxidant defenses, as measured by plasma GSH concentrations, were decreased in ART-animals. The current data also suggest that ART may have unanticipated effects on antioxidant defenses that should be further explored. Monitoring changes in antioxidant enzymes may be useful in such studies.

P11.9

Ecto adenosine deaminase in aortic wall correlates with development of atherosclerosis in ApoE/LDLr (--/--) mice

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Extracellular adenine nucleotides and adenosine could affect development of atherosclerosis by mediating vascular inflammation and thrombosis. Nucleotides and nucleosides concentration in blood is strictly regulated by membrane-bound enzymes. This work aimed to clarify changes in extracellular nucleotide metabolism in vessel wall and plasma in a genetic model of atherosclerosis.

Intact fragments of aorta from an Apolipoprotein E/LDL receptor double knock-out mice (ApoE/LDLr --/--) or from a wild type mice (WT) at 1, 3, 6 and 10 month of age were incubated with substrates for extracellular enzymes and its conversions were measured by HPLC. Progression of atherosclerosis was quantified in aortas by Oil-Red O staining and immunostaining for CD68. Additionaly, plasma adenosine deaminase activity and adnosine concentration were measured by HPLC or LC/MS.

Ecto-adenosine deaminase activity rises progressively from 10.7±2.4 nmol/min/cm^2 at 1 month to 26.1±2.9 nmol/min/cm^2 at 10 month ApoE/LDLR (--/--) mice aorta while in WT mice a decrease was observed from 3.28±0.57 to 1.71±0.22 nmol/min/cm^2. Ecto-nucleotidase activities were 2–3 times lower in mutants and age related changes were minor. Plasma adenosine deaminase activity increased from 0.59±0.04 in WT to 1.05±0.22 μmol/min/l in ApoE/LDLr (--/--) in 10 month mice. Plasma adenosine concentration in 10 month mice decreased from 76.3±11.3 nM in WT to 36.2±5.8 nM in ApoE/LDLR (--/--). Changes in plasma and vessel wall adenosine metabolism and adenosine concentration correlated with plasma Oil-Red stained lessions and CD68 areas that increased progressively with age of ApoE/LDLR (--/--).

These results indicate that extracellular nucleotide breakdown is adversely modified in atherosclerosis. In addition to substantial increase of overall rate of adenosine deamination this process seems to shift from plasma to vessel wall surface. Our observations suggest that changes in adenosine deaminase activity could serve as marker of atherosclerosis and highlight this enzyme as potential target for therapy.

P11.10

Metabolic disturbances in tissues of rats undergone intermittent alcohol intoxication

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Intermittent alcohol intoxication (IAI) is the newly worked up rodent model which allows studying different aspects of alcohol-related behaviors. Our research was aimed to compare metabolic disturbances in different tissues of the rats that were undergone two types of IAI.
In the first model (IAI – 1) in rats was brought about by intragastric infusion of 25% ethanol solution in the dose of 3.5 g/kg of body weight, twice daily, within 4 days. Then the alcohol infusions were followed by the 3 days' alcohol-free periods. In sum 4 cycles of IAI were used, and rats were sacrificed 3 days after the last alcohol ingestion.
In the second model (IAI – 2) there were 2 cycles of intermittent alcohol administration according to the scheme "7 days alcohol + 7 days of alcohol-tree periods". The animals were sacrificed 7 days after the last alcohol ingestion.
The contents of free amino acids and their derivatives were assayed in the deproteined homogenates of liver, skeletal muscle, and myocardium by HPLC method. The activities of diagnostically important enzymes and substances were assayed in blood serum.
The metabolic disturbances observed in different tissues were highly dependent upon the type of IAI and tissue localization.
The I type of discontinuous alcohol exposure was followed by hypeglycemia, and lower blood urea and creatinine, whereas the II type of IAI led to reliable activation of the liver enzymes in serum.
In general, both types of intermittent alcohol exposure caused significant metabolic misbalance in serum the tested tissues. The second model of IAI was found to be more toxic for the liver, whereas the first model was accompanied by significant disturbances in the amino acid levels in myocardium and skeletal muscles.

