ACTA PHYSIOLOGICA

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ABSTRACTS

FEPS Keynote lecture

KNL

The developing pain system: a lifelong story

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Pain in infancy influences pain reactivity in later life, but how and why this occurs is poorly understood. In this lecture, I will review the evidence for the long term effects of pain in early life upon the developing central nervous system from both clinical and laboratory studies. The peripheral and central mechanisms that underlie this developmental pain plasticity will also be discussed. Our research on human infants in intensive care and on laboratory rat pup models have demonstrated that while basic nociceptive pathways are functional at birth, the endogenous control systems in the central nervous system that normally modulate pain experience do not develop until the postnatal period. We propose that it is these control systems that are vulnerable to early life pain experience. This area of research is of fundamental importance for the treatment of pain in infancy and in understanding how individual pain phenotypes are shaped in early life.

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FEPS Plenary lectures

PL-1

The physiological functions of astrocytes is controlled by the sleep-wake cycle

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In vivo awake 2-photon imaging of astrocytes has in recent years turned the field of neuroglia signalling upside-down. Recent data has shown that astrocytic calcium signalling is not initiated by glutamatergic transmission but rather by alpha1 adrenergic receptors. Astrocytes also drive exchange of cerebrospinal fluid with interstitial fluid, but only during sleep. The new and rapid development in the field would be discussed.

PL-2

Towards a 4-dimensional analysis of vascular growth

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We are interested in the development, function and pathologies of the vascular systems. Like the neuronal system, the vascular systems function in tissue space, hence a comprehensive 3-dimensional interrogation of their structure and development is paramount to understanding vascular biology.

A few years ago, we started to use Ultramicroscopy to visualize both the developing blood and lymphatic systems in intact wholemount-stained midgestation mouse fetuses. This analysis revealed that lymphatic endothelial progenitor cells emerge from the largest fetal venous vessel, the cardinal vein, as streams of connected but non-lumenized cells. Our analysis not only resolved a long standing dispute on the mechanism of lymph vessel formation, but also identified novel structures in the developing lymphatic system that had so far eluded

the analysis by tissue sections and are only recognized in 3-dimensional renderings.
More recently we have employed ultramicroscopy to analyse pathologies of the mature vascular system like atherosclerotic plaques. Atherosclerosis is a chronic inflammatory disease that develops over several decades. Triggered by hyperlipoproteinemia, macrophages accumulate in the wall of large arterial vessels and initiate as complex set of events that result in medial thickening, formation of calcium and cholesterol-rich deposits and the accumulation of necrotic cells, which ultimately leads to the formation of the prototypic atherosclerotic plaque. While macrophages have been clearly identified as the driving force behind this pathogenesis, many details of their migratory behaviour during plaque formation remain unknown. Again, we have resided to use Ultramicroscopy of cleared whole aorta preparations to quantitatively assess the immigration of labelled macrophages at different times or under different conditions of plaque formation.
Light sheet microscopes are generating notoriously large data volumes that are prohibitively resource demanding to process and store. In light of the scarcity of suitable software, we have adopted and tailored the open source volume rendering engine (VOREEN) for the use with large data stacks of optical sections. The resulting program suite VOREEN Biology not only offers a graphical user interface suitable for the life-science community but is also been developed towards automated segmentation and quantification routine for biological structures. As an example, these latest developments will be illustrated at the quantitative analysis of macrophage migration into atherosclerotic plaques.

PL-3

Control of cAMP signaling and excitation-contraction coupling by phosphodiesterases in normal and diseased heart

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Cyclic AMP regulates a multitude of cellular responses and orchestrates a network of intracellular events. In the heart, cAMP is the main second messenger of the β-adrenergic receptor (β-AR) pathway producing positive chronotropic, inotropic, and lusitropic effects during sympathetic stimulation. These effects involve mainly the activation of cAMP-dependent protein kinase (PKA) and the phosphorylation of several key proteins involved in the excitation–contraction coupling, such as the L-type Ca^{2+} channel, phospholamban, ryanodine receptor and troponin I. The stimulation of β-AR can also act via another pathway that implicates the Ca^{2+}/calmodulin kinase (CaMKII). Whereas short term stimulation of the β-AR/cAMP cascade is beneficial for the heart, chronic activation of this pathway triggers pathological cardiac remodeling, which may ultimately lead to heart failure (HF). In the light of the knowledge accumulated over the years, it becomes clear that intracellular cAMP is not uniformly distributed within cardiomyocytes and that cAMP compartmentation is required for adequate processing and targeting of the information generated at the membrane. Localized cAMP signals may be generated by interplay between discrete production sites and restricted diffusion within the cytoplasm. In addition to specialized membrane structures that may limit cAMP spreading, degradation of the second messenger by cyclic nucleotide phospho-diesterases (PDEs) appears critical for the formation of dynamic microdomains that confer specificity of the response to the diversity of external stimuli. The large number of PDE families and isoforms, their different localization within the cell, and their organization in macromolecular complexes leads to a high level of compartmentation, both in space and time, of cAMP signaling in cardiac myocytes. However, such organization is fragile and any change in the localization, translational and posttranslational modifications of these proteins will affect the cellular response to hormones and neuromediators. Here, we present some of the functional properties and roles of two different PDE families expressed in heart muscle, PDE2 and PDE4, and the changes that occur in cardiac hypertrophy and HF.

PL-4

The role of serine protease inhibitors (serpins) in cell signaling

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Serpins *(ser*ine *p*rotease *in*hibitors) are a large family of glycoproteins consisting of protease inhibitors and non-inhibitory members. They are widely distributed in nature, and occur intracellularly as well as extracellularly. Inhibitory serpins form covalent complexes with target proteases while undergoing a major conformational change. Well known examples of inhibitory serpins are alpha-1-antitrypsin, antithrombin III, or C1 inhibitor.

Deficiency or dysfunction of these inhibitors is associated with COPD and liver disease (alpha-1-antitrypsin), thromboembolic disease (antithrombin III), or hereditary angioedema (C1-inhibitor). Non-inhibitory members of the serpin family have other functions such as acting as hormone precursor (angiotensinogen), as hormone transporters (corticosteroid-binding globulin, thyroxin-binding globulin) or as tumor suppressor (maspin). While it seems evident that serpins, which act as hormone precursors or hormone binding proteins, influence cell signaling, the role of protease inhibition and possible other properties of serpins for signal transduction is less clear. One possibility, how serpins could influence cellular signaling, is by inhibiting proteases that cleave/activate protease-activated receptors (PARs). However, at present very little is kown about the biological relevance of this pathway.

My group has been analyzing the biochemistry and biology of serpinA5 (protein C inhibitor), a secreted, heparin-binding serpin with broad protease reactivity and wide tissue distribution. We have shown that this serpin can bind anionic/oxidized phospholipids. It can be internalized by cells in an endocytosis independent manner and translocate to the nucleus. By binding to phosphoinositides serpinA5 may influence phosphoinositide-dependent signaling. In the nucleus internalized serpinA5 inactivates cathepsin L and thereby prevents cleavage of histone H3 by this protease and rescues other histone modifications. Based on theses data we propose a new mechanism how a secreted serpin might influence intracellular functions.

PL-5

Physiological studies of muscle in zebrafish larvae – exploring mechanisms in human muscle disease

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The zebrafish (*Danio Rerio*) has become an important tool in developmental biology, since most organ systems are established during the larval stage (< 6 days after hatching). The genome is characterized, and orthologues of human genes can be identified. Gene expression can be manipulated and several mutated strains exist. However, the zebrafish larvae also provide unique possibilities for functional (physiological) studies, and we have during recent years developed techniques for studies of zebrafish larval muscle, with a focus on understanding muscle function and human muscle disease. Zebrafish larval muscle is isolated and analyzed with mechanical measurements of contractile function (active force, shortening velocity) in combination with structural studies (microscopy and synchrotron light based small angle x-ray diffraction). Using knock-down strategies we have examined the function of the intermediate filament protein desmin, and shown that knock down results in a desminopathy with impaired muscle force, increased interfilament spacing and resistance to stretch-induced injury. Following knock down of the fast skeletal myosin binding protein C, a new myopathy was identified with affected force and muscle structure. The *Sapje* strain is a zebrafish model of Duchenne muscular dystrophy, with a missense mutation resulting in a stop codon in the dystrophin gene. These animals have impaired force generation and can be successfully treated using novel read-through compounds (Ataluren). This presentation will describe physiological studies of zebrafish larval muscle, and argue that this approach can be a fast, relevant and economical model for examining structure/function of normal and mutated muscle proteins and for developing novel treatment strategies for muscle disease.

Workshop

WS

Open Access Publishing: Friend or Foe to Physiologist

Susan Wray

Editor-in-Chief, Physiological Reports; University of Liverpool, UK

In this workshop I want us to explore

- What is meant by open access publishing?
- What are the differences between gold level and others?
- How can it help your career to publish in open access journals
- Can it damage your career!? Are there sharks in the water?
- Is it all about the money? Who makes the money and who pays?
- How can physiologists afford open access fees?
- What does *Physiological Reports* have to offer and why is it supported by The Physiological Society, the American Physiological Society, and now by FEPS and the Scandinavian Physiological Society

EYPS Keynote Lecture

KN_Y

Decoding the synaptic organization of appetite circuits

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New tools for mapping and manipulating molecularly defined neural circuits have improved the understanding of how the central nervous system regulates appetite. Activation of starvation-sensitive AGRP neurons can rapidly elicit behavioral state similar to food deprivation, which present an entry point for reverse-engineering neural circuits for hunger. We mapped functional synaptic interactions of AGRP neurons with multiple cell populations in mice and probed the contribution of these distinct circuits to feeding behaviour using optogenetic and pharmacogenetic techniques. We have also developed tools for detailed structural analysis of AGRP neuronal connections using serial-section electron microscopy. Our results characterized some basic features of functional and anatomical circuit organization for AGRP axon projections.

EYPS Plenary Lecture

PL_Y

Go for it! Why a PhD should be the path to success

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The PhD, otherwise known as the doctor of philosophy or Dr. Phil., is an internationally recognized degree, indicating that the PhD graduate has received training in research under supervision. Traditionally, the PhD was the route to an academic career, with most successful PhD graduates receiving tenured university positions. However, over the past 20–30 years, and particularly the past 10 years, the situation has changed dramatically. Governments in many countries – encouraged by the European Union – have invested massively in PhD education, believing that trained researchers will contribute to the 'knowledge society', and thus increase the competitiveness of their countries in the future economies of the world. Thus, only a small fraction of PhD graduates now end up in academic research. Yet, the PhD remains a research degree, and indeed, institutions have become heavily dependent on PhD students for their research output. The situation has thus created a paradox. On the one hand, it has become essential for institutions to have many PhD students and for the research performed to be of the highest level. On the other hand, the careers of PhD students are not necessarily going to be directly related to the research performed during their PhD studies. This lecture will explore how this seeming paradox is being addressed in biomedicine and to show that far from being inconsistent the two aspects are in fact complementary. The PhD is a degree that is relevant both for those seeking an academic career, as well as for those who will use their talents outside of academia.

FEPS European Young Physiologist Symposium: Oral Presentations

Y_1

Intestinal epithelial barrier and liver dysfunction after hemorrhagic shock in a rat model

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Aim: Hemorrhagic shock is a frequent complication in trauma patients, after gastrointestinal bleeding and major surgery. It is associated with end organ damage, caused by hypoperfusion and local and systemic inflammation. The aim of this study was to investigate the effect of the hemorrhagic shock on intestinal epithelial barrier and liver functions in a rat model.

Methods: This study was carried on 40 male Wistar albino rats 4-6 months old with body weight (250-300g). Rats were divided randomly into four main groups: Group 1 (Control group): It consisted of 10 normal healthy rats served as control and sacrificed without intervention. Group 2, 3 and 4 consisted of 10 rats in each, subjected to a non-lethal hemorrhagic shock and sacrificed in 30, 60, 90 min after induction of hemorrhagic shock. At the end of the study: Blood samples were collected for measurement of serum TNF-α, serum LPS, liver enzymes and albumin. Liver and ileum were excised immediately for histopathological study and measuring ZO-1 and TLR-4 genes expression.

Results: TNF- α, LPS, ALT were increased significantly and albumin level showed significant decrease in the all groups of shock. PCR for both ZO-1 and TLR-4 showed significant increase in all groups of the study.

Conclusions: This study gives more insight into the sequence of events in the gut after hemorrhagic shock. Hemorrhagic shock leads to intestinal tight junction integrity loss. This is followed by bacterial translocation, liver dysfunction and systemic inflammation.

Y_2

The protective effects of obestatin on oxidative brain damage of rats with pentylentetrazol (PTZ)-induced epileptic seizures

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Aim: In generalized epilepsy, increased generation of reactive oxygen metabolites (ROM) triggers convulsions by inactivating glutamine synthase and glutamate decarboxylase, increasing glutamate and decreasing GABA. The present study was planned to elucidate effects of obestatin treatment on the severity of seizures, memory performance and oxidative brain injury.

Methods: Following a learning-trial using passive-avoidance test, Wistar male rats were injected with saline (n=24) or obestatin (1 mg/kg, ip; n=24) and 30 min later pentylentetrazol (PTZ; 45 mg/kg; ip) was injected to induce seizures. In the control group (n=12) PTZ was not administered. Seizures were video-taped and evaluated by using Racine's scoring (0-5) method. Rats were decapitated on 24^{th} and 72^{nd} hours of PTZ injection. Memory performances of the rats on 72-hour group were evaluated by passive-avoidance test. Brain malondialdehyde (MDA), glutathione (GSH) levels, myeloperoxidase (MPO) activity and chemiluminescence, showing generation of ROM, were measured and histopathological analysis was made on groups which were perfused with formaldehyde. ANOVA and Student's t tests were used for statistical analysis.

Results: Generation of tonic-clonic seizures was reduced and seizure scores were lower in obestatin-PTZ group as compared to saline-PTZ ($p<0.01$). Compared to control group, reduced memory performance, increased brain MDA, luminol/lucigenin chemiluminescence, MPO activity ($p<0.01$-0.001) in saline-treated PTZ rats were reversed by obestatin ($p<0.05$). Brain GSH contents in both PTZ groups were similar and higher than the control group. Microscopically, neuronal damage observed in the cortex and hippocampus was alleviated in obestatin-treated PTZ group.

Conclusions: Obestatin, which reduced the severity of PTZ-induced seizures, improved memory dysfunction and neuronal damage, appears to act by inhibiting the generation of ROM, neutrophil infiltration and oxidative damage.

Y_3

Dependence of hippocalcin signaling on the lipid composition of the plasma membrane

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Aim: To reveal a relationship between plasma membrane lipid composition and signaling properties of hippocalcin

Methods: cell culture, transfection, fluorescent microscopy, calcium uncaging

Results: At this stage of research we managed to conduct primary experiments on hippocampal neurons, where dependence between lipid composition and hippocalcin signaling properties was found. We set few techniques to investigate that dependence in PC12 cell line:

1. Local and whole cell calcium uncaging to elevate calcium concentration in live cell.
2. Dephosphorilation of PIP2 with voltage sensitive phosphatase in order to regulate lipid
3. Calcium dependent translocation of fluorescently tagged hippocalcin in PC12 cells.

Hippocalcin signals in the hippocampal neurons by means of Ca^{2+}-dependent translocation from the cytosol to the plasma membrane that can be induced by depolarization-induced activation of voltage-gated Ca^{2+} channels. It was suggested that the lipid composition of the plasma membrane influences Ca^{2+}-dependent hippocalcin translocation being in this way extremely important for intracellular signaling.

Here we studied whether hippocalcin translocation depends upon PIP2 concentrations in the plasma membrane. For that voltage-sensitive phosphatase (Dr-VSP), converting PIP2 into PIP, was co-expressed with Pleckstrin Homology Domain of Phospholipase C tagged by Cyan Fluorescent Protein. The latter has high affinity to PIP2 and translocates from the plasma membrane to the

cytosol upon a decrease in PIP2 concentration in the membrane.

Conclusions: We demonstrated a fast transient decrease of PIP2 concentration in the plasma membrane when Dr-VSP was activated by means of depolarization. Now we study hippocalcin translocation in PC12 cells and hippocampal neurons managing PIP2 concentration using Dr-VSP in order to reveal a relationship between plasma membrane lipid composition and signaling properties of hippocalcin.

Y_4

Measurement of intracellular concentrations of fluorescently-labeled targets in living cells.

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Aim: The groundbreaking event in the protein distribution studies was the discovery of green fluorescent protein. Genetically encoded fluorescent reporter-tagged proteins have been developed as the tracing tool for localization of the expressed proteins within a cell. Here we propose simple, universal method for quantitative analysis of intracellular concentration of fluorescently-labeled proteins expressed in single living cells.

Methods and Results: The method exploits a simple fact - fluorescence detected from a preparation depends on concentration of the fluorescent molecules, their optical properties, spectral properties of the optical equipment used to record fluorescent signals. We demonstrate that knowing the optical functions equipment and loading the cell with a reference dye of known concentration it's possible to calculate the concentration of the expressed fluorescent protein based on a ratio of reference dye to protein fluorescence. We have introduce an equipment factor, that embodies optical function of the imaging system and optical properties of the reference dye and fluorescent protein that's a constant for a given equipment and pair of fluorophores. The concentration of a certain fluorescent protein in a given cell can be easily estimated by multiplication of reference dye concentration by the equipment factor. We describe how to calculate the factor, how to use it for protein concentration measurements in living cells. The method requires patching the cell for filling it with the reference dye of known concentration. Therefore, this method is ideally suited for electrophysiological research.

Conclusions: In this work we develop a fast, simple, precise method to estimating intracellular concentrations of fluorescently-labeled targets in living cells.

Y_5

A role of locally expressed AMP/AngIV/IRAP on glucose transport across jejunal epithelium of healthy subjects

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Aim: Recently it was presented that the renin-angiotensin system (RAS) is locally expressed in the enterocytes and Angiotensin II (AngII) type 2 receptor (AT2R) activation enhance the glucose transport, mediated via the sodium-glucose transporter 1 (SGLT1). The aim of this study was to investigate key components for AngIII and AngIV formation enzymes in the healthy jejunal mucosa and to assess AngIV effects on the glucose transport *in vitro*.

Methods: Enteroscopy with mucosal biopsy sampling was performed in 16 healthy volunteers. ELISA, western blotting and immunohistochemistry was used to assess the protein levels and localization of the enzymes and receptor. The functional effect of AngIV was examined in Ussing chamber experiments.

Results: The peptide AngII, the enzymes aminopeptidases-A, B, M as well as IRAP were detected in the jejunal mucosa. Immunohistochemistry localized the aminopeptidase-A, B, M preferably to the apical brush border membrane whereas IRAP was localized in the subapical cytosolic compartment in the enterocyte. AngIV increased the glucose induced electrogenic transport *in vitro*.

Conclusions: The present study indicates that a local alternative RAS i.e. all enzymes necessary for AngIII and AngIV formation and IRAP exist and may exert regulatory impact on glucose uptake in healthy human small intestine.

Y_6

Magnesium sulphate inhibits spontaneous and oxytocin induced contractions of mouse myometrium

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Aim: Magnesium sulphate is currently used in obstetrics for treatment of seizures in women with preeclampsia. It is also used for neonatal protection and given to women who are anticipated to deliver before 32 weeks of gestation. The use of $MgSO_4$ as a tocolytic is however questioned. The aim of this study was to assess *in vitro* the inhibitory effects of $MgSO_4$ in term pregnant and non-pregnant mice myometrium

Methods: Myometrial strips obtained from term pregnant (18 days) and non-pregnant mice were superfused in physiological saline solution at pH 7.4 and 37 °C. After steady contractions were achieved, the effect of different concentrations of $MgSO_4$ (2 – 12 mM) was examined on spontaneous and oxytocin induced (0.5 nM) myometrial contractions.

Results: $MgSO_4$ had an inhibitory effect on mouse myometrium. Compared with controls (100%), 10mM $MgSO_4$ produced a significant ($P < 0.001$) decrease in force integral of spontaneous (6.14 ± 3.18%, n=9) and oxytocin-induced (50.5 ± 3.93%, n=11) contracting pregnant myometrium, mean ± SEM. 50% reduction of force integral was achieved in spontaneous and oxytocin induced contractions at 4mM and 10mM respectively. In non-pregnant myometrium, the effect of $MgSO_4$ was less than that observed in pregnant, 50% reduction was seen at 8 and 10mM of spontaneous and oxytocin induced contractions respectively and further decreased with increased concentrations.

Conclusion: Our *in vitro* data demonstrated that $MgSO_4$ had a dose dependent inhibitory effect on pregnant and non-pregnant mouse myometrial contractility. Oxytocin decreases its effectiveness which may affect its tocolytic ability *in vivo*.

Y_7

Pharmacological influence of myometrial aquaporin 5 expression in pregnant rat

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Aim: The aquaporins (AQPs) are a family of integral membrane channel proteins that facilitate rapid passive movement of water. Earlier we proved the dominance of AQP5 subtype in late pregnant rat uterus. The expression of this subtype dramatically decreased on the last day (day 22) of pregnancy and in preterm delivery. Moreover, we also found that the AQP5 expression is regulated by oxytocin. Our recent aim was to study the pharmacological influence of myometrial AQP5 expression at the end of pregnancy.

Methods: Changes in AQP5 mRNA and protein expression were measured by real-time PCR and Western blot analysis, respectively. The hormonal influence was investigated after progesterone (0.5 mg/animal, s.c.) and estrogen (5 ug/kg bw, s.c.) treatment. The effects of myometrium-relaxing agents terbutaline (10 mg/kg bw, sc) and doxazosin (30/kg bw, p.o.) were also tested on the AQP5 expression.

Results: After 7 days of progesterone treatment the AQP5 expression significantly increased on pregnancy days 18 and 22. Four days of estrogen treatment was less effective for the AQP5 expression. The selective alpha1A-adrenergic receptor blocker doxazosin increased the AQP5 expression after single and multiple doses (3x) on the day 22. After single dose of beta-mimetic terbutaline the mRNA and protein expression did not change, while the repeated doses elicited a significant increase in the AQP5 expression at the day of term.

Conclusions: In the light of our results we suppose that there is a progesterone dominance in sexual hormonal control of AQP5 expression. All the investigated inhibitors of pregnant uterine contraction (terbutaline, doxazosin) enhanced the myometrial expression of AQP5. We presume that the uterine-relaxing effect is accompanied with the enhanced expression of this AQP.

Y_8

Prediction of apelin-13 concentration based on the levels of thyroid hormones in rats

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Aim: Apelin is a peptide hormone known as the ligand for the G protein-coupled APJ receptor. Apelin and APJ intervene an extensive variety of physiological activities comprising regulation of energy metabolism, nourishment intake and liquid homeostasis. The objective of the current study was to predict apelin-13 concentration based on thyroid hormones, TRH, TSH, T3 and T4 levels using multilayer perceptron (MLP) artificial neural networks (ANN).

Methods: In this study, 30 Sprague Dawley male rats were utilized, and were separated randomly into three groups (n=10). Sham and experiment groups received ceaselessly intracerebroventricular infusion by means of osmotic mini pump filled artificial cerebrospinal fluid or apelin-13 at concentration of 1 and 10 nmol (10ul/h) for seven days. Toward the end of infusion, the rats were sacrificed and their brain and blood tissues were taken. TRH mRNA levels in hypothalamus were determined by RT-PCR and serum TSH, T3 and T4 levels were measured by ELISA technique. MLP ANN modeling was constructed to predict apelin-13 concentration based on the TRH, TSH, T3 and T4 levels, and was assessed via sum of squares error (SSE) and relative error (RE).

Results: While 18 (64.3%) samples were used in training, 10 (35.7%) samples were employed in testing process. MLP ANN model had 4.65 of SSE and 0.55 of RE for training, and 3.21 of SSE and 0.56 of RE for testing, respectively. Normalized predictor importance values were 25.3% for TRH, 18% for TSH, 100.0% for T3 and 21.7% for T4 levels.

Conclusions: The suggested MLP ANN is a promising and successful system for predicting apelin-13 concentration based on the TRH, TSH, T3 and T4 levels.

Y_9

Skeletal muscle-derived stem/progenitor cells: a potential strategy for the treatment of acute kidney injury

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Introduction: The skeletal muscle-derived stem/progenitor cells (MDSPCs) have been thoroughly investigated and already used in preclinical studies. However, therapeutic potential of MDSPCs for acute kidney injury (AKI) has not been evaluated.

Aim: In our study we aimed to characterize rat MDSPCs, compare them with the bone marrow mesenchymal stem cells (BM-MSCs) *in vitro* and evaluate the feasibility of the MDSPCs therapy for the gentamicin-induced AKI in a pilot study in rats.

Methods: The characteristics of rat BM-MSCs and MDSPCs were assessed by the population doubling time, flow cytometry, immune-histochemistry, multipotent differentiation capacity and RT-PCR. A gentamicin-induced AKI model in rat was used to examine the MDSPCs therapeutic effect. Physiological and histological kidney parameters were determined.

Results: MDSPCs exhibited similar immune-phenotype, stem cell gene expression and multilineage differentiation capacity as BM-MSCs, but demonstrated higher proliferation rate. MDSPCs accelerated functional kidney recovery and regeneration, as reflected by significantly lower serum creatinine levels and renal injury scoring, higher urinary creatinine and GFR levels ($p<0.05$). PKH-26 labelled MDSPCs were present in the renal cortex 2 weeks after injection, indicating MDSPCs' capacity to migrate and populate the renal tissue.

Conclusions: In conclusion, MDSPCs are capable of mediating functional and histological recovery

and can be considered as a potential strategy for the treatment of AKI.

Acknowledgement: This research was funded by a grant (No. MIP-83/2013) from the Research Council of Lithuania.

Y_10

The effect of visfatin and dyslipidaemia on uterine contractility

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Aims: Adipose tissue secretes adipokines which have been linked to the pathophysiology of pregnancy-related complications. Visfatin is a recently discovered adipokine whose levels were reported to be increased during obesity and pregnancy. The aim of this study is to examine the effect of visfatin on mouse myometrial contractility, both wild type and APOE knockouts (hyperlipidaemia model).

Methods: Myometrial strips from term pregnant and non-pregnant wild type (WT) and APOE knockout (KO) mice were dissected, superfused with physiological saline, and the effects of visfatin (10nM–150nM), on spontaneous and oxytocin-induced contractions (0.5-1nM) were studied. After regular contractions were established, contractility was examined for control (100%) and test response at 37 °C for 10 min.

Results: Visfatin had a relaxant effect on mouse myometrium. This effect was small in non-pregnant myometrium and in pregnant tissue contracting spontaneously. For example, in the pregnant WT myometrium, 10-150 nM visfatin produced a reduction in the 5 min area under the curve (AUC) of 95 ± 3%, (n = 8), However, under more physiological conditions, oxytocin-induced contractions, a larger decrease was found (AUC = 76 ± 9%, n = 4), mean ± SEM. In the dyslipedimic APOE KO, the stimulation by oxytocin was reduced the AUC by (97 ± 6%, n = 4) compared to spontaneous contractions (104 ± 4%, n=5).

Conclusion: These data add to our earlier data in human myometrium showing that visfatin can reduce myometrial contractility, especially under physiological conditions. We also show that dyslipidaemia affects the tissues ability to respond to oxytocin. Together these data suggest that increased output of visfatin and dyslipidaemia in obese pregnant women may impair uterine contractility resulting in labour related complications.

FEPS Teaching Physiology Symposium

T_1

Blended Learning in Teaching Physiology: An Introduction

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Blended learning by strange definition is the mode of education in which a part of the education is provided using electronic means. For some this means that blended learning is synonymous to e – learning, while for others it is the blend of different educational tools. These different views on blended learning make it difficult to obtain and quick overview over publications in this educational field. For physiology education at the Maastricht University, blended learning is literally a blend of educational means to a determined, six year, end of medical education. In The Netherlands the physiology learning outcomes of the medical schools are drafted in a document called: “The framework of medical education in the Netherlands” and are comparable to the ones posted on the FEPS physiology teaching website. The Maastricht Medical School makes use of blended learning to stimmulate constructive and collaborative learning by students of the different semester learning outcomes and to safe guard the adherence to the Framework document for every student. For this second objective it becomes interesting to add learning analytics to the blended learning environment. Learning analytics allows students and staff to monitor progress and identify strengths and weaknesses in study performance in accordance to the framework document. To facilitate this blended learning one can use different computer interfaces that are accessible to students and staff in which the track record of the student is kept together with highlights of the students strengths and weaknesses and recommendations to change study behavior. Since most study resources are available as e-book, online libtaries or other e-type it is possible to monitor individual study behavior and combine these with the students academic performance. This allows individual guidance of students even in large year groups (200+). Next to these student centered blended learning possibilities, modern means pave the way for online courses or long distance online collaborations UnColleging physiology education. Using the internet it becomes possible to take part in long distance educational activities with partner universities. In other words, blended learning makes it possible to design, organise, and execute

joined physiology BSc, MSc, and PhD programs with FEPS and IUPS as certifying bodies.

T_2

Common understanding of PhD training: the ORPHEUS experience

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Although the PhD was traditionally the route to an academic career, the situation has changed dramatically over the past 10-20 years. In many countries, governments and other funding bodies have invested massively in PhD education, and now most professors have several PhD students. Thus relatively few PhD graduates find permanent employment in academic research. Yet, the PhD remains a research degree, and indeed institutions have become heavily dependent on PhD students for their research output. In institutions in some countries, this challenge, at least in biomedicine and health sciences, has been met by maintaining the traditional concept of the PhD as a degree of individual scientific excellence, but setting it in a structured environment with the offer of courses in generic skills. In other countries, consistent with the Salzburg principles, institutions have placed substantial emphasis on the responsibility of the institution for enhancing the employability of their PhD graduates outside academia, and require that significant parts of their PhD programmes provide the necessary skills that PhD graduates will require for non-academic employment. The organization ORPHEUS (Organisation for PhD education in biomedicine and health sciences in the European system), which has about 100 institutional members from across Europe and worldwide, has addressed this question. Through the development over many years of mutually agreed standards (www.orpheus-med.org) ORPHEUS has developed a common understanding for PhD training. These standards show how it is possible to safeguard the reputation of the PhD as a research degree while also strengthening career opportunities for PhD graduates.

T_3

Maastricht approach for teaching circulatory physiology: Enabling role of computer simulations in instruction and self-learning

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Recognition and management of hemodynamic disorder in patients requires insight into the dynamic and adaptive interactions of the circulatory system. Textbook concepts and illustrations are useful to define terms and relationships, but there are limits to address combined effects of multiple factors on circulatory dynamics. Hence, for students training on complex cases may be insufficient when using textbooks alone.

To address these limitations, we introduced computer simulations to (1) allow exploration of dynamic and adaptive aspects and (2) offer versatility in building complexity of problems. Students collaboratively work on predefined problems during instruction, with access to support from teachers to refine their questioning and understanding. Students retain access to the simulation tool to work on self-identified problems.

The software used is called CircAdapt Simulator, freely downloadable from *www.circadapt.org*. This tool enables real-time simulation of circulatory hemodynamics and displays tracings of blood pressures, volumes and flow velocities around the heart, blood vessels and shunts, if required. Patho-/physiological conditions are created by manipulating the properties of myocardial walls, heart valves and blood vessels, whether in isolation or in combination. In addition, systemic pressure and cardiac output regulation can be switched on and off.

Our 4-year experience is that most students rate the tool and overall approach very positively, because they are stimulated to learn interactively with peers and teachers. In learning group discussions, students use the tool to communicate or challenge their own insight and that of the group.

Current challenges are (a) to further facilitate and enhance self-learning, (b) to develop more tutorials, and (c) to critically identify whether and where students have gained competency.

T_4

Teaching physiology to medical students in Estonia: has this task become easier in last three decades?

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University of Tartu is the only university in Estonia where students can study medicine. Therefore, most of the current teaching staff in the Faculty of Medicine have first been the students in the same faculty and can compare the teaching and learning in their student years with the situation nowadays. In my presentation, I will first describe aspects that have remained almost the same over the last three decades, e.g. we have three separate courses of physiology for students of medicine, dentistry and pharmacy; our courses follow the system-based approach with lectures, practical classes and seminars that are often supervised by different faculty members; we teach in two languages. However, there are several sides in our teaching that have changed considerably over the 30 years, like replacing animal experiments with simulation programs, attempts to use more active learning in our classrooms and to introduce students to online textbooks, quizzes etc. The success and difficulties of our teaching staff in the last decades will be compared to recent studies about changes in teaching physiology.

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T_5

Preclinical sciences in the light of curriculum innovation and PBL introduction in LUHS Faculty of Medicine: where are we now?

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Problem based learning (PBL) was introduced in LUHS through medical studies curriculum reform in 2007. Program was reorganized from discipline-oriented to integrated modules, hybrid model of combining PBL tutorials and traditional classes was chosen, with biggest changes made in preclinical disciplines. Formerly taught in pure-discipline cycles of a few months duration, subjects like anatomy, biochemistry, physiology, etc. in reorganized curriculum were integrated on thematic basis and spread throughout 2 – 3 study years.
Teacher competencies had to be broadened to support newly introduced tutorials. Changes, made since PBL introduction, were perceived by teachers in several areas: pedagogical, including role shift from presenter to facilitator of students' (self) learning, changing focus from formerly mostly theory-oriented to more practically-applicable; personal development – “more motivation to master material from other subjects”; organizational – regular intra- and inter-departmental faculty meetings.

Student acceptance of the new system and their study results are encouraging. According to inquiry into student attitude to learning, made in LUHS department of Languages and Education, deep approach to learning was more frequently observed among the students of Faculty of Medicine, where PBL system has been introduced, students more frequently stated, that learning is a pleasurable activity, placed more emphasis on internal aspects of evaluation, than in other faculties. Long term retention of preclinical knowledge is not noted to be worse when compared to traditional learning. Teachers and supervisors at residency training express opinion, that specific knowledge of preclinical subjects, like physiology and pharmacology, do not seem to differ compared to before PBL introduction, however of notice is augmented ability to integrate processes into the whole and use them to make diagnostic and treatment decisions.

Remaining areas for development include more PBL-oriented assessment system, assurance of

tutorial quality, in terms of faculty competencies and tutorial process itself, improving integration of disciplines.

T_6

Study of motivational factors in Lithuanian University of Health Sciences

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Aim: To assess factors that influences the level of students' motivation the most in Lithuanian University of Health Sciences (LUHS).

Methods: The study was performed in three stages. First, LUHS students were asked to identify factors which influence their motivation using open-label questionnaire. In the second stage, the most influential factors were differentiated using semi-qualitative survey method. These factors were used in the final survey. The factors were divided into categories of: "academic environment", "associated with teachers", "physical environment" and "generally demotivating". Students were asked to assess these factors using Lickert's scale, rating their influence towards motivation to study from "-5" to "+5". Respondents also asked to assess their own learning outcomes, level of personal motivation and emotional atmosphere in the learning environment, and indicate their average study grade.

Results: Factors from groups "academic environment" and "associated with teachers" were indicated as most influential towards motivation to study (3.31 (± 0.34) and 2.73 (± 0.83), respectively). Of these, teachers' inner culture, feedback, professional competence and relevant elective course were the most influential. Mean rating of personal motivation was 2.74 (±2,03). Mean rating of emotional atmosphere in the learning environment was 1.64 (±2,5). 24.6% respondents rated emotional atmosphere in the learning environment negatively. Ratings of personal motivation correlated statistically significantly with ratings of emotional atmosphere in the learning environment.

Conclusions:
LUHS students find motivational factors associated with teachers most influential. This finding signifies the teacher as most valuable academic resource. Identified correlation, between emotional atmosphere and personal motivation ratings, mark the importance of social wellbeing in academic background.

T_7

Motivating health sciences students to learn actively and think critically about physiology

Ivna Kocijan

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With new information and new discoveries being made on a daily basis throughout the world, teaching physiology has become a challenge for teachers and a learning challenge for students probably greater than ever before. In order for students to manage new information, teachers not only need to convey the ever growing knowledge to students efficiently in a limited amount of time, they also need to provide them with methods essential for critical thinking that students can apply in the classroom and during self-learning. One such method is a ERR framework (Evocation, Realisation of meaning and Reflection) that motivates students to think critically and learn actively about physiology. ERR framework consists of three phases in which students actively participate: in the first phase, called evocation, different methods are used to recall knowledge about a subject that students already have; in the second phase (realisation of meaning) students obtain new knowledge using different learning techniques; and in the third phase (reflection) they connect knew and previous knowledge in a meaningful collection of ideas and practical application. In each of the three phases the teacher applies techniques which encompass individual work and team work, reading, writing, debate, brainstorming etc. In this way, students not only learn about physiology but at the same time they become active learners and critical thinkers. We use the ERR framework for our health studies students in groups as large as 150 and as small as 20, during lectures, seminars and lab-work. The framework is motivating and encouraging for students as well as the teacher.

T_8

Teaching and research physiology at the medical faculties in the Czech Republic

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The length of instruction on physiology is usually two semesters to four semesters. Physiological discipline students learn basic functions of individual organs and organ systems using knowledge of biochemistry, molecular biology, and genetics. Introduces students to the management functions in the organism.
Teaching of Physiology at individual institutions are closely connected with the scientific issues. Department of Physiology, 1st Medical Faculty in Prague. Ontogenetic aspects of the internal environment of the brain. Electrophysiological properties of the myocardium.Department of Physiology, 2nd Faculty of Medicine in Prague: pulmonary circulation and hypertension, fetal-placental vascular regulation.
Department of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine in Prague: pain, addiction, neuropsychopharmacology, hypoxia in relation to epilepsy. More than 12 years, taught physiology in the course structure and function of the human body (composed from histology and embryology, biochemistry and physiology).
Department of Physiology, Faculty of Medicine in Pilsen, has long focused on the function of the cardiovascular system.
Department of Physiology, Faculty of Medicine in Hradec Kralove has guiding themes: experimental hepatology, protein and amino acid metabolism and cardiovascular system.
Department of Physiology, Faculty of Medicine Masaryk Unoversity in Brno is engaged in the activity of the autonomic nervous system in cardiovascular diseases and diabetes mellitus. Also adverse effects of psychotropic drugs, anesthetics, and cytostatic drugs on the heart muscle is studied.
Department of Physiology Faculty of Medicine of Palacky University in Olomouc is focused on diabetes mellitus and cardiovascular system. Department of Physiology Faculty of Medicine, University of Ostrava has obesitological center.

Symposia

Symposium 1: Calcium regulation in striated muscle; failure and fatigue

S1-1

New insights into fatigue and failure in myometrium

Susan Wray

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We do not usually associate smooth muscle with the strenuous performances demanded of striated muscles. Perhaps for this reason the concept of fatigue and failure are less well explored in smooth muscles. Labour lasts for hours and has been likened to a marathon. Should we therefore be surprised that 10% of labours fail? Can some of the causes of these failed (dysfunctional) labours be explained by fatigue? We found that lactate is increased in myometrial capillary blood from women in dysfunctional labours, suggesting it is detrimental to uterine contractions. However no studies had directly examined its effects on myometrium. We have found that lactate inhibits Ca transients and force, due to induced acidification. Thus accumulation of extracellular lactate will reduce myometrial contractions and could therefore contribute to labour dystocia.
A puzzling feature of labour is that contractions become progressively stronger, as the myometrium experiences repetitive metabolic stress from hypoxia. This occurs as contractions briefly compress the uterine blood vessels. Transient decreases of oxygenation, pH and ATP, which if sustained can decrease contractile activity, occur *in vivo* with each contraction. Hypoxia regulates genes including those governing metabolism and function in many tissues, and changes in genes associated with hypoxia have been identified in transcriptomic studies of poorly labouring women. There is however no evidence showing how such changes could be important to successful labours, and no existing mechanism linking hypoxia to an *increase* in contractions. We have found a novel mechanism, hypoxia-induced force increase, HIFI, is switched on selectively, at term, by brief, repetitive, hypoxia. The increases in contractility are long-lasting, oxytocin-independent, intrinsic mechanism. HIFI explains how labour can progress despite paradoxical metabolic challenge.

S1-2

Transverse tubules and cardiac calcium handling

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Contraction of the heart results from the coordinated shortening of cardiac muscle cells, called cardiomyocytes. Contraction of cardiomyocytes is, in turn, dependent on sub-cellular structures called dyads which are functional junctions between invaginations of the surface membrane (t-tubules) and the sarcoplasmic reticulum. Well-organized dyads enable efficient triggering of Ca^{2+} release during the action potential, and powerful contraction. Dyads are formed gradually during development, with progressive assembly of both t-tubules and sarcoplasmic reticulum and precise trafficking of Ca^{2+} handling proteins including the L-type Ca^{2+} channel and Ryanodine Receptor. During diseases such as heart failure, dyads are broken down with a reversion to an immature phenotype. These alterations include changes in t-tubule morphology and altered localization of L-type channels and Ryanodine Receptors. Resulting disruption of Ca^{2+} homeostasis critically reduces contractile power in heart failure patients. Importantly, new data indicate that dyadic disruption varies regionally across post-infarcted, failing hearts, as t-tubule loss and *in vivo* contractile dysfunction are most prominent at sites proximal to the infarct. Our data indicate that loss of the t-tubule anchoring protein junctophilin-2 is key to such structural changes, and triggered by elevated ventricular wall stress. Wall stress is high proximal to an infarction due to local thinning of the wall, and in severe systolic heart failure wall stress is elevated due to ventricular dilation. Thus, these data provide a mechanistic link between dilation and the accompanying contractile dysfunction that occurs during heart failure, and provide a possible mechanism by which unloading of the heart may be beneficial in these patients. Alternative future strategies for heart failure patients may aim to protect t-tubule integrity by increasing expression of junctophilin-2 or inhibiting the mechano-transduction which leads to its downregulation.

S1-3

The role of calcium in the atrium

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Historically atrial cells have generally been assumed to lack transverse (t)-tubules which are deep invaginations of the sarcolemma important for Ca^{2+} entry. The L-type Ca^{2+} current is located on t-tubules and trigger release of Ca^{2+} from the SR which is responsible for subsequent cellular contraction. Thus t-tubules allow a rapid and synchronous rise in Ca^{2+} throughout ventricular cells whereas in atrial cells which lack t-tubules the rise in Ca^{2+} is slow. We have shown however that t-tubules are present in the atria of large mammals including human and they are important for normal function.

Heart Failure (HF) is associated with t-tubule loss in the ventricle but we have found this effect to be much more severe in the atria. T-tubule loss disrupts Ca^{2+} handling resulting in a decreased and asynchronous rise of intracellular Ca^{2+}. Thus, we have determined if it is possible to restore t-tubules to the atria and thence normalise cellular Ca^{2+} homeostasis. Our work shows recovery from HF is associated with restoration of atrial t-tubules although these structures are extremely disordered. Despite this disorder Ca^{2+} handling is surprisingly restored. We have identified amphiphysin II as a key component of t-tubule biogenesis which along with other proteins may be involved in t-tubule restoration.

