Oral Presentations

OL1

Role of kidneys in heme detoxification and iron metabolism during neonatal jaundice – research based on laboratory mouse model

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Neonatal jaundice (neonatal hyperbilirubinemia) is a physiological process caused by degradation of foetal erythrocytes. In humans, it starts in the second day of life and finishes at about day 10. During this short period in the organism of neonate large amounts of bilirubin – product of heme decay - are generated as an effect of haemolysis of foetal erythrocytes. Because of impaired bilirubin clearance, excess of this compound circulates in the body, infiltrates to the skin and mucosa, giving them characteristic, yellow colour. Accumulation of unconjugated bilirubin is highly toxic, because this compound can diffuse through blood-brain barrier and cause damage of brain structures, especially the basal nuclei.

According to the present knowledge, liver and spleen are the main organs, which participate in clearance of haemoglobin degradation products formed during haemolysis. In this project we show that also kidneys are involved in the process of heme detoxification and iron utilization in neonatal individuals.

In our study we used 3, 5, 7, 9 and 11 day-old male mice as a mammalian model organisms. To confirm that tested animals undergo neonatal jaundice and haemolysis we measured bilirubin, haptoglobin and hemopexin levels in plasma. Kidneys were analysed for a wide panel of proteins involved in iron metabolism, including iron importers (DMT1, TfR1), heme transporters (HCP1, HRG1, FLVCR2), heme oxygenase 1 and 2 (HO-1, HO-2) and ferroportin (FPN), iron exporter. In this study we report that heme and iron transporters and heme oxygenase 1 are highly expressed in the cells of the renal tubules. Our results suggest that kidneys may play an important role in postpartum iron recirculation when the metabolism of this element depends primarily on iron stored in the liver.

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OL2

Homocysteine-induced changes in iron metabolism are mediated by Akt kinases and transcription factor FOXO3a in endothelial cells

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Hyperhomocysteinemia is an independent risk factor for cardiovascular diseases such as ischemic heart disease, stroke, peripheral vascular disease, atherosclerosis as well as dementia, brain atrophy and others. However, molecular mechanism of homocysteine (Hcy) action has not been well understood. There is evidence that Hcy toxicity is mediated by iron ions. The main goal of the study was to evaluate the effects of Hcy on iron metabolism in HUVEC cells. We observed that 3 mM Hcy induced upregulation of mRNA and protein level of ferritin L and H in HUVEC cells in a time-dependent manner. The change in ferritin expression was preceded by a significant decrease in the cellular level of the active form of Akt kinase (P-Akt). Thus, we hypothesized that the observed changes in iron metabolism could be caused by the impairment of insulin signaling pathways involving Akt kinases. The role of Akt in modulation of ferritin level was confirmed in cells with the expression of Akt silenced by specific siRNA species (Akt1, Akt2 and Akt3), which led to an increase in ferritin protein level. In addition to that, Hcy-induced increase in ferritin protein level was attenuated by insulin. Moreover, in the cells transfected with siRNA against FOXO3a a decrease in mRNA and protein level for ferritin L and H was observed. Cytotoxicity of hydrogen peroxide was significantly decreased in cells pre-treated with Hcy. In conclusion, these results clearly indicate that impairment of Akt signaling pathway and subsequent activation of the transcription factor FOXO3a leads to adaptive changes manifested by an increase in the expression of ferritin L and H.

OL3

Interrelations between vitamin A and iron – modeled and analyzed using Petri nets

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Vitamin A and iron deficiency are major nutritional problems that collectively affect billions of people worldwide. The influence of vitamin A deficiency on body iron status have been widely studied, however, the mechanism of the relationship between vitamin A and iron metabolism remains unclear. The results of the recent studies demonstrate that the HJV-BMP6-SMAD signaling pathway, that normally activates the expression of hepcidin in a course of the iron deficiency, is impaired by vitamin A deficiency. To better understand this complex biological phenomenon and to systematize the existing knowledge, a Petri net based model of this complex issue has been created and then analyzed. This analysis has been based on t-invariants and MCT sets. It allowed to determine a biological meaning of components of the modeled system and has enabled for an in-depth analysis of the studied phenomenon.

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OL4

Cardiac involvement in hereditary hemochromatosis – what we know today?

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Hemochromatosis is a clinical syndrome caused by abnormal accumulation of iron in parenchymal organs. Iron overload syndromes are heterogenous and may be hereditary or acquired. Hereditary hemochromatosis (HC) is a very common inherited disease, based in more than 80% on *HFE* gene mutation. Abnormally increased intestinal iron absorption and accelerated recycling of iron by macrophages lead to progressive body iron accumulation and the generation of oxidative stress in tissues. Iron overload of the heart can lead to the left ventricular (LV) dysfunction. Cardiac hemochromatosis is initially characterized by diastolic dysfunction and arrhythmias and in later stages by dilated cardiomyopathy. Patients should undergo comprehensive 2D and Dopler echocardiography to evaluate their systolic and diastolic function. Newer modalities like *speckle-tracking* echocardiography hold promise for earlier detection of cardiac involvement. Cardiac magnetic resonance imaging with measurement of *T2 relaxation* times can help quantify myocardial iron overload. These diagnostic methods seem to be clinically important in the early stages of hereditary HC before a substantial damage of the LV.