P11.11

Adaptive changes in the systemic iron metabolism in response to iron deficiency and hemolytic anemias

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Iron is required for heme formation and is the most common limiting factor in erythropoiesis. Most of iron recycled from senescent erythrocytes by macrophages and absorbed by duodenal enterocytes is destined to be utilized by erythrocyte precursors in the bone marrow. It is therefore not surprising that iron deficiency remains the main cause of anemia called iron deficiency anemia(IDA). Newborn piglets are a suitable model to explore the multifaceted etiology of IDA in mammals, as IDA is the most prevalent deficiency disorder throughout the early postnatal period in this species and frequently develops into a critical illness. Iron scarcity in piglets is the result of interplay of several distinct risk factors such as low level of iron stores, increased iron requirements and limited external supply. We investigated the role of hepcidin, the systemic iron-regulatory hormone, in the regulation of intestinal iron absorption in anemic piglets, especially under conditions of morphological and functional rebuilding of the pig duodenal mucosa during the early period after birth. Intramuscular administration to piglets of large amounts of iron dextran few days postpartum is current practice in the swine industry, and has been proven to rectify the hematological status of piglets. Our findings show that although such routine iron supplementation prevents anemia, it increases the extent of oxidatively damaged DNA in the liver and induces hepcidin expression. We therefore proposed new innovative strategies for iron supplementation of newborn piglets improving the piglets' hematological status, attenuating the induction of hepcidin expression, and minimizing the toxicity of the administered iron. Our studies also concentrate on hemolytic anemia (HA), which is the third most prevalent form of anemia after IDA and anemia of chronic disease. During several pathological conditions, red blood cells undergo hemolysis and hemoglobin and heme are released into the circulation. We analyzed iron metabolism in hemolytic states resulting from a deficiency in a copper-containing, antioxidant defense enzyme – superoxide dismutase (Cu,Zn-SOD). Precisely, we examined the consequences of HA on systemic iron metabolism in 2 mouse models: 1) 1-year-old mice lacking Cu,Zn-SOD1 activity; 2) 14-day-old copper-deficient *mosaic* mutant mice with dysfunction of the ATP7A copper transporter, showing decreased Cu,Zn-SOD activity. Our results clearly indicate that the enhanced destruction of circulating red blood cells in these mice induces substantial changes in the expression of hepatic and renal genes involved in heme and iron homoeostasis.

P11.12

CoA biosynthesis system in the regulation of cellular redox-potential

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The role and level of CoASH are considered to be the key ones in the integral mitochondrial function in which CoASH sequestration, toxicity and redistribution of CoA ↔ acyl-CoA cause dramatic metabolic changes as the CASTOR syndrome (coenzyme A sequestration, toxicity or redistribution). The property of CoA biosynthesis to stabilize glutathione level and redox potential, which has been confirmed many times, is of a more extended character. The differences in molar intracellular concentrations of CoA (up to 0.15 mM) and glutathione (up to 10 mM) suggest participation of redox signaling similar to the deinhibitory effect on CoASH on pantothenate kinase manifested by disulfide forms of glutathione. The process of redox signaling mediated by transformation of CoASH ↔ CoASS may be characteristic of all cellular compartments and participants of thiol disulfide interaction, but it is especially pronounced in respect to SH-and SS-groups of proteins, with the extent of oxidation depending on the initiation of CoA biosynthesis. Formation of CoA-SS-protein complex (56 kDa) in the cytosol may be a possible buffer in implementation of this function. Another feature of this process may be conjugation of CoA biosynthesis and protein glutathionylation, which stipulates increase in free glutathione after administration of pantothenate-containing coenzyme precursors. In addition to the redox component transferred by proteins and glutathione, other participants in formation of an effective redox potential can manifest dependence on CoA-modulating effects, which results in a wide range of pharmacotherapeutic effects of pantothenic acid derivatives under oxidative stress, reperfusion-reoxygenation syndrome, neurodegenerative pathology, etc. The vitamin status of pantothenic acid can contribute to maintenance of redox-balance in living systems and corresponds to its universal distribution in animal world and vegetable kingdom. The possibility of application of CoA biosynthetic precursors for modulation of cellular redox-potential and tissues was demonstrated in experiments with neurodegenerative pathology initiated by aluminium neurotoxicosis and (or) bacterial lipopolysaccharide, choline deficiency, immobilization stress and after administration of antitumor antibiotics. Thereby new vistas are opened up for use of the CoA biosynthetic system in achievement of a redox-pharmacotherapeutic effect on the processes of redox signaling and protein post-translation modification.