S1-4

Calcium and fatigue in skeletal muscle

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Intense, repeated activation of skeletal muscles causes a decline in performance known as muscle fatigue. Fatigue involves impaired neural activation of muscle cells (central fatigue) as well as impairments intrinsic to the muscle cells (peripheral fatigue). Peripheral fatigue may include defects in action potential propagation, in sarcoplasmic reticulum (SR) Ca^{2+} handling and/or in the function of the contractile elements. Factors that may contribute to fatigue include changes in ionic and metabolite concentrations, and increased production of reactive oxygen/nitrogen species

(ROS/RNS). Moreover, the recovery after induction of fatigue can be very slow and factors causing this delayed recovery may differ from those that induce fatigue (Allen et al., 2008).

In our studies of fatigue, we have identified two major mechanisms underlying decreased force production during acute fatigue and recovery:
(1) Increased concentration of inorganic phosphate ions (Pi), due to breakdown of phosphocreatine, is a major cause of decreased force production during ongoing fatiguing stimulation. Increased Pi first decreases myofibrillar force production and subsequently it contributes to decreased SR Ca^{2+} release when muscle fibres become exhausted.
(2) Increased ROS/RNS contribute to the prolonged (hours) force depression after fatiguing stimulation. ROS/RNS then either decrease the SR Ca^{2+} release or reduce the myofibrillar Ca^{2+} sensitivity.

Many different activities cause fatigue and a major challenge is to identify the relative importance of various mechanisms in different conditions. Most of the mechanistic fatigue studies have been performed on isolated muscle and another major challenge is to use the knowledge from these studies to identify the mechanisms of fatigue and recovery in humans under normal conditions and in association with various diseases.

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S1-O1

Characterization of the effects of pro-inflammatory cytokines on energy metabolism in human myogenic cells

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Aim: Skeletal muscle health is dependent on function of its mitochondria. Sarcopenia during aging has been attributed to the low grade inflammation, suppressed regenerative potential of muscle precursor cells. The aim of the study was to investigate mitochondrial function in primary cell cultures derived from bioptates taken from young and old individuals.

Methods: Primary muscle cell culture myoblasts obtained from biopsies of v. lateralis in young and old subjects were stimulated with differentiation media supplement in addition with one of cytokines IL1-β, IL6 or TNF-α. The function of respiratory chain complexes (OXPHOS) was assessed by high-resolution respirometry.

Results: The myoblasts cultivated from old individuals differentiated into myotubes markedly slower than myoblasts from young individuals in ITS medium. Treatment of human myoblasts with TNF-α and IL-1β increased the proliferation and blocked differentiation in the presence of ITS. The data about mitochondrial respiration revealed that IL-1β caused a significant decrease in mitochondrial respiration normalized on protein content both in the myotubes of old and young individuals. TNF-α, on the contrary, caused a significant increase in mitochondrial normalized on protein or citrate synthase in myotubes of old and young subjects. The mode of action of these pro-inflammatory cytokines on OXPHOS of muscle cell cultures was the same in both groups - obtained from of the young or old persons.

Conclusions: The myoblasts cultivated from biopsies of old individuals differentiate into myotubes slower than myoblasts obtained from young individuals. IL-1β decrease, TNF-α stimulate but IL-6 exert no alteration on the OXPHOS activity, both in old or young individuals.

S1-O2

Emergence of Orai3 activity during cardiac hypertrophy

Acknowledgement:
Youakim Saliba[1,2], Mathilde Keck[1], Alexandre Marchand[1], Fabrice Atassi[1], Aude Ouillé[3], Olivier Cazorla[3], Mohamed Trebak[4], Catherine Pavoine[1], Alain Lacampagne[3], Jean-Sébastien Hulot[5], Nassim Farès[2], Jérémy Fauconnier[3], Anne-Marie Lompré[1]

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Aims: Stromal interaction molecule 1 (STIM1) has been shown to control a calcium (Ca^{2+}) influx pathway that emerges during the hypertrophic remodeling of cardiomyocytes. Our aim was to determine the interaction of Orai1 and Orai3 channels with STIM1 and their role in the constitutive store-independent and the store-operated, STIM1-dependent, Ca^{2+} influx in cardiomyocytes.

Methods and Results: We characterized the expression profile of Orai proteins and their interactions with STIM1 in normal and hypertrophied adult rat ventricular cardiomyocytes (ARVC). Orai1 and 3 protein levels were unaltered during hypertrophy and both proteins co-immunoprecipitated with STIM1; however, the levels of STIM1 and Orai1 were significantly greater in the macromolecular complex precipitated by Orai3. We then used a non-viral method to deliver cy3-tagged siRNAs *in vivo* to ARVC and silence Orai channel candidates. Cardiomyocytes were subsequently isolated then the voltage-independent, i.e. store-independent and store-operated Ca^{2+} entries were measured on fura-2 loaded cy3-labelled and control isolated cells. The whole cell patch-clamp technique was used to measure Orai-mediated currents. Specific Orai1 and Orai3 knockdown established Orai3, but not Orai1, as the critical partner of STIM1 carrying these voltage-independent Ca^{2+} entries in ARVC. Orai3 also drove an arachidonic acid-activated inward current.

Conclusion: Cardiac Orai3 is the essential partner of STIM1 and drives voltage-independent Ca^{2+} entries in cardiomyocytes. Arachidonic acid-activated currents, which are supported by Orai3, are present in cardiomyocytes and increased during hypertrophy.

S1-O3

Action potential clamp data analysis reveals large contribution of sarcoplasmic reticulum in excitation-contraction coupling of trout cardiomyocytes

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Aim: The significance of the different calcium influx pathways like L-type Ca^{2+}-channels (LTCC), reverse Na^{+}/Ca^{2+}-exchange (NCX_{rev}) or Ca^{2+}-induced Ca^{2+}-release (CICR) varies with species and the state of a cell. For instance, in case of heart failure, CICR decreases and influx via NCX_{rev} increases. The aim of this study is to develop a method to quantify calcium fluxes in cardiac excitation-contraction coupling under physiological conditions.

Methods: We used action potential clamp to measure I_{LTCC} and I_{NCX} currents in rainbow trout ventricular myocytes. Fluorescence changes induced by Ca^{2+}-transients were recorded using 1 µM Fluo-4 AM. To measure I_{LTCC} and/or I_{NCX}, we used 10 µM nifedipine and 1 or 5 mM $NiCl_2$ in extracellular solution. Ryanodine receptors and SR calcium ATPase were inhibited by incubating cells in the presence of 10 µM ryanodine and 2 µM thapsigargin. We used 240 µg/ml amphotericin B in pipette solution for the perforated-patch clamp configuration. Also, we composed a simplified mathematical model of Ca^{2+}-dynamics. By fitting model solution to the recorded experimental data, we determined contribution of LTCC, NCX and CICR currents in trout.

Results: From experiments where CICR was inhibited we determined the calcium buffering capacity in the cells that is 57 µM (95% confidence interval 41-77 µM). With this method we quantify the contribution and kinetics of LTCC (10-15%), NCX (15-25%) and CICR (60-75%).

Conclusions: We have developed a method to quantify calcium fluxes under physiological conditions in cardiomyocytes. Furthermore, we found that SR plays a significant role in trout EC coupling. This is in sharp contrast with previous estimates. Also, the method is not specific to trout and is easily applicable to different species.

Symposium 2: Role of glial neurotransmitters receptors in central and peripheral nervous system development and regeneration

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S2-1

Role of $P2X_7$ receptors in the development, maintenance and repair of peripheral nerves

Alessandro Faroni

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In recent years, using *in vivo* and *in vitro* techniques, we studied the role of $P2X_7$ receptors in both Schwann cell (SC) fate and cell death.
In particular, we showed that $P2X_7$ receptors are expressed in SC-like cells obtained by adipose-derived stem cells. These cells hold great promise for cellular therapies for nerve repair; nevertheless their survival rate following transplantation in PNI is extremely low, probably due to the hostile injury milieu, which includes high ATP concentrations. We have shown that these receptors can be pharmacologically targeted to prevent cell death, and we propose $P2X_7$ receptors as novel target to increase the survival of stem cells in cellular therapies for nerve repair.

Furthermore, using $P2X_7$ receptor knockout mouse models, we have investigated morphological and molecular characteristics of peripheral nerves in these animals. We found that $P2X_7$ knockout mice had an abnormal sciatic nerve phenotype and concluded that absence of the $P2X_7$ receptor in these nerves was affecting the developmental fate of SC, shifting them from a myelinating to a non-myelinating phenotype.
We then decided to determine whether the effects of the $P2X_7$ receptor on SC fate were conserved during regeneration following PNI. Using different sciatic nerve injury models in our $P2X_7$ knockout mice, we assessed regeneration and re-myelination of the nerves, together with molecular changes in the damaged sensory neurons. We found evidence of abnormal re-myelination in our knockouts, with molecular and morphological differences when compared to controls. These findings suggest that ATP signalling, working through the $P2X_7$ receptor, has a crucial role in SC fate determination during development and regeneration and may be a novel target for the improvement of peripheral nerve repair following injury.

S2-2

GABAergic control of myelination and nociception in peripheral nervous system

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Recent evidences demonstrated that Schwann cells (SCs) of the peripheral nervous system (PNS) synthesise γ-amino butyric acid (GABA), and express its receptors, the ionotropic GABA-A and the metabotropic GABA-B. Both receptors play important roles in SCs biology. GABA-A activation leads to an autocrine increase in GABA synthesis, myelin proteins expression and SCs proliferation. Indeed, GABA-B activation decreases proliferation and myelin proteins expression, playing a role in SCs differentiation, toward the state of non-myelinating SCs. During PNS development, GABA-B receptor appeared mostly localised in the pre-myelinating and non-myelinating SCs. However, a small population of peripheral myelinated fibres display GABA-B localisation at the node of Ranvier, indicating an important role for this receptor in axon-glial communication. Studies on conditional knockout mice, specifically lacking GABA-B1 receptor in SCs (P0-CRE/GABA-$B1^{fl/fl}$ mice), confirmed the alterations in the PNS myelination process and revealed that these mice are hyperalgesic and allodynic. In P0-CRE/GABA-$B1^{fl/fl}$ mice the morphological and behavioural changes are associated with a downregulation in neuregulin 1 (NRG1) type I levels, concomitantly with an upregulation of ErbB2/ErbB3 and pErk2, which is a key regulator of SC differentiation towards the pro-myelinating state. Interestingly, both GABA-A and GABA-B receptors are likely to be cross-regulated one to another. To corroborate this hypothesis, we have been studying the expression levels of different GABA-A subunits (e.g. alpha 2-3, beta 1-3, etc.) in SCs and dorsal root ganglia neurons of P0-CRE/GABA-$B^{fl/fl}$.
In conclusion, we suggest a crucial role for the GABA-ergic system in PNS. This may be relevant for the identification of new therapeutic strategies targeting peripheral GABA receptors, which may be suitable for peripheral neuropathies and associated chronic pain treatments.

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S2-3

A structured synaptic connectivity between GABAergic interneurons and oligodendrocyte precursors in the developing somatosensory cortex

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Oligodendrocyte progenitors, named NG2 cells, constitute the source of myelinating oligodendrocytes in the brain. In the developing neocortex, NG2 cells transiently receive a major synaptic input from GABAergic interneurons (1, 2). However, the operating modes and functions of these neuron-glia synapses are unknown. We recently examined the properties of individual interneuron-NG2 cell synapses from the subcellular to the network level during the critical period for oligodendrogenesis. We used paired recordings, holographic photolysis for circuit mapping and immunohistochemistry. Our findings show that the GABAergic innervation of OPCs by cortical interneurons form a structured synaptic network that is temporally and spatially regulated in coordination with the onset of oligodendrogenesis. Fast-spiking interneurons are highly connected to NG2 cells whereas Non-fast-spiking interneurons are poorly connected to these cells. These two different types of interneurons discriminate NG2 cell postsynaptic sites, targeting anatomically segregated subcellular domains, containing distinct $GABA_A$ receptors. This subcellular arrangement of presynaptic inputs is extended at the network level. Holographic photolysis revealed that interneuron-

NG2 cell connections exhibit very local connectivity maps, forming a specific network characterized by a local microarchitecture. Moreover, interneuron-NG2 cell synaptic connectivity is transient and reaches a peak at PN10, coinciding with a switch to a massive NG2 cell differentiation onto oligodendrocytes (3). In conclusion, NG2 cell synaptic connectivity is highly regulated in time and space during cortical development. This regulation is correlated with important oligodendrocyte developmental processes, suggesting implications of these neuron-glia synapses in the fate of NG2 cells.

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S2-4

Neurotransmission in CNS white matter: where are synapses?

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The massive computing power of the brain depends on myelinated fibres that are bundled together into the white matter (WM) – 'superhighways of information' that interconnect widely dispersed grey matter (GM). Myelin is produced by oligodendrocytes, which are derived from oligodendrocyte precursor cells (OPCs), a significant population of cells throughout WM and GM. OPCs form neuron-glial synapses and respond to neurotransmitters released by neurons via a range of receptors, which are proposed to regulate their differentiation into oligodendrocytes. However, WM is largely devoid of neuronal cell bodies and synapses. Nonetheless, neurotransmitter signalling is highly prominent and diverse in WM, with a predominance of glutamatergic, purinergic (ATP and adenosine) and GABAergic signalling. Experimental studies support a model of neurotransmitters being released from axons and astrocytes during action potential propagation to regulate myelination. Notably, there is life-long generation of oligodendrocytes from OPCs, which is required for replacement of myelin lost through natural 'wear and tear', for myelination of new neuronal circuits formed in response to new life experiences and regeneration of oligodendrocytes following pathological demyelination, such as occurs in multiple sclerosis (MS). However, myelination declines in the ageing brain, which may be important in the ultimate failure of remyelination in MS and the loss of WM in Alzheimer's diseases (AD). Significantly, we provide evidence that neurotransmitter signalling is dysregulated in ageing WM of the mouse optic nerve, comparable to that described in the GM of human ageing brain and AD. This lead us to propose a vicious cycle in the ageing brain, whereby disruption of neurotransmitter signalling results in impaired OPC regenerative potential and subsequent loss of oligodendrocytes and myelin, which is aggravated in diseases such as MS and AD.

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S2-O1

The K^+ efflux through postsynaptic NMDA receptors regulates astrocytic glutamate uptake

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Aim: Recently, we have demonstrated that intasynaptic K^+ accumulation depolarizes presynaptic terminal and increases glutamate release probability (Shih et al., 2013). Here we investigated the effect of K^+ accumulation on astrocytic glutamate uptake.

Methods: Glutamate transporter currents were recorded in voltage-clamped CA1 *str.radiatum* passive astrocytes in mouse hippocampal slices (C57BL/6J, P28-P35) in response to electrical stimulation of Schaffer collaterals (5 stimuli at 50 Hz) or local glutamate uncaging (200 µM). Our observations also were complemented with predictions of a detailed biophysical model.

Results: Increases in the extracellular K^+ concentration from 2.5 mM to 7.5 mM or to 20 mM significantly reduced the amplitude of uncaging induced transporter currents. Mimicking this depolarization by voltage clamp reduced the transporter currents to the same extent, reflecting the fact that astrocytic depolarization but not K^+ dependence of glutamate transporters suppresses glutamate uptake during K^+ accumulation. We also found that D-APV reduced the progressive increase in the decay time of the transporter currents produced by repetitive stimulation.

Conclusion: We suggest that the NMDA receptor dependent accumulation of intracleft K^+ during repetitive synaptic activity inhibits local glutamate uptake, which could extend glutamate presence in the synaptic cleft thus boosting glutamate spillover effects.

Reference:
Pei-Yu Shih, Leonid P. Savtchenko, Naomi Kamasawa, Yulia Dembitskaya, Thomas J. McHugh, Dmitri A. Rusakov, Ryuichi Shigemoto, Alexey Semyanov Retrograde Synaptic Signaling Mediated by K^+ Efflux through Postsynaptic NMDA Receptors / Cell Reports 2013 5:941–951

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Symposium 3: Molecular mechanisms of endothelium physiology in health and diseases

S3-1
On the physiology of vascular permeability

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Vascular permeability is induced by certain growth factors and by inflammatory cytokines. While the initiating factors have their unique receptors, the downstream mechanisms appear to be a large extent shared. Two major models exist for vascular permeability, formation of transcellular pores and opening of paracellular junctions. Moreover, the blood pressure and blood flow, regulated via the vessel vasotone is an important factor in regulation of vascular permeability. The consequences of permeability depend on whether the extravasation involves solute molecules and cells. Delineation of the mechanisms underlying molecular extravasation has been facilitated by the creation of a number of genetic loss-of function models. In contrast, there is less consensus on how cellular extravasation is mediated; possibly different classes of inflammatory cells extravasate differently, transcellularly or via the junctions. Vascular permeability in diseases is often an aggravating condition, leading to edema and increased interstitial pressure, promoting a disease progression. In the healthy individual vascular permeability is less well understood, however, the constant sieving of solute and molecules is most likely essential in tissue homeostasis. The role of vascular permeability in health and disease will be discussed.

S3-2
Regulation of vascular development and angiogenesis by the ETS transcription factor ERG

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Many diseases are associated with destabilisation of blood vessels, resulting in increased permeability, dysfunctional angiogenesis and haemorrhage; therefore, understanding the regulation of vessel stability could have important therapeutic applications. The ETS transcription factor ERG is highly expressed in the endothelium throughout development and adult life, and is one of the determinants of the endothelial lineage. ERG plays an important role in endothelial homeostasis and angiogenesis by controlling multiple endothelial functions such as cell survival, junction stability and cell migration. We have used Cre/loxP technology to generate endothelial-specific ERG deficient mice and showed that ERG is required for vessel stability during developmental and pathological angiogenesis. We demonstrate that ERG controls Wnt signalling in endothelial cells by regulating β-catenin protein degradation and by driving expression of the Wnt receptor Frizzled-4. Activation of Wnt signalling by stabilising β-catenin levels corrects the angiogenic defects associated with endothelial ERG deletion *in vitro* and in vivo. In addition, over-expression of ERG in vivo results in increased angiogenesis and reduced permeability of vascular endothelial growth factor (VEGF)-induced blood vessels. These results demonstrate that ERG is an essential transcriptional regulator of angiogenesis and vascular stability, and identify a novel candidate pathway to restore vascular integrity in disease.

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S3-3

microRNAs in tissue regeneration and revascularization

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Hind limb ischemia (HLI), resulting in muscle damage followed by vessel and muscle regeneration is associated with changes in microRNA expression. Here we investigated the role of miR-378a in ischemic muscles, a molecule considered to be significant in metabolism, myoblasts differentiation, tumor growth and tumor angiogenesis, (Skrzypek et al, Antioxid Redox Signal, 2013).

In wild type (WT) mice miR-378a expression decreases in acute phase of HLI. However, restoration of blood flow after HLI was inhibited in miR-378-/- mice (129SvEv/C57BL/6 background) in comparison to WT of mixed background and 129Sv36/SvEv strain. In miR-378a-deficient ischemic muscles more potent inflammatory response as assessed by inflammatory cells infiltration and expression of IL-1β, TNF-α, MCP-1, KC and VCAM-1 was observed. Moreover, FACS analysis revealed higher percentage of macrophages, granulocytes and dendritic cells in ischemic muscles of miR-378a-/- mice compared to WT. VEGF and angiopoietin-2 levels were elevated, while vessel-stabilizing angiopoietin-1 expression was transiently decreased after HLI in miR-378a-/- animals. Formation of capillaries was not, however, different in WT and miR-378a-/- mice, but the number of arterioles appears to be lower in miR-378a-/- animals. Interestingly, intramuscular or intravenous AAV-miR-378a gene therapy accelerated additionally the blood flow in WT animals, while it was ineffective in miR-378 KO mice.

miR-378a appears to affect hind limb revascularization, which is also associated with modulation of inflammation. Lack of improvement of revascularization after AAV-miR-378a transfer in miR-378a-/- mice suggests that basal expression of miR-378a in some, not efficiently transduced cells may play a role in proper revascularization.

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S3-4

Angiogenic Gene Therapy

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Therapeutic angiogenesis is a potentially useful therapeutic strategy for ischemic heart disease and peripheral arterial occlusive disease. It involves generation of new capillaries, collateral vessels or both in ischaemic muscles using either recombinant growth factors or their genes. Most commonly used growth factors for therapeutic angiogenesis are vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGF). Some other cytokines and growth factors may also have angiogenic effects *in vivo*. Improved perfusion can be achieved by angiogenesis and arteriogenesis. Arteriogenesis is a process caused by increased sheer stress at the arteriolar level resulting in the formation of large conduit vessels from preexisting small vessels. Local devices which improve gene transfer efficiency can be used for the treatment of arterial or vein graft stenosis after vascular manipulations. Most promising results have so far been obtained with direct catheter-based intramyocardial injections of VEGF genes with adenovirus and AAV vectors.

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S3-O1

Deletion of the NADPH oxidase organizing protein NoxO1 promotes angiogenesis

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Reactive oxygen species contribute to angiogenesis and vascular repair. NADPH oxidases are the main source of ROS in the vasculature. NoxO1 is a cytosolic protein facilitating assembly

on the constitutively active NADPH oxidase of epithelial cells. Being constitutively active, we speculate that NoxO1 contributes to basal ROS formation in the vascular system and modulates angiogenic responses. This hypothesis was tested in NoxO1 knockout mice and cells obtained from these animals.
Blood flow recovery after femoral artery occlusion was better in NoxO1-/- as compared to WT animals. Similar, ex vivo spheroid outgrowth assays revealed increased tube formation capacity in lung endothelial cells obtained from NoxO1-/- mice as compared to WT animals. In a spheroid confrontation assay, in which colour-labelled cells from WT and NoxO1-/- animals are directly studied within the same spheroid, the number of NoxO1-/- cells at the tips was higher than that of wild type cells. These results suggest that deletion of NoxO1 favours the expression of a tip cell like phenotype.
The NOTCH pathway is one of the main switches for an endothelial cell from a tip cell into a stalk cell phenotype and activation of the NOTCH pathway results in expression of a stalk cell phenotype. Physiologically, NOTCH mediated signalling requires proteases, among them the alpha-secretase ADAM17, to eventually result in the formation of the active NOTCH intracellular signalling domain. Importantly, ADAM17 activity was indeed reduced in NoxO1-/- cells when compared to wild type as measured by the degradation of an artificial substrate.
We conclude that NoxO1 controls alpha-secretase activity. Deletion of NoxO1 therefore promotes a tip cell phenotype which results in increased angiogenesis.

S3-O2

K_{ATP}, K_v7 and BK_{Ca} channels in the regulation of contractile responses of rat saphenous arteries during early postnatal ontogenesis

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Aim: Activation of K^+-channels is a key mechanism of vascular smooth muscle hyperpolarization and relaxation. We tested the hypotheses that the role K^+-channels play in the regulation of vasocontractile responses varies with postnatal maturation.

Methods: Segments of saphenous artery were isolated from young (10-15-days old) and adult (2-3-month old) male rats and studied using wire myography. We used blockers of K_{ATP} (glibenclamide, 3 microM), K_v7 (XE991, 3 microM) and BK_{Ca} (iberiotoxin, 0.1 microM) channels to evaluate their influence on basal tone and contractile responses to the alpha1-adrenoceptor agonist methoxamine. mRNA contents were determined by qPCR.

Results: Glibenclamide had no effect on basal tone and contractile responses of arteries in either young or adult rats. XE991 caused a significant increase of contractile responses and basal tone in young, but not adult rats; this was consistent with higher expression levels of $K_v7.1$ and $K_v7.5$ alpha-subunits in young rat arteries. Iberiotoxin augmented vasocontractile responses in both groups, in accordance with no between-group difference of BK_{Ca} (alpha-subunit) expression levels. Notably, endothelium denudation enhanced the effects of XE991 and iberiotoxin on arteries of young but not adult rats.

Conclusions: We demonstrated that K_{ATP} channels have no impact on contractile responses to alpha1-adrenoceptor agonist in both age groups; the influence of BK_{Ca} channels is not dependent on age, while the contribution of K_v7 channels decreases with maturation. The endothelium modulates the activity of BK_{Ca} and K_v7 channels in arteries of young rats.

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Symposium 4: Vascular Smooth Muscle Cell (Patho)Physiology 2015 – An Update

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S4-1

On the anti-mitogenic effects of cyclic AMP in vascular smooth muscle cells

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Aim: Vascular smooth muscle cell (VSMC) proliferation contributes to restenosis after angioplasty, vein graft intimal thickening and enlargement of atherosclerotic plaques. Prostaglandins acting through elevation of cyclic 3'-5'AMP (cAMP) reduce VSMC proliferation while sparing and even promoting protective endothelial cell (EC) functions including proliferation. Understanding the mechanisms underlying these highly divergent effects on ECs and VSMCs may open new therapeutic windows.

Methods: We conduct studies on isolated rat and human VSMCs and ECs, with emphasis on adenovirus mediated gene transfer: also studies of balloon injury in rats and vein grafting in mice.

Results: Elevation of cAMP through synergistic activation of protein kinase A and exchange protein directly activated by cAMP (Epac) inhibits the cell cycle in VSMCs but not ECs at 'classic' steps including mitogen activated protein kinase and induction of cyclin D. However, induction of the transcription factor early-response gene-1 is an earlier target and degradation of cyclin dependent kinase inhibitors a later independent step in the G1 phase of the VSMC but not EC cell cycle. In seeking a unifying mechanisms to understand these disparate effects we have focussed recently on the almost instantaneous differential rearrangement of the cytoskeleton (transduced in part through small GTPases) that cAMP provokes in VSMCs and ECs.

Conclusions: Cytoskeletal rearrangement is likely to provide a unifying explanation for the differential effects on VSMCs and ECs of cAMP and indeed other cues, including contacts with the extracellular matrix. These pathways almost certainly contain opportunities to discover new drug targets.

S4-2

Role of inflammation and vascular tone in flow-dependent arterial remodeling

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Local blood flow is regulated by networks of millions of small arteries and arterioles. Such regulation involves adaptation of both the level of vasoconstriction (tone) and structure (remodeling). Many cell signaling pathways have been found to contribute to vascular control. Despite the exploding understanding at the level of vascular biology, views on the integration of flow control over space and time remain limited.
Using various *in vitro* and *in vivo* approaches, we demonstrated that vascular tone and remodeling are causally linked: continuous deep vasoconstriction causes inward remodeling. We found this process to depend on the transglutaminases TG2 and FXIII, derived from SMC and TH2-activated macrophages. Flow is well known to cause both vasodilation and outward remodeling. Both processes are believed to depend on wall shear stress (WSS), and to contribute to the regulation of WSS. We created low flow and high flow pathways in rat mesenteric small artery arcades. This caused immediate adaptation of tone, followed after several days by inward remodeling in low flow vessels and outgrowth to larger calibers in high flow vessels. This was accompanied by recruitment of macrophages, as well as by upregulation of many cytokines. Yet, expression profiles were identical for high flow and low flow vessels. We concluded that inflammatory processes are crucial for induction of remodeling, but that the level of vascular tone sets the inward or outward direction of such remodeling.
We are building simulation models for spatial and temporal integration of flow control. Tone/remodeling models identified novel critical parameters with relevance for e.g. hypertension. Network models showed that communication between segments is needed for proper flow control, and requires not only shear stress and pressure sensing but also direct cell-cell coupling.

S4-3

Role of smooth muscle microRNAs in the regulation of calcium signaling and vascular tone

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The myogenic response is the intrinsic ability of small arteries to contract in response to increased internal pressure. Although microRNAs have been shown to play an important role in vascular smooth muscle function their importance for the regulation of the myogenic response has not been investigated previously. In this study we used smooth muscle-specific and inducible Dicer KO mice, which are deficient of most microRNAs, and found that the myogenic response was ablated in the small mesenteric arteries of these mice. This was associated with an increased PTEN expression, possibly due to deletion of miR-26a. Increased expression of PTEN, a negative regulator of PI3K/Akt pathway may reduce pressure-induced Akt-phosphorylation and pressure-induced calcium influx through voltage gated L-type calcium channels. Furthermore, myogenic tone was restored by the L-type channel agonist BayK 8644 or by transient stimulation with Angiotensin II (Ang II). The effect of Ang II was dependent on AT1-receptor stimulation and activation of the PI3K/Akt pathway. These results suggest a novel mechanism for regulation of myogenic tone in vascular smooth muscle by microRNAs.

S4-4

G-protein signaling in smooth muscle cell differentiation and vascular remodeling

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Pathological changes in the vascular system, such as formation of atherosclerotic plaques or an increase in arterial stiffness as a consequence of hypertension, pose as main risk factors for cardiovascular diseases including myocardial infarction, stroke or heart failure. Among others, reorganization of the vascular wall (vascular remodeling) is attributed to long-term changes in hemodynamic forces or an elevation of blood pressure. Initially, short-term alterations in these parameters are compensated by contraction or dilation of blood vessels in order to maintain blood supply to the organs. Such responses are mainly regulated by G protein coupled receptors (GPCRs) and the signal-transducing heterotrimeric G-proteins. GPCRs in the plasma membrane of vascular smooth muscle cells (VSMCs) are activated by contractile agonists but have also been described as mechanosensors mediating myogenic vasoconstriction. In particular, the activity of the G-protein α-subunits is precisely controlled by endogenous inhibitory proteins belonging to the family of the regulators of G-protein signaling (RGS). Interestingly, RGS proteins appear to be involved in VSMC responses elicited by chronically elevated wall stress as it may occur during hypertension. Prolonged distension of the vessel wall results in an increase in wall tension and eventually to compensatory wall thickening. This is mainly accomplished by VSMCs which undergo a phenotype switch from a quiescent to an activated state. The molecular mechanisms underlying VSMC activation as a result of increased wall tension and the involvement of RGS proteins regulating G protein signaling therein are largely unknown. In this context, we demonstrated that the VSMC phenotype switch depends on RGS5 activity which mediates RhoA activation in arterial VSMCs *in vitro* and in vivo. This seems to be a prerequisite for (stretch-dependent) vascular remodeling processes and might serve as a potential target for therapeutic intervention.

S4-O1

Vascular Notch3 deficiency induces polyuria in mice

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Aim: Notch3 is predominantly expressed by vascular smooth muscle cells (VSMC). Renal vasculature contributes to Na^+ and water homeostasis. Our aim was to analyse the consequences of Notch3 deficiency on water and sodium excretion.

Methods: Urine volume, osmolality, natriuresis, blood pressure were measured in basal conditions and after reversible water or salt restrictions, in Notch3 KO mice and control WT. In addition, using the approach of conditional induction of loss of Notch3/RBP-JK signaling in VSMC, activity of vascular Notch3 was genetically inhibited either at the end of renal development (1 month after birth) or at the early adulthood (3 months).

Results: In basal conditions, Notch3-deficiency was associated with polyuria (2.4±0.9 *vs* 1.4±0.5 mL/24h in WT; $p<0.01$) and reduced urine osmolality (742±185 *vs* 953±148 mOsm/kg; $p<0.01$). Importantly, after water restriction, Notch3 KO mice presented persistent increased diuresis ($p<0.01$), reduced urine osmolality ($p<0.01$), increased plasma osmolality ($p<0.05$). Differences between KO and WT mice remained after treatment by desmopressin and antagonists of endothelin or angiotensin II. Interestingly, Notch3/RBP-JK pathway inhibition in smooth muscle cells induced polyuria in mice when it was induced 1 month (but not 3 months) after birth. Na^+ excretion was higher in Notch3 KO mice at baseline and remained elevated during sodium restriction, associated with blood pressure reduction.

Conclusions: Notch3 is implied in the regulation of natriuresis and diuresis. Pharmacological and genetic approaches suggest that the tubulopathy induced by Notch3 deficiency is related to the persistent defect of Notch3 activity in renal vasculature, independently of tubular development and of vascular reactivity to endothelin or angiotensin II.

S4-O2

The effect of progesterone on the expression and function of the different alpha2-adrenergic receptor subtypes in late pregnant rat myometrium

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Aim: The adrenergic system and progesterone (P4) play a major role in the control of uterine function. The alpha2A- and alpha2C-ARs decrease the contractile response to noradrenaline, while the alpha2B-ARs mediate contractions. Our aims were to clarify the change in function and expression of the alpha2-AR subtypes after P4 pre-treatment in late pregnancy.

Methods: SPRD rats from pregnancy day 15 were treated with P4 for 7 days. The myometrial expressions of the alpha2-AR subtypes were determined by RT-PCR. *In vitro* contractions were stimulated with noradrenaline, and its effect was modified with the selective antagonists BRL 44408 (alpha2A), ARC 239 (alpha2B/C) and spiroxatrine (alpha2A). The accumulation of cAMP was also measured. The activated G-protein level was investigated by GTP binding assay.

Results: P4 pre-treatment decreased the contractile effect of noradrenaline in the presence of all the alpha2-AR subtype antagonists and BRL 44408 + spiroxatrine combination, which means decreased contractile response through the alpha2B-ARs. All of the alpha2-AR subtypes mRNA was increased, while there were no changes in the intracellular cAMP levels. P4 pre-treatment increased the G-protein-activating effect of NA in the presence of ARC 239, spiroxatrine and the spiroxatrine + BRL 44408 combination, but it was not changed in the presence of BRL 44408.

Conclusions: Our results suggest that the expressions of the alpha2-AR subtypes are P4 sensitive, which might contribute to the decrased myometrial contraction through the alpha2A- and alpha2C-ARs. Additionally, P4 may modify the G-protein coupling of alpha2B-ARs which can change the function of the receptor in contractility.

S4-O3

Lentiviral magnetic microbubbles enable targeted vascular gene transfer

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Aim: Site-specificity and efficiency remain the two most important challenges for targeted vascular gene transfer. We established a gene transfer technique using magnetic microbubbles (MMB) enriched with lentiviruses (LV).

Methods: MMB magnetic moments and velocities were assessed by magnetic responsiveness measurement. LV binding to MMB was verified by FACS. Transduction efficiencies of endothelial cells under static and flow conditions were quantified by reporter-gene expression. *In vivo*, LV-MMBs were targeted to dorsal skin vessels in mice by magnetic field (MF) and ultrasound (US; 1MHz, 2W/cm^2, DC50%, 30 s) exposure. Reporter-gene expression in organs was assessed by qRT-PCR. Viral particles in body fluids were analyzed with p24-ELISA and reporter-gene expression. MNP biodistribution was measured by MPS.

Results: Microbubbles containing negatively charged SO-Mag5 magnetic nanoparticles (MNP) exhibited higher magnetic moments and velocities compared to positively charged PEI-Mag4 MMB (n=3), whereas both MMBs were equally effective in binding GFP-coupled LV (n=4). LV-MMB exhibited a higher transduction efficiency under MF and US application *in vitro* compared to LV alone, whereby SO-Mag5 MMB were more efficient than PEI-Mag4 MMB ($p<0.05$;n=4). *In vivo*, MF and US exposure effectively targeted MMB to vessels of the dorsal skin (n=4). MNP accumulated mainly in lung and liver (n=5) 1h after treatment and were eliminated within 96h (n=4). No residual LV were detected in body fluids 48-72h after LV-MMB application (n=3).

Conclusions: Gene transfer via MMBs *in vitro* could be significantly improved by using negatively charged MNP. Combination of magnetic targeting and US application yielded site-specific targeting of skin vessels *in vivo*. Thus, LV-MMB mediated gene transfer may represent a valuable tool for future gene therapy.

Symposium 5: New forms of cell death in physiology and pathology

S5-1

Many ways to die, and mitochondria in the centre

Vilmantė Borutaitė

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S5-2

Regulation of RIPKs in cell survival and cell death by apoptosis and necroptosis, insights and therapeutic potential

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The discovery of regulated cell death has created many possibilities for gaining control over the life and death decisions made by cells during many inflammatory, degenerative and infectious diseases. For many years, apoptosis has been the focus of drug discovery. However, cell death research recently identified regulatory mechanisms and signaling pathways of non-apoptotic forms of regulated cell death, called regulated necrosis including necroptosis, parthanatos, cyclophilin-D-mediated necrosis, ferroptosis or oxytosis, MPT-dependent necrosis, pyroptosis and pyronecrosis, and NETosis/ETosis. Regulated necrosis (RN) is now defined as a genetically controlled cell death process morphologically characterized by cytoplasmic granulation, cellular swelling ("oncosis"), eventually resulting in plasma membrane permeabilization. Several of these cell death modalities share morphological features of necrosis, in particular the cellular swelling ("oncosis") and the eventual plasma membrane permeabilization.
We focus on the mechanisms that regulate the pro-survival and pro-cell death function of RIPK1 kinase during apoptosis and necroptosis *in vitro* and *in vivo*. We will also briefly discuss the execution mechanism of necroptosis involving MLKL. Several small molecules have been identified that can inhibit these non-apoptotic forms of cell death. The availability of these drugs will allow to develop strategies for translating the fundamental understanding of cell death pathways and their targeting into new therapeutic strategies for inflammatory, degenerative and infectious experimental disease contexts.

S5-3

MLKL mediates necroptosis in kidney tubules

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The pseudokinase MLKL is an essential downstream mediator of necroptosis, but the clinical and preclinical relevance of MLKL and the human relevance of necroptosis in general have hardly been explored. Here we present the results obtained from MLKL-deficient mice. Surprizingly, and in contrast to RIPK3-deficient tissues, MLKL-deficient freshly isolated renal organoids exhibit a strongly delayed onset synchronized necrosis ex vivo, resulting in overall protection from hypoxia-reoxygenation. MLKL-ko mice, in double blinded comparison with littermates, were characterized by increased capillary flow in intravital microscopy, a unique feature that might explain the stronger protection from renal ischemia-reperfusion in MLKL-ko mice compared with RIPK3-ko mice, whereas MLKL-ko mice were not protected from cisplatin- or folic acid-induced acute kidney injury (which are instead predominated by ferroptotic cell death). In the well-established model of TNF-induced SIRS, in our hands, MLKL-ko mice that were carefully matched to RIPK3-ko mice, exhibited a similar level of protection. In human, rat and mouse kidney transplantation, we provide evidence for the in situ detection of phosphorylated MLKL (pMLKL). To our knowledge, this is the first direct detection of activity of the necroptosis pathway in humans, as demonstrated by a highly specific monoclonal antibody against pMLKL in immunofluorescence and immunohistochemistry.

S5-4

Phagoptosis – cell death by primary phagocytosis – contributes to inflammatory neuronal loss and defence against cancer

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A cell may die as a result of being phagocytosed by another cell, a form of cell death we have called 'phagoptosis' with the defining property that inhibition of phagocytosis prevents cell death. Phagocytosis of an otherwise-viable cell may occur

because the cell is stressed, activated, senescent, damaged, recognised as non-self or misrecognised. Phagoptosis is probably the most common form of cell death in the body as erythrocytes turnover by this means. And phagoptosis eliminates pathogenic cells during infections and cancer.
CD47 is a don't-eat-me signal overexpressed on cancer cells. We found that function-blocking antibodies to CD47 on leukemic cells induced phagocytosis of the live cells by macrophages, resulting in their death and clearance.
We found that activation of microglia with LPS, Aβ of TNFα caused co-cultured neurons to expose phosphatidylserine, which bound MFG-E8, provoking microglial phagocytosis of neurons via the vitronectin receptor. However, if phagocytosis was blocked or microglia removed, then the phosphatidylserine exposure was reversed and the neurons remained viable.
In vivo LPS caused progressive neuronal loss by microglial phagocytosis, prevented by MFG-E8 knockout or phagocytosis inhibitors, leaving viable neurons. Delayed neuronal loss and motor deficits after transient brain ischaemia were prevented by knockout of phagocytic genes (MFG-E8 and MerTK), indicating that phagoptosis is detrimental in stroke. Thus phagoptosis may be important in a variety of inflammatory conditions and pathologies, inside and outside the brain.

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S5-O1

Short-term hypoxia and vasa recta function in kidney slices

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Aim: Acute Kidney Injury (AKI) is caused by changes in local renal blood flow and subsequent hypoxia. Vasa recta supply the inner part of outer medulla, an area at risk for hypoxic damages. Contractile pericytes are important mediators of vasa recta function. We hypothesize that a dysfunction in renal microcirculation, in particular of vasa recta under hypoxic conditions is responsible for a reduced medullary perfusion in AKI.

Methods: The effect of hypoxia on vasa recta was studied in a living rat kidney slice model. The kidney slices were subjected to control conditions (95 % O_2, 5 % CO_2) and hypoxia (1 % O_2, 5 % CO_2, 94 % N_2) for 60 and 90 minutes, respectively followed by a re-oxygenation step. The pericyte-mediated real-time changes in vasa recta were analyzed by DIC video-imaging after addition of angiotensin II (100 nM). The viability of the kidney slices and smooth muscle cells was verified using Click-IT Plus TUNEL assay and immunohistochemistry.

Results: The pericyte-mediated functional response of vasa recta to angiotensin II was not significantly altered following hypoxia for 60 and 90 minutes, respectively, if compared to control conditions. We found a significant increase of apoptotic cells in the medulla due to hypoxic conditions ($p<0.05$). However, apoptotic cells were neither detected in vascular structures nor in cells co-expressing alpha smooth muscle actin.

Conclusions: Short-term hypoxia has no effect on vasa recta function and survival of vascular cells. Our findings suggest the existence of mechanisms protecting renal vessels from hypoxic damage.

S5-O2

The Investigation of Astaxanthin Effect on Fatty Acid Values in Liver and Kidney Tissues of Rats which Exposed to Nicotine

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Aim: Nicotine cause to dependency on the living and lipid peroxidation in various tissues. Astaxanthin known as antioxidant that indicates therapeutic effect according to lipid peroxidation on the living. Thus, effect of antioxidant was investigated to fatty acid ingredient in liver and kidney tissues of rats which exposed to nicotine in this study.

Methods: Twenty Sprague-Dawley rats (200-250 g weight) were used in experiment. The animals were divided into four groups: control group (C, physiological solution, n=5), nicotine group (N, 5 mg/kg + nicotine – daily, n=5), astaxanthin group (A, 20 mg/kg + astaxanthin – daily, n=5) and nicotine + astaxanthin group (N+A, 5 mg/kg + nicotine – 20 mg/kg + astaxanthin – daily, n=5). Experimental applications were made as intraperitoneal during thirty days. Fatty acid values of liver and kidney tissues were analysed in Gas Chromatography (GC).

Results: ∑Saturated and fatty acid value in A group was determined statistically significant decreasing according to N group in liver ($p<0.05$). But, ∑Unsaturated fatty acid and ∑MUFA (monounsaturated fatty acid) in A group were determined statistically significant increasing according to N group in liver ($p<0.05$). ∑PUFA (polyunsaturated fatty acid) in N+A group was determined statistically significant decreasing according to N group in liver. Other than these values, some various fatty acid values were determined statistically significant in liver. On the other hand, statistically significant value in kidney was not determined without palmitoleic acid (16:1 n-7) value of A group.

Conclusions: Only nicotine, only astaxanthin and nicotine+astaxanthin influenced fatty acid ingredient of liver and kidney. But, nicotine and astaxanthin effected experiment groups in liver more than experiment group in kidney. Our results suggest that astaxanthin reduced the effect of nicotine on fatty acid composition in some groups of both tissues.

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S5-O3

Cardioprotective effect of beta-glucan against isoproterenol induced myocardial infarction in rats

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Aim: The present study was designed to investigate the cardioprotective activity of beta-glucan against isoproterenol (ISO) induced myocardial infarction (MI) in rats by studying electrocardiographic, biochemical and histo-pathological changes.