KL1

Iron, infection and immunity

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Iron is required for the growth of almost all infectious organisms but is also needed for host immune function. The iron regulatory hormone hepcidin controls both total body iron levels and the distribution of iron. Hepcidin expression is regulated by the balance of several signals, chief among them being iron status, inflammation, and erythropoietic drive. Interestingly, iron appears to be the only nutrient that is controlled by a hormone that responds both to nutrient levels and to infection, underscoring the importance of iron in host-pathogen interactions. Furthermore, hepcidin is evolutionarily related to microbicidal defensins that target bacteria and yeast infections. Here, I will discuss the role of hepcidin and iron in infectious diseases and the immune response. Emerging evidence is revealing marked heterogeneity in how hepcidin is regulated during different types of infection, and the effect of hepcidin and altered iron distribution on the progression of infections is also highly variable. One of the best-studied infections in this field is malaria. Epidemiological evidence in humans shows that iron is a critical determinant for the outcome of malaria, and experiments in mice show that hepcidin controls growth of the liver-stage of *Plasmodium* infection. Ongoing work is examining how iron availability and hepcidin influence the *Plasmodium* blood-stage, development and recovery from anaemia, and malarial transmission. The innate immune response to most infections (including malaria) involves an acute and profound hepcidin-mediated decrease in serum iron levels. Furthermore iron deficiency is the most common micronutrient deficiency worldwide; recent genetic evidence links lack of iron acquisition by lymphocytes from serum to severe immunodeficiency. Therefore, a currently underappreciated important aspect of iron and hepcidin in the context of infection is that iron levels may directly regulate the adaptive immune response.

OL5

Molecular chaperones involved in Fe/S biogenesis

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Iron–sulfur (Fe/S) clusters are important cofactors of numerous proteins involved in electron transfer, metabolic and regulatory processes. In eukaryotic cells, known Fe/S proteins are located within mitochondria, the nucleus and the cytosol. Over the past years the molecular basis of Fe/S cluster synthesis and incorporation into apoproteins in a living cell has started to become elucidated. Biogenesis of these simple inorganic cofactors is surprisingly complex and, in eukaryotes such as *Saccharomyces cerevisiae*, is accomplished by three distinct proteinaceous machineries. The 'iron–sulfur cluster (ISC) assembly machinery' of mitochondria was inherited from the bacterial ancestor of mitochondria. ISC components are conserved in eukaryotes from yeast to man. The key principle of biosynthesis is the assembly of the Fe/S cluster on a scaffold protein before it is transferred to target apoproteins. The chaperone machinery, which participate in transfer of synthesized Fe-S clusters from scaffold protein Isu to an apo-protein, include mitochondrial Hsp70 protein, the J-domain protein Jac1 and the nucleotide release factor Mge1 protein. Jac1 facilitates the interaction between Hsp70 and Isu, by first binding and targeting scaffold protein to the Hsp70 and subsequently stimulating its ATPase activity. The biochemical properties of Isu interactions with above described proteins, functioning in mitochondrial stage of Fe-S clusters biogenesis are still unrevealed.

OL6

Effect of daily alcohol and caffeine intake on serum hepcidin levels in healthy men

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There is clear evidence that caffeine drinking limits the absorption of nonhaem iron and alcohol increases body iron stores. However, there are few studies which have assessed the influence of caffeine and alcohol on indicators of iron status. We aimed to investigate the associations between daily caffeine and alcohol intake and iron metabolism parameters.
The study enrolled 88 men ($x \pm$ S.D. age: 24.22 ± 5.26 y; range: 22–40 y). Fasting blood samples were collected. ELISA kits were used to determine ferritin, soluble transferrin receptor, hepcidin, hemojuvelin, erythropoietin and erythrofferone and C-reactive protein levels. Caffeine and alcohol consumption were evaluated by using a food-frequency questionnaire. Participants were then divided into heavy drinkers (daily ethanol consumption >40 g/day n=23), moderate drinkers (daily ethanol consumption between 1 and 40 g/day; n=40) and abstainers (n=25).
Heavy alcohol drinkers had significantly higher hepcidin (Me=8.49 ng/l, IQR=5.26 ng/l) and ferritin (Me=57.36 mg/l, IQR=72.11 mg/l) when compared to both abstainers (hepcidin: Me=4.02 ng/l, IQR=3.31 ng/l; p=0.0002; ferritin: Me=25.58 mg/l, IQR=20.53 mg/l; p=0.0017) and moderate drinkers (hepcidin: Me= 5.28 ng/l, IQR=4.18 ng/l; p=0.0006; ferritin: Me=25.33 mg/l, IQR=25.02 mg/l; p=0.0053). Lower CRP levels were observed in moderate drinkers (Me=0.24 ng/l, IQR=0.26 ng/l) when compared to abstainers (Me=0.77 ng/l, IQR=0.88 ng/l; p<0.0001) and heavy drinkers (Me=0.98 ng/l, IQR=1.32 ng/l; p=0.0003) and abstainers did not differ significantly in CRP levels from heavy drinkers (p=0.9998). Caffeine consumption correlated positively with hepcidin (r=0.516, p=0.001), ferritin (r=0.281, p=0.008) and hsCRP (r=0.508, p=0.001).
A relation may exist between high alcohol and caffeine consumption and increased inflammation process. This relation could explain, in part, the effect of alcohol and caffeine intake on iron metabolism.