P11.13

Development of protective composition "tritarg" and its effects in conditions of lead intoxication

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Amino acids play a key role in the development and maintenance of homeostasis as on the one hand they are precursors in the biosynthesis of protein, and the on other hand they are the precursors of a large number of nitrogen-containing compounds (hormones, biogenic amines, neurotransmitters and neurotransmitter). Simultaneously amino acids (leucine, arginine, glutamine, methionine) are endogenous regulators of plastic processes and energy balance modulator (alanine, proline, aspartate). It is evident that change in free amino acid concentrations of in the tissues and cells of the body (especially the immune system), under the influence of negative environmental factors may reflect their metabolic and functional status.

The aim of the study was the development of protective properties of the "tritarg" composition exposed lead intoxication.

Experiments were conducted on white female rats weighing 160–190 g. Experimental groups were treated intragastrically with lead acetate in a total dose of 150 mg/kg or in combination with lead acetate and “tritarg” (10 times at a dose of 30 mg/100 g).

Animals treated with Pb^{2+} have manifested increased concentration of glutamate and proline as well as reduced BCAA/AAC ratio in the spleen. Increased consumption of glutamate and proline indicates activation of energy metabolism in spleen cells. 10 day administration of "tritarg" (a composition containing an organic zinc salt, arginine, taurine and tryptophan) has resulted in enrichment of the spleen cells with free amino acids and their nitrogen-containing metabolites (51629±2246 against 43064±1220 nmol/g in the control group). Concentration of asparagine, glutamine, threonine, arginine, phenylalanine and leucine (1.6 times), and aspartate (1.3 times), glutamate (1.2 times), alanine (1.3 times), tyrosine (1.5 times), valine (1.4 times), methionine (1.8 times), isoleucine (1.5 times), proline (1.9 times), β-alanine (1.4 times), taurine (1.2 times) and ethanolamine (1.4 times) has increases significantly.

Thus, course administration of "tritarg" to rats with lead intoxication increases mobilization of free amino acids, especially sulfur containing: methionine, cystathionine, taurine in spleen cells, which promoter the rate of antioxidant protection and stimulation of the adaptive response.

P11.14

Influence of caloric restriction and n-3 PUFAs supplementation on insulin sensitivity in prediabetes

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Introduction: Metabolic complications in obese may lead to type 2 diabetes development and some studies suggest that glucose-dependent insulinotropic polypeptide (GIP) participate in the pathogenesis of metabolic disorders in prediabetic state. It is also reported that caloric restriction protects from some of metabolic complications. In addition, diet enriched with *n*−3 PUFAs prevents from progressing of prediabetes to type 2 diabetes.
The aim of the study was to assess the influence of low calorie diet and three months of omega-3 PUFAs supplementation on insulin sensitivity markers (HOMA-IR) and incretin (GIP) output measured after oral glucose tolerance test in patients with obesity.
Methods: Obese patients with BMI 30–40 kg/m^2 aged 25–65 yrs. (n=50) included into the double-blind randomized EU BIOCLAIMS trial were put on low calorie diet (1200–1500 kcal/day) supplemented with capsules either with 3x600mg/day DHA:EPA (5:1) (EPAXTG, Norvay) or with placebo for three months. Blood concentrations of triglycerides, glucose, insulin and GIP were analyzed from samples obtained in the fasting state and during OGTT before and after supplementation.
Results: Significant decrease of insulin sensitivity index (HOMA-IR) value in the n-3 PUFAs supplemented group (n=26) was observed. DHA/EPA supplementation also resulted in significant reduction of insulin output (AUC) with decreased GIP secretion in patients supplemented with n-3 PUFAs during OGTT. Lower fasting and postprandial (OGTT) blood triglyceride level was observed independently after n-3 PUFAs as well as after caloric restriction.
Conclusion: Three months of DHA/EPA supplementation exerts the beneficial effect by reduction of insulin resistance and triglyceride level. Decrease of insulin output after OGTT may be result of the lower postprandial GIP release after n-3 PUFAs supplementation.