Methods: The present study was designed to investigate the cardioprotective activity of beta-glucan against isoproterenol (ISO) induced myocardial infarction in rats by studying electrocardiographic, biochemical and histopathological changes. The rats were divided into four groups of ten rats each. Control rats were given 2 ml of saline orally by intragastric gavage daily for a period of 10 days; beta-glucan group were treated with beta-glucan (50 mg/kg) dissolved in 2 ml of saline orally by intragastric gavage daily for a period of 10 days; Isoproterenol group were injected with ISO (100 mg/kg/day, s.c.) at interval of 24 h for 2 days and beta-glucan+ ISO group received beta-glucan treatment (50 mg/kg /day, p.o.) for 10 days and were injected with ISO (100 mg/kg/day, s.c.) on 9 th and 10 th day.

Results: Isoproterenol administration significantly elevated ST segment, reduced RR interval and increased heart rate on ECG, and also increased lipid peroxidation and decreased antioxidant enzyme activities in myocardial tissue. By contrast, pretreatment with beta- glucan produced a significant decrease in lipid peroxidation and improved the antioxidant status by increasing the activities of antioxidant enzymes. Moreover, pretreatment of beta-glucan significantly prevented the ISO induced alteration in ECG and decreased the severity of pathological changes and apoptosis in heart tissue.

Conclusions: As a conclusion, this study demonstrates that beta-glucan has a significant effect in the protection of heart against MI induced by ISO.

Symposium 6: Novel and old drivers of browning

S6-1

The interaction of genetics and environment in the realization of brown adipocytes

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Evidence is emerging that brown adipocytes (BA) in humans resemble the types found in white fat of mice, the so-called brite cells and those found in interscapular brown fat. The significance of this finding is that in mice the capacity for heat production by brown fat in response to the cold reduces excessive fat stores and improves type 2 diabetes. In mice the amount of brown fat that can be activated determines the effectiveness of the

anti-obesity action. Given that the amount of brown fat in humans ranges from undetectable to levels capable of contributing up to 12% of basal metabolic rate, it behooves us to establish how induction of brown fat and its thermogenic capacity can be maximized. The number of brown adipocytes depends in the type of fat depot, an environment that induces expression, generally by stimulation of the sympathetic nervous system, and mechanisms of brown adipocyte involution that affects stability of the tissue over time and its capacity for thermogenesis. I will describe studies on the interaction of the ambient temperature and the genetic background on developmental processes affecting brown adipocyte induction. Our studies indicate that the environment, while able to modulate the brown fat phenotype, is entirely dependent upon genetic mechanisms acting during early post-natal and perhaps fetal development to control the number of brown adipocytes in an individual.

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S6-2

Cross-talk between adipokines and myokines in fat browning

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Skeletal muscle is the largest organ determining whole-body insulin sensitivity and metabolic homeostasis. Adaptive changes of skeletal muscle in response to physical activity include adjustments in the production and secretion of muscle-derived bioactive factors, known as myokines, such as myostatin, that not only act locally in the muscle in an autocrine/paracrine way, but also are released to the bloodstream as endocrine factors to regulate physiological processes in other tissues. Irisin, derived from the cleavage of FNDC5 protein, constitutes a recently discovered myokine that induces myogenesis, through the activation of muscle growth-related genes and the repression of muscle atrophy markers, such as MAFbx and MuRF1. In this regard, both exercise and PGC-1a activation induce *FNDC5* expression and irisin secretion from skeletal muscle in rodents and humans. In addition, irisin induces fat browning (switch of white adipocytes to brown-fat-like cells) together with a concomitant increase in energy expenditure. Besides being a target for irisin actions, the adipose tissue also constitutes a production site of FNDC5. Interestingly, irisin secretion from subcutaneous and visceral fat depots is decreased by long-term exercise training and fasting, suggesting a discordant regulation of FNDC5/irisin in skeletal muscle and adipose tissue. In this regard, our group recently reported that the adipokine leptin differentially regulates FNDC5/irisin expression in skeletal muscle and fat, confirming the cross-talk between both tissues. Moreover, the secretion of irisin is regulated by other myokines, such as follistatin or myostatin, as well as by other adipokines, including fibroblast growth factor 21 (FGF-21). Taken together, myokines have emerged as novel molecular mediators of fat browning and their activity can be modulated by adipokines, confirming the cross-talk between skeletal muscle and adipose tissue in order to regulate thermogenesis and energy expenditure.

S6-3

Immunodetection of plasma irisin

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Irisin is a recently discovered peptide hormone which has a proposed action as a white adipose tissue browning agent. The peptide is suggested to be cleaved from a skeletal muscle membrane protein, *fibronectin* type III domain containing 5 (FNDC5), which in turn is stimulated by physical exercise-induced expression of transcriptional coactivator, peroxisome proliferator-activated receptor-gamma coactivator alpha (PGC1α), or cold exposure. From the muscle tissue irisin is travelling via circulation and acting on it´s, so far unknown, receptor(s) on beige precursor cells, interspersed within white adipose tissue. The resulting beige cells are brown adipocyte-like cells which are capable to produce heat.
Concerns have been raised about the poor quality of available antibodies used in western blotting and immunoassays (i.e. EIA, ELISA and RIA) in irisin detection from circulated blood. Plasma/serum irisin levels measured by different immunoassays have varied from 24 pg/ml to as high as 2000 ng/ml. Recent publications, however, have suggested that irisin circulates only in low levels. In addition, irisin antibodies seem to detect a wide variety of other proteins with high molecular sizes, which may affect significantly the obtained results. Better laboratory tools are needed to achieve reliable results to investigate thoroughly the possible physiological functions of irisin.

S6-4

Browning of white fat: What is the driving force?

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Two types of UCP1-expressing thermogenically active adipocytes are currently known: Classical brown adipocytes derived from the myogenic lineage and brite (brown-in-white) or beige adipocytes which can be induced in WAT depots in response to certain stimuli. Both, classical brown and brite adipocytes are present in adult humans. Promoting the recruitment and activity of these adipocytes may increase energy expenditure and represent a strategy to counteract the development of obesity. As part of the search for approaches promoting white-to-brown conversion in human adipocytes, we studied the role of irisin and LC-PUFAs in this process. Recently, the novel myokine irisin gained considerable interest as a potent inducer of WAT browning. Irisin is the cleavage product from the transmembrane protein FNDC5 and has been described in 2012 as an exercise-regulated myokine inducing the browning of WAT in mice. Our data indicate that irisin is not expressed in humans and does not trigger brite adipogenesis in human adipose-derived stem cells (hASCs). In addition to the energy content of diet, food composition and the presence of certain fatty acids may impact on WAT and BAT function. In particular, long-chain polyunsaturated fatty acids (LC-PUFAs) from the n-3 family exert anti-obesity effects in rodents, while n-6 LC-PUFAs are thought to promote obesity. However, the underlying mechanisms and the role of individual n-3 and n-6 LC-PUFAs in the white-to-brown conversion remain unclear. Our data reveal that the n-3 LC-PUFA eicosapentaenoic acid (EPA) and the n-6 LC-PUFA arachidonic acid (ARA) differentially regulate white versus brite adipocyte formation in hASCs. EPA induced a brite phenotype and improved mitochondrial function in hASCs, providing a novel mechanism for the anti-obesity effects of n-3 LC-PUFAs.

S6-O1

Anti-inflammatory effects of nesfatin-1 in rats with acetic acid induced colitis: role of ghrelin receptors

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Aim: Nesfatin-1 is an anorexigenic, neuroprotective, and anti-apoptotic peptide. Previously we have shown the protective effect of nesfatin-1 on colitis. Our aim was to investigate the potential underlying protective mechanism of nesfatin-1 in colitis.

Methods: Male Spraque-Dawley rats were were intracerebroventricularly (icv) cannulated for nesfatin-1 and antagonist applications. In colitis group, intrarectally (ir) 4% acetic acid solution (1 ml) and 10 minutes later icv nesfatin-1 (0.05 µg/5 µl) or vehicle (5 µl) were administered. Treatments continued for 3 days. In control group, physiological saline solution was used intrarectally. To identify the underlying mechanism, rats were divided into 2 subgroups, 5 minutes following colitis induction; icv SHU9119 (melanocortin receptor antagonist) or GHSR-1a antagonist (ghrelin receptor antagonist) were administered, 5 minutes later nesfatin-1 was administered for 3 days. On the fourth day, rats were decapitated, and colon tissues were sampled. Macroscopic and microscopic damage scores, malondialdehyde, glutathione, myeloperoxidase, superoxide dismutase, catalase, luminol and lucigenin chemiluminescence measurements were analyzed.

Results: The increased myeloperoxidase activity, malondialdehyde levels, luminol and lucigenin chemiluminescence measurements, macroscopic and microscopic damage scores with colitis induction ($p<0.05$-0.001) were decreased with nesfatin-1 treatment ($p<0.05$-0.001). GHSR-1a administration alleviated the protective effect of nesfatin-1 from microscopic and oxidant damage parameters and lipid peroxidation ($p<0.05$-0.001).

Conclusions: The results of the study suggest that the anti-inflammatory and antioxidant effects of nesfatin-1 on colitis might occur via ghrelin receptors.

S6-O2

Na^+/H^+ exchanger NHE1 and NHE2 activities have an opposing effect on experimental wound healing velocity in the rat gastric surface cell line RGM1

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Backround and **Aim:** Following superficial injury, neighboring gastric epithelial cells close the wound by rapid cell migration and electrical sealing, a process called epithelial restitution. Na/H exchange activity is necessary during this process, but the role of the different Na/H exchanger isoforms expressed in gastric pit cells has remained elusive. The role of NHE1 and NHE2 in wound healing velocity was therefore investigated in the nontransformed rat gastric surface cell line RGM1.

Methods: Restitution velocity was assayed by loading the confluent cells with the fluorescent dye DiR, making a longitudinal wound and fluorometrically following wound closure over time. RGM1 cells expressed virtually exclusively NHE1 mRNA and transport activity; NHE2 was therefore introduced by lentiviral gene transfer.

Results: At medium pH 7.4, RGM1 cells displayed wound healing even in the absence of growth factors, where migratory speed was independent of NHE activity. Growth factors or fetal calf serum (FCS) induced wound healing in a partly NHE1-dependent fashion. Wound healing was also stimulated by preincubation with acidic pH 7.1, and this stimulation was abrogated by NHE1 inhibition. Lentiviral NHE2 expression increased steady-state pHi and caused a dramatic reduction of restitution velocity after low pH preincubation, which was reversible by pharmacological NHE2 inhibition.

Conclusions: Migratory velocity during wound healing is increased by NHE1 activation in gastric surface epithelial cells, while NHE2 activity inhibits this process. Possibly, NHE2 is not localized to the leading edge during migration, and proton extrusion via NHE2 interferes with the local pH-gradient that facilitates directed migration.

S6-O3

Loss of carbonic anhydrase IX expression impairs gastric mucosal defense against luminal acid

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Aims: Carbonic anhydrase IX (CAIX) is ubiquitously expressed during embryogenesis but is down-regulated postnatally. CAIX expression persists in the stomach predominantly in the surface mucous cells. We hypothesize that this exoenzyme helps to protect the gastric mucosa against strong luminal acidity.

Methods: The cellular differentiation pattern, acid secretory capacity, the ability of the surface epithelial cells to withstand a luminal acid load, the mucus layer buildup and the cytokine profiles were assessed in CAIX KO and WT mice from newborn ages to late adulthood.

Results: The ability of gastric surface mucous cells to withstand luminal acid exposure and to generate an alkaline microclimate was significantly impaired in the stomach of CAIX KO mice compared to WT mice. This was accompanied by an increase in IL1ß prior to the gradual expansion of the mucous cell zone, regression of the parietal cell zone to the base of the glands accompanied by chief cell loss. Mild chronic proton pump inhibition from the time of weaning reduced the parietal cell loss in CAIX KO mice.

Conclusion: CAIX at the basolateral membrane of the gastric surface cells rapidly converts protons extruded by the surface cell basolateral membrane together with blood borne HCO3 to CO2 and H2O, and thus contributes to interstitial buffer capacity, surface cell pHi regulation, and maintenance of the pH microclimate in the mucus layer. Lack of CAIX results in chronic acid damage with a gradual regression of the parietal cell zone to the lower gland area.

Symposium 7: Monitoring interstitial fluid accumulation: from fundamental physiology and experimental evidence to clinical implications

S7-1

Clinical implications of inflammatory states in connection with fluid overload

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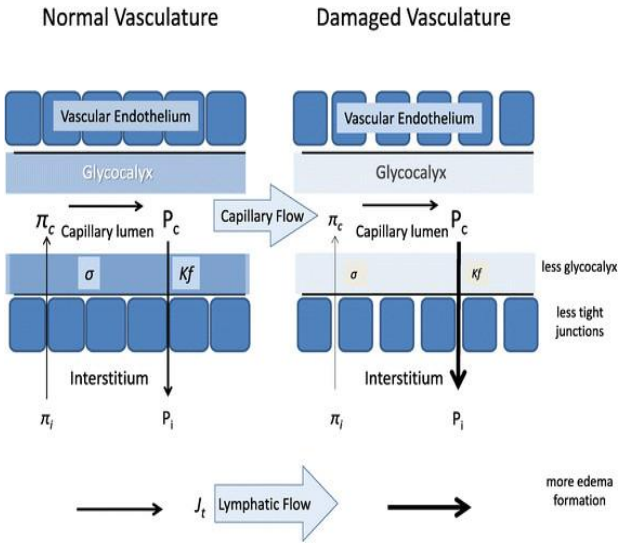
This talk will focus on the *clinical implications of fluid overload* during inflammatory conditions such as surgery, trauma and septicemia. In these conditions pertinent changes to the vascular endothelium will occur. See Figure.



The endothelial glycocalyx is important for maintaining the vascular wall on the transmission of shear stress, on the maintenance a permeability barrier, and on attenuating firm adhesion of blood leukocytes and platelets. Major constituents of the glycocalyx, including syndecans, heparan sulphates and hyaluronan, are shed from the endothelial surface under various acute and chronic *clinical* conditions, such as ischemia and hypoxia, sepsis, atherosclerosis, diabetes, renal disease and hemorrhagic viral infections.

Appropriately, pharmacological agents such as inhibitors of inflammation, antithrombin and inhibitors of metalloproteases display potential to attenuate shedding of the glycocalyx in various experimental models. Also, plasmacomponents, especially albumin, stabilize the glycocalyx and contribute to the endothelial surface layer. Though symptoms of the above listed diseases and conditions correlate with sequelae expected from disturbance of the endothelial glycocalyx (edema, inflammation, leukocyte and platelet adhesion, low reflow), therapeutic studies to prove a causal connection are rare.

In surgery and intensive care fluid therapy is a cornerstone and the amount and the rate has yet to be decided. There is a clinical discussion going on whether the increase of atrial natriuretic factor (ANP) will imply shedding of then glycocalyx. Thus, the question is will the fluid load and rate per se have implications for the increased permeability during inflammatory conditions.

S7-2

The interstitium - anatomy and physiology during normal and inflammatory states

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The interstitium constitutes the loose connective tissue that surrounds virtually all cells of the organism and at the same time provides these cells with the framework that is required to organize the cells of the body into organs constituting the organism. The interstitium has four principal components in addition to the cells: First, collagens constituting the stiff scaffolding for organs and organisms and second, elastic fibers and microfibrils. Third, there is the ground substance composed from the glycosaminoglycan hyaluronan as well as proteoglycans. Finally, the interstitium contains the interstitial fluid, which is an ultrafiltrate of plasma and the ECM, providing the route of transport for nutrients and waste materials. The interstitium contains 80% of the extracellular fluid volume and this volume is normally tightly controlled by autoregulation of the fluid fluxes across the capillary wall so that a change in one of the colloid osmotic or hydrostatic pressures acting across the capillary wall will set off changes in the interstitial colloid osmotic and hydrostatic pressures to counteract the changes in the interstitial volume. In inflammation the properties of the interstitium changes to promote transcapillary fluid flux by the interstitial pressure becoming more negative to enhance the transcapillary fluid flux across the capillaries to become an ”active” pressure in generating capillary filtration rather than normally counteracting and limiting the transcapillary fluid flux. This lowering is associated with alterations in the cellular attachments to the extracellular matrix fibers, the integrins, releasing tension the extracellular fiber networks and is associated also with the ability of glycosaminoglycans, in particular

hyaluronan, to swell when giving free access to fluid.

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S7-3

A non-invasive method to detect interstitial overload

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Fluid therapy plays a fundamental role in the management of hospitalized patients (Hoste *et al*. *Br J Anaesth* 2014; 113:740-7). Mistakes in the assessment of fluid requirements may cause organ dysfunction and failure because of both hypoperfusion and tissue oedema. It is estimated that in 2014 up to 20% of patients were subject to inappropriate fluid therapy (Kellum *et al*. *Br J Anaesth* 2014;113:729-31.). As part of Fast Track protocols, individualized fluid administration, namely the Goal Directed Fluid Therapy (GDFT), is among the 'best evidence based practices' for helping patients to recover faster after surgery (Mythenen *et al*. *Perioper Med (Lond)* 2012;1:2). All recent guidelines suggest that individualized fluid therapy has the potential to reduce risk to patients, enhance recovery after major surgery and improve overall outcomes of treatment. The GDFT is probably the most validated method for individual optimisation of circulation in the intensive care and perioperative settings. Infused crystalloid can either be rapidly eliminated as urine in healthy, awake and normotensive subjects or accumulate in interstitial tissues, especially during anesthesia, surgery or inflammatory conditions. Haemodynamic response to fluid challenge during GDFT protocols does not reliably reflect the distribution of a crystalloid during stepwise infusion and cannot either evaluate the state of interstitial expansion by fluids (hydration status), or detect imminent, or existing occult oedema in tissues. Evolving conceptual approaches such as mini volume loading test (mVLT) need validation in fundamental future research projects based on a rigorous methodological approach aiming to define the tools which may help to decide to stop fluid administration earlier, before overloading and to add other therapies such as vasoactive drugs. The mVLT method (Andrijauskas A *et al*. *Medicina* 2015; 51:81-91.) and it's physiological background, as well as techniques for monitoring interstitial fluid expansion are reviewed.

S7-4

Role of ion channels in lung oedema and pulmonary circulatory collapse

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The endothelial calcium/calmodulin-regulated K^+-channel with intermediate conductance, KCa3.1, and Na^+/Ca^{2+} permeable channels of the transient receptor potential (TRP) gene family are regulators of arterial tone by stimulating Ca^{2+}-entry-triggered and hyperpolarization-mediated dilation to mechanical stress and stimulation of G-protein-coupled receptors. Besides these roles, some TRP channels control endothelial/epithelial barrier functions and vascular integrity, while KCa3.1 provides the negative driving force required for Cl^- and water transport in some cells and most secretory epithelia. Among the endothelial Ca^{2+}-permeable TRPs, (vanilloid) type 4 (TRPV4), is best characterized and produces arterial dilation by stimulating Ca^{2+}-dependent NO synthesis and endothelium-dependent hyperpolarization. Stretch activation of endothelial TRPV4 channels followed by pulmonary vascular pressure-mediated Ca^{2+} uptake is also thought to be involved in acute lung injury. We have found that TRPV4 co-activates the calcium-sensing KCa3.1 in pulmonary endothelium and that KCa3.1-deficiency protects against TRPV4-induced pulmonary arterial relaxation, fluid extravasation, hemorrhage, pulmonary circulatory collapse, and cardiac arrest in vivo. These data identify KCa3.1 channels as crucial molecular components in downstream TRPV4-signal transduction and as potential target for the prevention of undesired fluid extravasation, vasorelaxation, and pulmonary circulatory collapse.

S7-O1

The volume-regulated anion channel (VRAC) and its associated SWELL1 are essential for mouse salivary gland development

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Aims: Pre-natal salivary gland morphogenesis and growth can be influenced by variations in extracellular osmolality, indicating that cell volume regulatory mechanisms are important during organogenesis. The volume-regulated anion channel (VRAC) and its associated SWELL1 are

important for regulatory cell volume decrease in various cell types. Here we study how inhibition of VRAC, and thereby SWELL1, affects salivary gland development.

Methods: Submandibular salivary glands (SMGs) were dissected from mice at embryonic day 13.5 (E13.5) and cultured in standard conditions in DMEM/F12 in microwell dishes. The is considered to be a Glands were cultured for 24h in normal media with DMSO (control) or media with 10 µM or 20 µM of the specific and potent VRAC inhibitor 4-(2-butyl-6,7-dichlor-2-cyclopentylindan-1-on-5-yl)-oxobutyric acid (DCPIB). The glands were photographed after 1 and 24 h. qPCR was used to analyse SWELL1 expression. MetaMorph software was used to measure gland area and bud development during morphogenesis.

Results: SWELL1 mRNA was present at E13.5 (early stage of glandular development). Glandular growth was similar in both control and media with 10 or 20 µM DCPIB. However, glandular budding disappeared completely in glands treated with DCPIB, most likely due to swelling of the gland. Furthermore, SWELL1 expression showed a 85% and 90% downregulation in glands cultured in 10 or 20 uM DCPIB, respectively ($p<0.05$).

Conclusions: VRAC and SWELL1 seem to be important at an early stage of glandular development. Impairing VRAC function and SWELL1 expression most likely reduces normal regulatory cell volume changes, which in turn will lead to impaired development of the gland. Thus, the ability of cells to volume regulate must be established early in fetal life.

S7-O2

SLC26a3 (DRA)-deficient mice display low surface pH, loss of firmly adherent mucus layer, altered colonic microbiome, and intestinal inflammation

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Aim: Patients with congenital chloride diarrhea (CLD), caused by loss of function mutations of SLC26a3 (DRA), display a propensity for acute and chronic intestinal inflammation. In this study, we investigated the effect of genetic ablation of Slc26a3 (DRA) on intestinal barrier function and microbiome composition.

Methods: DRA-deficient mice, an animal model for congenital Cl- diarrhea (CLD) were utilized to study the surface pH and the dynamics of mucus layer buildup in vivo by two photon microscopy, the intestinal microbiome by 16S rRNA sequencing, and the inflammatory state of the mucosa by qPCR and immunohistochemistry.

Results: DRA-deficient mice displayed strongly abnormal low surface pH and loss of fluid absorption in the mid-distal colon, but a virtually normal mucus layer buildup rate. However, they lacked a firmly adherent mucus layer. In addition, they displayed altered microbiota composition with a decrease in the firmicutes and an increase in the bacteroidetes in all parts of their colon. However, only the distal colon, which displays the thickest mucus layer and the highest DRA expression levels in WT mice, showed evidence for inflammation in the DRA KO mice, indicated by an increase in infiltrating mononuclear cells and increased TNF-alpha expression.

Conclusions: Apart from absorbing Cl-, Scl26a3 (DRA) is involved in the maintenance of intestinal barrier properties such as high colonic surface pH, firmly adherent mucus layer, and microbiome composition. The reason for inflammation in DRA-deficient colon may be a combination of weakened barrier properties with a more aggressive microbiota.

S7-O3

Association of surfactant protein B haplotypes with respiratory distress syndrome in neonates

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Aim: Genetic changes in surfactant protein B can cause respiratory disorders in neonates. The present study focuses on the possible relationship between haplotypes of surfactant protein B and the development of respiratory distress syndrome (RDS) in neonates.

Methods: Peripheral blood samples (0.5 ml) of 129 neonates were analyzed: premature infants with and without RDS (n=89), term neonates with respiratory problems of unknown etiology (n=20) and infants with non-respiratory disease (controls, n=20). After determination of the functional polymorphisms in gene for SP-B which are associated with RDS, the samples were genotyped and the haplotypes were assembled and assigned to the patients.

Results: There was a significant correlation between the administration of surfactant and gestational age (g.a.) ($P<0.000005$) and the administration of surfactant after 2 h with g.a. ($P<0.01$). Gestational age was related to infection ($P = 0.037$) and the need for repetitive surfactant administration ($P<0.000005$). Early infection is related to the early surfactant administration within 2 h ($P = 0.015$). The rs762548 was associated with the repeated administration of surfactant ($P<0.033$). Relatively common haplotypes of SP-B were not associated with RDS.

Conclusions: There is no link between common haplotypes of SP-B with respiratory distress in neonates. The results warrant further study of rare haplotypes by next generation sequencing.

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Symposium 8: Calcium signalling in astrocytes in health and disease

S8-1

Spatial and temporal properties of calcium events in single astrocytes

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Calcium activity in astrocytes is involved in the number of functions of these cells. Astrocytes express heterogeneous calcium events of different durations and spreads which thought to be triggered by local synaptic events. We developed novel method of whole event analysis in the time-lapse images of calcium activity in cultured astrocytes transfected with calcium sensor GCaMP2 or in astrocytes of mouse hippocampal slices expressing genetically encoded GCaMP2. We found that astrocytic calcium events do not categorize into different classes (e.g. local, expanded or global) but rather belong to the same skewed distribution, described by the power law. Power law distribution indicates that long and large events although very infrequent are responsible for larger proportion of overall calcium dynamics (in our case 30% of largest events were responsible for 70% of calcium activity). Activation of metabotropic glutamate receptors with selective agonist or by stimulation of glutamatergic Schaffer collaterals changed the power law exponent so the proportion of longer and larger calcium events increased. However the total number of calcium events did not increase, argue against triggering of new calcium events by the synaptic activity. We propose that change in the durations and the sizes of pre-existing calcium events is a mechanism by which neuronal activity affects astrocytic calcium dynamics. The discrepancy of our finding with the previous reports suggesting triggering of new calcium events may be due to the method of data analysis. For example, an increase in spread of calcium events originating in processes may make them detectable in soma. This leads to reporting of increased number of somatic calcium events in astrocytes in response to neuronal network activity.

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S8-2

Neuroglial interactions and activity-dependence of connexin channels in the olfactory bulb

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Because they express a high rate of connexins (Cx43, Cx30) astrocytes have the capacity to communicate through gap junction (GJ) channels and hemichannels (HCs). This adds another level of complexity when considering the contribution of neuroglial interactions to brain functions. We studied how neurons and astrocytes reciprocally modulate their network properties in olfactory glomeruli where neuronal connectivity is highly ordered. We observed: (1) a compartmentalized organization of GJ-mediated astroglial networks that overlaps neuronal functional units; (2) that these networks are plastic and modulated by neuronal activity. Interestingly, this modulation specifically targets Cx30-mediated GJ communication within glomeruli and depends on extracellular potassium generated by neuronal activity. Then, we addressed the contribution of astroglial Cxs to neuronal network activity. We observed that mitral cells (MCs) exhibit spontaneous and periodic activity (<1Hz) similar to the 'slow' wave oscillations recorded during slow wave sleep. UP and DOWN state fluctuations of MC membrane potential depend on glutamatergic interactions that take place at the level of the glomeruli. Importantly, these slow oscillations are modulated by the expression of Cxs. Indeed, in mice devoided of astroglial Cxs, the amplitude of slow oscillations is impaired. Also TTX treatments affect the UP state amplitude and firing rate of MCs. Those alterations are mimicked by a blockage of Cx43 HCs in the Cx30 KO mice. These results indicate that Cx43 HC function is promoted by neuronal activity and modulates neuronal network activity. Altogether these results indicate that astroglial Cxs play a role in bidirectional interactions between astroglial and neuronal networks.

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S8-3

Homer1 proteins and the tuning of astrocytic calcium signaling pathways

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In astrocytes, the intracellular calcium (Ca^{2+}) signalling mediated by activation of metabotropic glutamate receptor 5 (mGluR5) is crucially involved in the modulation of gliotransmission. Here we find that the mGluR5-mediated Ca^{2+} signalling leading to release of glutamate is governed by mGluR5 interaction with Homer1 scaffolding proteins. We show that in the critical postnatal period the long splice variants Homer1b/c are expressed in astrocytic processes, where they cluster with mGluR5 at sites displaying intense local Ca^{2+} activity. We show that the structural and functional significance of the Homer1b/c-mGluR5 interaction is to relocate endoplasmic reticulum (ER) to the proximity of the plasma membrane and to optimize both local and global Ca^{2+} signalling. We also show that in reactive astrocytes the short splice variant Homer1a is upregulated. Homer1a, by precluding the mGluR5-ER interaction disrupts Ca^{2+} signalling and exocytosis of glutamate, might thus limit the intensity and duration of gliotransmission in neuroinflammatory conditions.

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S8-4

Deregulation of astroglial calcium signaling by beta-amyloid: is it relevant to early neuronal dysfunction in Alzheimer's disease?

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Alzheimer's disease (AD) is a most common age-related neurological disorder with an enormous social and economical impact. AD is characterized by progressive loss of memory, social deficit and dementia. Currently, there is no cure or preventive therapy for AD and therefore novel approaches are desperately needed. The most accredited, amyloid, hypothesis of AD suggests that soluble beta-amyloid (Abeta) initiates pathogenic cascade, involving deregulation of calcium homeostasis, which leads eventually to neurodegeneration. Yet,

the possibility that Abeta affects calcium-signaling in astrocytes, and this may affect neuronal functions, at the early phases of the disease has been largely over-looked. In our lab we explore a scenario in which astrocytes may be first to react on Abeta and, through deregulation of astroglial calcium signaling, contribute to AD pathogenesis. First, we dissected cascade by which Abeta deregulates calcium homeostasis in hippocampal astrocytes. We found that calcium-dependent activation of calcineurin (CaN) and NF-kB mediates remodelling of astroglial calcium signaling toolkit. Similar changes were found in astrocytes from 3-Tg-AD mice. Next, using astrocyte-neuronal co-cultures and conditioned medium transfer, we found that Abeta42-exposed astrocytes, as well as astrocytes form 3xTg-AD mice, produced alteration of dendritic spines and reduction of synaptic proteins in cultured hippocampal neurons. Looking for mediators of such an effect we performed a whole-genome microarray study and compared changes in gene expression in astroglial cultures from non-treated wild type (Wt), Abeta42-treated Wt and 3-Tg-AD mice. We found altered expression of genes implicated in transcription, proteostasis and genes that may be involved in neuronal homeostasis and plasticity. Our data suggest that in early AD, astrocytes fail to maintain their homeostatic and signaling functions progressively leading to deregulation of synaptic transmission and plasticity.

S8-O1

Communication of remote tumor cells through the membranous tunneling tubes

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Aim: Membranous tunneling tubes (TTs) are recently discovered distinct form of communication between remote cells. The aim of this study was to identify the modes of formation and to examine the electrical and permeability properties of TTs between laryngeal squamous cell carcinoma (LSCC) cells.

Methods: Primary cultures of LSCC cells were prepared from tumor tissue samples taken during hemilaryngectomy surgery. Time-lapse and fluorescence imaging was used for examination of cell mobility, TT formation and their permeability to fluorescent materials. Electrical conductance of TTs was measured using dual whole-cell patch-clamp technique.

Results: We found that LSCC cells communicate with each other over long distances (up to 1 mm) through TTs that can be open-ended or contain functional gap junctions (GJs). In LSCC tissue and cell culture, we identified three types of connexins (Cxs), Cx43, Cx26 and Cx30, among which only Cx43 formed functional GJs between adjacent and connected through TTs cells. We identified 5 modes of TT formation and performed quantitative assessment of their electrical properties and permeability to fluorescent dyes of different molecular weight and charge. We observed cargo movement either inside the TTs or along their outer surface. TTs, containing F-actin and α-tubulin, transported mitochondria and were capable of transmitting double-stranded small interfering RNA. Finally, in the microsections of LSCC tissues, we identified the intercellular structures similar to those found in the primary LSCC cell culture.

Conclusions: Results of this study contribute to better understanding of intercellular communication between LSCC cells and provide more definite conception on mechanisms of tumor growth and spreading.

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Symposium 9: Re-examination of human reflexes using the discharge rate of single motor units

S9-1

Discharge rate method for error free estimation of the synaptic potentials in human motor neurons

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Aim: To discover a method for error free estimation of synaptic potentials in human motor neurons.

Methods: Since it is not possible to record directly from human neurons, indirect methods have been developed to study the synaptic potentials in these cells. The change in the electrical activity in a muscle in response to a stimulus has been used for the indirect estimation. However, the limitations of these classical (probability-based) techniques in estimating synaptic potentials were recognized and

reports have claimed that they contain significant errors in estimating the underlying potentials.

Results: To illustrate the errors in the currently used techniques directly and also to test a new hypothesis that claims that a frequency-based analysis for estimating synaptic potentials in neurons minimizes these errors, we have recently studied this problem in regularly discharging motor neurons in rat brain slice preparations.

Conclusions: In these studies, we have illustrated that the currently used methods for estimating pathways in the central nervous system do in fact contain significant errors and that these errors are minimized when the discharge rate information is used in a peristimulus frequencygram (PSF). It is crucial now that the PSF approach be used to reassess previously established pathways. We have now used this new method in several studies and 're-wired' many reflex circuitries (one example is shown below).

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S9-2

Discharge rate and synaptic noise affect reflex response regime of motor unit population

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Simulation and experimental studies have shown that the amplitude (probability) of the reflex response of motor neurons (MNs) to composite excitatory post synaptic potentials is dependent on the background discharge frequency and synaptic noise. In the present study, we hypothesized that the discharge frequency and synaptic noise may be the factors that determine the reflex response regime of a MN population activated at a given force level. For this purpose, the mono-synaptic H-reflex and the bi-synaptic reciprocal inhibition of a relatively large population of motor units were recorded from the tibialis anterior muscle of 12 subjects at 10% and 20% of maximum voluntary contraction using high density EMG electrodes. During the task, the subjects received electrical stimulation of tibial and peroneal nerves in different sessions. The hypothesis was additionally tested using a model of 100 MNs for a specific case as the membrane resistance of MNs changed gradually while the afferent input received was kept constant. The reflex amplitudes were measured using the cumulative sum of peri-stimulus frequencygram. For both simulated and experimentally recorded MNs, the correlation between reflex amplitude and the pre-stimulus mean discharge rate were estimated. A significant positive correlation was found between the discharge rate and the reflex amplitude for 10 subjects at 10% MVC (4 in excitation: $p<0.05$; 6 for inhibition: $p< 0.05$) and for 8 subjects at 20% MVC (3 in excitation: $p<0.05$; 5 for inhibition: $p< 0.05$). Moreover the correlation coefficients (r) decreased with decreasing coefficient of variation for the inter-spike-intervals ($p < 0.05$), that was used as a measure of synaptic noise. The simulations supported the experimental results. The results suggest that the MNs that fire with higher frequency in a population activated at moderate contraction forces have higher responses to inhibitor or excitatory postsynaptic potentials. However this is dependent on synaptic noise.

S9-3

The new technique for accurate estimation of the spinal cord circuitry: recording reflex responses of large motor unit populations

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We propose and validate a non-invasive method that enables accurate detection of the discharge times of a relatively large number of motor units during excitatory and inhibitory reflex stimulations. HDsEMG and intramuscular EMG (iEMG) were recorded from the tibialis anterior muscle during ankle dorsiflexions performed at 5%, 10%, and 20% of the maximum voluntary contraction (MVC) force, in 9 healthy subjects. The tibial nerve (inhibitory reflex) and the peroneal nerve (excitatory reflex) were stimulated with constant current stimuli. In total, 416 motor units were identified from the automatic decomposition of the HDsEMG. The iEMG was decomposed using a state-of-the-art

decomposition tool and provided 84 motor units (average of two recording sites). The reflex responses of the detected motor units were analyzed using the peri-stimulus time histogram (PSTH) and the peri-stimulus frequencygram (PSF). The reflex responses of the common motor units identified concurrently from the HDsEMG and the iEMG signals showed an average disagreement (the difference between number of observed spikes in each bin relative to the mean) of 8.2±2.2% (5% MVC), 6.8±1.0% (10% MVC), and 7.5±2.2% (20% MVC), for reflex inhibition, and 6.5±4.1%, 12.0±1.8%, 13.9±2.4%, for reflex excitation. There was no significant difference between the characteristics of the reflex responses, such as latency, amplitude and duration, for the motor units identified by both techniques. Finally, reflex responses could be identified at higher force (four of the nine subjects performed contraction up to 50% MVC) using HDsEMG but not iEMG, because of the difficulty in decomposing the iEMG at high forces. In conclusion, single motor unit reflex responses can be estimated accurately and non-invasively in relatively large populations of motor units using HDsEMG. This non-invasive approach may enable a more thorough investigation of the synaptic input distribution on active motor units at various force levels.

S9-4

Re-examination of the periodontal reflexes using frequency analyses

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Aim: Since direct measurement from the neuronal pathways is impossible, researchers used indirect methods to discover the feedback control of human mastication. We used a novel discharge rate analysis method, peristimulus frequencygram (PSF), to examine neural pahtways of the human masticatory system. In brain slice experiments while clearly illustrating that the currently-used methods contain serious errors, it was shown that the discharge frequency based analysis do not contain such erroneous information and that it indicates the properties of the synaptic potential and hence the neuronal pathway with confidence (Türker and Powers, 2005).

Methods: A periodontal mechanoreceptor (PMR) system was mechanically stimulated using fast-rising and slow-rising stimuli and the resultant response was recorded from the masseter muscles. To record the reflex response, either surface electromyogram (SEMG) or single motor unit (SMU) methods were utilized.

Results: Using the error free analyses method PSF, we have shown that the fast-rising mechanical stimulus on a tooth stimulated both PMRs and muscle spindles which resulted in substantial inhibition. However, when the stimulated tooth was locally-anaesthetized to overcome the activity of the PMRs, the same stimulus generated excitation (spindle response). When using slow-rising mechanical stimulus on a tooth, a dominant excitatory response is elicited.

Conclusions: Our studies highlight the importance of using the error free method to understand the feedback control of jaw muscles which are essential to develop scientifically based methods for the diagnosis and treatment of the jaw-related neuromuscular disorders.

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S9-O1

Electromyographic characterization of the gastrointestinal activity in rats

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Aim: Detection and interpretation of the in vivo myoelectric activity of the gastrointestinal (GI) tract is not fully solved. Our aim was to differentiate and characterize the signals from the different sections of GI tract with smooth muscle electromyographic method.

Methods: Male SPRD rats were anaesthetized with ketamine-xylazine. The total GI tract has been removed except one section (stomach, ileum or caecum). A pair of filament electrodes was inserted into the section, while a pair of disk electrodes was placed subcutaneously above the section. A strain gauge sensor was fixed on the surface of organ for the parallel detection of mechanical contractions. The electric signals were recorded by an online system and were analyzed by fast Fourier transformation. The software filtered the electric signals from the heart and the brain. The frequency of the electric activity was characterized by cycle

per minute (CPM), the magnitude of the activity was expressed as power spectrum density (PsD).
Results: Both types of electrode revealed the characteristic CPMs for stomach, ileum and caecum at 3-5, 20-26 and 1-3 cycle per minute (CPM), respectively. After neostigmine or atropine administration, the PsD values were increased (90-160%) or decreased (30-50%), respectively. However, the CPMs remained unchanged. A good correlation was found between the PsD values and the smooth muscle contractions.

Conclusions: We are able to detect the section-specific myoelectric activity of the GI tract in vivo. These signals are detectable even on the surface of the abdomen and correlate with the real contractions. Our method may serve as a new, non-invasive tool for pharmacological and clinical investigation of GI tract function.

S9-O2
Identification of M4 muscarinic receptors role in nocturnal activity

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Aim: M_4 muscarinic receptors have been shown to play role in many CNS functions, like learning, memory, autonomic processes, and regulation of motor activity. Previous studies identified overall increase in basal locomotor activity, but backcrossing studies doubted this conclusion.

Methods: We therefore studied the locomotor activity together with temperature using longitudinal telemetric measurement in eleven times backcrossed mice generation (C54Bl – WT *vs.* M_4 KO). Mice were anesthetized; the telemetric probes (Vital View, Starr Life, USA) were implanted into peritoneal cavity. Mice were left at least for two weeks to recover and then the spontaneous motor activity and temperature were analyzed using Chronos-Fit software. Statistical differences among WT and KO animals were determined using Student t-test, $p<0.05$ was considered as statistically significant.

Results: The M_4 KO mice showed increase in overall activity (weighted total, weighted mean in 24 hour period and total mean activity, $p<0.01$ in all parameters) but no change in the light (day) phase while there was an increase in dark (night) phase motor activity (difference between day-night mean, night mean (also weighted), mesor, peak, peak trough, and amplitude in 24 hour rhythm, $p<0.01$ in all parameters). In contrast, the temperature did not differ between WT and KO groups.

Conclusions: These results unmask differences in motor activity between WT and M_4 KO when studied in dark (night) phase while previous data obtained in light phase indicated similar activity. Thus it shows the importance of longitudinal observation in activity studies.

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Symposium 10: Sports performance, sports medicine and rehabilitation, public health

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S10-1
Changes in skeletal muscle calcium handling: trigger for beneficial adaptations vs. cause of overtraining

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Skeletal muscle weakness and premature fatigue development are major health problems associated with normal ageing as well as with numerous common diseases. Physical exercise can prevent and/or reverse these muscle problems and training improves the general health status in numerous diseases (1). Conversely, excessive muscle usage can induce muscle weakness, which may set the limit on performance of top athletes as well as patients with inherent neuromuscular disorders.
Recent studies indicate a key role of the sarcoplasmic reticulum (SR) Ca^{2+} release channel, the ryanodine receptor 1 (RyR1), in the muscle weakness observed, for instance, after strenuous endurance training, in normal ageing, in generalized inflammatory disorders, and in inherited conditions such as malignant hyperthermia (2,3). There is often a link between the impaired RyR1 function and increased levels of reactive oxygen/nitrogen species (ROS/RNS) (4). Conversely, altered RyR1 function may also be beneficial by increasing the cytosolic [Ca^{2+}], which may stimulate mitochondrial biogenesis resulting in increased fatigue resistance. Intriguingly, effective antioxidant treatment hinders positive adaptations of endurance training (5), and this effect might involve prevention of RyR1 modifications.

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S10-2

Exercising, obesity and diabetes – is mitochondrial citrate synthase a potential target?

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Citrate synthase (CS) is a key mitochondrial enzyme which is of importance for metabolic health and disease. The main aim of our study was to investigate if low CS activity impairs metabolic heath of mice fed high fat diet (HFD). We have also investigated if low CS expression can afffect cell viability. C57BL/6J (B6) mice and congenic B6.A-(rs3676616-D10Utsw1)/KjnB6 (B6.A) mice with low CS activity were fed HFD (45% kcal from fat) for 12 weeks. In the second study, we investigated effects of shRNA mediated knockdown of CS expression on fatty acid oxidation and viability of C2C12 muscle cells incubated with 0.8 mM palmitate. HFD feeding led to glucose intolerance. After glucose injection, the area under the blood glucose-time curve (AUC) increased ($P < 0.001$) during HFD feeding. AUC increased more ($P < 0.05$) for B6.A mice compared to B6 mice. In the second study, C2C12 mouse muscle cells showed 50% knockdown of CS activity when treated with shRNA targetting Cs mRNA. These cells showed reduced ($P < 0.05$) rate of palmitate oxidation and reduced viability compared to the control cells when incubated in media with 0.8 mM palmitate and 5.5 mM glucose. Muscle cells with CS knockdown also showed an increased ratio of proton production to oxygen consumption when incubated with 10 mM glucose. In summary, low CS activity is associated with a fast increase in glucose intolerance in mice subjected to HFD feeding. Low CS activity leads to a shift towards anaerobic metabolism, reduction in fatty acid oxidation and reduced viability of muscle cells incubated with high concentrations of saturated fatty acids.