OL7

The role of NCOA4 in ferritinophagy and ferroptosis

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Ferritin stores and detoxifies cellular iron by sequestering up to 4000 Fe atoms in its large cavity. The recycling of this iron is crucial and it involves the Nuclear Receptor Coactivator 4 (NCOA4), that acts as a selective cargo-receptor mediating ferritin autophagy (ferritinophagy). NCOA4 is a 70 kDa protein of 614 amino acids, predominantly localized in the cytoplasm, involved in regulation of nuclear hormone receptors, microtubule activity, transcription and ferritinophagy.
We cloned and expressed in *E. coli* the recombinant NCOA4 domain (383-522aa), known to be involved in NCOA4-Ferritin interaction, for its biochemical characterization. In an electrophoretic mobility shift assay and an ELISA, NCOA4 (383-522) showed specific binding to H-ferritin forming highly stable complex, whereas it did not bind to the L ferritin and Arg23Ala H-mutant.
In a second line of experiments, we modified NCOA4 expression in HeLa cells. As expected, the knocking-out of NCOA4 with CRISPR/Cas9 technology and overexpression of the full-length human and mouse NCOA4 altered the level of ferritin and ferritin iron. These cell lines allowed us to analyze the role of NCOA4 in ferroptosis, a programmed cell death unrelated to caspases that is dependent on iron availability and reactive oxygen species. By inducing ferroptosis with erastin and RSL3, compounds that alter cytosolic redox status, we found that NCOA4 knocking-out protected HeLa cells from ferroptosis, while NCOA4 overexpression made them more sensitive.
In conclusion, we found that NCOA4 domain(383-522) binds strongly the H-, but not the L-ferritin and the R23A-H-mutant. Moreover, the level of NCOA4 expression controls cytosolic ferritin abundancy and iron availability. The data support the hypothesis that NCOA4 has an important role in the regulation of ferritin and cytosolic iron homeostasis.

OL8

Insulin signaling and iron metabolism in skeletal muscle of transgenic animals bearing SOD1 G93A

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In the present study, we studied the role of impaired Akt signaling pathways and iron metabolism in skeletal muscle of transgenic animals bearing human mutated *SOD1* gene, which is an animal model of Amyotrophic Lateral Sclerosis. So far, the results of our preliminary studies indicate, that an increase in iron and ferritin concentrations in skeletal muscle of transgenic animals occurs well before the first symptoms of the disease, such as limb paralysis and muscle degeneration. Interestingly, we have also shown that ferritin undergoes increased ubiquitination, accompanied by iron-dependent oxidative stress. The results of these studies suggest that the disorder of iron metabolism may be the cause of disease, rather than a consequence of it.
Thus, we hypothesized that overexpression of SOD1 G93A in cell lines C2C12 and SH-SY5Y leads to disruption of the IGF/insulin signaling pathway, which can induce phenotypic changes manifested by an increase in the import of iron into the muscle cells.
So far, we showed that significant decrease in P-Akt level and changes in iron metabolism were already observed in pre-symptomatic animals. This was accompanied by an increase of active form of FOXO3a and upregulation of atrogin-1 and catalase. Increase in ferritin L and H was accompanied by rise in PCBP1 and APP proteins. In SH-SY5Y cells stable expressing SOD1 or SOD1 G93A elevated level of ferritin L and H has been observed. Interestingly, insulin or IGF-1 treatment significant downregulated ferritin L, H, and PCBP1 proteins in these cells. Conversely, cells transfected with siRNA against Akt 1, 2, 3 respectively showed significant increase in both forms of ferritin. To assess the role of FOXO3a in ferritin expression, we generated a line of SH-SY5Y cells that express a fusion protein consisting of FOXO3a fused at its COOH terminus to the ligand-binding domain of the estrogen receptor (TM-ER) which is activated by 4-hydroxytamoxifen (4OHT). Treatment of this cells with 4OHT significantly upregulated ferritin H protein level. In conclusion, our data suggest that impairment of iron metabolism in transgenic animal bearing SOD1 G93A may be caused by changes in Akt/FOXO3a signaling pathways.