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P11.15

Development of tautsin(K): background and results

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Zinc is a component of more than 300 different enzymes isolated from various sources, as well as a structural element of more than 2000 transcription factors. Zinc cations are involved in the regulation of secretion of several hormones (insulin, glucagon) and gene expression In contrast to other nutrients including essential amino acids or vitamins. These common biochemical functions of zinc are difficult to correlate with the physiological manifestations of micronutrient deficiencies. Numerous examples of beneficial effects of zinc supplementation in the most common "diseases of civilization": cardiovascular diseases, diabetes, immunodeficiency disorders of various origins, liver lesions of various etiologies have been proved. We were the first to claim the increase of digestive enzymes activity in newborn after administration of zinc sulfate.
In clinical medicine the effects of taurine on metabolism of carbohydrates, lipids, amino acids and peptides are commonly used. The diet for pregnant women and newborns enriched with taurine promotes optimal metabolic programming and prevents the development of a number of "diseases of civilization" - diabetes, obesity, hypertension. Nonspecific functions of taurine (including osmoregulation) are conceded to be key functions of the stabilization life cycle of cells. We were first created and studied the biological activity of amino acid-micronutrient compositions "tautsin(k)", "talerin", "tritarg", "sibitatsin", "titatsin", "amyuram". The prospective of this approach in the development of the treatment for metabolic therapy have been shown. Combination of taurine and zinc salts (zinc sulfate or zinc aspartate) in various proportions has created a complex treatment with immune modulating and a hepatoprotective property which was confirmed by the patents obtained. At the same time specific metabolic effects on the metabolism of various zinc salts have been shown. "Tautsin" composition has successfully gone through the stage of preclinical test.

P11.16

Expression levels of gelatinases, their inhibitors and the related genes in leukocytes of children with primary hypertension (PH), non-alcoholic fatty liver disease (NAFLD) and obesity

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Primary hypertension (PH) in childhood and non-alcoholic fatty liver disease (NAFLD) syndromes often share the same abnormalities, such as systemic low-grade inflammation, vascular remodeling, visceral obesity, insulin resistance, and dyslipidemia. However, NAFLD children rarely develop primary hypertension.

The aim of this study was to assess the peripheral blood leukocytes (PBLs) gene expression profile of mediators involved in the extracellular matrix degradation, that results in vascular remodeling and inflammation. We tested matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) PBLs expression of MMP-9, MMP-2, MMP12, MMP14, TIMP-1, TIMP-2, and other mediators: insulin like growth factor-1 receptor (IGF-1R); transforming growth factor beta (TGF-beta); and interleukin-6 (IL-6).

Material & methods: The mRNA expression levels were measured by quantitative RT-PCR (real-time reverse transcriptase-polymerase chain reaction) in the PH, NAFLD and normotensive, non-NAFLD obese patients PBLs, aged 9–16 yrs, compared to healthy, age and SDS-BMI-matched (for obese — normal weight) control group. Relative target gene expression level was normalized by expression of the reference gene – G3DPH.

Results: Leukocytes from **PH** but not NAFLD and obese children had very high levels of MMP-2, low TIMP-1 but increased TIMP-2, MMP-14, IGF-1R and IL-6 gene expression

NAFLD children leukocytes showed high TGF-beta gene expression levels (like PH children) but low MMP14 and IL-6 levels. However, IGF-1R expression was only slightly increased. **Obesity** was characterized by higher than in the PH and NAFLD leukocyte expression of genes of MMP-9 and MMP-12; however, the expression of other genes was low indicating that MMP-2, TIMP-2, IGF-1R, TGF-beta and IL-6 are independently up-regulated.

Conclusions: 1. The PH leukocytes MMPs (MMP-2, MMP-14) and TIMP-2 gene up-regulation possibly predisposes to increase in elastase proteolytic activity in the early PH stages; it may result in increased vascular remodeling and subsequent elevation of arterial stiffness due to vessel wall elastin degradation and immature collagen deposition. 2. In contrast, lower and more balanced expression of MMPs and TIMPs in NAFLD children together with decreased IL-6 and IGF-1R gene expression levels may reflect less remodeling of arterial wall and low leukocyte activation and less adhesiveness to the vascular endothelium.

P11.17

Identification of functional networks between Sirtuin 1 (*SIRT1*) and oxidative stress-related genes in leukocytes of women with gestational diabetes mellitus (GDM)

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Background: Sirtuin 1 (SIRT1) is a NAD^+-dependent deacetylase that regulates oxidative stress — one of major contributors to diabetes including gestational diabetes mellitus (GDM). In this study, we aimed to measure changes in oxidative stress-responsive genes associated with *SIRT1* overexpression in leukocytes of women with GDM using the qRT-PCR array technology. Additionally, using gene network analysis we identified possible biological interactions of *SIRT1* with the set of differently expressed genes.