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S10-3

Exercising until exhaustion: dynamic integration of brain, muscle and cardio-respiratory functions

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Endurance performance involves both psychological and physiological processes, but it is far from clear how they interact during exercise. As endurance depends on the interaction between vast number of system's components distributed at many levels, it is impossible to systematically deduce the macroscopic action behaviour from biochemical microscopic processes. Thus, our aim is to study endurance at macroscopic action level through the variables that best capture the dynamic of interactions between micro and meso-components: the order parameters or collective variables. A set of experiments have been designed to explore psychomotor (elbow angle and pedalling frequency), psychological (attention focus and perceived exertion); and physiological collective variables while cycling, running and weightlifting until exhaustion. A similar macroscopic nonlinear dynamics has been observed during these different types of exercise performed by different populations. The nonlinear effects correspond to those found in other studies that deal, for example, with gene expression cell dynamics which may generate and modify the phenotypic properties of athletes. They include bi-multistability, metastability, criticality and interaction dominant dynamics, as well as noise-induced transitions. With accumulated effort the system reduces the number of degrees of freedom and loses its initial flexibility on all studied levels, leading finally to task disengagement. The general 'loss of stability' mechanism is produced by a shift in the coordination between the basic biological processes of excitation and inhibition toward larger time scales. A redefinition of endurance and exhaustion is proposed on the basis of the nonlinear dynamic approach to psychobiological integration, and some practical applications for training interventions and training monitoring are suggested. It is likely that the application of nonlinear dynamics and statistical physics approaches may in the future form a comprehensive theory of endurance, encompassing multiple levels of biological organization within the same formal framework.

S10-4

Complex system approach in evaluation of persons physical activity features

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In modern medicine and sport many methods of investigation are involved covering organ physiology and pathological processes at different levels. Body system interaction effects are also included in these processes, but they are weakly described and explored. Investigations of these interactions require a big amount of data to describe those processes. To assess this, investigators require special analysis methods. Data integration usually is performed with mathematical methods. In real situations, when one organ activity causes changes in other system to cover the whole mosaic of existing mathematical tools are extremely complex. In every situation, when we have interactions of the target organ, the investigator faces at least several problems: 1) what main organ systems are included in specific research area (the size of holism), 2) what is potential context of selected working systems, 3) the methods of analysis applied in the present situation, including a possible link-ups. In human organism states, based on Vesalius, we have to include at least three holistic systems as a whole and the links between them. This are musculoskeletal, cardiovascular and regulatory (including CNS) systems. In any human body's adaptation to external or internal factors all those three are activated, although with different activity. Evaluated interaction between systems and determines the body's level of health, work performance, sports achievements, but here context plays as well very important role.

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S10-O1

Artificial selection for high aerobic capacity is protective against weight gain via high thermogenesis

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Aim: Thermogenesis may play a key role in dissipating extra energy as heat thus preventing extra weight gain. We studied the association of intrinsic aerobic capacity and physical activity (PA) on body temperature (BT).

Methods: We utilized rat strains that differ from their intrinsic aerobic capacity; high capacity (HCR) and low capacity runners (LCR). BT of HCRs and LCRs was measured before and after one year voluntary running or sedentary life. We also measured their spontaneous activity.

Results: HCRs have higher BT compared to LCRs ($p<0.001$). Aging decreased BT of HCRs to the level observed in LCRs, whereas running retained BT at initial level ($p<0.001$). Compared to LCRs, HCRs were spontaneously more active, had higher relative gastrocnemius muscle mass, and higher muscle PGC1a and Cyt c contents ($p<0.05$). Neither aging nor voluntary training had a marked impact on BT of LCRs.

Conclusions: These results suggest that the higher PA level together with greater relative muscle mass and higher mitochondrial content/function contribute to the more efficient thermogenesis of HCRs. The higher heat production can be considered as a protective metabolic trait that helps HCRs to resist excess body weight gain and to maintain better metabolic health compared LCRs. Voluntary running helped HCRs to maintain this positive trait during ageing addressing the importance of PA.

S10-O2

Usability of energy substrate during increasing workload exercise in woman with different body mass index and body fat mass

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Aim: It has been hypothesized that overweight and obese individuals have decreased lipid oxidation rate, which contributes to weight gain. The aim of this study was to compare the usage of energetic substrate components (lipids/carbohydrates) in increasing workload exercise for women with different body fat percentage (BF%) and body mass index (BMI).

Subjects: 22 young healthy, physically untrained women with different BMI participated in the study: 6 subjects were overweight or slightly obese (BMI=25.0-2.0 kg/m^2), 10 subjects with normal BMI (BMI=19.0-24.0 kg/m^2) and 6 subjects with reduces BMI (BMI<18.5 kg/m^2).

Methods: Energy expenditure, respiratory exchange ratio and usage of energetic substrate were measured by indirect calorimetry during the increasing workload treadmill exercise.

Results: Our results concluded the highest lipid oxidation rate consumed at low physical load intensity (20-35 Watts) were independent of the participant's body fat mass. But this highest lipid oxidation rate was reached at different oxygen consumption values within the three groups: in the reduced BMI/BF%, participants used 48-62% of the maximal oxygen consumption (%VO_{2max}). In the normal BMI/BF%, participants used 55-62% of the %VO_{2max}. In the increased BMI/BF% group maximal lipid oxidation was presented in wide %VO_{2max} range, namely, at 39-74% of %VO_{2max}. However, carbohydrate oxidation during increasing physical load correlates with increasing of %VO_{2max} in all investigated persons.

Conclusions: The lipid oxidation rate in overweight and slightly obese individuals might affect additional factors which result in greater and/or faster weight gain compared with reduced and normal BMI individuals. And people with higher BMI must seek a individualized exercise intensity to reach a maximal lipid oxidation rate.

S10-O3

Storage conditions affect electromechanical, histological and histochemical properties of osteochondral allografts

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Aim: Storage temperature and nutrient medium composition are the most critical factors for sustained biological activity of osteochondral allografts before implantation. The present study investigated the effect of various storage conditions on electromechanical, histological and histochemical properties of articular cartilage.

Methods: Osteochondral grafts derived from goat femoral condyles were frozen at -70ºC or stored at 4°C and 37°C in the medium supplemented with or without insulin-like growth factor-1 (IGF-1). After 14 and 28 days the cartilage samples were quantitatively analysed for electromechanical properties, glycosaminoglycan distribution, histological structure, chondrocyte viability and apoptosis.

Results: Storage at -70°C and 37°C significantly deteriorated cartilage electromechanical, histological and histochemical properties. Storage at 4°C maintained the electromechanical quantitative parameter (QP) and glycosaminoglycan expression near the normal levels for 14 days. Although hypothermic storage revealed reduced chondrocyte viability and increased apoptosis, these parameters were superior compared with the storage at -70°C and 37°C. IGF-1 supplementation improved the electromechanical QP, chondrocyte viability and histological properties at 37°C, but the effect lasted only 14 days. Electromechanical properties correlated with the histological grading score ($r = 0.673$, $p<0.001$), chondrocyte viability ($r = -0.654$, $p<0.001$) and apoptosis ($r = 0.416$, $p<0.02$). In addition, apoptosis correlated with glycosaminoglycan distribution ($r = -0.644$, $p<0.001$) and the histological grading score ($r = 0.493$, $p=0.006$).

Conclusions: Our results indicate that quality of osteochondral allografts is better preserved at 4°C

storage temperature. Storage at -70ºC or at 37°C is unable to maintain cartilage function and metabolic activity. IGF-1 supplementation at 37°C can enhance chondrocyte viability and improve electromechanical and histological properties of the cartilage, but the impact persists only 14 days.

Symposium 11: A cross talk between calcium and sodium in cell function, signalling and disease

S11-1

Regulatıon of CRAC channels by mıtochondrıa through Na^+ and redox sıgnalıng

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Mitochondria exert crucial control over plasma membrane (PM) Orai1 channels mediating store-operated Ca^{2+} entry (SOCE) and its biophysical correlate, the Ca^{2+} release-activated Ca^{2+} (CRAC) current. Yet, although the sensing of endoplasmic reticulum (ER) Ca^{2+} stores by STIM proteins and coupling to Orai1 channels is well understood, how mitochondria communicate with Orai1 channels to regulate SOCE activation remains uncompletely understood. Here, we reveal that SOCE is accompanied by a rise in cytosolic Na^+ that activates the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) causing enhanced mitochondrial Na^+ uptake and Ca^{2+} efflux. Omission of extracellular Na^+ to prevent the cytosolic Na^+ rise inhibits NCLX activity and impairs SOCE and CRAC currents. SOCE activates a mitochondrial redox transient which is dependent on NCLX and we reveal that this redox transient is required to sustain SOCE and CRAC currents. Thus, mitochondrial targeting of catalase is sufficient to rescue redox transients, SOCE and CRAC currents in NCLX-deficient cells. Our findings identify a *hitherto* unknown NCLX-mediated pathway that coordinates Na^+ and Ca^{2+} signals to effect mitochondrial redox control over SOCE.

S11-2

Properties and function of the lysosomal channels TPCs and TRPML1

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Acidic organelles such as lysosomes and endosomes store and release Ca^{2+} in response to cell stimulation. Endolysosomal Ca^{2+} has a role in several cellular functions, including hydrolytic activity, apoptosis, trafficking, energy metabolism and fusion/fission events. The endolysosomes express two Ca^{2+} permeable channels, the TRPMLs and the TPCs. The role and mechanism of activation of each channel in lysosomal Ca^{2+} release is not well understood. The messenger NAADP appears particularly important in mobilizing acidic Ca^{2+} stores while deletion of TRPML1 anso impair lysosomal Ca^{2+} release. Although the TPCs are essential for NAADP-mediated Ca^{2+} release from acidic stores, the molecular basis for triggering Ca^{2+} release by NAADP however is unclear. This presentation will discuss properties of the TPCs channels and their role in the response to NAADP and the potential role of TRPML1 in Ca^{2+} release and regulated exocytosis.

S11-3

Sodium signalling in neurons and glial cells of the CNS

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Aim: A basic characteristic of animal cells is the maintenance of a steep inward electrochemical gradient for sodium. In neurons, the sodium gradient energizes ion regulation and provides the basis for action potentials and excitatory postsynaptic currents. The sodium gradient also drives reuptake of transmitters, a task mainly performed by astrocytes. Because of its vital importance, intracellular sodium of both neurons and astrocytes was thought to be kept at a stable level. Contrary to this, our recent work has uncovered the existence of dynamic sodium changes with neural activity. Our our aim is to unravel the underlying mechanisms and consequences for brain function.

Methods: We combine dynamic imaging of intracellular sodium with the sodium-sensitive fluorescent indicator SBFI with whole-cell patch-clamp in mouse hippocampal tissue slices. Synaptic activity is induced by Schaffer collateral stimulation.

Results: Active neurons experience significant transient sodium increases upon excitatory synaptic transmission due to influx of sodium through glutamate-gated ion channels. Excitatory activity also evokes long-lasting sodium transients in astrocytes, which mainly arise due to sodium-dependent glutamate uptake. While there is no clear evidence for buffering of sodium, the

properties of activity-related sodium transients are fundamentally different from those described for calcium signals.

Conclusions: The functional consequences of sodium transients are just coming into view. Our own work shows that sodium elevations diminish glutamate uptake capacity by astrocytes. Furthermore, sodium increases promote reversed uptake of calcium by the Na^+/Ca^{2+}-exchanger, thereby contributing to calcium transients in neurons. Intracellular sodium changes might thus serve as signals themselves, influencing and regulating important cellular functions and playing a role in neuron-glia interaction.

S11-4

Crosstalk between TRPV1 and mitochondrial Na^+/Ca^{2+} exchanger controls pain signalling

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The pain receptor TRPV1 is acting by conducting Ca^{2+} and Na^+ influx in the nerve fibers of primary sensory neurons while the mitochondrial NCLX shapes the cytosolic and mitochondrial Ca^{2+} and Na^+ transients. The functional interplay between TRPV1 and NCLX in controlling the cytosolic and mitochondrial Ca^{2+} and Na^+ transients and subsequently the cell fate are however poorly understood. We combined fluorescent Ca^{2+} and Na^+ imaging technique with electrophysiology and molecular tool as siRNA NCLX, to investigate the communication between TRPV1 and NCLX. Activation of TRPV1 by capsaicin induced cytosolic Ca^{2+} and Na^+ fluxes via TRPV1 that were transmitted and integrated by NCLX into mitochondria. Knock down of NCLX expression was followed by a decrease in rate and amplitude of capsaicin dependent cytosolic Ca^{2+} influx and currents. TRPV1 currents were however fully rescued following the application of the cell permeable Ca^{2+} chelator BAPTA, indicating that NCLX by activating mitochondrial Ca^{2+} shuttling reduces cytosolic Ca^{2+} and thereby Ca^{2+} dependent inactivation of TRPV1. Furthermore, the enhanced Ca^{2+} shuttling by NCLX promotes cell death. Altogether our results indicate that the cross talk btween NCLX controls the mitochondrial Ca^{2+} and Na^+ transients initiated by TRPV1. Furthermore, NCLX modulates the cytosolic Ca^{2+} signals thereby controlling the TRPV1 channels activity and cell viabilty.

S11-O1

Effect of c-Abl gene silencing by siRNA on proliferation of mouse granulosa cells

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Aim: Granulosa cells (GCs) play a curicial role in folliculogenesis. c-Abl tyrosine kinase is the key factor in maintaining cell proliferation or differentiation, survival or death, retraction, or migration in response to growth factors, cell adhesion, DNA damage, oxidative stress, and other signals. We have investigated effects of *c-Abl* gene silencing, by small interfering RNA (siRNA), on the telomerase activity of mouse GCs.

Methods: The granulosa cells were isolated by puncturing the ovaries. They were grown in DMEM, 10% fetal bovine serum, for 24, 48, 72 and 96 h. Afterwards cells were treated ds-SiRNAs against: c-Abl. A SiRNA against cyclophilin B was used as control. No SiRNA treated cells were used. We used *qReal-Time PCR* for detecting *c-Abl, mTERT* (mouse telomerase catalytic subunit) and *β-actin* (internal control) in mRNA level*; Western blotting* for c-Abl, mTERT and β-actin. We showed c-Abl and mTERT protein localization by *immunohistochemistry* in mouse ovary tissue and i*mmunocytochemistry* in mouse GCs in cell culture.

Results: Results indicate that the *c-Abl* siRNA caused specific inhibition of *mTERT* mRNA expression after transfection. Knockdown of the *c-Abl* gene significantly decrease *mTERT* mRNA and mTERT protein expression in mouse ovary and GCs ($P<0.05$).

Conclusions: These findings suggest an important role of c-Abl in the regulation of mTERT expression during folliculogenesis in mouse ovary. Thence, identification of c-Abl-mTERT interaction may provide opportunities for the development of new therapeutic strategies for treatments for infertility and ovarian cancer.

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S11-O2

Protective effect of alpha lipoic acid, aerobic or resistance exercise from colitis in passive cigarette smoking young rats

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Aim: The role of passive cigarette smoking (PCS) and resistance exercise are not known. Our aim was to examine the effects of antioxidant ALA , chronic aerobic (AE) or resistance exercise (RE) on PCS rats with colitis.

Methods: Sprague-Dawley male rats (150-200g, n=54) were exposed to PSS (6 days/week, 4 cigarettes/day) while several groups were assigned to RE (climbing with weight; 3 days/week), others to AE (swimming; 3 days/week) whereas several groups were not exercised. Following 5 weeks, colitis was induced by intrarectal acetic acid. Subcutaneous ALA (50 mg/kg/day) or corn-oil were injected 3 days. Following decapitation, colon tissues were sampled to examine malondialdehyde (MDA) levels, myeloperoxidase (MPO), glutathione (GSH), superoxide dismutase, catalase activities, luminol and lucigenin chemiluminenscence, macroscopic scoring, histologic examination. ANOVA and student's t test were used for statistical analysis.

Results: The increased macroscopic and microscopic scores, MPO, MDA, luminol, lucigenin measurements in colitis and PSS-colitis groups were decreased via ALA. ($p<0.05$-0.001). AE declined macroscopic and microscopic scores, MDA, lucigenin compared to colitis and PSS-colitis groups ($p<0.01$-0.001). AE with ALA decreased luminol ($p<0.05$). RE reduced microscopic score, MPO, MDA, luminol, lucigenin ($p<0.05$-0.001) that were increased with colitis. GSH levels were decreased ($p<0.01$) in PSS-colitis group while approaching control levels by ALA.

Conclusions: PSS and colitis induction increased inflammatory parameters and oxidant damage. Our results suggest that ALA, AE or RE might be protective in ulcerative colitis.

Symposium 12: Chloride homeostasis and neuronal function

S12-1

Setting neuronal chloride gradient: a new role for extracellular matrix

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Neuronal signaling relies on ion fluxes through membrane-bound channels. Such fluxes are allowed due to the transmembrane ionic gradients created and maintained by membrane transporters. The neuronal gradient for chloride, an anion mediating most of the inhibition in the mature CNS, is the result of activity of two chloride transporters working in opposite directions, NKCC1 moving chloride in and KCC2 moving chloride out of the cell. The KCC2 function may be both up- and down-regulated through a number of mechanisms which allow for the fine-tune adaptation to a varying transporting load which mostly depends on chloride influx through both synaptic and extrasynaptic inhibitory chloride-permeable ion channels such as GABAA- and glycine-receptors.
Besides intraneuronal factors affecting KCC2 function and, thus, the chloride gradient, there is a number of extra-neuronal factors suggested to have a great influence over the distribution of chloride across the neuronal membrane. The very recent experimental findings point to the extracellular matrix as an important player in regulation of intracellular chloride. It has been claimed that large anion groups located within the extracellular matrix set the chloride gradient due to the Gibbs-Donnan effect, thus, effectively sidelining KCC2 as a major contributor to the neuronal chloride homeostasis. However, our own experimental findings concerning chloride homeostasis in anterior hypothalamic neurons suggest quite a different mechanism of involvement of the extracellular matrix.

S12-2

Impermeant anions, fixed charges, and the driving force of $GABA_AR$-mediated Cl^- currents

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It has been recently suggested[1,2] that electrostatic (Donnan) effects of immobile anions, not the cation-chloride cotransporters[3] (CCCs) KCC2 and

NKCC1, determine the driving force (DF_{GABA}) and polarity of GABAergic Cl^- currents in neurons. Here we disprove these ideas by analysing the underlying misconceptions and flaws. To this end we use basic biophysics and thermodynamics to analyse the effects of fixed charges and microdomains on neuronal Cl^- regulation. Fixed charges can alter the concentrations of mobile ions in aqueous microdomains where binding of ions to CCCs takes place, but this has no effect on the free energy of ion transport[3,4]. Donnan mechanisms acting across a plasma membrane or along cytosol[1,2] can cause local changes in E_{Cl} but not in DF_{GABA}, i.e. they cannot account for observations such as depolarizing and hyperpolarizing GABAergic Cl^- currents in the axon initial segment and in dendrites, respectively[5,6]. Currently available evidence does not support the idea that KCC2 expressed in CNS neurons transport ~500 water molecules along with one K^+ and one Cl^- ion, and basic thermodynamic principles rule out microdomains of restricted water[2] as a source of energy for Cl^- transport. Thus, the recently suggested ideas[1,2] on the role of immobile anions and restricted water in determining the polarity of channel-mediated Cl^- currents conflict with fundamental laws of thermodynamics and with empirical evidence demonstrating the role of CCCs in neuronal Cl^- regulation.

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S12-3

Presynaptic action potentials and GABAA receptors

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Neurotransmitter release depends steeply on presynaptic calcium influx, which in turn is affected by the amplitude and duration of the presynaptic action potential. Although presynaptic GABA(A) receptors occur at many synapses, how they affect neurotransmitter release is poorly understood. I shall discuss recent work from my laboratory on presynaptic recordings from excitatory and inhibitory nerve terminals in acute brain slices and in neuronal cultures. These recordings from previously inaccessible structures are revealing how sodium and potassium channels interact to shape the action potential. At hippocampal mossy fibre synapses presynaptic GABA(A) receptors are tonically active and can sense spillover of GABA from neighbouring synapses. Because the chloride reversal potential is relatively depolarized this leads to inactivation of potassium channels, broadening of the presynaptic action potential and increased calcium influx, resulting in facilitation of neurotransmitter release and presynaptic long-term potentiation. Whether these principles operate at other synapses remains to be fully resolved.

S12-4

Maternal administration of bumetanide rescues altered GABA developmental sequence and the autistic phenotype in rodent offsprings

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Autism is a developmental disorder characterized by restricted interest and communication impairment generated by genetic and environmental factors. It has been recently shown that the diuretic NKCC1 chloride importer antagonist bumetanide reduces the severity of autism in children. In present study we analyzed the cellular and network alterations that occur during the transition from fetal to postnatal life in animal models of autism. We focused in GABAergic inhibition, as it is deficient in autistic human and in animal models of autism and leads to an imbalance between excitation and inhibition. Electrophysiological recordings in CA3 region of hippocampus in brain slices, *in vivo* intracranial EEG recordings and behavioral tests were performed in two animal models of autism: rats exposed in utero to valproate and mice carrying the fragile X mutation. We show that the oxytocin-mediated neuroprotective g-aminobutyric acid (GABA) excitatory-inhibitory shift during delivery is abolished in both rodent models of autism. During delivery and subsequently, hippocampal neurons in these models have elevated intracellular chloride levels, increased excitatory GABA action, enhanced glutamatergic activity, and elevated gamma oscillations. Maternal pretreatment with bumetanide restored in offspring control electrophysiological and behavioral phenotypes. Our results suggest a chronic deficient chloride regulation in autism and stress the importance of oxytocin-mediated GABAergic inhibition during the delivery process. Our data validate the amelioration observed with bumetanide and oxytocin and point to common pathways in drug-induced and genetic rodent models of autism.

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S12-O1

Effects of treatment of paroxetine, bupropion or agomelatine on puberty onset, gonadotrophin and estradiol levels in female rats

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Aim: There are few studies in literature about how antidepressants affect the puberty onset in female rats. In this study, we aimed to explore the effects of paroxetine, bupropion and agomelatine, having different mechanism of action and used as antidepressants, on puberty onset and serum gonadotrophin and estradiol levels.

Methods: For the experimental studies, totally 40 female Sprague Dawley rats were used. All animals were randomly divided into four groups (control, paroxetine, bupropion and agomelatine) and each group consisted of 10 rats. The animals started to receive daily oral paroxetine (3.6 mg/kg), bupropion (17 mg/kg) or agomelatine (10 mg/kg) from post-natal day 21 to 90 day. The control group received only vehicle.

Results: There were significantly advanced on puberty onset days for paroxetine and agomelatine groups ($p<0.05$). Median of puberty onset in control, paroxetine, bupropion and agomelatine groups was 48, 43, 43 and 43.5 days, respectively. There was no significant difference in pubertal weight between control and all antidepressants groups. There was no any significant change in serum LH and FSH levels. There was significant increase in serum estradiol level in agomelatin-treated group compare to control group (127.47±10.22 and 52.69±7.46, pg/mL, respectively, $p<0.001$)

Conclusions: The present findings suggest that chronic peripheral administration of paroxetine, bupropion and agomelatine advance puberty onset as estimated from the date of vaginal opening.

Symposium 13: Microglia in the healthy brain and influence of their dysfunction

S13-1

Control of microglial responses to CNS infection and injury

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Aim: Microglia are principal innate immune cells of the CNS, serving the maintenance, protection as well as restoration of its structural and functional integrity. Microglia can sense infection and damage through overlapping sets of receptors. Toll-like receptor (TLR) 4 is known for recognizing lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria. Yet it also signals to multiple endogenous factors, which are generated, released or modified upon cell impairment and tissue injury. We focused on the organization and function of TLR4 in microglia.

Methods: Responses of microglia, extraneural macrophages and the CNS tissue to challenges with LPS, endogenous ligands, mechanical and ischemic injuries were determined and compared *in vitro* and *in vivo*, based on induction of cytokines/chemokines, expression of surface molecules, recruitment of immune cells and examination of tissue damage.

Results: The TLR4 co-receptor CD14 serves three non-redundant functions in microglia. First, it confers an up to 100-fold higher LPS sensitivity compared to peripheral macrophages to enable efficient pro-inflammatory cytokine/chemokine induction. Second, CD14 prevents excessive responses to massive LPS challenges via an interferon type I-mediated feedback. Third, CD14 is mandatory for microglial reactions to tissue damage-associated signals. In mice, these functions are essential for balanced CNS responses to bacterial infection, mechanical trauma and ischemic insults, since CD14 deficiency causes either hypo- or hyperinflammation, insufficient or exaggerated immune cell recruitment or worsened stroke outcomes. While CD14 orchestrates functions of TLR4, it is itself subject to massive regulations by TLR and non-TLR systems.

Conclusions: These regulations may operate to fine-tune microglial damage-sensing capacity upon infectious and non-infectious challenges. The CD14/TLR4 complex may thus serve as a sensory system that integrates most diverse signals to

instruct adapted responses to exogenous and endogenous threats.

S13-2

Microglia and neuroglia in ALS and spinal muscular atrophy (SMA)

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Amyotrophic lateral sclerosis (ALS) and Spinal Muscular Atrophy (SMA) have been traditionally considered pure Motor Neuron Diseases. Recent evidence from genetic models and from patients supports the view that this is not the case. Motor neuron decline and death are accompanied by alterations occurring in non-neuronal cells such as astrocytes, microglia, Schwann cells and oligodendrocytes. Since glial cells actively contribute at least to the progression of symptoms, MNDs should be regarded as multi-systemic diseases.
Although the ultimate mechanisms by which neurodegeneration and neuroinflammation initiate and propagate are still unclear, growing attention is lately focusing on the inflammatory component of MNDs as a target for treatment aimed at both increasing motor neuron survival and slowing down the progression of symptoms.

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S13-3

Of ghost cells and ravenous microglia: coupling between apoptosis and microglial phagocytosis in health and disease

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Phagocytosis is a crucial component of the regenerative response that remains notably unexplored in the brain. In physiological conditions in the adult neurogenic niche, where the excess of newborn cells undergo apoptosis, phagocytosis is efficiently executed by surveillant, ramified microglia. To test whether microglia are efficient phagocytes in the diseased brain as well, we confronted them with a series of apoptotic challenges and discovered a universal response. When challenged with excitotoxicity *in vitro* or chronic and acute inflammation in vivo, microglia proportionally increased their number and/or their phagocytic capacity to counteract the increased number of apoptotic cells, thus maintaining phagocytosis and apoptosis tightly coupled. Unexpectedly, this coupling was lost in a mouse model of mesial temporal lobe epilepsy (MTLE), a major neurological disorder characterized by seizures, excitotoxicity, and inflammation. This phagocytic blockade was not directly mediated by glutamate receptors on microglia but by the hyperactivity of the hippocampal network, which disrupted local ATP microgradients, reduced microglial motility and surveillance, and prevented the efficient targeting of apoptotic cells. Remarkably, phagocytosis remained chronically impaired in the experimental model as well as in hippocampal tissue resected from patients suffering from MTLE. The impairment of phagocytosis correlated with the expression of microglial proinflammatory, epileptogenic cytokines, contributing to the pathophysiology of epilepsy. Importantly, KA-induced seizures triggered an early increase in the number of apoptotic newborn cells in the hippocampal neurogenic niche that was not due to their decreased survival but to the accumulation of the non-phagocytosed cells undergoing apoptosis in physiological conditions. Thus, the efficiency of microglial phagocytosis determines the dynamics of apoptosis in the healthy and diseased brain and that phagocytic efficiency should be routinely assessed in neurodegenerative disorders.

S13-4

Sex- and age-dependent effects of thyroid hormone on glial function and morphology

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Aim: L-tri-iodothyronine (3, 3', 5–triiodothyronine; T3) is an active form of the thyroid hormones (THs) essential for the development and function of the central nervous system (CNS). Although the importance of THs in the CNS has been reported, precise actions of THs on microglia have not yet been well explored. In this study, we investigated the effects of T3 on microglial migration and phagocytosis *in vitro* and *in vivo*. In addition, we investigated the morphological changes in microglia and astrocyte in hypo- and hyper-thyroidism model mice.

Methods: Microglial migration and phagocytosis were investigated in both *in vitro* and *in vivo* using C57/BL6 mice. Hypo- and hyper-thyroidism were induced by injection of either anti-thyroid drug (propylthiouracyl; PTU) or thyroxine (T_4) to young (8

week-old) and old (>1 year-old) male and female mice. The effects of hypo- and hyperthyroidism on glial cells in the cerebral cortex and hippocampus were investigated by immunohistochemical analysis.

Results: Exposure to T3 increased migration, membrane ruffling and phagocytosis of primary cultured mouse microglia. Injection of T3 together with stab wound attracted more microglia to the lesion site *in vivo.* The T3-induced microglial migration or membrane ruffling was dependent on TH transporters and receptors, followed by non-genomic effects of T3 via $G_{i/o}$-protein, nitric oxide synthase, and subsequent complex signaling. Interestingly, effects of hyper- and hypothyroidism on glial cells were sex- and age-dependent.

Conclusions: TH affects microglial function and glia58_l morphology in sex- and age-dependent manner. These results may help to understand physiological and/or pathophysiological functions of THs in the CNS.

S13-O1

Hyperthermia: a systems approach in analysis of the effects on different organization levels

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Aim: Hyperthermia is a potent addendum to the existing cancer treatment modalities (surgery, chemotherapy and radiotherapy) that can improve clinical outcome up to 25%, however, the molecular and physiological mechanisms determining cellular fate during hyperthermia are still unclear. The present study is a systematic overview on effects of hyperthermic treatment on different organization levels (biomacromolecules, mitochondria, cells, liver tissue) considering cancer and normal tissues, different temperature ranges, gender, age, drugs and drug metabolites.

Methods: Parameters evaluated under the conditions of hyperthermia (37-51ºC) in biomacromolecules: protein denaturation and lipid phase transition; in isolated mitochondria: mitochondrial respiratory rate in different metabolic states, mitochondrial swelling, membrane potential, ROS generation; in normal and cancer cell lines: cell viability, plasma and mitochondrial membrane permeability, Ca^{2+}, adenine and pyridine nucleotides concentration; in liver perfusion and thermoablation experiments: NAD(P)H fluorescence, mitochondrial functions. The combined treatment of hyperthermia with cisplatin was evaluated in cell culture, with acetaminophen and its metabolite NAPQI in mitochondrial model.

Results: The obtained data are very complex, however some important insight in the mechanisms of response to hyperthermia can be summarized: age- and gender-dependent effect of hyperthermia and its combination with drugs in isolated mitochondria model (sexually immature organisms are more resistant to hyperthermia but more sensitive to drugs, the oxidative phosphorylation system is activated in females but inhibited in males at febrile temperature); thermosensitivity of tumour cell can be predicted from Ca^{2+} and free nucleotides concentration.

Conclusions: Our results give the fundamental knowledge on multifactorial action of hyperthermia in normal and tumour tissues. Obtained results can be further used in mathematical modelling of hyperthermic treatment mode and prediction of outcomes.

Symposium 14: Neurogenesis relevance and interaction in aging and neurodegeneration: new ideas for understanding and treating pathology

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S14-1

Stress and Neurogenesis: from mother to offspring

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Stressful events experienced during infancy including *in utero* stress events have been proposed as a major pathophysiological mechanism for developing vulnerability towards neuropathologies during adulthood and aging. One cardinal feature of such pathologies is impaired cognitive ability, which may in part rely on abnormal structure and functioning of the hippocampus. Our investigations over the last few years have focused on this relationship and more particularly on the role of adult hippocampal neurogenesis in these cognitive impairments. Prenatal stress was thus found to alter neurogenesis levels from adolescence till senesence and to induce deficits in neurogenesis-dependent behaviors such as spatial reference memory and pattern separation, i.e. the ability to form distinct memories of similar episodes, a process that is critical for reducing interference. In

line with this result, prenatal stress was found to predispose individuals to develop a PTSD-like memory profile, a pathology characterized by an altered pattern separation.
In our search for the etiological factors of such deficits, we have focused on mother-pup interactions, a fundamental shaping factor that we found to be altered by gestational stress. As maternal care, a crucial component of mother-pup interaction relies on olfactory function and adult bulbar neurogenesis, we hypothesized that gestational stress may interfere with motherhood-induced olfactory adjustments through a disruption of adult bulbar neurogenesis. Ongoing studies revealed that maternal stress dramatically disturbed social odor discrimination, and prevented the enhanced odor memory observed in lactating mothers, a phenotype that could be linked to alterations in the morphology of newborn neurons in the olfactory bulb.
Altogether, this set of data underlies the fundamental role of adult neurogenesis in the etiology of stress-related disturbances; hence it could represent an interesting target for alleviating stress-induced pathologies.

S14-2

Dentate gyrus neurogenesis in Alzheimer's disease. Focus on the neurovascular unit

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Aim: The neurovascular unit –composed by brain blood vessels, neurons and glia- play an essential role on health and disease. The blood brain barrier finely regulates the communication between the periphery and the CNS. Our aim was to address the involvement of the neurovascular unit during the neurodegenerative process, associated to normal and pathological cerebral aging. In particular, we focused on the dentate gyrus of the hippocampus and his neurogenic capability, highly reduced in aging and Alzheimer's disease (AD).

Methods and Results: We studied the microvasculature in the hilar region from control C57Bl/6 and PDAPP-J20 transgenic mice, model of AD, at different ages. In AD mice we found a reduction of vessel surface -as tomato lectin positive area-, in combination with increased number of vessel morphological aberrations. At early stages of the disease, we detected a low presence of the tight junction protein occludin in the hilus, suggestive of premature brain blood barrier disruption.
On the other hand, hippocampal neurogenesis was studied by assessment of number and arborization tree of young new neurons labeled with doublecortin. The neurogenic ability was already strongly affected at 3 months of age AD mice compared with control littermates.
Conclusions:
In my presentation during this symposium we will discuss the premise of a close relation between vascular and neurogenic perturbations in the dentate gyrus in the aging and AD context, proposing the hilar region as a susceptible area in neurodegeneration.

S14-3

Neurogenic and gliogenic alterations and recovery in Alzheimer's disease: a new view for understanding and tackling the disease

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Neurodegenerative diseases (ND) including Alzheimer's disease (AD) have been mainly associated with neuronal dysfunction and alterations ignoring the involvement of neuroglia in their apparition, evolution and treatment. Neuroglia is fundamental for brain homeostasis and represent the intrinsic brain defence system. Thus, ageing and all forms of ND inevitably involve glial cells. ND and AD disrupt brain connectivity affecting neuronal-neuronal, neuronal-glial and glial-glial interactions. Recent knowledge allows us to regard ND as potentially gliodegenerative processes, in which neuroglia determine their progression and outcome. We have recently probed this active pathological role, by showing: (i) an astroglial generalised atrophy with a concomitant astrogliosis just restricted to Ab plaques in the case of AD, whilst global hypertrophic behaviour in ageing processes, except in the entorhinal cortex, ii) alterations in glutamate glial metabolism and changes in S-100b trophic factor in both ageing and AD and finally (iii) an early resting microglial recruitment in AD affected areas, even before the presence of activated/macrophagic microglial cells. These neuroglial alterations appear in parallel with a marked reduction of cell proliferation and neurogenesis in both hippocampus and subventricular zone, appearing even earlier that the AD associated pathological hallmarks, plaques and tangles. Thus, concomitant glial and neurogenic alterations are key elements in the disruption of

neural networks connectivity, which associated with neurotransmitters imbalance; underlie the mnesic deficits associated with AD. Promisingly, we have also showed that they could be recovered by psychostimulation, therefore, new therapeutic approaches targeting simultaneously neurogenic and glial impairments might be of major relevance in the treatment of ND and AD.

S14-O1

Consumption of polyphenols can influence neurobiological mechanisms of cognitive functions: Possible role of nitric oxide (NO)

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Aim: Several controlled studies have shown the beneficial effects of flavonoids intake on cognitive functions. Our aim was to obtain the electrophysiological data characterizing the effects of single dose of polyphenolic substance administration (SDPSA) on selected higher brain functions.

Methods: SDPSA was administered to 30 volunteers performing the visually elicited and, successively, the memory guided saccadic eye movements either to visual targets or to their remembered position. EOG and EEG activity was registered just before and 2 hours after the SDPSA. The same procedure was repeated: with placebo and without any substance administration. The saccadic accuracy, saccadic eye movement related potentials and EEG power spectra were evaluated. The blood pressure was controlled also.

Results: Following SDPSA the memory guided saccades were accurate. No differences in saccadic eye movement related potentials were found. As for the EEG power spectra for saccades guided by memory information the significant decrease within the slow EEG bands was registered, alpha power mainly. The increased cortical activation appeared in areas having a role in attention, spatial orienting and memory. No changes in blood pressure were found.

Conclusions: The results revealed the overall activation of the brain cortex and more accurate space orientation or better attention performance after SDPSA administration. The increase of NO synthase activity induced by PS administration may activate the NO/sGC/cGMP/cGK pathway. The other components of cGMP signalling cascade are suggested to take part in neurobiological mechanisms of various cognitive functions. There is a reason to assume that long term consumption of polyphenolic rich diet can promote good execution in instrumental daily activities of seniors.

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S14-O2

Age-related differences in sensitivity of cortical and cerebellar mitochondria to ischemia induced permeability transition pore opening

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Aim: Ischemia induced damages to mitochondria might lead to cell death processes in neurons. That for we investigated the ischemic injury to brain cortex and cerebellum mitochondria functions and mitochondrial permeability transition pore (MPTP) MPTP opening at different age of rats.

Methods: Mitochondria were isolated from 7 days, 2-3 months or 7-10 months old rats cerebellum and cortex regions. Ischemia was induced for 90 min in hypoxic camera. MPTP opening was evaluated as mitochondria calcium retention capacity (CRC) using fluorescense dye Calcium Green 5N. Necrosis was evaluated by measuring activity of lactate dehydrogenase released in incubation medium.

Results: Ischemia had no effect on 7 days rats cerebellum and cortex mitochondria CRC, there was no statistically significant difference comparing with control. 2-3 month cortex mitochondria CRC after ischemia decreased 47% comparing with control. Ischemia reduced CRC of 7-10 month mitochondria isolated from both regions. Ischemia inhibited cortex and cerebellum mitochondria respiration rate at all ages of rats. Necrosis level was similar in cerebellum and brain cortex regions at all ages of rats.

Conclusion: Aging increases mitochondrial sensitivity to Ca induced MPTP opening. Cortex mitochondria are more sensitive to ischemia - induced MPTP opening than cerebellum, but at the age of 7-10 month effect of ischemia on cerebellar MPTP opening is the same as in the cortex.

Symposium 15: New insights in pain physiology: bases for therapies

S15-1

Opioid control of pain at the periphery: story of P2X receptors

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Small- and medium-sized peripheral sensory neurones localized in the dorsal root ganglia (DRG) initiate the sensation of pain and hence are classified as nociceptive neurons. Numerous studies have indicated that homomeric $P2X_3$ ATP receptors expressed in the membrane of sensory neurones contribute to various types of pain behaviour, as well as pathological forms of nociception represented by hyperalgesia and allodynia.

Opioids are commonly employed for pharmacological control of pain, although their therapeutic usage is limited by multiple adverse effects and societal issues. It is generally acknowledged that opioids induce analgesia through their action on the central nervous system. This perception, however, has been challenged with accumulation of evidence indicating that a considerable part of opioid analgesia is mediated by peripheral μ-, κ- and δ-opioid receptors.

In the present study, we show that homomeric $P2X_3$ receptors are regulated by opioid signalling in a subpopulation of sensory neurones. Furthermore, we discovered a dual link between purinoceptors and μ-opioid receptors: the latter exert both inhibitory (pertussis toxin-sensitive) and stimulatory (pertussis toxin-insensitive) actions on P2X3 receptors through phospholipase C (PLC)-dependent pathways. This dual opioid control of P2X3 receptors may provide a molecular explanation for dichotomy of opioid therapy. Pharmacological control of this newly identified facilitation/inhibition switch may open new perspectives for the adequate medical use of opioids, the most powerful pain-killing agents known today.

S15-2

Lymphotactin (XCL1) modulation of trigeminal subnucleus caudalis (vc) excitability *in vitro*

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Aim: Pro-inflammatory cytokines are implicated in chronic pain and processes linked to central sensitization through mechanisms that have not been fully elucidated yet. Lymphotactin (XCL1) signals via the receptor XCR1 but its role in modulation of central excitability is unknown. In this study, the potential contribution of XCL1 to central sensitization in the trigeminal subnucleus caudalis (Vc), an area linked to oro-facial pain was studied.

Methods: Firstly, immunohistochemistry was used to investigate if XCL1 can activate *in vitro* the immediate early gene c-fos and members of the Mitogen-Activated Protein Kinases (MAPK) family, namely phosphorylated P38 (p-P38) and phosphorylated extracellular signal-regulated kinase (p-ERK), all known markers of central sensitization. Secondly, electrophysiology in rat trigeminal brainstem slices *in vitro* was used to determine the effects of XCL1 on Vc intrinsic excitability. All procedures were carried out under anaesthesia and accorded with UK Home Office legislation.

Results: XCL1 increased activation of c-fos, p-P38 and p-ERK in the superficial layers of Vc, as assessed by semi-quantification of immuno-staining. Extracellular recordings made in Vc using transverse brainstem slices indicated that superfusion with XCL1 enhanced a form of rhythmic activity that can be quantified using spectral analysis. XCL1 significantly increased power area and power amplitude parameters, both indicating an enhanced level of intrinsic excitability. This is similar to 4-aminopyridine-induced augmented excitability in spinal cord which has been used as a model of enhanced central excitability.

Conclusions: These data suggest a possible role for this chemokine in the pathogenesis of chronic trigeminal oro-facial pain.

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S15-3

T-type calcium channels functioning in diabetic neuropathy

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Aim and Methods: Peripheral diabetic neuropathy (PDN) is one of the earliest, the most frequent and troublesome complications of diabetes occurring in about 60% - 70% of diabetic patients. Both upregulation of T-type channel density and changes in their biophysical properties have been shown in primary nociceptors isolated from diabetic animals having painful diabetic neuropathy. Moreover, selective local knock down or pharmacological block of T-type channels *in vivo* effectively reversed mechanical and thermal hyperalgesia in diabetic neuropathy. However, particular mechanisms linking T-type channels with PDN are poorly understood. 6-7 week diabetic rats developed either thermal hyper, hypo- or normalgesic types of PDN. These developmentally similar diabetic rats were studied in order to analyze T-type channel-dependent mechanisms underlying different thermal nociception.