OL9

Kinetics of interaction between protein involved in the biogenesis of iron-sulfur clusters

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Iron–sulfur (FeS) clusters are prosthetic groups present in proteins of organisms in all kingdoms. They are crucial for proper functioning of many pathways like those responsible for electron transfer, redox reactions, DNA synthesis and repair, ribosome assembly and cell metabolism regulation. In eukaryotic cells main Fe/S assembly and transfer machinery is located within mitochondria, inherited from bacterial ancestors and is highly conserved. It is called "iron-sulfur cluster (ISC) assembly machinery and its central point is the Isu1 protein scaffold. On this scaffold FeS clusters are synthesized when it is in complex with other dedicated proteins like Nfs1 cysteine desulfurase and Yfh1 yeast frataxin homologue. After synthesis of the cluster, it is transferred from Isu1 scaffold to target apoproteins by a system of mediators, of which most important is the chaperone machinery composed of mitochondrial Hsp70 protein, the J-domain protein Jac1 and the nucleotide release factor Mge1 protein. The kinetics of Isu1 interactions with above described proteins, are still not known and in our research, we want to describe those kinetics. We will use Bio-layer interferometry technique as it enables us to measure in real time the rates of association and dissociation of proteins to other components of FeS assembly and transfer machinery.

OL10

Characterization of iron metabolism-related genes in the Jackson Laboratory toxic milk mouse model of Wilson disease

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Wilson disease (WD) is an autosomal disorder of copper metabolism caused by mutations in the *ATP7B* gene and leading to the toxic accumulation of copper in the liver and brain. As a result of mutation, copper cannot be transported into the Golgi apparatus, where it is incorporated into apo-ceruloplasmin and released to the serum. Ceruloplasmin (Cp) is a copper-dependent enzyme possesing ferroxidase acivity, and thus provides a link between copper and iron metabolisms. Disturbances in Cp expression strongly affect iron homeostasis. Four rodent models with *ATP7B* gene defects have been described and one of them is the Jackson Laboratory toxic milk (tx-j) mutant mice (*Atp7b^{tx-J}*), not characterized yet in the context of interactions between Cu and Fe. Our results show decreased level of Cp and iron concentration in the serum of 6-month old mutant mice. Moreover, elevated non-heme iron content in the liver, increased expression of hepcidin (with no effect on ferroportin protein level) and associated upregulation of membrane-bound splice variant of Cp have been observed. Disturbances of iron metabolism-related genes observed in mouse model of Wilson disease (*Atp7b^{tx-J}*) can improve our understanding of complex interplay between copper and iron metabolisms.

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OL11

Nordic Walking training reduced oxidative stress and induced changes in iron metabolism in elderly people

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Regular exercise is applied in order to reduce risk of several morbidities, however its influence on body iron stores in elderly people remains unknown. At the same time, body iron accumulation is associated with high risk of acute myocardial infarction, diabetes and cancer.
Thus, the main goal of this study was to evaluate the influence of Nordic Walking training on lipid profile, oxidative stress, iron metabolism and physical fitness level.
A group of 35 elderly women participated in the study. All of them were older than 60 years (68±5.12 years old). All of the subjects underwent a medical check-up prior to the experiment.
The applied training program resulted in a significant change in iron metabolism. Significant decrease in blood iron and ferritin concentration was observed. All the components of physical fitness improved. Interestingly, a negative correlation between the blood ferritin and endurance test was recorded ($r=-0.34$, $p=0.03$). A statistically significant decreasing of malondialdehyde level (mean change=–21.52; 95% CI –36.64 to –6.39) and concentration of advanced oxidation products (mean change = –8.32; 95% CI –14.22 to –2.43) over time of follow-up was observed. Analyses of lipid profile show the only change in high density lipoprotein which was significantly higher after the training (mean change=4.51; 95% CI 1.47–7.56).
Nordic Walking training applied in elderly people significantly reduced levels of oxidative stress and this was accompanied by the drop-in body iron stores. It can be speculated that lowering iron stores leads to less iron-dependent ROS formation.

OL12

Serum levels of pro-hepcidin are higher in patients with Parkinson's disease treated with Deep Brain Stimulation