Methods: Total RNA was isolated from leukocytes obtained from 8 women with normal glucose tolerance (NGT) and 12 pregnant women with GDM at 24–33 weeks of pregnancy. Leukocyte *SIRT1* expression was determined by qRT-PCR and based on its level, the GDM group was stratified into 2 subgroups: GDM women with significantly increased *SIRT1* expression (n=6, *P*0.05 *vs* NGT), termed as GDM/*SIRT1*(↑) and women with unchanged *SIRT1* expression (n=6, *P*>0.05 *vs* NGT), termed as GDM/*SIRT1*(↔). Gene expression profiles from NGT, GDM/*SIRT1*(↑), and GDM/*SIRT1*(↔) subjects were evaluated with a 96-well RT^2 Profiler PCR Array containing 84 key genes related to oxidative stress. Functional relationships between *SIRT1* and differentially expressed genes were determined by the Ingenuity Pathway Analysis (IPA).

Results: PCR array analysis showed that 50 of the same genes were down-regulated at least 2-fold in the GDM/*SIRT1*(↑) and GDM/*SIRT1*(↔) groups compared to NGT control, except of the *MPV17* gene, and this effect was significantly smaller in the GDM/*SIRT1*(↑) than GDM/*SIRT1*(↔) group (*P*0.05). IPA revealed that some of these genes were involved in five crucial canonical pathways including: (*i*) glutathione redox reactions I (*GSTZ1, GPX1, GPX3, GPX5, GPX6, MGST3*), (*ii*) *NRF2*-mediated oxidative stress response (*HMOX1, PRDX1, CAT, NQO1, SQSTM1, AOX1, TXN, SOD3, MGST3*), (*iii*) superoxide radical degradation (*CAT, NQO1, SOD3*), (*iv*) mitochondrial dysfunction (*PRDX3, PRDX5, UCP2, CAT, TXNRD2*), and (*v*) thioredoxin pathway (*TXN, TXNRD2*).

Conclusions: This study shows significant quantitative differences in the 50 oxidative stress-responsive genes between the GDM/*SIRT1*(↑) and GDM/*SIRT1*(↔) groups and, additionally, indentifies networks of these genes with *SIRT1* in leukocytes of women with GDM thus providing a novel insight into a the role of leukocyte *SIRT1* in diabetic pregnant patients.

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P11.18

N1-methylnicotinamide salt of pyruvate attenuates hypoxic stress induced myocardial infarction in *ApoE/LDLR* double knockout mice

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Background: Pyruvate, as an intermediate in the Krebs cycle, is an important source of energy for myocardium and improves contractility of normal, hypoxic, and postischaemic animal myocardium. Furthermore, it was shown that application of pyruvate to patients with congestive heart failure resulted in improved hemodynamics and myocardial function. The main problem in its clinical application is hipernatremia caused by high doses of sodium pyruvate necessary for therapeutic effect. The way to overcome this effect is application of alternative pyruvate salts such as N1-methylnicotinamide (MNA). This compound could provide additional benefit due to its anti-inflammatory and platelet anti-aggregatory functions. The aim of the study was to investigate the effect of MNA pyruvatein mouse heart infarction model.
Methods: Seven months female ApoE/LDRr double knock-out mice were divided into three groups. Following anesthesia mice were intravenously injected with MNA pyruvate, sodium pyruvate or saline in the control group. In one set of experiments (n=5 in each group) hearts and plasma were collected after 10 min. In subsequent set of experiments 10 min after administration of MNA pyruvate and saline (n=7 in each group) mice were exposed to hypoxic stress (8 minutes of 8% oxygen in breathing air) followed by reoxygenation. After 4 hours blood was collected from the jugular vein and the heart was removed and prepared for histological analysis. Creatine kinase in plasma was analysed with HPLC based assay.
Results: Serum sodium concentration after 10 minutes from injection of sodium pyruvate was 153 mmol/l. This was significantly increased as compared to the control group (147 mmol/l serum) and group with MNA pyruvate (148 mmol/l). Four hours after hypoxia, creatine kinase activity in the serum of MNA pyruvate injected mice decreased to 1.3 nmol/min/ul from 4.0 nmol/min/ul in control. During hypoxia, control mice developed profound changes of STU area of ECG while no changes were observed in MNA pyruvate group.
Conclusion: This study demonstrated that exposure of mice to hypoxia with the administration of MNA pyruvate protects from cardiac infarction development. Results were evidenced by attenuation of ECG changes and reduction of creatine kinase release. This benefit was not accompanied by hypernatremia clearly visible after administration of equivalent dose of sodium pyruvate.