Results: We found a complex interplay between diabetes-induced changes in functional expression of T-type Ca^{2+} channels and modulation of their biophysical properties resulted in upregulation of these channels under hyper- and normalgesia and their downregulation under hypoalgesia in DRG thermal nociceptors. In longer-term diabetes thermal hyperalgesia is changed to hypoalgesia that is accompanied by downregulation of T-type current in these thermal nociceptors. At the same time pain symptoms of diabetic neuropathy other than thermal persist in diabetic animals and patients during progression of diabetes into later stages suggesting that other types of DRG neurons may be sensitized and contribute to pain.

Conclusions: It has been demonstrated that capsaicin-insensitive low-pH-sensitive type of DRG neurons shows diabetes-induced upregulation of Cav3.2 subtype of T-type channels. This upregulation results in the increased excitability of these neurons and may contribute to nonthermal nociception at a later-stage diabetes. Thus, these findings indicate that alterations in functioning of T-type channels, specific for each type of PDN, may underlie the variety of pain syndromes induced by type 1 diabetes.

S15-4

AMPA receptor trafficking in persistent pain

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AMPA receptors (AMPARs) are responsible for the majority of excitatory synaptic transmission and play a critical role in synaptic plasticity in the mammalian CNS. Here we have shown that AMPAR subunit GluR2 is internalized in dorsal horn neurons of the spinal cord during the maintenance period of CFA-induced persistent inflammatory pain. This internalization depends on spinal cord dorsal horn (DH) protein kinase C (PKC) activation and is causally linked to pain hypersensitivity during the maintenance period of persistent inflammatory pain. We have also shown that CFA-induced inflammation causes an increase in functional expression of extrasynaptic AMPARs in rat substantia gelatinisa neurons during the maintenance rather than development of persistent pain. This increase, revealed as a significant enhancement of AMPA-induced membrane currents and $[Ca^{2+}]i$ transients, was observed only in neurons characterized by an intrinsic tonic firing properties; whereas no changes were observed in neurons exhibiting a strong adaptation. The increase also accompanies by an enhancement of surface GluR1 expression and of the total amount of cobalt-positive neurons indicating an increase in a pool of GluR2-lacking AMPARs in extrasynaptic plasma membrane. Our recent results show that PKC inhibition by AS ODN (intrathecal administration) attenuate CFA-induced increases in the calcium permeability of AMPARs in the superficial dorsal horn neurons. Concomitantly, such inhibition resulted in major anti-hyperalgesic effects, suggesting that PKC plays a major pronociceptive role in chronic pain states. Taken together, the results provide direct evidence linking dorsal horn regulation of AMPAR trafficking by PKC to pain perception and suggest that it may offer a specific molecular target for the treatment of pain.

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S15-O1

Investigation of possible interaction between the noradrenergic system and enkephalinergic system in experimental acute pain model in pain modulation

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Aim: This study was performed to investigate possible interaction between the pain modulatory actions of noradrenergic and the enkephalinergic systems in experimental acute pain in rats.

Methods: Pain was induced in adult male BALB-C mice by applying infrared heat stimulus to the hind paw plantar surface and effects of treatments on pain latencies were measured at 15, 30, 60 and 90 minutes. Noradrenaline (NA), phenylephrine, prazosin, yohimbine and a combination of these were intraperiotenally administered and consecutive pain threshold measurement values were compared with basal values. Statistical analysis were performed by use of analysis of variance test was followed by Duncan's post hoc tests for normally distributed data. $P<0.05$ value was accepted as statistically significant.

Results: Administration of 0.1 mg/kg opiorphin provided significant analgesic effect 15 minutes after injection, and the effect was declined time dependently (2.61±0.41 sec, 3.91±0.54 sec, 3.18±0.90 sec, 2.74±0.52 sec and 2.98±0.70 sec, just before and 15, 30, 60 and 90 minutes after 0.1 mg/kg opiorphin, n=7 for each). Combination of opiorphin (1 mg/kg) with NA (0.1 mg/kg) caused significant analgesia at 15th minute ($P<0.01$).When opiorphin (0.1 mg/kg) was combined with alpha-2 antagonist yohimbin (2 mg/kg) a significant algesia was obtained. Administration of phenilephrine (10 mg/kg) caused no significant effect on pain threshold but its combination with opiorphin (0.1 mg/kg) caused significant analgesia at 15 minute. Combination of opiorphin (0.1 mg/kg) with alpha-1 antagonist prazosin caused a significant algesia that lasted up to 90 minute after their injections.

Conclusions:
Results from this study indicates some level of interaction between the noradrenergic and the enkephalinergic systems in pain modulation.

Symposium 16: Astroglia in neuropsychiatric diseases

S16-1

Neurological and psychiatric disorders as gliopathies

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Astroglial cells represent a main element in the maintenance of homeostasis and providing defense to the brain. Consequently, their dysfunction underlies many, if not all, neurological, neuropsychiatric and neurodegenerative disorders. General astrogliopathy is evident in diametrically opposing morpho-functional changes in astrocytes, i.e. their hypertrophy along with reactivity or atrophy with asthenia. Neurological disorders with astroglial participation can be genetic, of which Alexander disease is a primary sporadic astrogliopathy, environmentally caused, such as heavy metal encephalopathies, or neurodevelopmental in origin. Astroglia also play a role in major neuropsychiatric disorders, ranging from schizophrenia to depression, as well as in additive disorders.

S16-2

Astrocytes as targets for drugs used in psychiatric diseases

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Astrocytes contribute ≥20% of cell volume and oxidative metabolism in the brain *in vivo*, express multiple receptors for neurotransmitters, including the 5-HT_{2B} receptor, interact with neurons at the synapse and support CNS homeostasis elsewhere. Astrocytes also respond to the drugs used in the CNS diseases. The mechanisms underlying the actions of these drugs, however, are neither fully understood nor generally acknowledged. During recent decade, we have extensively studied effects of antidepressants and anti-bipolar drugs in astrocytes in primary cultures and in astrocytic and neurones freshly isolated from drug-treated mice. Deployment of cell cultures allow studies of molecular mechanisms, exclude effects secondary effects associated with neuronal networks, and, in the case of the serotonin-specific reuptake inhibitors (SSRIs) studies, exclude effects of the serotonin transporter, SERT, which is not expressed in astroglia. All SSRIs act on astrocytes as agonists of 5-HT_{2B} receptors, and all three classical anti-bipolar drugs cause an intracellular alkalinization in astroglial cells. Many effects of both antidepressant and anti-bipolar drugs are exerted through regulation of calcium-dependent phospholipase A_2 (that controls metabolism of arachidonic acid) or through Ca^{2+} homeostatic and signalling pathways. Sometimes anti-depressant and anti-bipolar drugs exert opposite effects, and some effects on gene expression in drug treated animals are opposite in neurones vs. astrocytes. Changes in intracellular pH induced by anti-bipolar drugs affect uptake of *myo*-inositol and thereby signaling via inositoltrisphosphate (IP_3), this being in accord with one of the main theories of mechanism of action for these drugs. Studies of antidepressants, however, question the predominant role of the inhibition of SERT, the notion which is, at present, almost universally accepted.

S16-3

Putative role of astrocytes in major neuropsychiatric diseases

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The last decade has witnessed exciting advances in the neuroscience of mental health including the mapping of neural circuitry and neurochemical mechanisms, identification of multiple genetic loci, and the application of novel technologies to both the pathophysiology and treatment of mental disorders. Despite these advances, mental health is experiencing a crisis in the development of new treatments. Several inter-related factors account for this failure. Research in the last decades realized that the high heterogeneity of cells populating the brain might have offered an invaluable source of new information on brain´s functions. Among these cells, glia in general, and astrocytes in particular, have appeared in all their multifaceted spectrum of activities. The information on these cells suggested that they become a priceless tool to refine pharmacological treatments. Astroglia are involved in pathogenesis of many (if not all) neuropsychiatric diseases. Another principal challenge lies in detailed characterization of remodeling of astrocytes in the disease-specific context. This is important when taking in account remarkable heterogeneity and plasticity of astrocytes. Neuropathological/neuroimaging evidence demonstrates that in most of neuropsychiatric

illnesses the astroglial atrophy and asthenia have the leading role, in contrast with other conditions in which astrogliosis prevails. In chronic diseases such as schizophrenia and major depressive disorders decrease in astroglial numbers and functional capabilities are fundamental for pathological development. In neurodegenerative diseases both atrophic changes and astrogliosis occur. The removal of glial dysfunction may be considered for future drug development in neuropsychiatry. Indeed, the multitude of molecules expressed by neuroglia, responsible for their homoeostatic and defensive functions, provides alternative approaches for novel treatments.

S16-4

Astroglial mechanisms of hyperammonemia - induced brain injury

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Ammonia plays a key role in the pathogenesis of hepatic encephalopathy (HE), a complex neurological syndrome related to liver damage. Astrocytes are the primary target of ammonia toxicity in the brain. Cytotoxic brain edema, the major cause of death in HE, is subsequent to ammonia- induced astrocytic swelling attributed to a vicious cycle of oxidative/nitrosative stress (ONS) and intracellular osmotic imbalance. The edema-inducing effect of ammonia is partly mediated by the excessive accumulation of its metabolite, glutamine (Gln). Toxic effects of Gln are due to: i) an increase of the intraastrocytic osmotic pressure ii) mitochondrial damage following its entry to the inside of the organelle, where it is degraded back to ammonia and glutamate (Glu) in a glutaminase-mediated reaction, (the “Trojan horse” mode of action, Albrecht J and Norenberg MD, Hepatology, 2006). Ammonia also evokes ONS in astrocytes directly; reactive oxygen species (ROS) are generated by NADPH oxidase, whereas nitric oxide (NO) formation results from overactivation of the astrocytic N-methyl-D-aspartate receptor and increased Arg uptake. Ammonia decreases the expression and activity of astroglia-specific GLU transporters GLAST and GLT-1. The ensuing decrease of astrocytic Glu uptake and its increased accumulation in the extrasynaptic space result in overactivation of neuronal Glu receptors followed by their inactivation, causing excitatory/inhibitory neurotransmission imbalance. Ammonia impairs astrocytic monovalent ion transport, mainly by interfering with the expression/function of the Na-K-Cl cotransporter-1 (NKCC1) and of the inward rectifying potassium channel Kir4.1, contributing to ion dyshomeostasis, resulting in both impaired Glu uptake and astrocytic swelling. Recent evidence implicates increased interaction of extracellular Glu with astrocytic NMDA receptors, in HE-induced downregulation of Kir4.1.

S16-O1

Assessment of trace elements in children with autism spectrum disorders

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Aim: It is hypothesized that high exposure to sulphydryl reactive metals (mercury, lead, and cadmium) might be involved in the etiopathogenesis of autism and autism spectrum disorders (ASDs). Another hypothesis claims that ASDs children might have a decreased detoxification capacity due to genetic polymorphism. The present study aimed to evaluate trace elements (Pb, Cd, Hg, Mn, Cr, Cu and Zn) status in children with ASDs.

Methods: In our Laboratory of Neurotoxicology we investigated trace elements status in hair, blood/plasma and urine of 11 children with ASDs and data compared to the control group of 9 children.

Results: Obtained data revealed that trace elements status was within physiological levels. Only one case had urinal Pb levels above 10 mkg/L. We found that ASDs children had higher (Mann-Whitney U test, $p<0.05$) Cu and Cr concentrations in hair. Therefore, Zn levels in all investigated media (hair, plasma and urine) were lower ($p<0.05$) as compared to controls, and in most cases were lower as advisable. Zinc is essential for the function of about 300 metalloenzymes. Clinical manifestations of severe zinc deficiency may vary at different ages, and leads to impaired cognitive function, behavioural problems, impaired memory, learning disability and neuronal atrophy.
We found that Hg concentrations in hair, blood and urine were lower as compared to control group. Moreover, our data support other findings that autistic children (Majewska et al. 2010, Kern et al. 2007) are poorer detoxifiers and subsequently have lower Hg levels in hair.

Conclusions: Larger and methodologically sound studies are needed to investigate all aspects of trace elements kinetics and their involvement into

pathogenesis and late outcomes in autism spectrum disorders.

Symposium 17: Calcium signalling micro-domains

S17-1

Calcium signalling in the endoplasmic reticulum/secretory granule micro-domain

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In 1990 we provided indirect evidence for the existence of Ca^{2+} signalling micro-domains in pancreatic acinar cells (Osipchuk et al 1990) and in 1993 we could directly demonstrate Ca^{2+} microdomains in the apical granule containing region of these cells (Thorn et al 1993). The pancreatic acinar cell is specialized for bulk secretion of digestive enzymes and therefore has a relatively large apical micro-domain, dominated by secretory (zymogen) granules, in which Ca^{2+} signalling is of crucial physiological significance because of the need to exercise precise control of the exocytotic secretory events. Local Ca^{2+} signalling in the apical domain occurs by repetitive episodes of Ca^{2+} release from a relatively small volume of endoplasmic reticulum (ER) terminals that are functionally fully connected to the bulk of the ER in the baso-lateral region, which is the quantitatively dominant Ca^{2+} store (Hegyi & Petersen 2013). Thus Ca^{2+} release from the small volume of the apical ER terminals can be sustained by intra-ER Ca^{2+} diffusion from the basal to the apical parts of the cells. The particular characteristics of the apical Ca^{2+} signalling domain will be discussed with special emphasis on its passive and active Ca^{2+} buffering properties and its ability to respond to local Ca^{2+} elevations by Ca^{2+}-induced Ca^{2+} release via a number of different channel types (Gerasimenko et al 2015). The functional significance of these characteristics for appropriate Ca^{2+} spiking will be discussed as well as the pathophysiological consequences of destroying the Ca^{2+} signalling microdomain.

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S17-2

Presenilin 2 modulates ER-mitochondria coupling by tuning the inhibitory effect of mitofusin 2 on organelle tethering

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Communication between organelles appears to play key roles in cell biology. In particular, physical and functional coupling of the endoplasmic reticulum (ER) and mitochondria is crucial for regulating various pathophysiological features. Here we discuss the role of two proteins, mitofusin 2 (Mfn2) and presenilin 2 (PS2), that have been shown to affect ER mitochondria interactions and the transfer of Ca2+ between the two organelles. As to Mfn2, we demonstrate that this protein plays an inhibitory role, unlike previously suggested, in the interaction between the two organelles and its down regulation causes an increase in the Ca2+ transfer between ER and mitochondria. As to PS2, whose mutations are responsible for some forms of familial Alzheimer's disease (FAD), we show that it promotes ER-mitochondria coupling only in the presence of mitofusin 2 (Mfn2). The two proteins interact in vitro and in living cells while their homologues Mfn1 and PS1 are dispensable for this interplay. We also show that forms of PS2 with FAD-linked mutations are more effective than the wild-type form in modulating ER-mitochondria tethering because they are more efficient at binding to Mfn2 in mitochondrial-associated membranes. We propose a revised model for ER-mitochondria interaction to account for these findings and discuss possible implications for FAD pathogenesis.

S17-3

Distinct spatial Ca^{2+} signatures selectively activate different NFAT transcription factor isoforms

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Protein isoforms are widely expressed in biological systems. How isoforms that co-exist within the same sub-cellular domain are differentially activated remains unclear. Here, we compare the regulatory mechanism of two closely related transcription factor isoforms, NFAT1 and NFAT4, that migrate from the cytoplasm to the nucleus following the increase in intracellular Ca^{2+} that accompanies opening of store-operated Orai1/CRAC channels. We demonstrate that NFAT1 has a private line of communication with Orai1, activating in response to Ca^{2+} microdomains near the open channels. By contrast, NFAT4 stimulation requires both local Ca^{2+} entry and a nuclear Ca^{2+} rise. We mapped differences in nuclear location to amino acids within the SP-3 motif of the NFAT regulatory domain. The different Ca^{2+}-dependencies enable agonist to recruit different isoform combinations as stimulus strength increases. Our data uncover a mechanism whereby co-existing cytoplasmic transcription factor isoforms are differentially activated by distinct sub-cellular Ca^{2+} signals.

S17-4

Calcium imaging in organelles with GAP (GFP-aequorin protein) probes

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Genetically encoded calcium indicators (GECI) permit monitoring subcellular Ca^{2+} signals inside organelles. Most GECI contain endogenous calcium-binding proteins whose functionality in vivo may be perturbed by competition with cellular partners. We describe here a novel family of fluorescent Ca^{2+} probes based on the fusion of two Aequorea victoria proteins, GFP and aequorin (GAP). Both proteins have been used extensively with no report of interferences with Ca^{2+} homeostasis or other secondary effects. GAP exhibited green fluorescence whose excitation spectrum was shifted by Ca^{2+}. The unique combination of features exhibited by GAP include: dual-excitation ratiometric imaging, high dynamic range, good signal-to-noise ratio, and insensitivity to pH and Mg^{2+}. Ca^{2+} affinity could be tuned by mutations in the aequorin EF hands. Ca^{2+} calibration was uncomplicated, with a maximum ratio increase of three to fourfold and a Hill coefficient of 1. We have targeted GAP to five distinct organelles and behaviour was as expected for a selective Ca^{2+} probe. Both, virus-induced expression as well as cell lines stably expressing targeted GAPs were successfully achieved. Transgenic mice for endoplasmic reticulum-targeted GAP exhibited a robust long-term expression and reproducible performance in various neural tissues including hippocampus, cerebral cortex, cerebellum, spinal motor neurons or dorsal root sensory neurons. Expression pattern in other tissues will be advanced. Proof of concept for measurements *ex vivo* and *in vivo* will be shown. The biosensors presented here fill a gap in the actual repertoire of Ca^{2+} indicators for

organelles and are especially valuable for Ca^{2+} imaging applications in the living animal.

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Symposium 18: Cardiovascular control by surrounding tissue

S18-1
Brain energy supply and metabolism in aging

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Brain's electrical activity correlates strongly to changes in cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen ($CMRO_2$). Brain oxygen consumption is correlated controlled by Na,K-ATPase activity driven by transmembrane currents while Ca^{2+}-signals control activity-dependent rises in CBF. During aging, global CBF is reduced, while $CMRO_2$ is relatively preserved. The neuronal substrate for this change in function is incompletely understood, but may in part be related to the occurrence of fast Ca^{2+} waves in astroglia, which produce protracted decreases in the tissue partial pressure of O_2. In addition, changes in cortical neuronal activity may contribute. We here examined the hypothesis that an overall reduction in activity of cortical interneurons explained the age-dependent decline in CBF with preserved $CMRO_2$. We focused on parvalbumin positive (PV) interneurons, which are fast spiking and induce neuronal network oscillations in the gamma frequency range (40-80 Hz) in adjacent interneurons and excitable pyramidal cells via perisomatic trajectories and $GABA_A$ receptors with fast kinetics. Disruptions in gamma rhythm and function of parvalbumin positive interneurons are associated with cognitive decline in Alzheimer's disease. We report, that evoked CBF responses, synaptic activity and gamma oscillations decreased in old mice as compared to adult mice. The decline in gamma activity was consistent with a decrease in Ca^{2+} activity in PV perisomatic boutons, and in postsynaptic neurons receiving presynaptic perisomatic innervation in old as compared to adult mice. In comparison, the stimulation-induced rise in $CMRO_2$ became larger; suggesting that the metabolic costs of evoked synaptic activity was increased in old animals. The age-dependent increase in energetic costs of synaptic activity is consistent with a decline in energetic reserve capacity and age-related cognitive decline.

S18-2
Metabolic regulation of vascular tone: novel signaling mechanisms in the heart and brain

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Adjusting blood flow to the local metabolic demand is a critical physiological function protecting against ischemia and tissue dysfunction. In contrast to heavily sympathetically innervated vascular beds (e.g., mesenteric arteries), coronary and cerebral arteries are controlled mostly by local mechanisms. Multiple possible signaling mechanisms between perivascular tissue and the vascular wall have been proposed but their respective contributions to metabolic regulation of vascular tone remain unclear.

Accumulation of metabolic waste products causes local acidification under conditions of increased metabolism and insufficient blood flow. Whereas H^+ is known to induce vasodilation via inhibition of voltage-gated Ca^{2+} channels in the vascular smooth muscle cells, the importance of altered concentration and protonation of buffer systems has been unclear. We find that changes in extracellular [HCO_3^-] *per se* modify the contractile state of cerebral arteries via endothelium-dependent vasorelaxant pathways: decreased [HCO_3^-] is sensed through a mechanism that requires receptor protein tyrosine phosphatase γ and limits vasorelaxation in response to metabolic acidosis. Thus, the anti-relaxant effect of low [HCO_3^-] maintains vascular responsiveness during metabolic acidosis and may protect against cerebral edema.

The presence of cardiomyocyte-rich perivascular tissue influences vasomotor tone in coronary arteries. Diffusible vasoactive factors from cardiomyocyte-rich perivascular tissue inhibits a) agonist-induced vasoconstriction via attenuation of rho-kinase-dependent smooth muscle Ca^{2+}-sensitivity and b) vasorelaxant responses to muscarinic stimulation by lowering endothelial Ca^{2+}-responses and limiting production of H_2S.

We propose that these novel mechanisms of vascular tone regulation by perivascular tissue are important for matching blood flow to local metabolic demand and therefore represent rational new targets for treatment of ischemia.

S18-3
PVAT-dependent relaxation: Quid Novi

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Cardiovascular and metabolic diseases are currently the primary cause of morbidity and mortality in the western world and are spreading to the rest of the world following globalization. Adipose tissue, in particular perivascular adipose tissue (PVAT) is recognized as an important player in the development of these diseases. The release of relaxing factor(s) from the PVAT has been a matter of interesting and highly spirited debates about its nature, the channels that govern its activities and its role in vascular dysfunction. Data from our laboratory indicate that Adipose-Derived Relaxing Factor (ADRF) is an important player, however the potential channels necessary for its downstream activities are still under study. Our recent research primarily focuses on Kv7.1 channels, which are known to be expressed in vascular smooth muscle cells.

S18-O1
BK channels limit nitric oxide-induced reduction of vessel contractility

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Aim: Activation of vascular smooth muscle high conductance calcium-activated potassium (BK) channels contributes to vasodilation induced by an acute exposure to nitric oxide (NO). In vivo, however, NO is often released continuously. Whether the vasoactive effect of continuously present NO is also dominated by BK channels is unknown. Thus, the hypothesis was tested that BK channels mediate the vasoactive effect of continuously present NO.

Methods: Experiments were performed on rat tail and saphenous arteries using isometric myography, FURA-2-fluorimetry and the patch-clamp technique. Vasoconstriction was evoked by the α1-receptor-agonist methoxamine (MX).

Results: Continuously present NO-donors (SNP, SNAP, DETA-NO) attenuated MX-induced vasoconstriction in a concentration-dependent manner, demonstrating their anticontractile effect. In contrast to our hypothesis, a considerable increase of this NO-induced effect was observed in the presence of the BK channel-inhibitor iberiotoxin (IBTX). IBTX augmented MX-induced contractions in the absence of NO-donors, revealing the existence of active BK channels under these conditions. However, IBTX was without effect on MX-induced contractions in the presence of NO-donors, pointing to a NO-donor induced deactivation of BK channels. Further, effects induced by the NO-donor SNP were abolished by hydroxycobalamin, a NO-scavenger; ODQ, a guanylate cyclase-inhibitor and Rp-8-Br-PET-cGMPs, a PKG-inhibitor. SNP attenuated the MX-induced calcium influx in smooth muscle cells of intact vessels without affecting calcium release or calcium store content. Furthermore, SNP stimulated BK currents in isolated smooth muscle cells in a PKG-dependent manner.

Conclusions: Our study demonstrates that in rat arteries continuously present NO exerts an anti-contractile effect that is limited by deactivation of smooth muscle BK channels and involves a GC/PKG-mediated reduction of calcium influx.

S18-O2
Connexin 43 expression and gap junction permeability is differently regulated by heat stress-activated c-Jun N-terminal kinase in HeLa cells and skeletal myoblasts

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Aim: Skeletal myoblasts have been applied for treatment of myocardial infarction; however, their survival and electrical incorporation is burdened by local inflammation. In this study we examined the effect of heat stress and a role of c-Jun N-terminal kinase in regulation of connexin43 expression and gap junction permeability in skeletal myoblasts and model HeLa cells.

Methods: Endogenous Cx43-expressing SkM culture was prepared from the femoral muscle of New Zealand white rabbit. Immunocytochemistry and Western blot assay were performed for the protein detection and quantification. Number of gap junction (GJ) channels in junctional plaques was evaluated using conventional fluorescent microscopy and the number of hemichannels in cell membranes - by TIRF microscopy. The permeability of GJs was examined by measuring

the scrape-loaded (SkMs) or loaded through patch pipette (HeLa cells) Lucifer Yellow transfer.

Results: In Cx43-EGFP expressing HeLa cells, hyperthermia down-regulated the F-actin network and caused the decrease in total amount of Cx43-EGFP protein by 36%, number of channels in GJ plaques by 24% ($p < 0.05$), density of hemichannels in the cell membranes by 52% ($p < 0.05$) and efficiency of GJs that was 2.9-fold lower compared to control ($p < 0.05$). In SkMs, F-actin network was resistant to hyperthermia that increased the total amount of Cx43 by 2.8-fold and the efficiency of GJs by 2-fold ($p < 0.05$). These effects were attenuated by a selective JNK kinase inhibitor XG-102.

Conclusions: Hyperthermia induces JNK- and F-actin-dependent cell type-specific effects on Cx43 expression, GJ and hemichannel formation and GJ permeability.

Posters

1. Cardiovascular physiology

P1-1

Human H2 receptors in a mouse model and the endogenous cardiac histamine content

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Aim: In transgenic mice, which express the human H2 histamine receptor in cardiac myocytes (TG) but not in wild type litter mice (WT), histamine induced positive inotropic and chronotropic cimetidine-sensitive effects. Here, we were interested whether it might be possible to release endogenous histamine from cardiac preparations which we had detected histologically in cardiac slices.

Methods: This release can be achieved in part by establishing concentration response curves to compound 48/80 in isolated left or right atrial preparations in the organ bath.

Results: In initial studies, we noted the presence of histamine and the expression of histidine decarboxylase in cardiomyocytes from WT and TG. Compound 48/80 (C) can release endogenous noradrenaline in isolated cardiac preparations, as increases in force and frequency were noted both in preparations from WT mice. Hence subsequently, left atrial preparations were studied in the presence of 10 µM propranolol, a β-receptor antagonist. Under these conditions, we noted a positive inotropic effect at 327 µM C in TG (to 350%) but not in WT preparations (n=4-10, $p<0.05$), which were sensitive to 10 µM cimetidine, a H2 receptor antagonist.

Conclusions: We noted *in vitro* that endogenous cardiac histamine content is probably releasable and the released histamine concentrations are sufficient to affect human H2 receptors in a mouse model.

P1-2

The effects of beta amyloid peptide 22-35 on isolated and perfused rat hearts

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Aim: Beta amyloid peptides are deposited as insoluble febriles in the brain parenchyma and cerebral blood vessels in Alzheimer's disease. In addition to neuronal degeneration, beta amyloid peptides may contribute to regulation of cardiovascular functions. The neurotoxic effects of beta amyloid peptides are well understood. However, little is known about the effects of these peptides on cardiovascular functions. Especially the influence of beta amyloid peptide 22-35 on cardiovascular functions of isolated rat heart has not been studied yet. Therefore, we studied the possible effects of this peptide on isolated rat heart.

Methods: According to Langendorff's method, the hearts were isolated and perfused with modified Krebs-Henseleit solution under constant pressure conditions. Left ventricular developed pressure (LVDP; an index of contractility), $+dP/dt_{max}$ (other index of contractility), heart rate (HR), coronary flow, monophasic action potential amplitude (MAPamp) and MAP duration at 90% repolarization (MAP_{90}) were measured.

Results: Beta amyloid peptide 22-35 in doses 1, 10 and 100 nM significantly decreased LVDP and $+dP/dt_{max}$ without affecting HR, coronary flow, MAPamp and MAP_{90}.

Conclusions: Our results indicate that 1, 10 and 100 nM doses of beta amyloid peptide 22-35 may exert a negative inotropic action. Furthermore, beta amyloid peptide 22-35 at all doses may not produce

any significant effect on HR, coronary flow, MAPamp and MAP_{90}.

P1-3

The Effects of beta amyloid peptide 1-42 on isolated rat hearts

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Aim:
The neurotoxic beta amyloid peptides are involved in the pathogenesis of Alzheimer disease. Beta amyloid peptides may play a role in the regulation of cardiovascular functions. However, there is little information about the actions of these peptides on cardiovascular functions. Furthermore, the action of beta amyloid peptide 1-42 on cardiovascular parameters of isolated rat heart has not been studied. Therefore, we investigated the possible effects of beta amyloid peptide 1-42 on isolated rat heart.

Methods:
The hearts were isolated and perfused with modified Krebs-Henseleit solution under constant pressure conditions. Left ventricular developed pressure (LVDP; an index of cardiac contractility), maximal rate of pressure development of left ventricle ($+dP/dt_{max}$; another index of cardiac contractility), heart rate (HR), coronary flow, monophasic action potential amplitude (MAPamp) and MAP duration at 90 % repolarization (MAP_{90}) were measured.

Results:
Beta amyloid peptide 1-42 in doses of 1, 10 and 100 nM significantly decreased LVDP, $+dP/dt_{max}$ and HR. This peptide in a dose of 1 nM did not affect coronary flow, but 10 and 100 nM doses significantly reduced this parameter. The peptide in doses of 1, 10 and 100 nM did not alter MAPamp, but increased MAP_{90}.

Conclusions:
These results indicate that beta amyloid peptide 1-42 may produce negative inotropic and negative chronotropic effects with an increase in MAP duration. Furthermore, this peptide at high doses may decrease coronary flow.

P1-4

Experimental diabetes mimics pulmonary hypertension induced by mild chronic hypoxia in rats

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Hypoxia induces radical stress that is crucial in the pathogenesis of the hypoxic pulmonary hypertension. As diabetes also induces radical stress, we hypothesized that the sensitivity of pulmonary blood vessels to chronic hypoxia is increased in diabetic rats.
Forty adult male rats were divided into 4 groups: H (hypoxia 2 weeks, FiO_2 =0.12), D (streptozotocine diabetes, 100 mg/kg s.c. in neonatal period), DH (diabetes and hypoxia), N (normoxic controls). The animals were anesthetized and blood samples were taken from jugular vein to measure the levels of biological antioxidant potential (PAT test; FRAS 4 Evolvo) and glycemia. Mean pulmonary artery pressure (PAP) was measured by catheterization via jugular vein (with chest intact). Heart ventricles were then weighted.
Glycemia was significantly higher in diabetic vs non diabetic groups (DH 15.9 ± 1.7(SE); D 15.9 ± 2; H 6 ± 0.2; N 6.2 ± 0.2). Exposure to mild hypoxia elicited a weak but significant pulmonary hypertension in normoglycemic rats. In normoxia, hyperglycemia elevated PAP to a similar extent as did hypoxia in normoglycemic rats. In contrast to our hypothesis, hyperglycemia did not increase PAP above the level found in hypoxic normoglycemic animals. Right to left heart weight index was significantly higher in the hypoxic compared to normoxic groups. PAT test values was significantly lower in the group HD vs. N.
Experimental diabetes and mild hypoxia have similar, non-additive effect on PAP but not on right-left heart weight index. The exhaustion of antioxidant mechanisms was demonstrated by lower values of PAT test in hypoxic group with diabetes.

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P1-5

Effects of diadenosine polyphosphates on bioelectrical and contractile activity of the rat heart

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Aim: Diadenosine polyphosphates (Ap(n)A) are naturally occurring molecules, but their physiological role has not been established. In several studies Ap(n)A were demonstrated to participate in smooth muscle tone regulation, however, effects of Ap(n)A in heart were not investigated. The aim of present study was to elucidate effects of diadenosine tetra- and diadenosine pentaphosphate (Ap4A, Ap5A) on bioelectrical and contractile activity of rat myocardium and to compare Ap(n)A-induced effects with effects of adenosine (Ado) and ATP.

Methods: Standard microelectrode technique was used to investigate influence Ap4A or Ap5A (1-10 μM) on action potentials duration (APD) in isolated, Tyrode perfused, electrically paced (3.3 Hz) multicellular preparations of left (LA) and right atria (RA), or ventricular wall (RV) of rat heart. Myocardium preparations were dissected from male Wistar (200-250 g) rats. Left ventricle (LV) contractility under Ap4A or Ap5A action was estimated with use of Langendorf perfused isolated rat hearts. All experiments were approved by local bioethical committee.

Results: Administration of Ap4A, Ap5A (10 μM) caused significant ($p(T)<0.05$, $n>6$ for all groups) decreasing of APD in rat LA (to 77±5% and 89±2 %), RA (78±4% and 71±5%, n=6) and RV (65±3% and 46±4%, n=6). APD decreasing, induced by Ap4A and Ap5A were similar with such of 10 μM Ado or ATP ($p(U)>0.1$). Both Ap4A, Ap5A induced significant dose-dependent multiphase changes of rat myocardium contractility.

Conclusions: Ap4A and Ap5A have profound effect on the bioelectrical activity of atrial and ventricular rat myocardium, contractile activity of left ventricle. Diadenosine polyphosphates action in heart may be mediated by the similar mechanisms with classical purinergic agonists - Ado and ATP.

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P1-6

Effect of oxytocin on L-NAME induced hypertension and chronic stress in rats

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Aim: Cardiovascular disease (CVD) represents the major cause of death worldwide; Hypertension (HTN) and stress are two major factors that increase the risk of CVD. Oxidative stress, mononuclear cell infiltration, reduced endothelial function, diminished nitric oxide bioavailability and impaired vasomotor responses are common manifestations. As a neurohormone and a neurotransmitter, oxytocin (OT) has beneficial effects on both risk factors. However, little is known about its effects on risk factor combinations (e.g., HTN+stress). We examined how the actions of OT on the HTN+stress differ from either risk factor alone.

Methods: Immunohistochemical staining and biochemical (Myeloperoxidase, Lipid peroxidation and glutathione) measurements were performed to assess functional and structural alterations in aorta and heart tissues of Wistar rats that were either administered L-NAME (50 mg/kg/day i.p.) (HTN), placed on 1-h water avoidance stress for 2 weeks (stress) or both (HTN+stress). Blood pressure (BP) was measured in non-anesthetized rats by tail plethysmography.

Results: Oxytocin injection (0,1 mg/kg/day i.p.) leaded BP decrease in both HTN and HTN+stress groups. Stress didn't have any impact on BP alone. OT application has shown favorable effects on increased tunica adventitia thickness, neutrophil infiltration and lipid peroxidation on HTN group. Stress-applied group, OT prevented neutrophil infiltration, edema and endothelial injury but inadequate to diminish bleeding and oxidative degradation of lipids. On the other hand exogen OT administration attenuated BP to the control levels but wasn't able to reveal any other beneficial affect on HTN+stress group.

Conclusions: The results of this study demonstrate that OT blunts the inflammation and oxidative stress elicited by HTN and stress; however didn't exposed any advantageous effect on combination of HTN and stress.

P1-7

Ketamine/xylazine anaesthesia in the chronobiological cardiovascular studies

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Aim: Administration of anaesthesia may influence specific aspects of *in vivo* animal experiments and is an especially important consideration for experiments conducted during the daytime. Although chronobiological studies investigating interactions between general anaesthesia and circadian rhythms are sparse, all suggest that general anaesthetic agents have a significant effect on circadian rhythms. To assess the suitability of ketamine/xylazine anaesthesia in chronobiological studies.

Methods: The present study was performed using ketamine/xylazine-anaesthetized (100mg/kg/15mg/kg. im) female Wistar rats after adaptation to a light-dark (LD) cycle (12h:12h). Heart rate, rectal temperature, electrocardiographic parameters, autonomic nervous system activity, acid-base balance, and plasma concentrations of Na^+, K^+, Ca^{2+} and Cl^- were evaluated for their dependence on the LD cycle.

Results: LD differences were found in heart rate and rectal temperature, measured before and after administration of the anaesthetic agent, in all electrophysiological parameters (except QT interval, R and P waves amplitudes) and ion concentrations (except K^+) but not in parameters of acid-base balance. Spontaneously breathing rats are in asphyxic conditions in in-vivo experiments independently of the LD cycle. LD differences were found also in parameters of heart rate variability where parasympathetic tone predominates and sympathetic activity is depressed independently of the LD cycle.

Conclusions: Ketamine/xylazine anaesthesia may be especially applicable in chronobiological studies because it does not alter the daily rhythmicity of followed parameters by marked manner. It modifies LD differences but probably without loss of rhythmicity. However, it is not too applicable for cardiovascular research because it can cause serious bradycardia resulting of dominant parasympathetic tone and of systemic asphyxia.

P1-8

Increased erythrocyte aggregation in patients with primary open angle glaucoma

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Aim: It was suggested that blood rheology had a significant effect on microcirculation and impaired rheological parameters are involved in many diseases. It is well known that retina is susceptible to ischemia and impaired hemorheological parameters might be involved in ganglion cell death, which is the primary mechanism in glaucoma pathogenesis. The aim of the current study was to determine alterations in blood rheology (plasma viscosity, erythrocyte aggregation and deformability) in patients with primary open angle glaucoma (POAG).

Methods: The study comprised 20 POAG patients (glaucoma group) (65.57±1.11 years) and 23 age and sex matched (66.66±2.12 years) healthy controls (control group) ($p>0.05$). Elongation index (EI) which is the indicator of erythrocyte deformability and erythrocyte aggregation were measured by an ektacytometer (LORCA). Plasma viscosities were determined by a cone-plate rotational viscometer.

Results: There was no statistically significant difference between the groups in terms of erythrocyte deformability ($p>0.05$). On the other hand, eythrocyte aggregation amplitude (AMP) (25.78±0.71 vs. 23.40±0.97, $p=0.028$); and mean corpuscular hemoglobin concentration (MCHC) (34.48±0.38 vs. 31.81±0.66, $p=0.002$) were significantly higher in the glaucoma group compared to those of the control group (respectively). However, plasma viscosity did not differ between the groups ($p>0.05$).

Conclusions: Increased erythrocyte aggregation in patients with POAG may indicate the role of impaired rheological features in glaucoma pathogenesis. A higher erythrocyte aggregation may contribute to the glaucomatous damage by affecting microperfusion of the optic nerve head. Modification of rheological parameters in glaucoma patients may be considered as an adjuvant therapy in glaucoma management.

P1-9

Investigation of hemorheological parameters in patients with interstitial lung diseases

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Aim: Although hemorheological alterations in various lung diseases (chronic obstructive pulmonary disease, silicosis, bronchitis vs.) have been well-defined, no information is available about the effects of interstitial lung diseases (ILD), which include more than 200 different entities, on hemorheological parameters. Nonspecific interstitial pneumonia (NSIP), organizing pneumonia (OP) and Idiopathic pulmonary fibrosis (IPF) are types of ILD. The aim of this study was to investigate hemorheological parameters (erythrocyte deformability, aggregation, and plasma viscosities) in NSIP, OP and IPF patients.

Methods: The study enrolled 26 patients (8 NSIP, 11 OP, 7 IPF) and 26 healthy controls. Erythrocyte deformability and aggregation were measured by an ektacytometer. Plasma viscosity (PV) was determined by a cone-plate rotational viscometer.

Results: There was no statistically significant difference between the groups with respect to age, sex, fibrinogen levels. Erythrocyte deformability measured at 1.69 Pa was lower in NSIP patients compared to control, and in OP patients compared to NSIP patients. On the other hand, eythrocyte aggregation was significantly decreased in the IPF patients compared to controls. Although statistically insignificat, PV of patients was higher than controls. Mean corpuscular hemoglobin concentration (MCHC) was significantly decreased in NSIP patients and red cell distribution width (RDW) increased in the IPF and NSIP patients compared to controls.

Conclusions:
It can be suggested that, altered hemorheological parameters in IPF and IIP may be important in both pathogenesis and follow up of these patients.

P1-10

In vitro effect of nesfatin-1 on contractions in the rat thoracic aorta

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Aim: Nesfatin-1 has been identified for the first time in the hypothalamus in 2006. It is a new metabolic hormone thought to be important endocrine and metabolic functions. In the light of this information, we investigated potential effects of nesfatin-1 on contractions of rat thoracic aorta.

Methods: The thoracic aorta of male rats were used in this study (n=7). Aortic vessel strips were removed from male Wistar rats, following decapitation and placed in a jacked tissue bath containing Krebs solution. Effects of different concentrations of nesfatin-1 (0.1 µM, 1 µM and 10 µM) on contractions were studied. 0.1, 1 and 10 µM doses of nesfatin-1 application on contractions dependent effects were tested using isolated organ bath also has been shown histologically by immuno-histochemical staining of changes in rat aortic tissue.

Results: Nesfatin-1 inhibited rat aortic contraction dose-dependently and it was observed that nesfatin-1 led to immunohistochemical changes in the rat aorta. While it does not create any difference statistically in 0,1 µM nesfatin-1 dose, the decreases in frequency and amplitude of contractions were statistically significant in 1 µM and 10 µM nesfatin-1 for strips ($P<0.05$). The findings of the study results nesfatin-1 was observed in a dose dependent manner to inhibit the contraction of rat aorta also dose-dependent effects of these practices have been identified in histologically at the tissue level.

Conclusions: The data suggest that in addition to the central control of nesfatin-1, showed effects on peripheral muscle contraction mechanism and suggest that regulates the peripheral blood pressure in this way.

P1-11

Assessment of Carotid Stiffness with Vector Velocity Imaging

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Aim: Vascular stiffness is a crucial cardiovascular variable and an independent marker of early atherosclerosis disease that correlates with cardiovascular morbidity and mortality. Evaluation of this biomarker in clinical practice could help to refine the individual phenotype of vascular risk. This study aimed to validate Vector Velocity Imagining (VVI), a non-invasive ultrasound speckle tracking technology versus the Echo-Tracking (ET) method considered the reference method.

Methods: Common carotid artery stiffness was assessed prospectively with ET (Esaote, QAS) and VVI-assisted (Siemens, Syngo) total radial strain measurement in 10 healthy patients, free of cardiovascular disease (8 men, mean age 31 ± SD 12 years) and at low cardiovascular risk (i.e. Framingham risk score 2 ± 3 %). Evaluation of cardiac function was systematically performed.

Results: Carotid stiffness assessed with VVI-assisted variables (i.e. radial systolic velocity, radial strain, radial displacement) correlated with ET for compliance($r=0.91$, $p<0.001$; $r=0.89$, $p<0.005$ and $r=0.94$ $p<0.001$ respectively), for beta stiffness index ($r=-0.93$, $p<0.001$; $r=-0.89$, $p<0.005$ and $r=-0.94$, $p<0.001$) and for distensibility ($r=0.82$, $p<0.01$; $r=0.94$, $p<0.001$ and $r=0.88$, $p<0.005$). Age was strongly linked to VVI-assisted variables ($r=-0.74$, $p<0.02$; $r=-0.73$, $p<0.05$; $r=-0.76$, $p<0.02$). These correlations were independent from cardiac variables.

Conclusions: Carotid stiffness assessed with VVI-assisted total radial strain are strongly correlated to ET in a population at low cardiovascular risk. This easy-to-use and affordable technology could advantageously replace ET, the reference technique, providing new informations for the personnalized characterization of the vascular impact.