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Hepcidin is a critical hormone responsible for the systemic metabolism of iron and simultaneously belongs to protein mediators of the acute inflammatory response primarily induced in response to interleukin 6. It can therefore be regarded as a link between oxidative stress processes, where iron plays an important role, and the processes of neuroinflammation – both considered as responsible for neurodegeneration in Parkinson's disease (PD).
The study assessed serum pro-hepcidin levels in patients with PD (52 patients) and in the control group (31 healthy volunteers, of age and sex matched and similar to the PD group, without evidence of neurodegenerative disorder in their family history). 37 patients with PD were treated only pharmacologically, whereas 15 were treated with DBS. The exclusion criteria of the study were anemia and co-existing diseases of possible inflammatory etiology. Peripheral blood samples received from patient's with PD and control group were collected and stored frozen with a clot activator at temperature −80°C, in 4 ml serum separation tubes. Quantitative measurements of serum samples received from both groups were assessed with the use of enzyme-linked immunosorbent assay (ELISA) kits for Hepcidin Prohormone *(DRG)* examination.
In the subgroup of patients with Parkinson's disease treated with Deep Brain Stimulation the serum concentration of pro-hepcidin was significantly higher compared to the control group ($p<0.001$), as well as compared to the group of patients with Parkinson's disease treated farmacologically ($p=0.02$). The obtained results suggest possible damaging role of prolongated high freqency stimulation and the implantation of the electrodes into the brain tissue of the host, in the form of incresaed production of inflammatory mediators, associated with activation of the astroglia and microglia.

OL13

Unbiased approaches to decipher genetic control of iron homeostasis

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Adequate body iron supplies are orchestrated by the hepcidin-ferroportin-transferrin axis. But human genetic studies suggest that additional mechanisms contribute to regulation of iron homeostasis. The main objective of our research is to better understand the molecular control of iron homeostasis. To this end we have previously designed and conducted unbiased large-scale RNAi screens for transcriptional regulators of hepcidin, the key liver hormone that adjusts iron availability to body iron requirements. This work demonstrated that hepcidin suppression is linked to the control of mitogen stimulation and nutrient status via components of Ras/RAF MAPK and mTOR signaling. Another follow-up analyses of the screening hits led us to identify a commonly used anti-hypertensive drug, spironolactone, as well as imatinib, a first-line, lifelong therapeutic option for some frequent cancer types as hepcidin-suppressive agents. We expect these results to be of relevance for patient management, which needs to be addressed in prospective clinical studies.

KL2

Regulation of iron homeostasis by the hepcidin/ferroportin system

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Imbalances of iron homeostasis account for some of the most common human diseases. Pathologies result from both, iron deficiency or overload and frequently affect the hepcidin/ferroportin regulatory system that balances systemic iron metabolism. The small hepatic peptide hormone hepcidin orchestrates systemic iron fluxes and controls plasma iron levels by binding to the iron exporter ferroportin on the surface of iron releasing cells, triggering its degradation and hence reducing iron transfer to transferrin. Hepcidin thus maintains transferrin saturation at physiological levels assuring adequate iron supplies to all cell types.
My presentation will focus on mechanisms that control hepcidin and ferroportin expression as well as on pathologies that arise when this key regulatory circuitry underlying systemic iron homeostasis is disrupted.

OL14

Copper transport in spermatogenesis

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Copper is a trace element essential for normal metabolic processes in every living cells and is necessary for normal growth and development for all the living organisms. Due to its ability for electrons transfer, copper is a cofactor in enzymatic proteins, which play an important role in key metabolic processes as cellular respiration, free radicals detoxification, neurotransmitters synthesis or iron metabolism. Disturbances in copper homeostasis lead to highly destructive processes in the organism. Copper deficiency results in decrease or lack of the activity of many enzymes but copper overload can be also dangerous because active copper ions are generators of free radicals in the cells. For many decades copper has been identified as a highly toxic element for spermatozoa but investigation of the last years showed that copper is also essential for the process of spermatogenesis. Our results shown that in the testis during the process of gametogenesis copper uptake, transport and excretion are precisely controlled by proteins involved in copper metabolism. In the seminiferous tubules copper uptake by germinal and Sertoli cells is mediated by membrane copper transporter CTR1 protein, which is highly expressed in spermatogonia, through preleptotene and leptotene spermatocytes till pachytene spermatocytes. Obtained results indicate that during the spermatogenesis, ATP7A and ATP7B Cu-Transporting ATPases play an important role in maintaining of copper homeostasis. Premeiotic germinal cells (preleptotene and leptotene primary spermatocytes) are protected from copper excess by ATP7A protein. While ATP7B protein expression was found in Sertoli cells and near to elongating spermatids, thus its function seems to be related with regulation of copper concentration during the process of spermiogenesis.

OL15

Petri net based approach to modeling and analysis of macrophage participation in iron metabolism

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Macrophages are important to immune function and also play an essential role in the regulation of iron homeostasis. Clearance of senescent/damaged red blood cells is the major function of macrophages involved in erythrophagocytosis in spleen red pulp and liver. These cells recycle iron derived from hemoglobin catabolism through the ferroportin. During inflammation, pro-inflammatory macrophages (M1) sequester iron into ferritin to reduce iron availability to pathogens. On the other hand, alternatively activated macrophages (M2), which are involved in inflammation resolution and repair of the tissues, internalizes iron, that afterwards is exported by ferroportin. In addition to their distinct roles in the inflammation, differently polarized macrophage populations appear to have specific functions in other pathological settings, such as obesity and atherosclerosis. In these phenomena a switch between M2 into M1 is observed, what leads to iron-retention, increasing inflammation and oxidative stress. In our study, using Petri nets theory, we have created a model that reflects the relationships between macrophages populations, central regulators of iron balance during inflammation. The analysis of the model has been based on t-invariants. They correspond to subprocesses which do not change the state of the modeled biological system. The analysis allowed for a more complete understanding of the studied process.