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P11.19

A novel *TAZ* gene mutation and maternal mosaicism in Polish family with Barth syndrome

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Barth Syndrome (BTHS) is a recessive X-linked disease characterized by hypertrophic or dilated cardiomyopathy, skeletal myopathy, chronic/cyclic neutropenia as well as growth retardation, respiratory chain dysfunction and 3-methylglutaconic aciduria in male patients.Prevalence is estimated at 1/300 000–400 000 in the USA and 1/140 000 in England. It is caused by mutations in *TAZ* gene coding for the tafazzin protein, responsible for cardiolipin remodeling. We present new pathogenic mutation of *TAZ* gene in Polish family which occurs as a mosicism in female members of the family. The proband suffered from various symptoms characteristic for Barth's syndrome and finally died at the age of 6 months. We performed Sanger sequencing of DNA from peripheral blood and epithelial cells in nine members of his family. Here we report a novel exonic mutation c.83T>A (p.Val28Glu) of *TAZ* gene. The mutation was passed through four generation in the family and the proband inherited it from his mother. The accurate molecular genetics examination revealed a mosaicism of the mutation in almost all female family members. This approach is very important for genetic counseling. Most of the genetic diagnosis is based only on samples from peripheral blood. In our female Barth's syndrome carriers the mutation was present only in the epithelial cells DNA. We concluded that genetic diagnosis of Barth's syndrome should be performed in women at least on the two or more types of cells drived from the different germ layers. The results of our study also point that the phenotype differs depending on degree of mosaicism and probably other factors.

P11.20

Does apolipoprotein E genotype and LRP1 polymorphisms influence the phenotype in patients with different clinical types of metachromatic leukodystrophy ?

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Metachromatic leukodystrophy (MLD) is a severe, neurodegenerative, metabolic disorder which is caused by deficient activity of arylsulfatase A (ARSA). MLD belongs to the group of rare, inherited, lysosomal diseases. Sulfatides and other substrates of ARSA are stored in central and peripheral nervous systems, and in some other organs. Accumulated sulfatides are especially toxic to oligodendrocytes and Schwann cells leading to progressive demyelination. The kind of apolipoprotein E (apoE) isoform is of essential significance for the modulation of sulfatides quantity in the brain as apoE4 contains more sulfatides than apoE3. Taking into consideration the fact that apoE4 leads to the loss of sulfatides in CSF of Alzheimer disease patients, we examined if apoE isoforms display any impact on clinical outcome in patients with different forms of MLD in whom sulfatides accumulate. The significant association of age at onset of MLD symptoms with *APOE ε2/ε3/ε4* and *LRP1* c.766C>T polymorphisms was shown in multivariate stepwise regression analysis, in which other factors known to affect age at onset of the disease, i.e. clinical type of MLD, family connection of the patient and sex were also analyzed. As expected, the clinical type of MLD explained about 80% of the variance of the dependent variable. The impact of both polymorphisms on age of onset of the disease was considerably lower: 2.0% in the case of *APOE* polymorphism and 1.0% in the case of *LRP1* polymorphism. Thus, the clinical outcome in MLD patients is related principally to the genotype of the *ARSA* gene, while the polymorphisms in the *APOE* and *LRP1* genes are most probably only slightly modifying factors.