P1-12

Adrenergic regulation of bioelectrical activity of rat pulmonary veins myocardium

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Aim: Recently, much attention is paid to the pulmonary veins myocardium (PV) as a region responsible for atrial fibrillation triggering. Arrhythmogenic properties of PV are partially associated with unique properties of cardiomyocytes which provides spontaneous or automatic activity in this structure. Adrenergic stimulation increases PV spontaneous activity, and, at least in rats, leads to shifting of resting potential (RP). The aim of the present study was to investigate mechanisms of RP alteration in rat PVs in response to adrenergic stimulation.

Methods: RP were recorded with use of standard microelectrode technique in isolated multicellular Tirode-perfused quiescent preparations, including PV and left atria (LA) myocardium. Male rats (250-300 g) after approved pretreatment and anaesthezation were used for preparations dissecting. RP was registered in distal region of PV of left lung lobe.
Results: alpha1-agonist phenylephrine (Phe, 10 μM) induced dapolarization of RP in PVs (20±3 mV, $n=11$, $p(T)<0.01$). Beta-agonist isoproterenol (Iso, 10 μM) led to hyperpolarisation of RP to 10±2 mV ($n=9$, $p(T)<0.01$). Phospholipase C (PLC) and proteinkinase C (PKC) inhibitors (U73122, 0,5 μM, $n=6$, chelerythrine, 1 μM, $n=5$, respectively) were unable to suppress phenilephrine-mediated depolarization in PV. Fasudil (10 μM), Rho-kinase and proteinkinase A (PKA) inhibitors, reduced Iso-mediated hyperpolarization by 60%. Forskolin - adenylyl-cyclase activator (AC, 50 μM) hyperpolarized RP in PV (11±2 mV, $n=6$, $p(T)<0.01$) and effect was similar to that of Iso.

Conclusions:
Activation of alpha- and beta-adrenoreceptors leads to opposite RP shifting in rat PV. Alpha-adrenoreceptor mediated depolarization probably independent of PLC and PKC activation. Beta-adrenoreceptors induced RP hyperpolarization most likely associated with activation of AC and PKA.

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P1-13

Molecular basis of sinus bradycardia in hypothyroidism

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Aim: Hypothyroidism in man and animal models manifests in a low heart rate (bradycardia) and cardiac output. We hypothesise that the bradycardia is due to remodelling of the pacemaker, the sinus node (SN). The aim of this study was to characterise the hypothyroid SN and identify the mechanism(s) underlying the bradycardia.

Methods: Hypothyroidism was induced in Sprague Dawley rats (male, 260-330 g, n=8) with 6-n-propyl-2-thio-uracil (PTU, 10 mg/kg/day for 15 days). The control cohort (n=8) received vehicle treatment. ECGs were recorded *in vivo*, in anesthetized (ketamine, 100 mg/kg) animals. SN, atrial and ventricular biopsies were collected and mRNA abundance was measured using qPCR.

Results: PTU administration for 15 days induced hypothyroidism and this was associated with a 6% increase in body weight and a 42% reduction in heart rate (both $p<0.05$). No changes were observed in the control cohort. Automaticity in the SN is due to the spontaneous diastolic depolarization initiated by the synergistic interaction between two clock mechanisms: the 'membrane-clock' and the intracellular 'Ca^{2+}-clock'. In the hypothyroid SN, mRNA expression levels of transcripts corresponding to key membrane-clock components: funny current (I_f; HCN1 and HCN4) and T-type Ca^{2+} current ($I_{Ca,T}$; CaV3.1) remained unaltered. The Ca^{2+}-clock involves the ryanodine receptor (RyR2), Na^{+}-Ca^{2+} exchanger (NCX1) and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a). Hypothyroidism did not affect RyR2 and NCX1 levels; however, SERCA2a mRNA levels were reduced by 37% ($p<0.05$). SERCA2a activity is negatively regulated by phospholamban and this transcript was upregulated by 289% in the hypothyroid SN ($p<0.05$).

Conclusions: PTU causes hypothyroidism with severe bradycardia in healthy rats. In the hypothyroid SN, SERCA2a downregulation coupled with phospholamban upregulation would compromise the Ca^{2+}-clock component, causing bradycardia.

P1-14

The effect of aliskiren, captopril and spironolacton on blood pressure and nitric oxide production in left ventricle of SHR

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Aim: The renin-angiotensin-aldosteron system is a cascade that governs cardiovascular, renal, and adrenal functions. Three important components to this system could be dysregulated. Therefore, we determined whether aliskiren as a direct renin inhibitor, captopril as an angiotensin converting enzyme and spironolactone as an aldosterone receptor antagonist, have different effect on hypertension, NOS activity and eNOS expression in the heart.

Methods: 12-week-old male SHRs were assigned to untreated group, group treated with aliskiren (either powdered or nanoparticle-loaded, 25mg/kg/day) by gavage, group treated with captopril (100mg/kg/day) and group treated with spironolactone (200mg/kg/day) in tap water for 3 weeks. Blood pressure (BP) was measured by the tail-cuff-plethysmography. NOS activity was determined by conversion of ^{3}[H] Arginine to ^{3}[H] Citrulline and eNOS expression by Western blot analysis.

Results: BP was reduced after treatment with all 3 classes of drug in comparison to controls. However, nanoparticle-loaded aliskiren decreased BP more effectively than powdered. Moreover, all treated groups, except powdered aliskiren group, reduced myocardial hypertrophy in comparison to untreated SHR and increased NOS activity in left ventricle. Surprisingly, only nanoparticle-loaded-aliskiren decreased eNOS expression in comparison to untreated SHR.

Conclusion: Despite decreasing of BP and myocardial hypertrophy with the 3 classes of drug, changes in eNOS expression varied. We supposed that studied drugs increased NOS activity rather than eNOS expression. Thus we conclude that a nanoparticle-loaded drug which is realized gradually may represent an effective approach for treatment of hypertension and related disorders. The encapsulation may protect drugs against degradation and thus increase their bioavailability in organs.

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P1-15

Effects of chronic and acute zinc sulphate supplementation on myocardial ischemia-reperfusion injury in rats

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Aim: The aim of this study was to investigate the effect of acute and chronic zinc-sulphate application on myocardial ischemia-reperfusion injury in rats.

Methods: Spraque-Dawley type 50 adult rats were devided into five groups.
Group 1, (n:10) Normal Control Group: Fed with normal diet
Group 2, (n:10) Simulation (Sham) Group: Undergone all surgical procedures under general anesthesia except myocardial I/R application
Group 3, (n:10) Myocardial Ischemia-Reperfusion Group: Fed with normal diet and undergone I/R under general anesthesia
Group 4, (n:10) Myocardial Ischemia-Reperfusion Chronic Zinc Group: Given 5 mg/kg i.p. zinc for 15 days and undergone I/R under general anesthesia at the end of applications
Group 5, (n:10) Myocardial Ischemia-Reperfusion Acute Zinc Group: Given 25 mg/kg i.p. zinc sulphate 1 hour prior to the operation and undergone I/R under general anesthesia
After applications blood samples collected by cardiac puncture, heart tissue samples, following heparin application were collected.
MDA and GSH were determined with spectrophotometric method.

Results: Highest MDA levels in both plasma and tissue samples were obtained in group 3 ($p<0.05$). MDA levels of Group5 were lower than Group3 and MDA levels of group4 were lower than group3 and 5 significantly ($p<0.05$). Highest eryhthrocyte GSH levels obtained in group4 ($p<0.05$). GSH levels of group5 were higher than Group3 ($p<0.05$). GSH level of Group3 was higher than control (G1) and sham (G3) groups ($p<0.05$). Highest GSH levels measured in heart tissue was observed in group4 ($p<0.05$). Heart GSH levels of other groups were not different from each other.

Conclusion: Results of present study has presented that suppressed antioxidant activity with increased oxidant damage during heart ischemia reperfusion in rats can be reversed partially by zinc application.

P1-17

The effect of central serotonin concentration changes on respiratory regulation

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Aim: Serotonin (5-HT) is involved in many aspects of the neural control of breathing. Fluoxetine is a selective serotonin re-uptake inhibitor that causes an increase in extracellular 5-HT transporter blockade at serotonergic cell bodies. We hypothesized that acute 5-HT re-uptake inhibition with Fluoxetine should also have significant effects on hypoxic and hypercapnic respiratory responses.

Methods: For this purpose, eighteen male albino rabbits were anesthetized with urethane (400 mg/kg i.v) and alpha-chloralose (40 mg/kg i.v). For intracerebroventricular (ICV) administration of fluoxetine (0.35 mg/kg) a cannula was placed in left lateral ventricle by stereotaxic method. When the rabbits were allowed to breathe hypoxic and hypercapnic gas mixtures before and after ICV administration of Fluoxetine, respiratory frequency (f_R), tidal volume (V_T), ventilation minute volume (V_E), systemic arterial bood pressure (BP), and heart rate (H_R) were recorded.

Results: ICV fluoxetine injection during normoxia caused significant increases in V_T and V_E ($P<0.01$) and significant decreases in BP and H_R. We observed the expected significant increase in ventilation during acute hypoxia and hypercapnia in rabbits. When the animals were switched to hypoxia following ICV administration of Fluoxetine, V_T, V_E and BP increased significantly ($P<0.05$).When the animals were switched to hypercapnia following ICV administration of Fluoxetine f_R, V_T, V_E and BP increased significantly ($P<0.05$).The increases in percentage values of V_T and V_E in Fluoxetine+Hypoxia phase ($P<0.01$, $P<0.01$) and Fluoxetine+Hypercapnia phase ($P<0.05$, $P<0.05$) were lower than those during hypoxia and hypercapnia alone.

Conclusion: Our results suggested that ICV Fluoxetine administration increased ventilation by the effect of 5-HT on respiratory neurons in normoxia but acute hypoxic and hypercapnic respiratory responses were suppressed probably through activating an inhibitory pathway to the respiratory neurons under the influence of medullar serotonergic neurons.

P1-18

Respiratory alterations elicited by chronic long-term intermittent hypobaric hypoxia in rabbits

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Aims. None of the studies carried out so far investigated the effect of denervation of peripheral chemoreceptors on basal ventilation and respiratory responses to acute hypoxia in subjects exposed to chronic long-term intermittent hypobaric hypoxia (CLTIHH). We aimed to research the effect of CLTIHH (430 mmHg, 5 h/day, 5 days/week, 5 weeks) on basal ventilation and respiratory responses to hypoxia and the effects of CLTIHH on central respiratory mechanisms after peripheral chemodenervation.

Methods. Sixteen adult albino rabbits were divided into two groups: CLTIHH (n = 8) and control (n = 8). The tidal volume (VT) and respiratory frequency (f/min) were initially recorded in both groups and respiratory minute volume (VE) was calculated. PaO2, PaCO2, and pHa values were determined.

Results. The initial values of f/min and VE in CLTIHH group were significantly higher (p <0.001, p <0.05) than that of control group. After exposure to hypoxic gas mixture (8% O2-92% N2) the elevations in f/min, VT, and VE in CLTIHH group were significantly higher (p <0.001, p <0.05, p <0.001) than those of control group. After denervation of peripheral chemoreceptors, the decrease in VE in CLTIHH group was found to be significantly less (p <0.05) than that of control group. When the animals in control group were allowed to breathe hypoxic gas mixture, f/min, VT, and VE decreased significantly (p <0.05) and hypoxic depression was obtained. In contrast, hypoxic depression did not occur in the CLTIHH group.

Conclusions. Our results suggested that CLTIHH increases the basal ventilation and hypoxic respiratory responses and that enhanced ventilatory responses were due not only to the augmentation of peripheral chemoreceptor activity but also to the augmentation of central respiratory activity.

P1-19

Optical cardiac action potential alternans formation in different myocardial layers during early regional ischemia

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Aim: Cardiac alternans in ischemic heart is known as a precursor of ventricular arrhythmias and sudden cardiac death. It was demonstrated that during acute regional ischemia the alternans can be driven by intramural 2:1 conduction blocks. The aim of this study was to evaluate the formation of optical action potential (OAP) alternans in different myocardial layers in ischemic rabbit heart.

Methods: Experiments were performed in Langendorff-perfused rabbit hearts stained with near-infrared (NIR) dye di-4-ANBDQBS. Regional ischemia was induced by occlusion of left anterior descending coronary artery for 15 min. To evoke cardiac alternans the pacing cycle length (PCL) was shortened from 300 ms to 200–150 ms. Two lasers of 532 nm and 660 nm wavelengths and two glass microelectrodes were used to register optical and electrical action potentials, respectively, from different layers of heart muscle.

Results: After artery occlusion the location of ischemic zone was similar at excitation of 532 nm and 660 nm vawelenghts, used to obtain signals from surface and almost all ventricular wall thickness, respectively. The decrease of PCL evoked formation of OAP amplitude alternans of different magnitude, depending on excitation wavelength. The differences were also dependent on applied PCL. This was confirmed by microelectrode recordings. It suggests that the influence of ischemia on epi- and endocardial action potentials was not homogenous.

Conclusions: Usage of green and red excitation wavelengths light in NIR dye stained heart revealed that optical cardiac action potential alternans formation during early regional ischemia is non homogenous in different myocardial layers due to different influence of ischemia on epi- and endocardial electrical behavior.

P1-20

The effects of copper application on oxidative and antioxidant systems in rats

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Aim: Copper is an essential trace element, but its redox reactivity leads to risks of damage to cell and tissues. Oxidative damage of copper has been reported in recent studies even though it is necessary for organism as a trace element. Present study was planned to determine the presence of oxidative damage of copper on blood cells, especially on erythrocytes and possible antioxidant defense mechanisms against it.

Methods: In this study, Wistar- albino type female rats weighing 180-220g were used in both control (n=9) and experimental (n=9) group. While control group rats were being fed with normal diet and water, experimental animals were fed by normal fodder but received 250 mg/l copper in their drinking water (dissolving approximately 1g $CuSO_4.5H_20$ in one liter water) for nine weeks. After experimental period blood samples were taken from the abdominal aorta under light ether anesthesia. Serum copper concentrations, erythrocyte superoxide dismutase (SOD) activity and glutathione (GSH) levels and plasma malondialdehyde (MDA) concentrations were measured in both groups.

Results: MDA concentrations and SOD activities of experimental group were found higher than controls ($p<0.05$) and GSH concentration in experimental group was found to be lower than control group animals ($p<0.05$).Serum Cu concentrations in rats that were exposed to the copper were found to be significantly higher than that of the control group ($p<0.05$).

Conclusions: The results of this study may show that copper may lead to increase in lipid peroxidation and SOD activity and GSH levels may also be decreased by effect of copper in blood.

P1-21

Current chronic heart failure treatment: comparisson of single Lithuania's university centre with other European centres

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Aim. To compare pharmacotherapy of ambulatory chronic heart failure (CHF) patients in single Lithuania's university centre with other European centres participating in EURObservational Research Programme (EORP) Heart failure Long-term registry (HFLTR).

Methods. In total, 240 patients were entered in the HFLTR registry on a one-day-per-week basis from our university centre and 7401 patients from other 211 European centres from May 2011 to March 2012.

Results. ACE inhibitors were prescribed to 67.1% of patients in the Lithuanian centre and 67% in other European cardiology centres (p=0.975). ARBs were prescribed more often in other European centres (23.9% vs. 16.7%, p=0.009).
BBs were prescribed significantly less in the Lithuanian centre (82.5% vs. 88.9%, p=0.002). Metoprolol was more often used in our centre (51.2% vs 11%, p=0,001) and contrarily, bisoprolol was more frequently prescribed in other European centres (40.8% vs. 1.3%, p=0.001), as well as carvedilol (40.7% vs. 25%, p=0.001).
Spironolactone was prescribed significantly less frequently in our centre – 58.6% vs. 67, p=0.025. Eplerenone was used just in 0.4% cases, while this medication was prescribed to 23.7% patients of other European centres (p=0.001).
Diuretics were still in use for stable CHF patients: 71.7% of patients were treated with diuretics in our centre and statistically significantly more frequently – 83.1% – in other European centres (p=0.001).

Conclusion: No difference in treatment of stable heart failure patients with angiotensin converting enzyme inhibitors have been defined in both centres. Prescription of beta blockers and mineralocorticoid receptors antagonists was lower in Lithuania. Single Lithuania's centre has significantly less stable heart failure patients on diuretic therapy as it is recommended by current guidelines.

P1-22

Trends in neurohormone up-titration for chronic heart failure treatment in single Lithuania's centre and other European centres

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Aim: to compare up-titration of neurohormone blockers for ambulatory patients in single Lithuania's university centres and comparison with other European centres participating in EURObservational Research Programme Heart failure Long-term registry.

Methods: Patients data were entered in the registry on a 1 d/week basis. 1 year follow-up data were registered based on a visit to the clinical centre after 12 months or by phone-calls. In total 240 patients from our centre were registered as ambulatory patients with chronic heart failure (CHF) from May 2011 to March 2012.

Results: In our centre 67.1% of patients received ACE inhibitors at target doses and just 29.3% – in other European centres ($p<0.001$). No significant difference in ARBs dose was defined (16.7% vs. 24.1%, $p=0.294$). The percentage receiving target doses of BBs was higher in our university center (82.5% vs. 17.5%, $p<0.001$). 52.1% of our centre patients treated with MRAs were on target doses and it differs statistically significantly– from other registry centres (52.1% vs.30.5%, $p<0.01$). Reasons for not reached the patients target doses in our centre were as follows: symptomatic hypotension (34.3%), worsening of renal function (4.6%), cough (1.9%), high potassium level (0.9%) for ACE inhibitors; symptomatic hypotension (23.5%), worsening renal failure (5.9%) for ARBs; symptomatic bradycardia (12.7%) and hypotension (10.7 %), bronchospasm (5.4%), worsening CHF (6.3%) for BBs; high potassium level (15.4%) for MRAs.

Conclusions:
Statistically significantly more patients in our centre received a target doses of ACE-inhibitors, BBs and MRAs. The main reasons why the target doses of neurohormone blockers have not been achieved were symptomatic hypotension for ACE-inhibitors and ARBs, symptomatic bradycardia for BBs and hyperkalemia for MRAs.

P1-23

A different marker to determine arrhythmia potential between elite active cyclists and veterans: T peak T end

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Aim: We aimed to investigate the potential arrhythmia risk of active sport trainings between the active elite cyclists and veterans by using the simple, non-invasive cardiac tests.

Methods: Groups were healthy volunteers (Group1, n=28, mean age 35.8±4.6 y), elite active cyclists (Group2, n=27, mean age 21±3.0 y), veteran cyclists (Group3, n=27, mean age 29.5±7.1 y). Groups were underwent 12 derivation ECG records, transthoracic echocardiography investigations. Tp-e interval, Tp-e dispersion, and Tp-e/QT ratio were measured from ECG records. Tp-e intervals were measured with Tangent method.

Results: Mean Tp-e interval results were as follows according to groups 1, 2, 3; 75.0±9.3 ms, 88.1±7.0 ms, 83.2±8.8 ms, respectively. Statistically significant differences were obtained between the groups 1 and 2 ($p<0.001$), groups 1 and 3 ($p=0.001$), group 2 and 3 ($p=0.035$). The mean Tp-e dispersion results in groups 1, 2, 3 were; 21.9±7.9 ms, 36.7±19.4 ms, 23.1±12.8 ms, respectively. Significant differences were reported between the groups 1 and 2 ($p=0.001$) and 2 and 3 ($p=0.001$). The mean Tp-e/QT ratios were as follows in groups 1, 2, 3; 0.21±0.02 ms, 0.22±0.02 ms, 0.22±0.02 ms, respectively. Statistically significant differences were observed between the groups 1 and 2 ($p=0.044$), 1 and 3 ($p=0.044$).

Conclusion: Long-term, intense, active sports may prolong the Tp-e interval, Tp-e dispersion, corrected Tp-e interval and Tp-e/QT ratio which are associated with potential arrhythmias either in active athletes or veterans. This may also be associated with left ventricular hypertrophy in active athletes and remnant left ventricular hpertrophy in veterans.

P1-24

Assessment of spontaneous baroreflex sensitivity in premature newborns

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Aim: In human newborns, little information was known about baroreflex sensitivity (BRS), because continuous non-invasive recording of blood pressure was not available. At present, a volume clamp method (Finometer, Portapres, FMS), after verified modification, can be used in preterm newborns. We aimed to determine the relationships between spontaneous BRS, blood pressure (BP), pulse/heart rate (HR) and parameters reflecting intrauterine/early postnatal growth and development.

Methods: BP signal was recorded in 57 preterm newborns (gestational age 25-37 weeks, birth weight 650 – 2730 g) using Portapres with a cuff placed around the child´s wrist. BRS was calculated by BeatScope 1.1a and PVRBRS software (Wesseling).

Results: HR and respiratory rate decreased with increasing parameters reflecting fetal and early postnatal development. BRS was lower in more preterm newborns (4.11±1.59) compared to the newborns of higher gestational age (8.12±5.00 ms/mmHg). Similar relationships were also between BRS and birth weigh, postconceptional age and actual weight. Multifactor analysis revealed 56% positive association between BRS and postconceptional age, birth weight as well as diastolic blood pressure (DBP).

Conclusions: Baroreflex sensitivity and through this parameter the maturation of the autonomic nervous system can be evaluated non-invasively even in preterm newborns. Baroreflexes in these infants are active, however baroreflex sensitivity is low and it increases progressively with postconceptional age and actual weight.

P1-25

Pro-contractile action of the Na,K-ATPase/*Src*-kinase signaling pathway in the vascular wall.

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Aim: Na,K-ATPase is essential for maintaining the transmembrane ion gradient and might initiate various intracellular signaling. These signals possibly act through a modification of the local ion concentrations or via *Src*-kinase activation. It is known that inhibition of the α-2 isoform of Na,K-ATPase by ouabain elevates blood pressure. Consequently, ouabain was shown to potentiate arterial contraction *in vitro*. In contrast, we have demonstrated that siRNA-induced down-regulation of the α-2 isoform Na,K-ATPase expression reduced arterial sensitivity to agonist stimulation and prevented the effect of ouabain. Here we demonstrate results of our research on the mechanisms involved in the modulation of vascular wall contractility by ouabain-sensitive Na,K-ATPase.

Methods: The experiments were performed using rat mesenteric arteries in isometric myograph conditions. To inhibit kinase activity a *Src*-family selective tyrosine kinase inhibitor, PP2, and pNaKtide - a membrane-permeable small peptide which antagonizes ouabain-induced activation of *Src*-kinase were used.

Results: The pro-contractile action of ouabain is associated with activation of *Src*. This is supported by Western blot analyses showing activation of *Src* by ouabain and its inhibition by pNaKtide. *Src* was also activated by agonist (noradrenaline) stimulation. *Src*-dependent potentiation of vasoconstriction was associated with sensitization of contractile machinery to $[Ca^{2+}]_i$, as evident from MYPT (myosin phosphatase targeting protein) phosphorylation assay. Down-regulation of the α-2 isoform Na,K-ATPase prevented the inhibitory effect of *Src* inhibitors on arterial contraction.

Conclusions: the pro-contractile action of ouabain-sensitive Na,K-ATPase inhibition is associated with *Src*-kinase inhibition suggesting the role of this signaling pathway in regulation of vascular tone and peripheral resistance.

P1-26

Preliminary study: What is the effect of darkness on electrophysiology of the heart in young people?

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Aim: Primary aim of the study is to evaluate effect of resting stay in absolute darkness on ECG parameters of young people. The results of treatment method, which is based on staying in absolute darkness to improve the health of people who are under constant stress, suggest that this method could affect electrophysiology of heart and decrease especially heart rate.

Methods: Evaluations were performed in 17 students aged between 19 and 26 years. They were placed for 72 hours in a special room with an absolute darkness. The room met the optimal conditions for a comfortable individual stay and it was located in a quiet place. In the room there was only one person per stay. Participants received food and drink according to their needs. RR, PQ, QT, QTc intervals and heart rate were evaluated from II. bipolar limb record of ECG one day before entering the room and 1 day, 3 days and 7 days after exiting every day at 07:30h a.m.

Results: RR, PQ and QT intervals nonsignificantly extended, with maximum during third day after exiting the stay. QTc interval nonsignificantly shortened and heart rate nonsignificantly decreased.

Conclusion: Resting stay in darkness for 72 hours is too short period to have effect on electrophysiology of the heart.

P1-27

Anthocyanins as electron acceptors at complex I

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Aim: Anthocyanins, a subclass of flavonoids, are known to protect against myocardial ischemia; however, little is known about their direct, acute effects on mitochondria injured by the ischemic insult. We aimed to determine whether the anthocyanins delphinidin 3-O-glucoside (Dp3G), cyanidin 3-O-glucoside (Cy3G) and pelargonidin 3-Oglucoside (Pg3G) can restore the function of the respiratory chain after ischemia-induced damage to isolated heart mitochondria, and the mechanism by which this could occur.

Methods: Experiments were performed on hearts from 2 to 4- month-old male Wistar rats. Mitochondria (control or after 45 min ischemia) were isolated by differential centrifugation. Mitochondrial respiration was measured with an Oxygraph-2k. The activity of complex I of the electron transport chain was measured in mitochondria isolated from control hearts and hearts subjected to ischemia for 45 min. Complex I activity was evaluated by subtracting the rotenone-suppressed NADH oxidation rate from the NADH oxidation rate. The ATP concentration in the supernatant was measured fluorimetrically with an ATP assay kit.

Results: Cy3G and Dp3G increased the activity of complex I, measured in the presence or absence of coenzyme Q1 (CoQ1), in ischemia-damaged mitochondria, whereas in nonischemic mitochondria the effect was observed only in the absence of CoQ1. Dp3G and Cy3G but not Pg3G increased state 3 respiration and ATP synthesis with NADH-dependent substrates in mitochondria after ischemia.

Conclusions: The results suggest that certain anthocyanins can act as electron acceptors at complex I, and bypass ischemia-induced inhibition, resulting in increased ATP production after ischemia. This study provides new information on a possible role of certain anthocyanins in the regulation of energy metabolism in mammalian cells.

P1-28

Association between pentraxin-3 and growth differentiation factor-15 in adolescent male swimmers

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Aim: The purpose of this study was to evaluate plasma pentraxin 3 (PTX3) and growth differentiation factor-15 (GDF-15) concentrations in adolescent male swimmers and compare any possible interactions with canonical biochemical and haematological parameters.

Methods: Twenty-six adolescent male swimmers and 29 gender- and age-matched sedentary controls participated in this study. Fasting blood samples were taken from the participants. Biochemical and haematological values, plasma PTX-3 and GDF-15 levels were measured.

Results: White blood cell count in the adolescent swimmers was significantly lower, but red blood cell counts, haemoglobin concentrations and haematocrit values higher than that in the sedentary controls. Plasma PTX-3 levels were markedly higher in the adolescent male swimmers than that in the sedentary controls (378.44 ± 173.93 vs 257.82 ± 103.20 pg mL^{-1}, $P < 0.05$). There was no significant difference in GDF-15 levels between the two groups (186.12 ± 40.65 vs 203.60 ± 36.77 pg mL^{-1} in the swimmers and the sedentary, respectively). However, it was tended to be lower in the adolescent swimmers. Relationship between PTX3 and GDF-15 was linear.

Conclusion: In conclusion, adolescent male swimmers had higher PTX3 levels than sedentary controls and there was a linear relationship between PTX3 and GDF-15.

P1-29

Effects of high fructose diet and exercise on glucose transporter 5 and putative glycerol-transporter aquaporin 7 in the *in vivo* rat heart

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Aim: Three hypotheses were tested in this study. H1) High-Fructose diet (HFD) increases cardiac GLUT5 expression; H2) Physical exercise (E) enhances cardiac AQP7 expression; H3) HFD supplied to rats subjected to exercise (HFD-E) blocks the E-induced increase in AQP7 expression.

Methods: Male Sprague Dawley rats were allotted to four groups: control (C; n=11), exercise (E; n=10), high fructose diet (HFD; n=12) and high fructose diet plus exercise (HFD-E; n=12) groups. HFD was started 28 days before euthanasia. At day 24-27, rats were subjected to moderate exercise, followed by vigorous exercise on day 28 (E and HFD-E groups). Cardiac GLUT5 and AQP7 mRNA levels were determined with RT-PCR. Protein content was assessed immunohistochemically.

Results: GLUT5 mRNA and protein expression did not differ between groups. mRNA levels of AQP7 increased significantly (4.8 fold) in group E compared to group C ($p<0.001$). However, AQP7 mRNA levels were similar in HFD and HFD-E groups compared to group C. The extent, expressed as percentage positive cardiomyocytes, and intensity of immuno-histochemical staining for AQP7 were significantly increased in group E compared to group C ($p<0.001$), group E ($p<0.001$) and group HFD-E ($p<0.001$).

Conclusions: Our study indicated that HFD did not increase cardiac GLUT5 expression. However, exercise enhanced cardiac AQP7 expression and protein content (H2 accepted). Additionally, HFD prevented the exercise-induced increase in cardiac AQP7 expression (H3 accepted). This inhibitory effect may be related to competition between fructose and glycerol as energy substrate in the heart subjected to five days of physical exercise.

P1-30

The pH sensitivity of TRPM7-like current in human atrial cardiomyocytes

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Aim: The pH sensitivity is a novel feature of TRPM7 channels. However, such information in native human cardiomyocytes is missing. In search for a role of this channel in the human heart under pathological conditions, such as ischemic heart disease, we examined TRPM7-like current sensitivity to changes in the extracellular pH (pH_o).

Methods: Under the whole-cell patch-clamp configuration, we used a modified K^+- and Mg^{2+}-free pipette solution to investigate the TRPM7-like current recorded by a 2-s symmetrical voltage ramp

from -120 to +80 mV from a holding potential of -80 mV in cardiomyocytes isolated from human atrial specimens.

Results: To examine how TRPM7-like current in human cardiomyocytes is affected by acidic pH_o, the membrane current was recorded under control conditions, i.e. in the presence of Ca^{2+} and Mg^{2+} in physiological Tyrode solution, and in divalent-free (DVF) extracellular medium, by altering pH_o of extracellular solution from 7.4 to 6.0, 5.0, and 4.0. It is interesting to note, under perfusion with a physiological Tyrode solution upon acidification of the extracellular medium has been reversibly increased both inward (at -120 mV) and outward (at +80 mV) TRPM7-like currents. While, in DVF Tyrode solution the acidification induces an opposite effect. After TRPM7-like current had reached steady-state level in DVF medium under pH_o 7.4, decreasing extracellular pH_o caused a reversible decrease of both inward (at -120 mV) and outward (at +80 mV) TRPM7-like currents.

Conclusions: These results indicate that the sensitivity of TRPM7 channels to extracellular pH in human atrial cardiomyocytes is not the same, when superfused with physiological or divalent-free Tyrode solution. However, the mechanisms underlying such the effects of extracellular pH remains largely unknown.

P1-31

Agomelatine has relaxant effect on isolated rat thoracic aorta through nitric oxide dependent mechanism

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Aim: Agomelatine, a new antidepressant with a unique receptor profile as a melatonin receptors' agonist and $serotonin_{2C}$ receptor antagonist. The purpose of the present study was to determine the effects of agomelatin on rat toracic aorta, paying special attention to the role of the endothelial nitric oxide in these effects.

Methods: Aortic rings obtained from female adult Wistar rats placed in organ baths containing Krebs–Henseleit solution. Changes in isometric tension of aorta rings were recorded using a force-displacement transducer and data acquisition system. Endothelium intact rings were used in first step of study. Rings contracted with serotonin (10^{-5} M) and then cumulative agomelatine concentrations (10^{-9}-$3x10^{-4}$ M) added into the organ baths. The same procedure applied in the presence of nitric oxide synthase inhibitor (L-NAME, 10^{-4} M) and also in endothelium-removed rings.

Results: Relaxation responses to agomelatine were expressed as percentages of the serotonin-induced contraction. Concentrations of agomelatine causing 50% of maximal response (IC_{50}) were calculated from each individual concentration-response curves. Maximal responses and -log IC_{50} values for curves obtained before and after L-NAME incubation were compared by using Student's t test.
After obtaining steady-state responses to serotonin, agomelatine (10^{-8}- 10^{-3} M) relaxed the thoracic aorta rings in a concentration-dependent manner ($p<0.05$). Denudation of vessel endothelium by mechanically or preincubation of thoracic aorta rings with L-NAME significantly shifted to the right the response to agomelatine.

Conclusions: In this study, agomelatine caused concentration-dependent relaxation in rat thoracic aorta rings constricted with serotonin. This effect may occur via nitric oxide dependent mechanism

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P1-32

Effects of nanoparticle-loaded aliskiren on structural alterations in the heart and aorta

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Aim: Aliskiren, the most recent inhibitor of renin, has been shown to exert cardioprotective effects independent of its blood pressure (BP) lowering activity. However, clinical use of aliskiren is limited by short lifetime of this drug. Therefore, we aimed to study effects of nanoparticle-loaded aliskiren, with gradually realized drug, on BP and structural alterations of the heart and aorta developed due to hypertension.

Methods: 12-week-old male SHRs were divided to the untreated group, group treated with powdered aliskiren (25mg/kg/day), group treated with nanoparticle-loaded aliskiren (25mg/kg/day), and group treated with nanoparticles only for 3 weeks by gavage. BP was measured by tail-cuff plethysmography. Collagen and elastin contents were determined by picro-sirius red staining. In the

aorta, wall thickness (WT), inner diameter (ID) and cross sectional area (CSA) were measured as well.

Results: At the end of experiment, BP was lower in both powdered and nanoparticle-loaded aliskiren groups with more pronounced effect in the second one. Moreover, nanoparticle-loaded aliskiren was able to decrease collagen content (by 11%) and CSA (by 25%) in comparison to the powdered aliskiren group, while it had no significant effect on the similar parameters in the heart. There were no significant changes in elastin content, WT and ID among aliskiren groups and control group. Polymeric nanoparticles, however, increased collagen and elastin content and WT of the aorta.

Conclusions: Nanoparticle-loaded aliskiren seems to be promising drug in large vessels protection, more suitable polymeric nanoparticles, however, are needed for better tissue preservation.

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P1-33

Effect of red wine extract on pro-inflammatory markers - nuclear factor-kappaB and inducible NOS in experimental metabolic syndrome

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Aim: We aimed to analyse the effects of alcohol free Alibernet red wine extract (AWE) on nitric oxide synthase (NOS) activity and pro-inflammatory markers like nuclear factor-kappaB (NF-kappaB) and inducible NOS (iNOS) protein expressions in experimental metabolic syndrome.

Methods: Young 6-week-old male Wistar Kyoto (WKY) and obese, spontaneously hypertensive rats (SHR/N-cp) were divided into control groups and groups treated with AWE (24.2 mg/kg/day) for 3 weeks (n=6 in each group). Total NOS activity and endothelial NOS (eNOS), iNOS and NF-kappaB (p65) protein expressions were determined in the heart left ventricle and aorta by Western blot and immunohistochemical analysis.

Results: All investigated parameter were significantly increased in the aorta of SHR/N-cp rats. Pro-inflammatory markers like NF-kappaB and iNOS were increased in the left ventricle as well. AWE treatment did not affect total NOS activity and eNOS expression in the aorta, however it was able to decrease NF-kappaB and iNOS protein expressions in both left ventricle and aorta.

Conclusion: In the cardiovascular system, Alibernet red wine extract decreased NF-kappaB and iNOS protein expressions elevated as a consequence of developed metabolic syndrome. This effect may represent one of the protective, anti-inflammatory properties of Alibernet red wine polyphenols on the cardiovascular risk factors related to the metabolic syndrome.

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P1-34

Effect of acute continuous and intermittent systemic hypoxia on serum cytokine concentrations

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Aim: Hypoxia induces vascular remodeling, which is result of synthesis and release of cytokines. This could change local vascular beds responses causing local inflammatory reactions, angiogenesis and vascular leakage. Study was designed to evaluate effect of hypoxia's type (continuous or intermittent) on serum cytokine levels.

Methods: Twenty four volunteers participated in acute 20 minutes continuous and intermittent (5 min x 4 times) hypoxic protocols. Venous blood samples were obtained from the cubital vein by standard venous puncture before (B) and after (A) intermittent and continuous hypoxia. Samples were coagulated (30 min), centrifuged and then stored at -80°C. Cytokine measurements (EGF, FGFb, IFN-γ, TNF-α, IL-10, IL-17, IL-8, IL-7, MCP-1, and VEGF) were performed with standard Millipore kits according to the manufacturer's instructions.

Results: In acute intermittent hypoxia (pO_2=12%) there were no significant changes in EGF, FGFb, IFNy, IL-17, IL-7, VEGF, IL-10, IL-8 serum cytokine levels; however significant ($P<0.05$) decrease were observed in MCP1 and TNF-α serum levels. In acute continuous hypoxia (pO_2=12%) there were no significant change in EGF, FGFb, IL-7, IL-10, IL-8, TNF-α serum levels, but were observed significant

(P<0.05) decrease in IFN-γ, IL-17, MCP-1, VEGF serum levels.

Conclusions: Our results suggest that hypoxias type causes different modulatory effect on cytokine production.

P1-35

Lung volumes related to physical activity, aerobic capacity and Body mass index in students

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Aim: The aim of this study was to estimate the correlation between the function parameters of the respiratory system and the amount of physical activity, body mass index and aerobic and physical fitness in Daugavpils University students. The study involved a group of 191 students of both sexes from Daugavpils University aged 20-36 years.

Methods: Students (n=191, 123 females and 68 males, mean age 24,02 ± 0,24 years) underwent measurement of anthropometric values (height, weight), physical (PWC170 index) and aerobic fitness (predicted VO_2max), spirometry (tidal volume, breathing frequency (BF), minute ventilation (MV), expiratory reserve volume (ERV) and vital capacity (VC) and filled in International Physical Activity Questionnaire (IPAQ).

Results: Both MV and VC were directly and statistically significantly associated with aerobic fitness, physical fitness and amount of physical activity. Vigorous PA independently was directly and statistically significantly associated with physical fitness and aerobic fitness. After adjusting for sex, BF was inversely and statistically significant associated with physical activity amount in female. Both female and male had inverse and statistically significant association of BMI and relative VO_2max.

Conclusions: Reduced lung volumes were associated with lower aerobic fitness, lower physical fitness and lower amount of weekly physical activity. Healthier body mass index was associated with higher aerobic fitness (relative VO_2max) in both female and male.

2. Muscular hysiology

P2-1

The effects of normobaric hypoxic exercise and docosahexaenoic acid in total protein and protein oxidation on plantaris muscle of rats

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Aim: In this study the effects of normobaric hypoxic exercise, Docosahexaenoic acid (DHA) and exercise on oxidative protein damage found in plantaris muscle of rats are investigated.

Method: 28 male Sprague Dawley rats of 4 months age are divided into five groups: control (K), hypoxia (H), hypoxia+exercise (HE), hypoxia+DHA (HD) and hypoxia+exercise+DHA (HED). Hypoxia was maintained by %14 O2 for 28 days. Exercise were made on treadmill (5 days a week, 20 minutes a day, with 1.8 km/s speed, 0% slope). DHA were gavaged everyday (36 mg/kg/day). After experimental period, plantaris muscles were removed and total amount of protein, advanced protein oxidation (AOPP) and thiols (T-SH, NSH and P-SH) were examined with spectrophotometric methods. The differences between groups were evaluated by Kruskal- Wallis and Mann Whitney U tests.

Results: The amount of protein in the tissues of H group decreased significantly when compared with the K group, total amount of protein in the tissues of HED group increased when compared with the H group. Taking DHA in hypoxia caused a significant decrease in AOPP level. But there is no difference in AOPP levels between HED and HE groups. Similarly taking DHA did not prevent decrease of total thiol (T-SH) and protein thiol (P-SH) amounts.

Conclusion: Applying DHA+exercise in hypoxia prevented decrease on the total protein amount in rat plantaris muscle and applying only DHA prevented oxidative protein damage caused by hypoxia.

Keywords:
Exercise, hypoxia, advanced oxidation protein product, thiols, total protein

P2-2

Can data smoothing influence the assessment of oxygen uptake kinetics?

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Aim: Increase in oxygen uptake (VO2) during the moderate-intensity exercise has three phases: after the short cardio-dynamic phase there is a rapid exponential increase until reaching steady state and finally, VO2 can slowly rise further (the slow component). The rapid phase is described by the time constant (tau) and the amplitude. The aim of our study was to assess the effect of two data smoothing methods on determining the time constant.

Methods: Ten men volunteered to participate in two tests at the 90% of the maximal aerobic power using the rowing ergometer. VO2 was registered with a portable oxygen analyzer. VO2 data were interpolated to 1 second intervals and then smoothed by two **Methods:** a) data points were averaged to 5-second intervals, b) the value of the middle point of 5-second moving window was replaced with the value of the fitted linear function in this point. Both VO2 data were fitted with the double-exponential models using MatLab and time constants of the rapid phase using both smoothing methods (tau-a and tau-b) were found.

Results: When comparing time constants from 20 tests the mean difference between tau-a and tau-b was 0.36 (95% CI: -0.38….1.10) seconds. However, when we compared results for one subject from two tests, the method of smoothing could change the results more, e.g in one subject tau-a increased by 2.1%, whereas tau-b increased by 32.5%.

Conclusions: As long as the method for VO2 data smoothing and fitting with exponential models has not been standardized, these steps have to be described thoroughly in publications and when comparing results from different publications the influence of smoothing has to be accounted for.

P2-3

Evaluation of the effects of simvastatin on energy metabolism in muscle tissue during early phase of sepsis

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Aim: Sepsis in animal models lead to energy supplies in muscles and result weakness and further occured mitochondrial dysfunction. We aimed to investigate the effect of simvastatin on levels of Creatinine, Creatinine Phoshate, AMP, ADP and ATP in muscle tissue in during early phase of sepsis.

Material and Methods: Male Wistar albino rats (200-250 g) were divided into four groups as control, LPS, simvastatin, simvastatin, simvastatin+LPS groups. Sepsis was induced by administration of Lipopolysaccharide (LPS) (O127:B8; 20 mg/kg, i.p). After four hours tissue samples were taken for investigating Creatinine, Creatinine Phoshate, AMP, ADP and ATP by HPLC method. We used one way variance analysis and Tukey test.

Results: There were no differences among experimental groups in levels of Creatinin. Creatinin phosphate levels were increased ($p<0.05$) also AMP levels was decreased in the experimental groups compared to control. In the simvastatin+LPS group, AMP levels were increased compared to simvastatin and LPS groups. There were decrements in the levels of ADP in both the LPS and simvastatin+LPS groups compared to others ($p<0.01$) In the simvastatin groups, ATP levels were higher than in the controls ($p<0.05$).

Conclusion: Although, We observed that Simvastatin increased levels of AMP, but it decreased significantly the levels of ADP and ATP. Pretreatment of Simvastatin may not be effective on low energy levels in early phase of sepsis.

P2-4

The effect of kinesio taping on functional performance assessment among young female basketball players with chronic ankle instability

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Aim: It has been known that repetitive ankle sprains cause chronic ankle instability (CAI) on basketball players. Kinesio taping is becoming popular as an injury prevention method over athletes. The effect of kinesio taping on functional performance of female basketball players with CAI is inspected.