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OL16

Evaluation of iron content in skeletal muscles of patients with hereditary hemochromatosis

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Hereditary hemochromatosis is one of the most frequent inherited diseases among Caucasians. In more than 80% of cases it is caused by HFE gene mutations which are responsible for pathological iron accumulation in parenchymal organs and their progressive damage. The diagnosis of this disease in a phase of early and partially reversible pathology may be difficult due to subtle and nonspecific symptoms. Patients frequently complain of generalized muscle weakness which has been associated with endocrinopathy. However, potential iron accumulation in muscles can lead to the reduced exercise capacity. The aim of the study was the evaluation of iron content in skeletal muscles of patients with early diagnosis of hereditary hemochromatosis.
Fifteen patients with the diagnosis of hereditary hemochromatosis (HH), mean age 45±15.18 years (age range 18–65 years) were enrolled to the study and compared to fifteen healthy controls. Ten patients were carriers of C282Y homozygous mutations, four were H63D homozygotes and in one patient combined C282Y/H63D heterozygous mutation was detected. All subjects presented abnormal, increased serum iron concentrations (mean value 208±39 mcg/dl) and increased transferrin saturation (mean value 73±22%), mean ferritin concentration was 650±315 ng/ml. In all cases, T2* mapping by MRI (MyoMaps application on Siemens Aera 1,5T scanner, Siemens AG„ Germany) was performed to assess for myocardial and liver iron overload. T2* maps were also assessed for skeletal muscle T2* values and compared to skeletal muscle T2* of healthy controls. All T2* measurements were performed in regions of interest (ROIs) with low residual error (<10%) as assessed by the Segment software (Medviso AB, Sweden). Statistical analysis was done using STATISTICA data analysis software, version 12 (StatSoft Inc., USA). All statistical data are presented as a mean ± standard deviation (median). Analysis of differences between variables was done by nonparametric statistics: U Mann-Whitney's test. The *p*-value less than 0.05 was considered as being significant. No unequivocal cases of cardiac iron deposits were identified in the study group, while two of the patients had myocardial T2* values below the reference range. While the control group was younger, T2* values showed no significant correlation with age in both groups ($p>0.05$ for all ROIs). and it confirmed the presence of iron deposits in the liver in the majority. T2* values in ROIs placed over the latissimus dorsi (LD) and pectoralis major (PM) muscles in HH patients were significantly lower compared to controls [T2* LD ROI for HH *vs* control: 26.62±2.86 ms *vs* 28.93±1.94 ms; $p=0.016$], [T2* PM ROI for HH *vs* control: 24.42±4.26 ms *vs* 29.04±1.64 ms; $p=0.001$], [T2* LD&PM ROI for HH *vs* control: 25.52±1.99 ms *vs* 28.98±1.67 ms; $p<0.001$].
Our results indicate higher iron content in skeletal muscles of HH patients. The assessment of skeletal muscle T2* values provides additional insight in muscle tissue involvement, may explain muscle weakness and fatigue observed in some, and potentially guide management decisions. Further investigation is warranted in larger patient cohorts.

OL17

Disorders of iron homeostasis in non-alcoholic fatty liver disease – what does a clinician want to know from a biologist?

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Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver pathology in modern world. NAFLD is now estimated to affect one billion people worldwide, and the number is predicted to be rapidly growing in the coming years. The presence of NAFLD is tightly linked with metabolic syndrome and insulin resistance. Additionally excessive accumulation of iron, diagnosed in 30% of NAFLD patients, is a serious risk factor for disease progression to cirrhosis and hepatocellular carcinoma. It is now widely recognized that body iron imbalance is tightly linked with pathogenesis of NAFLD, and that iron metabolism may appear a potential therapeutic target in this condition. Still however, mechanisms underlying association of iron metabolism with disease progression as well as pathways leading to excessive iron accumulation in NAFLD remain largely understudied. Authors summarize the current understanding of the role of iron in NAFLD.

OL18

Regulation of ferroportin expression by hepatic iron and hepcidin in pregnant females and their fetuses in response to normal and iron-deficient diet

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Iron deficiency anemia during pregnancy ravages both maternal and fetal health. Although the regulation of iron homeostasis during pregnancy still remains largely unexplored, it seems that it is directed to maintain fetal iron status at the expense of mother iron needs.

In our study we examined the impact of dietary iron deficiency on hepatic maternal and fetal iron homeostasis in mice. We compared the influence of changes in hepatic iron content and hepcidin expression on ferroportin protein level in livers of both pregnant females and their fetuses.