P11.21

Role of Heat Shock Protein 72 in the regulation of lipid-induced insulin resistance in skeletal muscle

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Heat shock protein 72 (Hsp72) is known to protect against obesity-induced insulin resistance, a condition in which cells fail to respond to insulin. Insulin resistance is associated with many health related complications, including type 2 diabetes and heart disease. It is also known that expression of Hsp72 in human skeletal muscle is positively correlated with insulin sensitivity but the underlying molecular mechanisms are not well understood. Therefore, the aim of this study was to investigate the molecular mechanisms involved in Hsp72-associated regulation of insulin sensitivity in skeletal muscle. Herein we show that overexpression of Hsp72 decreases both palmitic acid- (16:0) and C2 ceramide-induced insulin resistance in C2C12 cells. Additionally, C2C12 cells overexpressing Hsp72 are characterized by decreased lipid content and increased phosphorylation of 5'AMP-activated protein kinase (AMPK), as well as acetyl-coA carboxylase (ACC), its downstream target. Inhibition of AMPK with compound C attenuated the Hsp72-induced improvement in insulin sensitivity in C2C12 myotubes. We also observed that overexpression of Hsp72 with an inactive ATPase domain does not affect insulin sensitivity in C2C12 myotubes. Moreover, the phosphorylation of AMPK was significantly decreased in these cells. Overall, this study showed that overexpression of Hsp72 decreases lipid accumulation and improves insulin sensitivity *via* upregulation of AMPK pathway in C2C12 cells. Furthermore, active ATPase domain in Hsp72 is required to increase insulin sensitivity and AMPK phosphorylation in myotubes.

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The influence of selenitetriglycerides on the activity of superoxide dismutase in human prostate cancer

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Introduction: Prostate cancer is the most frequently diagnosed malignancy in men. There is a demand for new potent drugs which could enhance the therapy of androgen-dependent hPCa, and/or delay the recurrence of the lethal androgen-refractory form of the disease, and/or decrease chemoresistance of the androgen-insensitive form of hPCa. Data from epidemiological, nutritional and clinical trials suggest that selenium +4 (SeIV) can prevent hPCa. Selol, a new organic derivative of Se (IV), is a mixture of selenitetriglycerides. After *p.o.* treatment it is 56-times less toxic in mice than Na-selenite. It exhibits strong anticancer activity *in vitro.*
Study model: hPCa xenografted mice (LNCaP – androgen-sensitive cells), 5 weeks of tumor induction, 3 weeks *p.o.* treatment with sunflower oil or Selol (10% LD50) of control-mice (C) or tumor-bearing mice (Tb).
The aim of the work: 1) Assessment of the changes in the total activity of superoxide dismutase (SOD), its isoenzymes and redox state in hPCa tumors and selected mouse organs in the course of androgen-dependent hPCa in 2 variants: without Selol and after treatment with Selol. 2) An attempt to assess the potential use of Selol in hPCa treatment.
Results: 3 weeks of daily administration of Selol results in a 35 % decrease in large-size hPCa tumor mass with high PSA level (mean decrease in mass of all sizes of tumors was 17%). Selol does not change the total SOD activity, decreases Cu,ZnSOD activity while it increases moderately mitochondrial isoenzyme (MnSOD) activity in hPCa tumors. After Selol treatment intracellular redox state (E_h) in tumors increased from -223 mV to -175 mV. A moderate increase in Mn-SOD and strong increase in Eh in hPCa tumors after Selol treatment may facilitate an increase in apoptosis and inhibition of tumor growth, which is confirmed by a decrease in mean tumor mass. The changes in SOD activities in normal liver and brain, which accompany the changes in SOD in cancer tumor, suggest a tumor-organs interaction, and indicate the influence of prostate cancer on the entire organism. Therefore, the therapy should focus not only on the elimination of cancer tumor, but also on restoring redox homeostasis in the whole body.
Conclusions: Selol's multidirectional influence on cancer tumor cells as well as the cells of key organs may contribute to changes in redox state and tumor elimination by enhancing the natural mechanisms of cancer cells eradication.

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Cardiac metabolic substrate preference in the atherosclerotic *ApoE/LDLR* (--/--) mice model