Methods: Players from 1st League Basketball Team's youth squads were included to the study, without ankle instability (n=16) and with chronic ankle instability (n=15). Every athlete's proprioception and postural stability are assessed three times before, 10 minutes after the application and 48 hours after the application Kinesio- Taping. The joint position sense is assessed on 20º ankle dorsiflexion and 20º ankle plantar flexion with dynamometer (Cybex Humac Norm). The postural stability is assessed dynamically with both feet separately, between 12 and 4 levels with the balance device (Biodex Balane System SD). The single and double stance oscillation indexes are determined on both anterior/posterior and medial/lateral planes.

Results: The reduction between the anterior/posterior oscillation index, medial/lateral oscillation index and the oscillation rates on all planes (on both single and double stance, eyes are open) before and 10 minutes after and 48 minutes after kinesio taping, found statistically significant basketball players with chronic ankle instability ($p<0,05$, $P<0,001$ respectively). There was no any significant difference on oscillation index rates on participants without ankle instability. Also, no any significant difference found on joint position sense between participants with and without ankle instability.

Conclusion: According to our results, Kinesio taping is effective on supporting and stabilizing the ankles of the athletes with chronic ankle instability, but not effective on joint position sense.

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P2-5

Effects of the beta amyloid peptide 22-35 and 1-42 on the isolated rat ileum smooth muscle

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Aim: Alzheimer's disease is a common neurodegenerative disorder, characterized by depositing beta amyloid peptides in the brain. Beta amyloid peptides have also important effects except nervous system. Accumulation of the beta amyloid peptides have been observed on the skin and skeletal muscle of Alzheimer patients.The effects of the beta amyloid peptide 1-42 and beta amyloid peptide 22-35 on the contractility of smooth muscle have been not investigated. Therefore, here an investigation of the effects of the beta amyloid peptide 1-42 and beta amyloid peptide 22-35 on the contractility of ileum smooth muscle in rat has been aimed.

Methods: Longitudinal muscle strips of rat ileum were tested in the isolated organ baths with commercially obtained beta amyloid peptide 1-42 and beta amyloid peptide 22-35. Cumulative concentration response curves for beta amyloid peptide 1-42 (10-9 -10-6 M) and beta amyloid peptide 22-35 (10-9 - 10-5 M) on rat ileum strips were obtained. Data were statistically evaluated using one way variance analysis followed by Tukey's HSD for multiple comparison and ED50 values were calculated using R packages.

Results: Our findings suggest that both peptides did not statistically effect on ileum smooth muscle, but as shown in the graphical and median parameters of beta amyloid peptide 22-352, a dose-dependent inhibition was seen, although it is not statistically significant.

Conclusions: A few researchers reported that some amyloid peptides cause contraction in smooth muscle while some others not.We showed that amyloid peptide 1-42 did not have any effects whereas 22-35 caused an obvious inhibiton.These different responses to the peptides can be results of aminoacids number and order in the peptides.

P2-6

Effects of 12-week programme of spine-stabilizing exercises on trunk muscles area, strength and function in women with chronic low back pain

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Aim: To evaluate the effects of a program of lumbar stability exercises (PLSE) on cross-sectional area (CSA) and strength of trunk muscles in women with chronic low back pain (LBP).

Methods: Female volunteers with LBP were divided into an experimental group (EG; n = 25) and a control group (CG; n = 11). The EG subjects were enrolled in a 12-week PLSE to increase lumbar stability. Before starting the PLSE, after completing it and 1 and 2 months after the intervention the following tests were carried out: isometric force at an angular velocity of 60 deg/s was measured using an isokinetic dynamometer; measurement of the CSA of the multifidus muscle was performed using an ultrasound system.

Results: After completing the 12-week PLSE, trunk extension (41.25 ± 9.25%) and flexion (21.53 ± 4.48%) strength increased ($P < 0.001$) in the EG. This increase in strength remained after 1 and 2 months ($P < 0.05$). This increase in multifidus muscle CSA was maintained after 1 month (right side: 8.26 ± 0.6 cm^2; left side: 8.41 ± 0.7 cm^2) ($P < 0.05$) and 2 months (right side: 7.45 ± 0.6 cm^2; left side: 7.53 ± 0.7 cm^2) ($P < 0.05$).

Conclusions: The 12-week PLSE increased multifidus muscle CSA, trunk extension and flexion strength. This increase in strength was maintained for 2 months, but the decrease in LBP and improvement in functional condition continued for 1 month only.

P2-7

Comparison of the effect of local microvibration and pulsed electromagnetic field application on bone fracture

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Aim: The effectiveness of various therapeutic methods on bone fracture have been demonstrated in several studies. In the present study we tried to evaluate the effect of Low Magnitude High Frequency Vibration and Pulsed Magnetic Fields on rat tibia fracture during the healing process.

Methods: A linear fracture was applied on the tibias of 30 rats. We divided rats into three groups. The first one is low magnitude high frequency vibration group (VG), the second is pulsed electromagnetic field group (PEMFG) and the third is control group (CG). We applied vibrations to VG for 15 min/day by “bangle method” that we developed. We applied pulsed electromagnetic field for 3.5 hours/day with a faraday cage to PEMFG. Nothing was applied to control group. Serum osteocalcin levels of three groups were also compared.

Results: We took the x-rays of the tibias at 7 and 21 days after the end of the healing process. The x-ray results were evaluated whether there was callus tissue or not. There were statistically significant differences between PEMFG and VG when compared to CP about the existence of the callus tissue. VG and PEMFG groups were compared with each other and the difference was not statistically significant. There was also a statistically significant difference between the groups of PEMFG and CG.

Conclusions: Our results suggest that the application of direct LMHF vibration and PEMA on the fracture promoted bone formation and healing approximately equal levels.

P2-8

Analysis of energy expenditure in adults using Actiheart and self-administered questionnaire

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Aim: Variance of daily energy expenditure mainly depends on person’s energy expenditure during

physical activity. The aim of the study was to assess individual free living energy expenditure and physical activity obtained by Actiheart and International Physical Activity Questionnaire (IPAQ), and analyse activity level and energy expenditure differences in working days and weekend.

M**ethods:** Study involved 30 adults at the age of 21 to 43 years who rated their physical activity during last 7 days by self-administered short form IPAQ. All participants were equipped with combined heart rate and acceleration measuring device, Actiheart (CamNtech Ltd, UK). Data were registered during two working days and one weekend day in order to calculate their energy expenditure and physical activity level (PAL).

Results: Registered mean total energy expenditure was 43±8 kcal/kg/day, activity energy expenditure - 16±7 kcal/kg/day. Calculated PAL was in range from 1.50 to 2.62. According to the PAL the participants were divided in 3 groups – low, moderate and highly active - 30%, 37% and 33% of participants in each group respectively. No statistically significant difference of energy expenditure and PAL between working days and weekend in no one of PAL group was found. Results obtained by IPAQ identified negative correlation ($p<0.05$) of sitting time in working days and MET-minutes/week. Total energy expenditure per body mass kilogram registered by Actiheart positively correlated with MET-minutes/week ($p<0.05$).

Conclusions: Energy expenditure and PAL in working days and weekend do not variate significantly. Participants who present longer sitting time in working days have less MET-minutes/week. Persons who have more MET-minutes/week scored by IPAQ have higher total energy expenditure per body mass kilogram obtained by Actiheart.

P2-9

The evaluation of tibial torsion angle after anterior cruciate ligament reconstruction

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Aim: Traumatic, biological, operational and anatomical factors may influence the re-injury of operated anterior cruciate ligament (ACL). The excessive tibial torsion is one of the most serious reasons of lower extremity torsions. The purpose of this study was to investigate the variance of tibial torsion angle (TTA) after reconstruction of ACL according to non-operated knee and to realize the relationship between this angle and post-operative rehabilitation.

Methods: Mean age was and in these patients 21 ACL were reconstructed by hamstring auto-graft technique. The study was conducted during the period of 6-24 months following surgery. The physical assessment of both knees of ACL was done by Lachman test. KT-1000 measurements and Lysholm scores was noted even for normal and operated knees. Isokinetic test and TTA were measured by computer tomography and physical assessment methods.

Results: There were no differences between the normal and operated knees according to TTA variances ($p>0.05$). Statistically significant differences were not observed between the Lysholm, KT-1000 ve Lachman values and TTA variances ($p>0.05$). There was a correlation between the TTA variances of knees as the force variances occurred during isokinetic test ($p<0.05$). There was a significant loss of force during the flexion route at a speed of 180º/sec ($p<0.05$).

Conclusions: The accelerated rehabilitation protocols may cause prolongation of time to return to sports due to high rates of re-injury. Measurement of TTA may be useful as a criterion for decision of returning to sports. In rehabilitation programs, exercises including the persistence of force at the flexion direction may be helpful for lowering the TTA differences.

P2-10

Electromyographic detection of pregnant uterine activity

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Aim: The myoelectric processes are crucial for the initiation of myometrial contractions, especially in pregnancy. However, there is no reliable method to detect the electric activity of the myometrium to predict premature contractions or other disorders during pregnancy. Our aim was to develop a smooth muscle electromyography method for pregnant rat uterus in vivo.

Methods: Pregnant SPRD rats (21st and 22nd day of pregnancy) were anaesthetized with ketamine-xylazine and their stomach, small intestine and

large intestine were removed from the abdomen. A pair of filament electrodes was inserted into the uterus, while a pair of disk electrodes was placed subcutaneously above the pregnant myometrium. Additionally, we fixed a mechanical strain gauge sensor on the surface of the myometrium for the parallel detection of mechanical contractions by the evaluation of area under curve (AUC). The electric signals were amplified and recorded by an online computer system. The software automatically filtered out the electric signals from the heart and the brain. The recorded signals were analyzed by fast Fourier transformation. The frequency of the electric activity was characterized by cycle per minute (CPM), the magnitude of the activity was described as power spectrum density (PsD).

Results: The frequency of the pregnant uterine activity was found at 1-3 CPM. I.v. oxytocin (1 µg/kg) increased by 25-50%, while i.v. terbutaline (50 µg/kg) decreased the PsD by 25-40% measured by filament and disk electrodes. We found a strong correlation between the alterations of PsD values and the strain gauge sensor-detected AUCs.

Conclusions: Our electromyographic method is able to detect the myoelectric activity that reflects the mechanical myometrial contraction. This technique may have a great importance both in pharmacological and in clinical fields.

P2-11

The response of coenzyme Q10 and oxidative stress to exercise training alone and combined with coenzyme Q10 treatment in aged rats

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Aim: This study aimed to determine the effect of exercise training alone and in combination with coenzyme Q10 (CoQ10) supplementation on the CoQ10 level, oxidative damage, and antioxidant defense markers in blood and skeletal muscle tissue in young and aged rats.

Methods: The study included 4-month old (young) and 20-month old (aged) rats. Each group was further divided into control, exercise training, CoQ10 supplementation, and CoQ10 supplementation plus exercise training groups. The exercise training program consisted of swimming for 8 weeks, and CoQ10 or vehicle during the same period.

Results: The CoQ10 concentration in plasma ($P < 0.05$), but not in skeletal muscle ($P > 0.05$) increased significantly following CoQ10 supplementation in both the young and aged rats. The plasma 8-OHdG level was significantly lower, and plasma SOD and CAT activity were significantly higher in the aged rats in the CoQ10 and CoQ10 plus exercise training groups than in the other groups ($P < 0.05$); however, there wasn't a significant difference between the groups in skeletal muscle ($P > 0.05$). Plasma and skeletal GSH levels did not differ between the groups ($P > 0.05$).

Conclusions: The present findings indicate that CoQ10 supplementation increased the CoQ10 concentration in blood, and increased antioxidant enzyme activity.

P2-12

The effects of omega-3 fatty acids on exercise induced bronchospasm in nonasthmatic obese and non-obese children

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Aim: The aim of this study was to assess and compare the impacts of the fish oil-derived n-3 fatty acids (EPA and DHA group) on severity of exercise-induced bronchospasm (EIB) in obese and non-obese non-asthmatic children.

Method: Of the participants, 35 were obese (BMI percentile values of 95-98) while 38 were non-obese (BMI percentile value <85). Descriptive study was conducted with 73 subjects aged 10 to 13 years of both genders participants and these subjects were divided into eight groups. While some of the children took 2.4 g of EPA and DHA containing fish oil capsules daily for 8 weeks, the others took placebo supplements. At the beginning of the study and eight weeks later, exercise testing and pulmonary function tests were applied to all the participants.

Results: No significant difference was found in the baseline frequency of FEV_1, FVC and FEV_1 / FVC. At the end of 8 weeks of supplementation with fish oil supplements, the percentage fall in FEV_1 value significantly reduced in group one and five. The highest significant decrease was observed in the group 1 (15.6 %±6.3). Systolic blood pressure and resting heart rate in the same group (Before: 112.5±7.9 mmHg, After: 103.5±7.8; Before: 98.0±8.3, After: 89.5±6.6) was observed to decline

in value, while an increase occurred in the start and end load.

Conclusions: According to the obtained data from this study, fish oil-derived n-3 fatty acids can improve pulmonary function of obese and nonobese children and has therapeutic effect on EIB.

P2-13

The effect of melatonine on rats gastrocnemius muscle applied with carbontetrachloride

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Aim: In this study, the preventive antioxidant effect of the melatonin against the acute damage resulting from the toxic effect of the carbon tetrachloride (CCl_4) on the skeletal muscles of the Wistar albino rats has been studied.

Methods: In this study 24 Wistar Albino type of male rats that their have been used have been the experiment groups: Group 1 (Control group, Corn oil) Group 2 (Control group, ethanol+PBS) Group 3 (CCl_4) Group 4 (CCl_4+Melatonin). After the tissues determining, it has been passed through routine histologic processes, they were embedded into parafine and sections of 5µm were taken. Fibrosis formation was depicted with Masson's-Trichrome staining.

Results: No fibrosis has been found on the gastrocnemius muscle of the control group which have been stained with Hematoksilen-eosin and it has been observed that the muscle integrity was normal in this group. In CCl_4 group as a result of the hematoxylin - eosin staining, partial melting has been observed on the fibrosis ve muscle fibres. And it has been found in this group that as a result of Masson's-Trichrome staining there is increase in the amount of ligament tissue around the veins, and that there was orientation disorder in the muscle fibres and also there is hipertrophy. On the other hand, in CCl_4+melatonin group it has been observed that with Masson's-Trichrome staining there was a seemingly normal situation apart from the decrease in fibrosis.

Conclusions: In this study it is possible to say that when CCl_4+Melatonin group was compared to the CCl_4 group, melatonin has a preventive or even curative effect.

P2-14

The effects of anaerobic threshold on V_E/VCO_2 and V_E/VO_2 during incremental exercise and constant load exercise test.

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Aim: The relationship between ventilation (V_E) to CO_2 output (VCO_2) (i.e. V_E/VCO_2) is an important objective criteria to evaluate ventilatory efficiency during exercise. We comparatively examined the V_E/VCO_2 relationships during an incremental exercise and constant load exercise tests to evaluate the anaerobic threshold (AT) on ventilatory efficiency.

M**ethods:** After obtaining a signed informed consent which was approved by the local ethical committee, 11 male subjects (mean±SE, age: 21.0±0.7 yr; 74.9±1.7kg) performed an incremental exercise test (15 W/min) using an electromagnetically-braked cycle ergometer to determine AT, respiratory compensation point (RCP) and maximal exercise performance (Wmax). AT and RCP were estimated from ventilatory and respiratory gas exchange parameters. Each subjects also performed constant load exercise test work rate corresponded AT for approximately 20 min.

Results: Wmax and AT values were found to be 218±7 W and 130±6 W, respectively. During incremental exercise test, V_E/VCO_2 ratio was found to be 30.4±0.9 at warm up, 25.7±0.8 at AT, 26.3±0.9 at RCP and 29.8±1.1 at maximal exercise. During constant load exercise test V_E/VCO_2 was found to be 31.3±1 at warm up and 30.3±0.8 at end of test. There was significantly differences between V_E/VCO_2 at the AT from the incremental exercise and constant load exercise test ($p<0.05$).

Conclusions: During an incremental exercise test, the lowest V_E/VCO_2 obtained at the AT on RCP are clinically important parameters for evaluating ventilatory efficiency. However, measurement of V_E/VCO_2 in constant of exercise test, work load corresponded to AT, could provide long term effective ventilatory efficiency results rather than rapid incremental exercise test.

P2-15

Predictive models forecast relationships of apelin-13 concentrations with UCP1 in WAT and BAT, and UCP3 in muscle

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Aim: Apelin is a peptide hormone with an adipose tissue, and demonstrates its effects by binding to the APJ receptors. We reported that chronic apelin-13 dose dependent injection reduced uncoupling protein 1 (UCP1) in white adipose (WAT) and brown adipose tissue (BAT), uncoupling protein 3 (UCP3) mRNA synthesis in muscle. The present study aimed to forecast the relationships of apelin-13 concentrations (A13C) with UCP1 in WAT and BAT, and UCP3 in muscle.

Methods: In the present study, 40 Sprague Dawley male rats were utilized and separated to four groups (n=10 in each group). The rats in the experimental groups (apelin-13 at 1, 5 and 50 µg/kg doses) and the rats in control group were injected ip for 14 days. At the end of the experiment, UCP1 (BAT and WAT) and UCP3 (muscle) mRNA synthesis were measured by RT-PCR method. Exponential regression models were used to forecast the relationships of apelin-13 concentrations with UCP1 in WAT and BAT, and UCP3 in muscle.

Results: The estimated exponential regression models were (UCP1-in-WAT) = 0.6684exp(-0.022(A13C)) (coefficient of determination=0.74), (UCP1-in-BAT) = 0.6585exp(-0.016(A13C)) (coefficient of determination=0.61) and (UCP3-in-muscle) = 0.5568exp(-0.02(A13C)) (coefficient of determination=0.57), consecutively. The estimated models were statistically significant ($p<0.05$).

Conclusions: The results of the current study indicated that the predictive models were successful in forecasting the relationships of A13C with UCP1 in WAT and BAT, and UCP3 in muscle.

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3. Endocrine system physiology

P3-1

Protective effects of silymarin on methotrexate-induced testicular damage in rats

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Aim: Methotrexate (MTX) is widely used in the treatment of various cancers and nononcological diseases such as psoriasis and rheumatoid arthritis but it can lead to reproductive toxicity. Silymarin (SLY), the major component of milk thistle, has antioxidative, antiinflammatory and anti-apoptotic effects. This study was designed to explore to possible protective effects of SLY against MTX-induced testicular damage in rats.

Methods: Thirtysix rats were divided into six groups: Group 1 (saline, i.p., single injection), Group 2 (0.5% carboxymethyl cellulose (CMC), by gavage once daily for 5 consecutive days), Group 3 (SLY, 300 mg/kg per day, p.o., for 5 consecutive days) Group 4 (MTX, 20 mg/kg, i.p., single injection), Group 5 (MTX+CMC similarly as groups 2 and 4) and Group 6 (MTX+CMC+SLY similarly as groups 2, 3 and 4). Histopathologic alterations including apoptotic changes (TUNEL) and HSP 70 expression of the testes were evaluated.

Results: MTX administration caused distortion and disruptions of seminiferous tubule germinal epithelium. Apoptotic cell death was also increased in testis tissues after MTX administration. SLY treatment resulted in significant amelioration in the histological changes. SLY treatment reduced the number of TUNEL-positive cells as compared with the MTX treated rats ($P<0.05$). While HSP 70 expression was decreased in testis tissues after MTX administration, SLY treatment resulted in increase of HSP 70 expression ($P<0.05$).

Conclusions: SLY treatment decreased the destructive effects of MTX on testicular tissue of rats.

P3-2

The levels of NOS in rat testicular tissue damage created by diabetes and pentoxifylline therapy

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Aim: Diabetes is known to be associated with erectile dysfunction, retrograde ejaculation, testicular hormone level and a decrease in semen quality respectively. In this project, we aimed to investigate at the molecular level, the effect of NOS on testes pathology in diabetes and examine the effect of pentoxifylline on healing.

Methods: In this study, 50 wistar albino male rats were used. The rats were divided into 5 groups; Group I control; Group II only diabetes; Group III and IV diabetes + pentoxifylline; Group V only pentoxifylline. Group D+PI rats received 50 mg/kg/day pentoxifylline during two months. However, Group D+PII rats received saline in the first month and the 50 mg/kg/day of pentoxifylline for the following month. NOS expression in testicular tissue was assessed using qRT-PCR, western blot and immunohistochemistry analysis.

Results: At the end of the experiments, MSTD, JTBS values and serum testosterone levels were decreased compared to controls; however, the number of apoptotic cells, nNOS, iNOS and eNOS mRNA and protein levels were all increased when compared to the control values. We found that especially NOS expression improved with pentoxifylline therapy.

Conclusion: As a result, diabetes, a chronic disease that causes serious damage in the testicular tissue. NOS contributes to this damage and treatment with pentoxifylline may be effective in reversing this damage.

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P3-3

***In vıtro* effect of agomelatine on spontaneous and oxytocin-induced contractions ın the rat myometrium**

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Aim: Agomelatine is a new antidepressant with an innovative pharmacological profile. It is a potent synthetic melatonergic MT1 and MT2 receptor agonist and also has 5-HT2c antagonist properties. In humans, melatonin receptors were detected in several organs, including cardiovascular system, ovary/granulosa cells and myometrium. MT1 and MT2 melatonin receptor mRNA's are present in nonpregnant and pregnant rat myometrium cells. The aim of this study was to investigate the effects of agomelatine on spontaneous and oxytocin-induced contractility of rat myometrium *in vitro*.

Method: This study used in the female rat myometrium (n=7). Myometrium strips were removed from female Sprague-Dawley rats in dioestrus, following decapitation and placed in a jacked tissue bath containing Krebs solution. Effects of concentrations of agomelatine (50 μM, 100 μM and 200 μM) on spontaneous and oxytocin-induced contractions were studied. Also we used 2 μM luzindole (the melatonin receptor antagonist) before application of agomelatine to determine whether inhibition of agomelatine as melatonin receptor-mediated.

Results: Agomelatine inhibited spontaneous and oxytocin-induced contractions of rats myometrium in a dose-dependent manner. In this study, we demonstrate for the first time that agomelatine has inhibitory effect on spontaneous and oxytocin-induced uterus contractility in rats. The decreases in frequency and amplitude of contractions were significant in 100 μM and 200 μM agomelatine doses for strips. After application of luzindole, inhibition not reversed.

Conclusions: These results showed that agomelatine not bind the melatonin receptor. Because of reducing uterine contractions with agomelatine, it can be used to inhibit uterine contractions in pregnant women with abortion risk. These results imply that agomelatine may have the potential to modulate myometrial function in humans.

P3-4

The effects of zinc supplementation on oxidative stress and insulin secretion in B-cell with type-I diabetes mellitus-induced by streptozotocin

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Aim: Diabetes is one of the most common chronic diseases characterized by loss of insulin producing beta cells. Zinc is an essential trace element that plays a vital role in many biological processes including growth and development, immunity, and metabolism. Although zinc effect on insulin secretion is still unclear. The aim of the study was to investigate whether zinc can increase insulin secretion by its antioxdidants properties or not.

Methods: Human B-cell line was used in the present study. Four groups were generated as Control (C) Zinc supplementation (C+Z) Diabetes (D), Diabetes+Zinc (D+Z). The isolation of protein was measured total oxidant (TOS) and antioxidant status (TAS). Then oxidative stress index (OSI) was calculated by using TOS/TAS. Insulin secretion and content was evaluated.

Results: Diabetes caused to decrease insulin section, but increase to insulin content. However, D+Z group was restored insulin secretion, and content by zinc supplementation. Although D group gave rise to elevate TOS and OSI, TOS and OSI were lowered but not statistically important.

Conclusion: Diabetes provoked oxidative stress, finally decreased to insulin secretion and increased to insulin content at beta cell. Zinc supplementation after diabetes increased to insulin secretion and decrease to insulin content by diminishing oxidative stress at beta cell.

P3-5

The elevation of insulin secretion in streptozotocin-induced B-cell by zinc supplementation

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Aim: Diabetes mellitus is a common disease in the world. Oxidative stress is the most important mechanism of its pathogenesis. Zinc is essential trace element in the body. Studies provide information on the insulin mimetic action of zinc, but the mechanism of it is still unclear. The aim of the study was to investigate whether zinc is able to increase insulin secretion by its antiapoptotic effect, insulin and F actin distribution at B-cell or not.

Methods: The current study was used to human B-cell line. Total 4 groups were created as Control (C) Zinc supplementation (C+Z) Diabetes (D), Diabetes+Zinc (D+Z). Protein was isolated to measure some apoptotic markers. Immunofluorescence imaging was done to determine of insulin and F-actin distribution at beta cell.

Results: Diabetes was initiated to apoptosis by alteration of cytochrome-c, p53, apoptosis-inducing factor, caspase-3 protein levels. D+Z was decrease to loss B-cell by restoration of cytochrome-c, p53 caspase-3 protein levels. D caused destruction of F actin and insulin distribution, but D+Z restored F-actin and insulin distributions.

Conclusion: Diabetes leads to lose B-cell by apoptosis and destroy the F-actin and insulin distribution eventually to decline insulin secretion. B-cell with zinc supplementation after diabetes gives rise to modulate to apoptotic protein and F-actin, insulin distribution finally the elevation of insulin secretion.

P3-6

The effect of resveratrol supplementation on plasma leptin and liver glycogen levels in rats with acute swimming exercise

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Aim: Exercise increases free fat acide and glucose metabolism. Leptin reduce food intake and increase energy expenditure. The aim of the present study was to determine how resveratrol supplementation effect plasma leptin and liver glucogen levels.

Methods: Study was conducted on male Wistar-Albino rats. They were divided into 4 groups.
Group 1, (n:7) Control group: Fed with standart animal food.
Group 2, (n:7) Swimming group: Fed with standart rat food and exposed to 30 minutes swimming exercise.
Group 3, (n:7) Resveratrol Group: Fed with normal rat food plus resveratrol by drinking water (10 mg/kg/day) for 4 weeks.
Group 4, (n:7) Resveratrol Plus Swimming Group: Fed with normal rat food plus resveratrol by drinking water (10 mg/kg/day) for 4 weeks and exposed to 30 minutes swimming exercise till end of the 4 weeks study period.
Following 4 weeks experimental period animals were decapited, plasma and liver samples were taken for analyses. Plasma leptin was determined by rat leptin kit by RIA (ng/ml), liver glycogen by immunohistochemical procudure.

Results: The highest plasma leptin levels were determined in groups 1 and 3 ($P<0.05$). There were no differences between group 2 and 4 for leptin levels. The highest liver glucogen levels were determined in group 3 (resveratrol supplemented), ($P<0.05$), the lowest levels were determined in group 2 (swimming group). Liver glucogen levels in group 1 (control) and resveratrol swimming group (group 4) were not different.

Conclusion: The findings of present study show that resveratrol supplementation has no effect on plasma leptin levels, however, resveratrol supplementation has saving or regulatory effect on liver glucogen storage in rats with exercise or non-exercise procedures.

P3-7

Lithium-induced hypothyroidism: Oxidative stress and osmotic fragility status in rats

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Aim: The present study was conducted to explore the possible effects of different doses of lithium carbonate on thyroid functions, erythrocyte oxidant–antioxidant status, and osmotic fragility.

Methods: Twenty-four Wistar-type male rats were equally divided into three groups: groups I and II received 0.1 and 0.2 % lithium carbonate in their drinking water, respectively, for 30 days. The rats in group III served as controls, drinking tap water without added lithium. At the end of the experimental period, the erythrocyte osmotic fragility and the levels of triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) were measured in blood samples.

Results: Compared to controls, there was a statistically significant increase of TSH but decreases of the T3 and T4 levels in group II ($p<0.01$). Both experimental groups showed a statistically significant increase of the maximum osmotic fragility limit ($p<0.001$). The minimum osmotic fragility values of the animals in group II were statistically higher than those of controls ($p<0.001$). The standard hemolytic increment curve of both experimental groups was shifted to the right when compared to the curve obtained from the controls ($p<0.05$). Also, relative to controls, the activities of MDA and SOD were significantly higher ($p<0.01$) and the GSH level lower in group II ($p<0.05$), but not so in group I.

Conclusions: The results of the present study show that treatment with lithium carbonate may result in thyroid function abnormalities, increased oxidative damage, and possible compromise of the erythrocyte membrane integrity resulting from increased osmotic fragility.

P3-8

Bone metabolism in chronic ethanol treated ovariectomized rats: The role of nitric oxide

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Aim: Alcoholism reduces bone turnover whereas estrogen deficiency results in elevated bone turnover. Reduction of the in bone turnover is likely to reduce the risk of osteoporosis in postmenopausal women because these individuals are losing bone because of elevated bone turnover. We investigated the effect of NO on bone metabolism in ovariectomized rats following chronic ethanol treatment.

Methods: The rats were divided into two groups. The first group received sham operation (SHAM) and the rats in Group II were ovariectomized (OVX). Five weeks after the SHAM and ovariectomy, the rats in each group were treated with ethanol for 4 months. After ethanol administration, the NOS inhibitor, L-NAME, was given for three weeks along with ethanol to the same rats. Serum IL-1beta, IL-6, TNF-alpha, NO, Ca, P, PTH, 25(OH)D3, ALP, bone-ALP, levels were measured in different stages of the experiment.

Results: IL-1beta, IL-6, TNFalpha and NO levels increased after ethanol administration in SHAM and OVX rats. The decrease in Ca was significant while the changes in P, PTH and 25(OH)D3 levels were not. ALP and bone-ALP levels were significantly decreased. In ovariectomized and SHAM rats, administration of L-NAME together with ethanol produced a significant increase in IL-1beta, IL-6 and TNFalpha levels. In this state, Ca and P levels were significantly increased; PTH and 25(OH)D3 levels were significantly decreased. Also, there was a significant decrease in, ALP, bone-ALP levels.

Conclusions: NO increase due to alcohol intake may function as a protective mechanism preventing bone resorption in cases of estrogen insufficiency.

P3-9

Alteration in the expression and function of alpha1-adrenergic receptor subtypes in late pregnant rat uterine by progesterone and oestrogen pre-treatment

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Aim: The aim of the present study was to investigate the role of alpha1-adrenoceptor (AR) subtypes in the last day pregnant rat uterus by subtype-specific antagonists (alpha1A-AR antagonist WB 4101 and alpha1D-AR antagonist BMY 7378) after oestrogen (E2) and progesterone (P4) pre-treatment *in vitro*.

Methods: In the isolated organ bath studies contractions were elicited with noradrenaline (NA) (10^{-8}-$10^{-4.5}$ M) in the presence of propranolol (10^{-5} M) and yohimbine (10^{-6} M) in order to avoid beta-, and alpha2-adrenergic actions. The myometrial expressions of the alpha1-AR subtypes were determined by RT-PCR and Western blot techniques. The activated G-protein levels of the alpha1-ARs were investigated by a radiolabelled GTP binding assay.

Results: Both E2 and P4 pre-treatment changed the myometrial contracting effect of NA. In the presence of WB 4101, the P4 pre-treatment decreased the NA-induced myometrial contraction. In the presence of BMY 7378, both E2 and P4 pre-treatment reduced the effect of NA. The mRNA and protein expression of alpha1A-AR was decreased after E2 pre-treatment. NA increased the [^{35}S]-GTPgammaS binding of alpha1-ARs, which was most remarkably elevated by P4. Pertussis toxin inhibited the [^{35}S]-GTPgammaS binding stimulating effect of NA indicating the role of G_i-proteins in the signal mechanisms of alpha1-ARs.

Conclusions: The E2 pre-treatment blocks the expression of alpha1A-ARs, while does not influence the expression of alpha1D-ARs. The P4 pre-treatment does not have any effect on the myometrial mRNA and protein expression of alpha1-ARs, however, it alters the G-protein coupling of these receptors.

P3-10

Serum levels of apelin and visfatin in patients with obstructive sleep apnea syndrome

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Aim: Obstructive sleep apnea syndrome (OSAS) is characterized by an increased cardiovascular risk. Obesity is typical metabolical feature of OSAS. Apelin and visfatin are known as adipocytokines have roles in the cardiovascular system. Circulating level of visfatin is enhanced in metabolic disorders, such as type-2 *Diabetes mellitus* and obesity. The aim of this study is to determine serum level alterations of apelin and visfatin in OSAS.

Methods: The study was enrolled with OSAS (n=60) and healthy (n=28) subjects. Blood samples were collected in tubes without EDTA. Serum levels of apelin and visfatin were measured by ELISA kits. All results were given as means±standard error of mean (SEM). Statistical significances between the groups were performed by Mann-Whitney U tests. Differences were considered significant at $p<0.05$.

Results: Serum level of apelin was not change significantly in OSAS (60.0±5.26pg/ml) compared with control group (66.66±10.11pg/ml)(p=0.884). Serum visfatin levels were not change significantly in OSAS (0.07±0.005pg/ml) compared with control group (0.08±0.01pg/ml)(p=0.826).

Conclusions: We believe to elevate serum level of apelin may have therapeutic potential for OSAS because of its anti-inflammatory feature. On the other hand visfatin may have a pro-inflammatory role in the development of OSAS. We suggest that inhibiting the effects of visfatin or reducing the serum level of visfatin can be used as a novel treatment strategy for OSAS. However, further studies are needed to clarify our hypothesis.

P3-11

Serum levels of growth hormone in patients with obstructive sleep apnea syndrome

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Aim: It is very well known that obstructive sleep apnea syndrome (OSAS) alters the serum levels of several hormones by the effect of hypoxia on central neurotransmitters that causes changes in the hypothalamus-pituitary axes in relation with sleep fragmentation which is induced by apnea and hypopnea, sleep loss and naps. Growth hormone (GH) secretion is affected by several factors such as age, sex, obesity, body composition and sleep quality. The oxygen content of inhaled air affects the secretion of GH and insulin-like growth factor–1(IGF-1). Decreased serum level of IGF-I independent from serum level of GH is found in relation with chronic hypoxemia. On the other hand intermittent hypoxemia increases GH secretion. We would like to examine how changes occur in serum level of GH in Turkish patients with OSAS.

Methods: The study was enrolled with OSAS (n=60), and healthy (n=28) subjects. Blood samples were collected in tubes without EDTA. Serum level of GH was measured by ELISA kits. All results were given as means±standard error of mean (SEM). Statistical significances between groups were performed by Mann-Whitney U tests.

Results: Serum GH levels were found significantly higher in OSAS (0.04±0.002ng/ml) compared to control group (0.02±0.001 ng/ml) (p=0,000).

Conclusions: Alteration in serum level of GH can be related with OSAS. Additionally, circulating level of GH is effected by its polymorphisms. For this reason to clarify the role of GH axis in OSAS we believe that GH should be studied in patients with OSAS in different populations.

P3-12

Serum levels of leptin and nesfatin in patients with obstructive sleep apnea syndrome

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Aim: Obstructive sleep apnea syndrome (OSAS), is characterized by narrowing of the upper airway and it often causes a decrease in arterial oxygen saturation during sleep. Nesfatin-1 is responsible for the regulation of food intake. Its secretion is decreased during fasting. Leptin is in the pathogenesis of increased cardiovascular risk and obesity. The aim of this study is to determine the changes in serum leptin and nesfatin-1 levels in patients with OSAS.

Methods: The study was enrolled with OSAS patients (n=60) and healthy (n=28) subjects. Blood samples were collected in tubes without EDTA. Serum levels of leptin and nesfatin-1 were measured by ELISA kits. All results were given as means±standard error of mean (SEM). Statistical significances between groups were performed by Mann-Whitney U tests. Differences were considered significant at $p<0.05$.

Results: We observed increased serum level of leptin, although not statistically significant, in OSAS group (411.2±44.1pg/ml) compared to control group (324.5±50.9pg/ml) (p=0.329). Decreased serum level of nesfatin-1 was significant in OSAS group (2.31±0.14pg/ml) compared to group control (5.00±0.82pg/ml) (p=0.000).

Conclusions: We suggest that nesfatin-1 can be used as novel biomarkers in the diagnosis of OSAS in Turkish population. Leptin can be an anti-inflammatory drug target treatment for OSAS. Induction of nesfatin-1's action or level can provide new treatment strategies for OSAS. We believe that further studies are needed to clarify specific roles of peptides in OSAS.

P3-13

Serum levels of obestatin and adiponectin in patients with obstructive sleep apnea syndrome

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Aim: It is well known that obstructive sleep apnea syndrome (OSAS) alters serum levels of several hormones related with apnea, hypopnea, sleep loss and naps. Obestatin is a novel aminopeptide effecting food intake. Adiponectin is an adipokine which is secreted by adipose tissue. Its effects are widely known as antidiabetic, anti-inflammatory, antiatherogenic, and cardioprotective. The aim of this study is to determine alterations in serum levels of obestatin and adiponectin in OSAS.

Methods: The study was enrolled with OSAS patients (n=60), and healthy (n=28) subjects. Blood samples were collected in tubes without EDTA. Serum levels of obestatin and adiponectin were measured by ELISA kits. All results were given as means±standard error of mean (SEM). Statistical significances between groups were performed by Mann-Whitney U tests. Differences were considered significant at $p<0.05$.

Results: Serum obestatin levels were not significant in OSAS group (21259±740 pg/ml) compared with control group (22057±873 pg/ml) (p=0.773). Serum adiponectin levels were significantly lower in patients with OSAS (9.08±1.7 ng/ml) compared with control (22.6±3.6 ng/ml)(p=0.000).

Conclusions: In OSAS both interested anti-inflammatory adipokines were decreased. We believe targeting inflammatory processes can offer new and effective applications for treatment of OSAS.

P3-14

The evaluation of sexual function of men with type 1 diabetes mellitus

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Aim: The aim of the study – to compare sexual function between men with type 1 diabetes (T1D) and healthy partcipants. To assess the impact of disease duration and control, diabetic complications and age for sexual function of men with T1D.

Methods: Study subjects - 18-50 year old men with T1D and age-matched healthy men. Participants filled questionnaires about disease duration, complications and Brief Sexual Function Index (BSFI). All participants with T1D were evaluated for chronic diabetic complications: neuropathy, retinopathy, and nephropathy. Blood tests including HbA1c, creatinine, estradiol, testosterone, sex hormone-binding globulin (SHBG) levels were performed.

Results: 30 diabetic men (mean age 32.8±1.17) years) and 31 healthy men (mean age 34.3±7.43 years) years included to the study. Estradiol levels were significantly lower (p=0.010); SHBG - were higher (p=0.035) for men with T1D. Overall satisfaction was significantly lower of men with proliferative retinopathy and maculopathy compared with proliferative retinopathy alone (p=0.012). There were no significant differences between sexual function and diabetic nephropathy also neuropathy. Negative correlations between BSFI of erection (p=0,042) and ejaculation function (p=0.037), problem assessment (p=0.013) subscales and age of men with T1D were observed as well as relations between erection (p=0,025), ejaculation function (p=0.013), overall satisfaction (p=0.011) and duration of T1D. Negative correlation between erection function and SHBG levels was observed in men with t1D (p=0.019).

Conclusions: Sexual function of men with T1D is worse than healthy participants. Sexual function correlates with duration of the disease, age, advanced retinopathy, estradiol and SHBG level.

P3-15

Hypoglycemia features of type 1 diabetes patients with nephropathy

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Aim: The aim of the study - to compare the hypoglycemic events and fear of hypoglycemia between type 1 diabetes (T1D) patients associated with nephropathy (DN) and without DN.

Methods: 100 adult patients with T1D were included to the study. The patient cohort consisted of two groups: 50 patients (mean age 35.48±8.91 yrs) with DN and 50 patients (mean age 32.66±8.97 yrs) without DN. All patients filled out questionnaires about T1D, associated complications, treatment, hypoglycemia - frequency, symptoms and the fear of hypoglycemia.

Results: 28% of patients with T1D experieced hypoglycemia up to 4 times per month, and only 8% indicated that didn't experience hypoglycemia during the last month. No significant difference was found when assessing the frequency of symptomatic hypoglycemic conditions in T1D patients with or without DN.
31% of T1D patients indicated that asymptomatic hypoglycemia occured up to two times per month while 47 patients had constant symptoms of hypoglycemia. There was no significant difference between groups with or without DN (p>0.05) evaluating the frequency of asymptomatic hypoglycemia. 53% of all participants were afraid of hypoglycemic conditions. No significant differences between groups (with and without DN) were found assesing the fear of hypoglycemia. Older patients with DN (mean age 38.36±8.49 yrs) compared to younger participants with DN (mean age 32.6±8.53 yrs) are significantly more afraid of hypoglycemia (p<0.05).

Conclusions: Symptomatic and asymptomatic hypoglycemia occurs in a similar frequency between patients with or without DN. Older patients with DN are more afraid of the hypoglycemic conditions compared to younger participants with DN.

P3-16

The relationship between mitogen activated protein kinase and apoptotic proteins in pancreatic beta cell with type-I diabetes

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Aim: Diabetes mellitus (DM) is a common disease at worldwide. DM has been reported to relate oxidative stress resulting in initiating apoptosis. Zinc is a trace element in the body and has antiapoptotic and antioxidant effects. Moreover, zinc has been shown to restore the beta cell function, but limited data has been available the mechanism how to inhibit the apoptosis. The aim of the current study was to explore the supplementation of zinc on antiapoptotic effect via some mitogen-activated protein kinase.

Methods: The groups were created as Control (C), Zinc supplementation (C+Z), Diabetes (D), Diabetes+Zinc (D+Z). After created type-I diabetes by using STZ, zinc supplementation was incubated for twenty-four hours. The protein was isolated to measure extracellular-signal-regulated kinases (ERK)-1, ERK-2, stress-activated protein kinase (SAPK), P38, apoptosis inducing factor (AIF) and Bcl-2.

Results: Although diabetes decreased ERK-1 and ERK-2 levels versus (vs.) control, diabetes elevated the protein level of SAPK and p38 vs. control. D+Z group was decreased the level of ERK-1 and ERK-2 though D+Z group was caused to diminish SAPK and p38 protein level. D group was high AIF and Bcl-2 protein levels. D+Z was partially restored AIF and Bcl-2 levels.

Conclusion: Diabetes triggered apoptosis by increasing P38, SAP and decreasing ERK-1 and ERK-2, zinc supplementation after diabetes was declined the apoptosis by decreasing P38, SAP and increasing ERK-1 and ERK-2. Consequently, zinc may be an excellent candidate as an antiapoptotic agent to protect the mass of beta cell remaining in diabetes patients.

P3-17

What is normal sexuality of 26 – 36 year-old Lithuanian men?

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Aim. To investigate the structure of sexual function of 26–36-year-old Lithuanian men, 2003/2004 **K**aunas participants at the study "The reproductive function of **E**stonian, **L**atvian and **L**ithuanian **Y**oung men (**KELLY**) men" comparing with type 1 diabetic patients (T1D), using **E**uropean **M**ale **A**geing **S**tudy–**S**exual **F**unction **Q**uestionnaire, (**EMAS – SFQ**).

Methods. Eighty two KELLY men aged 26-36 years were recruited from the list of study participants. 129 T1D patients were randomly selected from Lithuanian register of diabetes. All the 211 individuals completed EMAS – SFQ.