Mouse females were fed a control or low iron diet for 2 weeks prior to and during pregnancy. Livers from mothers and fetuses were collected at day 18 of gestation for analysis of iron content and the expression of iron metabolism genes.

Exposure of females to iron-deficient diet resulted in the development of severe anemia as evidenced by the decrease in RBC indices and plasma iron parameters. Dietary iron restriction induced considerable decreases in hepatic maternal (5-fold) and fetal (2.5-fold) iron content. Hepatic hepcidin mRNA level in mothers receiving control diet was 23-fold as high as in fetuses. Under iron deficiency hepcidin mRNA was 5-fold decreased in females but was still higher compared with iron-replete fetuses. Iron-deficient fetuses showed residual hepcidin expression. Basal hepatic ferroportin protein level was higher in fetuses compared with mothers. Under iron deficiency it was strongly down-regulated in fetuses and to a much lesser degree in mothers. Our results suggest that hepatic iron outcompetes hepcidin in regulating ferroportin expression in livers of pregnant females and their fetuses in response to dietary iron restriction.

OL19

Iron nanoparticles: hope for treating neonatal iron deficiency anemia in piglets

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Iron deficiency anemia (IDA) has been recognized as a serious iron disorder in piglets. Both hepatic iron reserves and sow's milk are largely insufficient sources to meet iron requirements for erythropoiesis in suckling piglets. Moreover, the molecular machinery responsible for iron absorption in newborn piglets is not fully developed To counteract the development of IDA, supplementation with an exogenous iron must be applied to piglets. Intramuscular administration of large amounts of iron dextran on days 3–6 postpartum is a current practice in the swine industry. However, high parenteral intake of supplemental iron may easily perturb control of systemic iron metabolic processes through the rise of hepcidin level, which is known as key iron metabolism regulatory protein. With the development of nanotechnology, nanoparticles have shown application prospects since 1970s. Nanoparticles are ferrofluids consisting of an aqueous dispersion of magnetic iron oxides with diameters from 50 to 200 nm. Iron nanoparticles (IONPs) have become a powerful tool for several applications in various industry including medicine when may be used as a potential drug or gene carrier. The second promising supplement in curing IDA in piglets is Sucrosomial® Iron. It is combination of ferric pyrophosphate covered by a phospholipids plus sucrose esters of fatty acids matrix. Sucrosomial® Iron (SI) and IONPs have been shown to be directly transported into the bloodstream across enteric mucosa and thus, seem to be a promising compounds for the treatment of IDA in piglets. Based on available data and our pilot study performed on Polish Landrace piglets aged from 3 to 28 days, and compare with intramuscular injection of iron dextran showing high IONPs bioavailability, lack of their accumulation and toxicity in tissues and finally considering the evidence of their capacity to cross biological membrane it appears as potential tools for IDA treatment in neonates piglets.

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OL20

Glucose and fructose supplementation abrogates exercise-induced increase in hepcidin in young men

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Recently it has been reported that changes in gluconeogenesis may influence iron metabolism. Some kind of exercise can stimulate gluconeogenesis and possible through this influence iron metabolism. Thus, one of the goals of this study was to evaluate the effects of simple sugar supplementation, which can inhibit gluconeogenesis, on blood hepcidin level after exercise. A group of 17 physically active young men completed an incremental exercise test before and after a 3-day diet supplemented with fructose (4 g/kg BM) or glucose (4 g/kg BM). After a 1-week break, they crossed over to the alternate mode for the subsequent 3-days period. Venous blood samples were collected before and after 1 h exercise and were analysed for serum hepcidin, IL-6, CRP, iron, and ferritin. The physiological response to exercise was also determined.
The concentration of hepcidin increased 1 h after exercise for the baseline test ($p<0.05$), whereas no changes in hepcidin were observed in men whose diet was supplemented with fructose or glucose. Blood IL-6 increased significantly after exercise only in subjects supplemented with fructose. Changes in hepcidin did not correlate with shifts in serum IL-6.
At present, many young people experience too much body iron accumulation. The reason of this phenomenon is not clear. There is accumulating evidences that not proper diet and lack of exercise could be a main contributing factors. Our data suggest that protective effects of exercise on excess iron accumulation in human body which is mediated by hepcidin can be abrogated by high sugar consumption which is typical for contemporary people.