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Heart metabolism is characterized by a high energy demand. Cardiac metabolic substrate preference is coordinated by factors such as the availability of individual substrates or hormonal activity but also could be dysregulated in pathological conditions, such as atherosclerosis. *ApoE/LDLR* (--/--) mice are excellent model to study not only vascular alterations or thrombosis but also biochemical changes. This study aimed to characterize changes in cardiac metabolic substrate preference in cardiomyocytes in *ApoE/LDLR* (--/--) mice.
Two groups were included in the study: 6 month old *ApoE/LDLR* (--/--) mice (n=4) and wild type mice (n=4) at the same age. Cardiac metabolism was studied by subcutaneous administration of 1-^{13}C-D-glucose at 1.8 mg/g body weight dose, followed by collection of the heart and blood for LC-MS analysis after 90 minutes. Samples were analyzed by LC-MS to measure ^{13}C glutamate enrichment to estimate carbohydrate vs. fatty acid contribution to cardiac Krebs cycle. Furthermore the free fatty acids concentration in the *ApoE/LDLR* knock-out and control plasma was measured.
Our preliminary studies established that the ^{13}C glucose enrichment in blood was stable at about 50% from 30 min after injection and that cardiac ^{13}C glutamate enrichment increased up to 60 min and then remains stable. ^{13}C glutamate enrichment increased after insulin administration while decrease was noted following iodoacetate administration. ^{13}C glutamateenrichment after 1-^{13}C glucose injection decreased from 4.56±0.75% in wild type to 3.44±0.015% in *ApoE/LDLR* (--/--) mice. Concentration of free fatty acids in plasma of the *ApoE/LDLR* (--/--) mice was 1.98±0.06 mmol/l as compared to 1.31±0.06 mmol / l in control mice.
We conclude that cardiac carbohydrate use is decreased in atherosclerotic *ApoE/LDLR* (--/--) mice. This indicates enhanced use of fatty acids and enhanced oxygen cost of energy production. Increased free fatty acids concentration in plasma of *ApoE/LDLR* (--/--) mice may be key factor in its enhanced use. Our data are consistent with the concept of pharmacological inhibition of fatty acid oxidation to protect heart in the atherosclerosis.

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P11.24

Aortic valve dysfunction in ecto-5'-nucleotidase (CD73) knock-out mice

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Aortic valve stenosis (AS) is one of the most common cardiac defects in humans. The inflammation plays a key role in its pathophysiology. Adenine nucleotides and products of their metabolism, in particular adenosine, have an important role in regulation of inflammation. The decrease in the amount of adenosine may have severe implications, because of its anti-inflammatory and cardioprotective functions. The risk of progression of AS can be intensified by high fat diet. The aim of the study was to investigate the effect of high-fat diet and CD73 knock out (CD73-/-) on the development of aortic valve dysfunction in mice.

Mice were devided into groups: high-fat diet CD73-/-, high-fat diet WT, low-fat diet CD73-/- and low-fat diet Wild Type (WT). Animals were fed a special diets for 15 weeks with weight and blood glucose level monitoring. Then, mice were anesthetized and underwent Doppler ultrasound analysis with the 12 MHz probe for determination of the peak aortic valve flow. Subsequently, the aortic roots were collected and fragments were used for analysis of the extracellular catabolism of adenine nucleotides by incubation in HBSS with the substrates appropriate for ecto-nucleoside triphosphate diphosphohydrolase (eNTPD), ecto-5'-nucleotidase (e5'NT) and ecto-adenosine deaminase (eADA). Fragments of heart were also collected for histological analysis of aortic valves. Results are presented as mean±SEM.

CD73-/- mice were characterized by a lower weight and higher glucose level than WT (27.7±1.4 vs 31.3±2.3g; 156.6±7.5 vs 113.3±7.7 mg/dl). High-fat diet induced an increase in the blood glucose level in both group. High-fat diet caused notable increase in peak aortic flow indicating obstruction in comparison to low-fat diet (3.69±0.32 vs 2.85±0.25m/s). CD73-/- knock out on low-fat diet led to increase in peak aortic flow (3.73±0.21m/s) compared to WT. Highest values of peak aortic flow were observed in CD73-/- mice on high-fat diet (5.04±0.37m/s). Activity of e5'NT in CD73-/- mice was below 20% of WT. Activity of eADA on the aortic surface in the CD73-/- mice was lower compared to WT (0.72±0.14 vs 1.30±0.14 nmol/cm2/min respectively). There were no significant differences in eNTPD activity between both groups. High-fat diet had no considerable impact on the ecto-enzymes activities. There were no excessive accumulation of amorphous matrix, fibrin clots and calcium deposits in the analyzed aortic valves, but changes in their morphology may indicate a development of dysfunction.

Our results indicate that knock out of CD73 leads to aortic stenosis in mice similar to that induced by high-fat diet. Combination of high-fat diet with CD73 knock out leads to most severe valve dysfunction. Increased level of adenosine in pathological valves could be in the future an alternative method of treatment for aortic valve replacement.