KL3

Hepcidin: from discovery to therapeutics

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Iron is an essential biometal employed by almost all cells as a cofactor for fundamental biochemical activities.The molecular circuits that achieve iron balance, both at the cellular and systemic levels, begin to be well characterized.
At the systemic level, body iron homeostasis depends on the iron-dependent hormone hepcidin, a 25-aminoacid peptide produced mainly by the liver, that allows iron adaptation according to the body iron needs. The circulating peptide acts to limit gastrointestinal iron absorption and serum iron by binding to ferroportin, a transmembrane iron exporter, thereby inducing its internalization and subsequent degradation, leading to decreased export of cellular iron. Conversely, complete hepcidin deficiency, as well as liver specific hepcidin deficiency, in mice leads to progressive iron accumulation with predominant iron overload in tissues and iron sparing of the macrophages. Conversely, transgenic animals constitutively expressing the hepcidin gene display iron deficiency anemia.
Different aspects of hepcidin will be considered in this presentation, from the discovery of its function in mice, to its dysregulation in the pathogenesis of a spectrum of iron disorders.
The emergence of hepcidin as the pathogenic factor in most systemic iron disorders should provide important opportunities for improving their diagnosis and treatment. If further investigations are awaited concerning the molecular regulation and interaction of hepcidin and ferroportin to expand our understanding of iron disorders, there is no doubt that targeting the hepcidin-ferroportin axis constitutes an interesting alternative therapeutic for human application.

OL21

Role of duodenal cytochrome b (CYBRD1) in iron homeostatis and disease outcome in chronic hepatitis C patients

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Chronic hepatitis C (CHC) patients often exhibits iron overload phenotype, which is recognized as a negative prognostic factor of the disease. Duodenal cytochrome *b* (CYBRD1) is a ferroreductase involved in iron absorption by duodenal enterocytes, and its expression in the intestine is regulated by iron level in the organism. A single nucleotide polymorphism (SNP) rs884409 in *CYBRD1* is implicated in the pathogenesis of hemochromatosis. In this study we analyzed the impact of the *CYBRD1* polymorphism and its expression on iron overload and disease outcome in CHC patients.
A total of 243 patients with CHC were enrolled in the study. Liver biopsy specimens and blood samples were collected from all individuals. Genotyping and analysis of gene expression of the hepatic CYBRD1 were performed. Iron deposits in hepatocytes, serum markers of iron overload, and expression profile of gene-regulators of iron homeostasis were analyzed.
The single nucleotide variant rs884409 G is associated with elevated serum iron levels, increased markers of liver inflammation, and oxidative stress. Hepatic expression of *CYBRD1* is associated with the expression of *TFR2*, *ID1*, and *HO-1* genes, serum ferritin levels, and with iron accumulation in the liver. Moreover, carriers of variant rs884409 G allele are at increased risk for hepatocellular carcinoma development (age and sex adjusted TT *vs* GG, GT: OR=0.27, CI 95%=0.09–0.85, *P*=0.024).
The results of this study provide evidence for *CYBRD1* involvement in iron homeostasis and disease outcome of CHC.

OL22

Iron and the immune response in chronic hepatitis C

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Iron overload in chronic hepatitis C (CHC) is associated with unfavorable disease course and a risk of carcinogenesis. Its etiology and relationship with the immune response in CHC are not fully explained. Single-nucleotide polymorphisms (SNPs) within DNA region containing interferon lambda 3 (*IFNL3*) and *IFNL4* genes are prognostic factors of treatment response in CHC.

Our aim was to determine if *IFNL* polymorphisms in CHC patients associate with body iron indices, and if there is a relationship between iron and hepatic expression of genes involved in different immune response signaling pathways.

For 192 CHC patients four SNPs within *IFNL3-IFNL4* region (rs12979860, rs368234815, rs8099917, rs12980275) were genotyped. In all patients liver function tests and iron parameters in serum were analysed. Histopathological examinations of liver biopsy specimens was performed in 185 patients. Expression of 5 mRNAs and 6 long non-coding RNAs (lncRNAs) was determined with qRT-PCR in 105 liver samples.

Rs12979860 TT or rs8099917 GG genotypes and markers of iron overload associated with higher activity of gamma-glutamyl transpeptidase and liver steatosis. The presence of two minor alleles in any of the tested SNPs predisposed to abnormally high serum iron concentration, and correlated with higher hepatic expression of lncRNA NRIR. On the other hand, homozygosity in any major allele associated with higher viral load and viral clearance after Peg-IF +ribavirin therapy. Patients bearing rs12979860 CC genotype had lower hepatic expression of hepcidin (*HAMP*) (P=0.03). *HAMP* mRNA level positively correlated with serum iron indices and degree of hepatocyte iron deposits. Additionally, serum ferritin correlated with hepatic expression of genes involved in Th2 type of response (IL4R, TH2-LCR lncRNA) and IFN signaling (lncCMPK2, RSAD2), while serum iron level associated with Th1 and Th17-specific gene expression (AC096579.7, IFNg, lnc-DC). Expression of type Th1-specific, but not Th2-specific genes, associated with ALT, AST, GGTP levels in serum as well as with fibrosis, necroinflammatory activity and steatosis in liver biopsy samples.

Dysregulation of iron metabolism in CHC is linked with immune response to viral infection. *IFNL* polymorphisms influence regulatory pathways of cellular response to IFN, and affect body iron balance in chronic HCV infection. Further research is needed to confirm if ferritin and iron levels link with distinct immunological polarization pathways and if they could possibly serve as indicators of immune status in CHC patients.