Results. In KELLY men overall sexual functioning (OSF) was 11-27 scores (85%), when the possible score Sexual-function-related distress (SFD) 1-5 scores (the possible score range 0 to 20), Masturbation score (M) was high (once per week 45% and once to 2-3 times per month 30%, altogether 75%), when the possible score range 0 to 7.ranged from 0 to 33. In T1D males M is lower than in KELLY men from the beginning of disease. SFD becomes significantly higher in patients with disease duration 5 +, and OSF – significantly lower in 10 +. Capacity to achieve and maintain erections was statistically higher in KELLY than in D T1D males. More than 4 morning awaking erections per week were observed more frequently in KELLY men.

Conclusions. Sexuality of KELLY males after 9-10 years of investigation of their reproductive health is excellent in comparison with presumably disturbed sexuality of patients (T1D).
These results would be considered "normal sexuality" in "elite age" for sexuality. However, so far not all the diabetic patients suffer from sexuality disturbances even after a long duration of diabetes."

P3-18

Effects of exercise time of the day on oxidant and antioxidant capacity in sedentary and trained subjects.

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Aim: In this study, we comparatively examined the effects of exercise time of the day on body oxidant and antioxidant capacities in sedentary and trained subjects.

Methods: After obtaining a signed informed consent, 28 male subjects (14 trained and 14 sedentary) participated a football game (60 min) for tree times on different days: at 8:00 (E1), at 15:00 (E2) and at 23:00 (E3). Blood samples were taken before and at the end of the games and centrifuged immediately. Serum samples analysed for ADMA and MDA using HPLC and GSH using ELISA.

Results: There were increases in MDA and ADMA levels before and after games in both sedentary and trained subjects: 0.693±0.05 µmol/L vs 0.953±0.05 µmol/L - 0.689±0.07 µmol/L vs 1.08±0.13 µmol/L and 0.802±0.06 µmol/L vs 1.06±0.04 µmol/L - 0.324±0.06 µmol/L vs 0.567±0.06 µmol/L for E1; 0.728±0.05 µmol/L vs 0.993±0.07 µmol/L - ; 0.792±0.08 µmol/L vs 1.176±0.12µmol/L and 0.858±0.03 µmol/L vs 1.127±0.04 µmol/L - 0.394±0.07 µmol/L vs 0.479±0.07 µmol/L for E2; 0.820±0.03 µmol/L vs 0.995±0.05 µmol/L - 0.885±0.07 µmol/L vs 1.22±0.12 µmol/L and 0.870±0.04 µmol/L vs 1.529±0.06 - 0.378±0.06 µmol/L vs 0.474±0.07 µmol/L for E3, respectively. In addition, GSH levels decreased in sedentary and trained subjects: 4.479±0.4µM vs 1.982±0.4µM and 6.583±0.5µM vs 5.350±0.3µM in E1 and 2.354±0.1µM vs 1.670±0.1µM and 5.050±0.3µM vs 4.651±0.3µM in E2, respectively. However, there were significant increases in GSH levels in both sedentary 1.172±0.1µM vs 2.123±0.2µM and 3.794±0.3µM vs 5.050±0.3µM in E3.

Conclusions: Increased resting MDA and ADMA and decreased GSH levels in late night exercise should be consider when planning an exercise training program for improving health.

P3-19

The relationship between irisin and testicular functions: A morphological approach

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Aim: Irisin is a newly identified myokine and its presence has been shown in different tissues including testis. The present study was planned to display to effects of irisin on testicular morphology.

Methods: In this study, rats were randomly divided into four groups (n=10). First, brain infusion kits were surgically implanted in right lateral ventricle of rats in sham and two irisin infusion groups. While sham group were infused with artificial cerebrospinal fluid (vehicle), irisin treatment groups were infused two different concentrations (10 and 100 nM) of irisin. After infusions, all rats were sacrificed and testis tissues were taken for histological examinations. The testis tissues were fixed in 10% formalin solution, and then embedded in paraffin. The paraffin blocks were cut 5 µm and stained with haematoxylin and eosin (H&E).

Results: In all groups including the irisin groups, the seminiferous tubules were intact and germ cells organized in concentric layers except slight histological changes such as a few atrophied tubules or contain sloughed spermatogenic cells into the lumen. The mean of germinal cell layer thickness were increased in the 100 nM infused group compared to other groups ($p<0.05$). Although mitotic index of 100 nM group was higher than 10 nM group, the difference was not found statistically significant. No difference was found significant as statistically among all groups in term of the seminiferous tubule diameter ($p>0.05$). The highest the number of Sertoli was observed in 100 nM irisin group compared to other groups ($p<0.01$).

Conclusions: The observations indicate that irisin may have some effects on testicular functions by affecting testis morphology.

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P3-20

The alterations in serum ghrelin and leptin levels after intracerebroventricularly irisin infusion in rats

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Aim: Irisin, a novel exercise-induced myokine, activates thermogenesis in rodents through increasing beige fat cells abundance within white fat. Irisin, has broad implications for metabolism and energy homeostasis. We previously showed that intracerebroventricular (icv) irisin infusion caused to increases in food consumption in rat [1]. However, it is known that many hormones such as leptin, ghrelin, neuropeptide Y, agouti-related peptide, proopiomelanocortin and cocaine and amphetamine regulated transcript play active roles in the control of feeding behavior. Therefore, the present study was aimed to investigate possible effects of the irisin on eating behavior-related hormones including leptin and ghrelin.

Methods: In this study, 40 male Wistar-Albino rats were used. Rats were evenly separated into four groups (n=10). Osmotic mini-pumps were implanted to lateral ventricle and artificial cerebrospinal fluid (vehicle; sham group), 10 (physiologic) and 100 nM (pharmacologic) concentrations of irisin were infused for 7 days. After 7 days of icv infusion, animals were decapitated and blood samples were collected. Serum leptin and ghrelin levels were measured by ELISA.

Results: Icv infusion of irisin dose dependently decreased in serum leptin levels ($p<0.05$) but increased ghrelin levels ($p<0.05$).

Conclusions: The study results suggest that irisin affects food intake by increasing ghrelin and suppressing leptin production.

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Reference:
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P3-21

Investigation of effects of kisspeptin-10 in methionine induced lipid peroxidation in testis tissue of young male rats

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Aim: Oxidative stress can cause lipid peroxidation, protein degradation and DNA damage. Kisspeptin (metastin) is known to have a regulatory role in hypothalamic control of reproductive hormones. We have examined effects of kisspeptin-10 on methionine-induced lipid peroxidation in testis tissue.

Methods: Twenty four young male Wistar rats were divided into four groups (n=6). They were treated with vehicle (saline) for 30 days, L-methionine (1 g/kg, oral) for induction of injury by hyperhomocysteinemia for 30 days kisspeptin-10 (20 nmol, sc) starting on day 18 for 12 consecutive days, and L-methionine for 30 days and then kisspeptin-10 for the last 12 consecutive days. All animals were decapitated. The right testes were removed for apoptotic and morphological analysis by TUNEL and hematoxylen-eosin staining methods, respectively. Plasma samples were collected for luteinising hormone (LH) and testosterone measurement. The left testes tissues were used for enzyme activity assay, western blotting and RT-PCR.

Results: Kisspeptin-10 did not affect cell proliferation in seminiferous tubules, however, methionine caused damage and spermatogenetic cell loss and increased apoptosis ($p<0.05$). Administration of kisspeptin to methionine-receiving rats prevented apoptosis and morphological damage compared to control group. Testosterone levels declined in methionine group without statistical significance. LH levels decreased by methionine compared to control group ($p<0,001$) and increased by kisspeptin compared to methionine group ($p<0.05$). CATmRNA expression was significantly decreased by methionine ($p<0.05$). There was no significant difference in total SOD activity between among the groups.

Conclusions: The present findings suggest that kisspeptin treatment may morphologically protect spermatogenic cells against methionine-caused damage.

P3-22

Investigation of epigenetic changes in exfoliated mammary epithelial cells retrieved from lactating women

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Aim: DNA promoter methylation is an important biomarker for breast cancer risk. Bio-accumulated dioxins through environmental, occupational and dietary exposure may be linked with DNA methylation. This study was designed to investigate epigenetic changes in exfoliated human mammary epithelial cells.

Methods: Breast milk (10 ml) was collected from 13 nursing post-partum mothers living in Istanbul. Volunteers were asked to fill a questionnaire form including demographic, medical and nutritional information. The study protocol was approved by the local clinical ethics committee. Epithelial cells were isolated by centrifugation and magnetic antibody. DNA isolation from epithelial cells was performed with isolation kit. Promoter methylation of six tumor suppressor genes (RASSFF1, GSTP1, CDH1, SFRP1, RBP1 and PYCARD) was assessed by pyrosequencing of bisulfide-modified DNA. Dioxin level was evaluated by using reporter gene assay after extraction. Results were statistically analyzed with Pearson Correlation.

Results: Methylation on CpG island of CDH1 gene increased significantly with age ($p<0.05$). Low DNA methylation was observed in the lactating mothers who nursed more than one child ($p<0.05$). No significant correlation was seen between DNA methylation and dioxin levels.

Conclusion: These results suggest that nursing plays an important role to protect from breast cancer for women as mammary epithelial cells in breast lumen are renewed. Methylation risk on tumor suppressor genes increases with age. These are preliminary findings of an ongoing project in which a total of 200 breast milk samples will be analyzed to evaluate any relationship between DNA methylation and dioxin levels.

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P3-23

Epidemiological study of bone density in male and female medical students in Ostrava

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Aim: Osteoporosis is a systemic, progressive bone disease which is manifested by reduced bone density and by disorders in bone structure and bone micro architecture. This disease has become an epidemic of the third millennium. The main factors are inadequate diet, lack of activity and especially disorders of mineral metabolism. The total decreased physical activity and disorders in dietary habits are serious problem of the medical students. The aim of study was to compare differences in bone parameters between genders.

Methods: The primary objective of the study was to evaluate the bone density using a two X-ray absorptiometry (DXA) in 120 boys and 120 girls (18–26 aged). Bone mineral density (BMD), bone mineral content (BMC), T-Score and Z-Score were evaluated. All parameters were measured in the femoral neck (dbmdfn, dbmcfn, dtscorefn, dzscorefn), lumbal spine in L1-L4 (dbmdp, dbmcp, dtscorep, dzscorep), and a full body scan (dbmdt, dbmct, dtscoret, dzscoret) was eventually recorded. The results were compared between gender groups.

Results: The significant difference ($p<0.001$) was found in all monitored parameters for BMD, BMC, T-Score, Z-Score from femoral neck and full body scan between the genders. Lumbal spine densitometry parameters did not show any significant difference in T-Score–0.1905 and Z-Score–0.5991. Values of all measured bone parameters were higher in boys than girls.

Conclusions: It is concluded that female medical students have lower physical activity because they probably take more pride in study of medicine than the male ones. Of course, the dietary habits a genetic predispositions must be considered.

4. Gastrointestinal physiology

P4-1

Effects of silymarin on methotrexate-induced hepatotoxicity in rats

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Aim: Methotrexate (MTX) is a commonly used drug for treatment of many kind of cancers and also some inflamatory diseases. However, there are many side effects especially including hepatotoxicity. Silymarin (SLY) is widely used as a hepatoprotectant in the treatment of various liver disorders. The purpose of this study was to examine the protective effects of SLY on MTX-induced rat liver damage.

Methods: 36 Rats were divided into six groups: Group 1 (saline, i.p., single injection), Group 2 (0.5% carboxymethyl cellulose (CMC), by gavage once daily for 5 consecutive days), Group 3 (SLY, 300 mg/kg per day, p.o., for 5 consecutive days) Group 4 (MTX, 20 mg/kg, i.p., single injection), Group 5 (MTX+CMC similarly as groups 2 and 4) and Group 6 (MTX+CMC+SLY similarly as groups 2, 3 and 4). After the livers were removed, tissue samples were fixed and processed by using routine paraffine techniques. The paraffin blocks were then sectioned (5mm) and the sections were taken to slides with poly-L-lysine. The samples were immunohistochemically stained using avidin-biotin-peroxidase method for Bax and Caspase 3. Also, TUNEL method performed used to detect apoptosis.

Results: MTX injection exhibited vascular congestion, sinusoidal dilatation, periportal inflammation in the liver tissues. Increased number of TUNEL, Bax and Caspase 3 positive cells indicated that apoptotic cell death were also markedly increased in liver tissues after MTX administration. SLY treatment resulted in partially decrease of apoptotic cell death together with amelioration of the histological alterations.

Conclusions: In conclusion, SLY treatment leads to a reduction on methotrexate-induced hepatotoxicity in rats

P4-2

The protective effects of carnosine in carbon tetrachloride induced liver injury in rats: biochemical and immunohistochemical study

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Aim: In this study, the goal was to investigate the protective effect of carnosine on carbon tetrachloride-induced liver tissue damage.

Methods: A total of 32 adult male Wistar albino rats were used in this study. The rats were divided into 4 groups. The control group, group II was treated with 0.2 ml/kg/day carbon tetrachloride, group III was treated with 0.2 ml/kg/day carbon tetrachloride + 200 mg/kg/day carnosine and group IV received 200 mg/kg/day carnosine.

Results: Increased serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), liver malondialdehyde (MDA) levels and heat shock proteins (HSP-70) expression and steatosis were observed following treatment with carbon tetrachloride but decreased with carnosine treatment.

Conlusion: In conclusion, we observed that carbon tetrachloride results in very severe histopathological changes in liver tissue and treatment with carnosine partially prevents that damage. In addition, our results indicate that HSP-70 serves as a mediator of liver damage.

P4-3

Potential protective role of ethyl pyruvate on haemostatic disturbances in rats with acute liver injury

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Aim: The potential protective effect of ethyl pyruvate on CCl_4-induced haemostatic disturbances in rats was investigated in this study. The potential protective effect of ethyl pyruvate on CCl_4-induced haemostatic disturbances in rats was investigated in this study.

Methods: Forty male Sprague-Dawley rats were randomly divided into four equal groups including control (1 ml ringer's lactate solution at 0, 90 and 360 min, i.p.), CCl4 (a single dose of 1.6 g/kg, i.p.), EP (40 mg/kg, at 0, 90 and 360 min, i.p.) and EP +

CCl4 group (EP 30 min before, and at 60 and 360 min after CCl4 administration). Twenty-four hours after the last injection, blood samples were collected and fibrinogen levels, activated partial thromboplastin time (aPTT), prothrombin time (PT), platelet counts and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities were evaluated. The results were analyzed using Mann-Whitney U test.

Results: The results showed that CCl_4 alone treatment caused a significant ($p<0.05$) prolongation of PT and aPTT and decrease in plasma fibrinogen level compared to those of the control group. At the same time, CCl_4 treatment significantly ($p<0.05$) increased the serum AST, ALT, and ALP activities. On the other hand, the administration of EP intraperitoneally at 30 minutes before, and at 90 and 360 min after CCl_4 significantly ($P < 0.05$) decreased PT, aPTT, AST, ALT and ALP values and increased ($p<0.05$) fibrinogen level when compared with CCl4-treated only group

Conclusions: The results of our study suggest that ethyl pyruvate treatment plays a protective role in coagulation disturbances associated with CCl4 administration in rats. This effect of ethyl pyruvate may be useful for preventing haemostatic disturbances associated with liver diseases.

P4-4

DSS-induced inflammation and regulation of reelin gene expression in mouse colon

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Aim: We reported that the myofibroblasts of mouse colon express reelin and that its colonic mRNA abundance is increased by experimental colitis (1). In this work the mechanism(s) involved in the inflammation-induced reelin expression are investigated.

Methods: Inflammation was induced in mice by oral administration of 3% dextran sulfate sodium in the drinking water, during 3, 6 or 9 days. Colonic mRNA and protein were measured by real-time PCR and Western Blot, respectively. Methylation of reelin promoter region was examined by bisulfite modification of genomic DNA and sequencing.

Results: Reelin protein abundance increases with the time of treatment, whereas that of alpha-SMA (myofibroblasts marker) reaches the maximum value at day 3, indicating that the inflammation-induced increase in reelin expression is not due to increased number of myofibroblasts. Inflammation significantly increases the mRNA levels of ApoER2 and TGF-beta1, decreases that of CASK and has no effect on that of Sp-1 transcription factor. In other tissues, however, ApoER2 (2) and TGF-beta1 (3) down-regulate reelin gene expression, whereas it is up-regulated by Cask (4) and Sp-1 (5). In addition, inflammation reduces the methylation of reelin promoter region and DNMT1 mRNA levels.

Conclusions: The observations suggest that the inflammation-induced increase in reelin expression results from an epigenetic regulation of the reelin promoter region.

Abbreviations:
ApoER2, apolipoprotein E receptor 2; TGF-beta1, tumor growth factor-beta1; CASK, calcium/calmodulin-dependent serine protein kinase; DNMT1, DNA methyltransferase1, alpha-SMA, alpha-smooth muscle actin.

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P4-5

Effects of reelin and DSS-induced inflammation on gene expression and that of reelin on tissue reparation in mice colon

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Aim: We reported that, in mice, the absence of reelin (*reeler*) results in greater susceptibility to DSS-induced inflammation (1). The impacts of inflammation and absence of reelin on genes involved in intestinal homeostasis and the role of reelin on intestinal recovery from mucosal injury are investigated.

Methods: Control and *reeler* mice were administered 3% DSS in the drinking water during 9 days. For recovery experiments, DSS was administered for 5 days followed by either 1 or 3 days with drinking water. Distal colon, real-time PCR and immunohistochemistry were used.

Results: DSS-induced inflammation down-regulates CDX2, HES1 and ATOH1 mRNA

expression and up-regulates that of SOX9 in the colon of both, control and *reeler* mice, whereas that of SMAD5 is up-regulated only in the *reeler* colon. Despite the increase in SOX9 mRNA levels, the immunoassays reveal disappearance of SOX9 positive cells, in both types of mice. Neither inflammation nor the lack of reelin modifies the levels of Cyclin D1 mRNA. The results also reveal that, as compared with control, the *reeler* mice exhibit poorer recovery from the DSS-induced mucosal injury. After 1 day recovery the colonic SOX9 mRNA levels are maintained high only in control mice but SOX9 positive cells reappeared in both control and *reeler* mice, being their abundance lower in the latter. Following 3 days recovery, SOX9 mRNA decreased in both, control and *reeler* mice.

Conclusions: During inflammation reelin affects the expression of genes that control intestinal cell proliferation and differentiation and it could be involved in the mucosal healing.

Reference:
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5. Physiology teaching

P5-1

Continuous and noninvasive recording of cardiovascular parameters enhances understanding of physiology

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Over the last decades, there has been a shift away from the use of animals to the use of human subjects in practical classes. Cardiovascular parameters, such as blood pressure (BP) and heart rate (HR), can be measured by students on each other using relatively inexpensive equipment. In addition to teaching the traditional palpatory, auscultatory and oscillometric BP measurement methods, we have also used the continuous recordings of BP and HR. For this purpose we have successfully applied the differential oscillometric device which measures the mean blood pressure (MBP) at fingers in every cardiac cycle and was primarily designed for scientific research.
Our main focus for the first year students has been to demonstrate that HR and MBP change from beat to beat. These changes cannot be discovered by traditional methods of measurement. To the second year and master students we have demonstrated HR and MBP changes in response to the various tests (e.g., hand elevation, physical or emotional load, different breathing patterns).The ability to continuously measure variables and to display them to students is very important to their general understanding of cardiovascular physiology.
Introducing the Powerlab system with several identical measurement complexes enables students to practice how to set up the measurement system and use software for registration and analysis. Korotkoff sounds can be recorded by a cardiomicrophone and peripheral pulses by a finger pulse transducer. The envelope curve of oscillometric pulses can be obtained from the cuff pressure recording by means of high pass filtering. Moreover, it is possible to demonstrate how different algorithms can give different values for systolic and diastolic pressures.

P5-2

How to conduct seminars in integrative physiology?

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Aim: The aim of this presentation is to give an overview of different methods to conduct seminars of integrative physiology in our courses at the Department of Physiology, University of Tartu.

Methods: We decided to analyze the feedback from both students and faculty members to find advantages and disadvantages of different teaching methods.

Results: The general current trend in university teaching has been to move away from traditional lectures toward instructions that provide students opportunities to actively engage with content material and promote self-directed learning. Our university began to use the European Credit Transfer and Accumulation System (ECTS) in 2006. That process included an increase of the proportion of independent work by students. Therefore, we have chosen six topics of integrative physiology covered in seminars (in groups of 16-18 students). Due to the implementation of Moodle e-learning environment, some faculty members started to use PowerPoint presentations from students as a method for preparing the topic and also presenting it to the group in the seminar, whereas other faculty members still prefer to use only discussion in the seminars. It has been concluded repeatedly, there is no single best way of learning for everybody.

Student's feedback was taken from the opinion poll during the last weeks of the course and electronic questionnaire data provided after the completion of the course, feedback from the faculty was collected from six teachers involved in integrative physiology seminars during last years.

Conclusions: We found that both methods had positive and negative aspects. Therefore, we consider combining of both methods, and hope to achieve the best possible outcome to make medical physiology more understandable and interesting for our students.

6. Neurophysiology

P6-1

The interference of tonic muscle activity on the EEG signal: Single motor unit study

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Aim: Electroencephalography (EEG) is an important measurement tool of the cerebral electrical activity. The EEG signal is recorded via placing recording electrodes over the scalp. However, considering the anatomical locations of facial or masticatory muscles surrounding the skull, the electrical activity of muscles can interfere with the EEG. Temporalis muscle (*m. temporalis*) keeps the mandible in the physiological rest position and it covers a large area under the frontal EEG electrodes. In this study, we evaluated the possible interference of the resting activity of the temporalis muscle on the EEG under conventional EEG recording conditions.

Methods: In 9 healthy adults EEG activity from 19 scalp locations and single motor unit (SMU) activity from anterior temporalis muscle were recorded in three relaxed conditions; eyes open, eyes closed, jaw dropped. The EEG signal was spike triggered averaged (STA) using the action potentials of SMUs as triggers to evaluate their rejections at various EEG recording sites.

Results: Resting temporalis SMU activity generated prominent Macro-electro-myo-encephalogram (Macro-EMEG) potentials with different amplitudes, reaching maxima in the proximity of the recorded SMU. Interference was also notable at the scalp sites that are relatively far from the recorded SMU and even at the contralateral locations.

Conclusions: Considering the great number of SMUs in the head and neck muscles, prominent contamination from the activity of only a single MU should indicate the susceptibility of EEG to muscle activity artifacts even under the rest conditions. This study emphasizes the need for efficient artifact evaluation methods which can handle muscle interferences.

P6-2

The effect of exercise modalities on D-galactose induced rat Alzheimer model

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Aim: The present study was designed to investigate the effect of different exercise modalities on D-galactose induced Alzheimer model.

Methods: D-galactose (100 mg/kg) or saline was administered by intraperitoneally for 6 weeks to ovariectomised or sham-operated Wistar rats. Aerobic (AE; swimming), resistance (RE; climbing with weight) or combined exercise (CE) types were performed for 3 times a week for 6 weeks. Hippocampal mRNA levels of nerve growth factor (NGF), Amyloid precursor protein 695 (APP) were determined by RT-PCR. Novel object recognition test was performed. Malondialdehyde (MDA), glutathion (GSH), lucigenin and NO levels of brain tissue, the levels of circulating IGF-I (Elisa) were measured.

Results: Although the time spent with novel object of Alzheimer sedentary group was decreased according to sham sedentary group, it was increased back in all exercised groups ($p<0.05$-0.01). Increased MDA levels of the Alzheimer sedentary rats were decreased via all exercise types ($p<0.05$). Lucigenin levels were risen in Alzheimer sedentary and AE groups ($p<0.05$). Serum IGF-I levels were increased in all exercised groups, while there was an additional increase in Alzheimer RE group ($p<0.05$-0.001).The increased hippocampal NGF mRNA levels via Alzheimer model were declined by CE ($p<0.01$-0.001). APP

mRNA levels of Alzheimer sedentary group were increased compared to sham sedentary group and decreased with CE ($p<0,05$). Brain NO levels were increased in all Alzheimer groups compared to sham groups ($p<0.01$). GSH levels were risen in all exercise groups and were declined in sedentary Alzheimer group ($p<0.05$).

Conclusions: Different exercise modalities may have ameliorative effect in Alzheimer's disease by reducing oxidative stress parameters.

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P6-3

Olfactory sensitivity in autism spectrum disorder

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Aim: Abnormalities in olfactory sensitivity have been previously reported in individuals with Autism Spectrum Disorders (ASD) (1, 2). However, little is still known about odour threshold, identification and discrimination in ASD. Aim of this study was to examine odour threshold, identification and discrimination in a group of individuals with ASD, compared to typically developing (TD) controls.

Methods: olfactory function were studied in 10 participants with ASD (mean age 19.3±2 years, 8 boys) and 10 controls (mean age 22±2 years, 8 boys). We tested olfactory threshold, identification and discrimination wit the Sniffin' Sticks test. Participants with ASD had an IQ mean 103.2±18.5. All controls were not reported for intellectual disabilities.

Results: Our results indicated that, while all the participants with TD had an odour threshold in the normal range, all the participants with ASD showed different levels of hyposmia: moderate (50%), mild (40%) and severe hyposmia (10%). Moreover, participants with ASD were significantly impaired regarding odour detection threshold in comparison with TD controls ($U=13.00$; $p<0.05$) and odour discrimination ($U=10.00$, $p<0.05$). There were not significant differences between participants with ASD and TD in olfactory identification ($U= 24.00$, $p>0.05$).

Conclusion: Our results indicated impaired odour perception in individuals with ASD, specifically in olfactory threshold and in odour discrimination.

P6-4

The influence of 29 weeks of social isolation on basic behavioral and somatic parameters in Wistar Kyoto rats

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Aim: Modeling of psychiatric conditions in animals is challenging. Chronic stress is a major risk factor of developing severe mental illness such as schizophrenia or depression. Social isolation is a strong stressor in social animals including rodents. We investigated the impact of postweaning social isolation in Wistar Kyoto rats (WKY).

Methods: Upon weaning (postnatal day 21), animals were randomly divided to two groups of 8 rats. Rats in the first group were housed singly (isolation reared, IR), but could smell, hear, and see other rats. Rats in the second group were reared 3 or 2 per cage (socially reared, SR). After 29 weeks we assessed body weight, blood pressure, behavior in open field, acoustic startle reactivity, prepulse inhibition (PPI) and habituation of acoustic startle reflex.

Results: The body weight was significantly higher in IR compared to SR rats. No significant group difference in blood pressure was found. IR rats showed higher locomotor activity and lower exploratory behavior, but the difference to SR rats was not significant. Habituation of startle reflex was significantly lower in IR rats, but no significant group difference was found in startle reactivity and PPI.

Conclusions: Our findings suggest that postweaning isolation rearing for 29 weeks results in some behavioral and bodily changes in WKY rats. However, the alterations seem not to be very severe.

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P6-5

Satiety state dependent metabolic sidedness in the hypothalamus of male rats

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Aim: Hypothalamus is a mid-line brain structure with a symmetric organization of neuronal populations regulating homeostatic processes. Earlier, we described an estrous cycle- and estrogen-dependent metabolic lateralization in female rats which is further modulated by the satiety state of the animals. In the present study, our aim is to determine whether the metabolic sidedness is influenced by the satiety states and castration in male rats.

Methods: After quick decapitation, the hypothalami of male wistar rats were removed. Mitochondrial activity was measured by a Clark type electrode separately from isolated mitochondrial fractions of the left and right hypothalamic sides. The following groups were used: 1) *ad libitum* fed, intact males 2) *ad libitum* fed, castrated males 3) fasted, intact males 4) fasted, castrated males. The experiments were carried out 3 weeks after castration, and the animals were fasted for 24 hours.

Results: In *ad libitum* fed animals (regardless of reproductive status), we measured higher metabolic activity in the right hypothalamic sides exclusively (right sided dominance). On the other hand, 24 hours food deprivation decreased the differences between the two sides, moreover, in 50% of the fasted animals, left sided dominance was detected.

Conclusions: Using male animals, we provided evidence that satiety-dependent hypothalamic regulatory mechanisms show a lateralized distribution between the two hypothalamic sides, and the presence or absence of testes do not influence the metabolic lateralization in male animals.

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P6-6

Effects of bisphenol-A on cell viability in developing cerebellar cell culture

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Aim: Estrogen (E2) and thyroid hormones (THs), and their receptors play a key role during development of the central nervous system. Some molecules - called endocrine disruptors, like bisphenol-A (BPA) - have the ability to interfere with hormonal regulatory pathways, for example during cerebellar development. BPA can be found in many household and industrial products, and it can be easily accumulated by the organisms. Here we want to demonstrate how BPA modifies estrogen- and thyroid hormone receptor (ER, TR) expression on transciptional and translational levels, and how BPA affects the viability of individual cells.

Methods: We treated primary cerebellar cell cultures (originated from postnatal 7 days old rat pups) with E2, THs, BPA and combinations of the three substances. After incubation for different time periods, ELISA test for serum lactate dehydrogenase (LDH) levels, PCR for mRNA, and Western blot for protein measurement was performed. Results were compared to non-treated controls.

Results: Our results show that BPA decreases the receptor number, and the cell-viability in each group, as opposed to the untreated cell cultures and to those treated with hormones only.

Conclusions: Both E2 and THs, in adequate concentrations, are required for the precise orchestration of cerebellar development. Our results show that BPA decreases the receptor expression of the above hormones. Without ER and TR, the vitality of the cells reduces, and a large number of “damaged” cells will undergo necrosis, as seen in the results.

Acknowledgement:
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P6-7

The proportion of neuron specific neuronal protein (NeuN) positive neurons in hippocampal CA regions of adult female rats fed with low-calorie diet during adolescence; levels of NOS isoforms in hippocampus

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Aim: Calorie restriction (CR) without malnutrition extends longevity, delays ageing and protects also cognitive functions at later age. Nitric oxide (NO) is believed to act as an intercellular signal that regulates synaptic plasticity in hippocampal neurons, which is critical for memory. NO also regulates neurotrophic factor expression, and neuronal survival. However, NO synthesis decreases with age in brain. CR increases bioavailability of NO. In this study we hypothesized that 15% low-calorie diet (LCD) in adolescence may protect or enhance NO levels and neurons in hippocampus in adulthood.

Methods: Twenty-eight days old Sprague-Dawley female rats were separated into 4 groups: 4 weeks standard diet (SD); 4 weeks low-calorie diet (LCD); 8 weeks SD; 4 weeks LCD+4 weeks SD. Three isoforms of NOS (endothelial, neuronal, inducible) in hippocampus and NeuN (Neuronal nuclear antigen, neuronal marker)-stained cells in hippocampal CA regions were evaluated, immunohistochemically.

Results: Despite the insignificant increase in iNOS and nNOS, eNOS levels significantly increased in hippocampus of adult rats fed with LCD during adolescence. NeuN-immunoreactivity significantly has increased in all hippocampal CA regions, but the increase was most prominent in the hippocampal CA1 neurons.

Conclusions: Our original findings suggest LCD in adolescence is important for protecting hippocampal neurons in adulthood. Correlation between the increased neuron number and eNOS in hippocampus suggests that the protection of hippocampal neurons may be through eNOS.

P6-8

Effects of calorie restriction in adolescence on brain and serum BDNF levels and serum cholesterol levels in adulthood

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Aim: It was reported that calorie restriction (CR) retard aging, prevent neurodegeneration due to aging and it makes these by reducing the metabolic risk markers. Brain-derived neurotrophic factor (BDNF) exerts multiple biological functions in the CNS. Besides its well-known role in nerve cell survival and adaptive plasticity, suggest a novel role BDNF regulates cholesterol metabolism for synapse development. For this reason, we investigated the effect of 15 % low-calorie diet in adolescence on brain and serum BDNF levels and serum lipid level in early adulthood.

Methods: Twenty-eight days old Sprague-Dawley female rats were separated into 4 groups: 4 weeks standard diet (SD); 4 weeks low-calorie diet (LCD); 8 weeks SD; 4 weeks LCD+4 weeks SD. Rats were decapitated after related nutrition. The brain was removed. BDNF levels in brain and serum were examined by ELISA method and serum lipid profile also examined by ELISA method.

Results: Our results indicated that, LCD in adolescence increased BDNF levels in brain significantly but not in serum. Triglyceride and cholesterol levels decreased significantly in LCD4 compared to ND4. In LCD4+ND4, cholesterol and triglyceride increased significantly compared to LCD4, both triglyceride and cholesterol were low though the decrease in cholesterol was significant compared to ND8.

Conclusions: The findings suggest that BDNF may contribute to lipid metabolism. It is known that cholesterol is harmful to brain function but it has a synergistic effect with BDNF. Therefore we suggest that it may be important to investigate the effects of LCD in adolescence on brain lipid levels, as LCD caused an improvement in brain functions in adulthood.

P6-9

Effect of chronic exercise on central monoamines synthesis and cognition in aged rats

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Aim: Cognitive frailty is one of the greatest health threats. The present study was designed to evaluate the effects of the exercise on brain functional decline that accompanies the aging process.

Methods: Old male Wistar rats (18 months) were used to study the effect of chronic exercise (36 days, during 5-10 min/day, at a speed of 4-15 rpm) on central monoaminergic neurotransmitters serotonin, noradrenaline, and dopamine in rat brain, quantified by electrochemical HPLC. The accumulation of 5-hydroxytryptophan and L-3,4-dihydroxyphenylalanine after decarboxylase inhibition was also analyzed as a measure of the rate of tryptophan and tyrosine hydroxylation. Furthermore, we investigated possible changes on memory processes using the spatial working memory 8-arm radial maze. In this test we analyzed the time necessary to complete the trial and the number of errors committed.

Results: Compared to control group (standard conditions), results from spatial memory test showed an improvement after the chronic exercise in trial time and total errors (reduction of 65-61%, respectively). Concerning the neurochemical changes on monoamines neurotransmitters, which are linked to cognitive processes, exercise program significantly reversed the age-induced deficits in all monoaminergic neurotransmitters studied, enhancing the levels of hippocampal serotonin (36%) and noradrenalin (76%) and striatal serotonin (86%) and dopamine (49%), largely due to an increased activity of tryptophan and tyrosine hydroxylase (103-104% in hippocampus, 64-25% in striatum).

Conclusions: Our results suggest that chronic exercise can be useful to recover monoaminergic neurotransmitter systems from the age-associated deficits and enhance cognitive functions, suggesting the use of exercise as a therapy to protect against the cognitive impairment in normal brain aging.

P6-10

Effect of early long term enrichment program on central monoamines synthesis and cognition in aged rats

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Aim: The aim of this study was to analyze the effects of environmental enrichment program as an anti-aging therapy that promote neuronal plasticity, delaying the cognitive decline associated with aging.

Methods: Male Wistar rats exposed to standard conditions or environmental enrichment (EE, changing objects each week) from 1 month until 20 months. We quantified by HPLC central monoaminergic neurotransmitters serotonin, noradrenaline, and dopamine in hippocampus and striatum. The accumulation of 5-hydroxytryptophan and L-3,4-dihydroxyphenylalanine after decarboxylase inhibition was also used as a measure of the rate of tryptophan and tyrosine hydroxylation. To evaluate memory processes, a hippocampus-dependent test was used: the spatial working memory 8-arm radial maze, analysing the time necessary to complete the test and the number of errors committed.

Results: At 20 months, rats submitted to EE showed an improvement on spatial memory compared to control group, reducing the time (38%) and total errors committed (39%). Concerning monoaminergic neurotransmitters, EE program enhanced the levels of hippocampal serotonin (37%) and noradrenalin (34%) and striatal serotonin (119%) and dopamine (46%), largely due to an increased activity of tryptophan and tyrosine hydroxylase (51-112% in hippocampus, 56-80% in striatum). These monoamines neurotransmitters are linked to cognitive processes; thus, these results are in good correlation with the restorative effect of EE program on spatial memory in the radial maze on aged rats.

Conclusions: Results demonstrate the high effectiveness of the early long term enrichment program to delay the age-related monoaminergic neurotransmission decline and enhancing cognitive abilities in old rats, which can be due to an increase of brain neuroplasticity.

P6-11

Effects of resveratrol on SIRT1 expression and NF-kB acetylated levels in aged rats

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Aim: Resveratrol is an antioxidant and sirtuin-activating compound, which exhibit neuroprotective effects in aged rats. Some of their effects may be mediated by SIRT1 deacetylation of NF-kB, which also might regulate the efficiency of NF-kB signalling. We have studied the expression of SIRT1 and the levels of NF-kB acetylated in hippocampus of young and aged rats, and after resveratrol in aged rats.

Methods: Aged (20 month-old) Wistar male rats were treated with resveratrol (20 mg/kg, i.p.), and their corresponding controls (20 month-old) plus a group of young rats (3 month-old) were treated with corn oil (1 ml/kg, i.p.), during 28 days. The levels of SIRT1 (110 and 75 kDa) and NF-kB acetylated were quantified in the hippocampus by Western immunoblot analyses with specific antibodies.

Results: The content of SIRT1 protein decreased in rat hippocampus with age (110 kDa: 35%; 75 kDa: 14%). Chronic resveratrol prevented the deleterious effect of aging on SIRT1 expression, recovering the content in hippocampus (110 kDa: 90%; 75 kDa: 105%). The content of NF-kB acetylated in the hippocampus of aged control rats was 15% higher than in young rats. Resveratrol treatment returned the content of hippocampal NF-kB acetylated protein to similar values than in young rats (94%).

Conclusions: The reduced expression of SIRT1 in hippocampus of old rats can be reverted by resveratrol treatment. Furthermore, the results suggest that resveratrol treatment induced SIRT1-dependent NF-kB protein deacetylation at lysine 310 in hippocampus of aged rats.

P6-12

SIRT1 and monoamine levels in hippocampus of aged-rats after chronic polyphenol treatments

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Aim: Polyphenols such as sylimarin, quercetin, and naringenin are antioxidants, which exhibit neuroprotective effects in aged rats. Their action mechanisms are not fully elucidated, but some of their effects may be mediated by SIRT1 expression. We have studied the expression of SIRT1, and the levels of monoamine neurotransmitters in the hippocampus of aged rats after chronic polyphenols treatments.

Methods: Aged (18 month-old) Sprague male rats were treated with sylimarin, quercetin, and naringenin (20 mg/kg/day, i.p., during 28 days), and their corresponding control and a group of young rast (3 month-old) were treated with corn oil (1 ml/kg/day, i.p., during 28 days). Effects of aging and polyphenol treatments were evaluated by the measurement of SIRT1 (110/75 kDa) in hippocampus by Western immunoblot with specific antibody. Besides, we quantify the levels of serotonin, noradrenaline, their precursors and metabolites in the same region by HPLC-ECD.

Results: We observed an age-related decline in SIRT1 protein levels in hippocampus of aged rats (110 kDa: 35%; 75 kDa: 14%). Sylimarin, quercetin, and naringenin prevented the deleterious effect of aging on SIRT1 expression, recovering the content in hippocampus with values close to those of young rats (110 kDa: 76%, 85%, 91%; 75 kDa: 104%, 117%, 112%; respectively). Regarding monoamine levels, sylimarin, quercetin and naringenin, enhanced the levels of serotonin (49%, 53%, 54%, respectively) and noradrenaline (42%, 32%, 40% respectively) in hippocampus of aged rats.

Conclusions: The recovery of SIRT1 and monoamine levels in the hippocampus of aged rats by polyphenols treatments correlated with the restorative effects previously observed on cognitive abilities.

P6-13

BDNF modulates neural network activity via TrkB-mediated launching of intracellular signaling cascades

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Aim: Brain-derived Neurotrophic Factor (BDNF) is an important signaling molecule involved in neurogenesis, growth and survival of neurons in the central nervous system. This protein can participate in synaptic transmission and in fast activation of Nav1.9 ion channels. The present study was focused on investigation the effect of BDNF on spontaneous neural network activity of dissociated hippocampal cultures in different stages of their development *in vitro*.

Methods: Hippocampal cells were dissociated from E18 embryonic mice and plated on multielectrode arrays (Alpha MED Science, Japan). A single BDNF (0,1 ng/ml, 1 ng/ml, 10 ng/ml) application into culture medium was conducted on 7, 14 and 21 days of culture development *in vitro* (DIV). Spontaneous bioelectrical activity of neural networks was recorded in original value, within 40 minutes, 2 and 24 hours after BDNF application.

Results: BDNF modulates the spontaneous bioelectrical activity of mature neural networks from 14 DIV. This effect was observed 10-15 minutes after application and manifested in the increasing of network burst duration and in restructuring the pattern of spontaneous network activity without changes in the number of spikes in burst.

Conclusions: Our studies revealed that single BDNF application has neurotropic effect on the spontaneous bioelectrical activity of mature neural networks via interaction with TrkB receptor leading to the activation of intracellular signaling cascades and is not associated with fast activation of ion channels.

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P6-14

Effect of gamma-glutamyl cysteinyl ethyl ester on apoptotic miRNA expressions in kainic acid induced excitotoxicity model

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Aim: The expression patterns and functions of miRNAs in neurodegenerative diseases have not been fully understood yet. Targeting miR-34a, miR-132 and miR-184 has been reported to alter seizure-induced neuronal death. Kainic acid (KA) is an analogue of glutamate and can induce excitotoxicity. In our study we aimed to investigate the effects of gamma-glutamyl cysteinyl ethyl ester (GCEE), neuroprotective agent, on miRNA profiles in KA induced excitotoxicity.

Methods: In our study, Sprague-Dawley rats were used. Control group (n=4) was given saline, KA group (n=4) was given 10 mg/kg KA and KA+GCEE group (n=4) was given 10 mg/kg KA and 10 mg/kg GCEE intraperitoneally. miRNA expressions were evaluated by Real-Time PCR in striatum and cerebellum.

Results: KA+GCEE and KA treatment significantly increased expressions of miR34a, miR132 and miR184 in cerebellum when compared to control ($p<0.05$) . In striatum, expressions of miR34a and miR132 in KA and KA+GCEE group were significantly increased when compared to control ($p<0.05$). Also the levels of expression were found higher in KA+GCEE group. However miR184 expression was decreased by both KA and KA+GCEE treatment in striatum ($p<0.05$).

Conclusion: It is observed that expressions of miR34a, miR132 and miR184 influenced by excitotoxicity. Decreased miR184 expression with KA traetment may show that miR184 can be associated with neuronal survival. It is considered that increased miR34a and miR132 expressions can be caused by increase in oxidativestress that results in apoptosis and neurodegeneration. Our results showed that neuroprotective effect of GCEE was not related with effect on miRNA profiles in KA-induced excitotoxicity.

P6-15

Effect of gamma-glutamyl cysteinyl ethyl ester on mitochondrial DNA damage in kainic acid-induced excitotoxicity model

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Aims: Glutamate induced excitotoxicity is a triggering factor that causes neurodegeneration underlying nervous system disorders like epilepsy and stroke. Overstimulation of glutamate receptors results in production of reactive oxygen species (ROS). Kainic acid (KA), analogue of glutamate, can induce excitotoxicity in brain regions. Glutathione (GSH) is crucial for the cellular defence against ROS. It is suggested that deficiency of GSH and excitotoxicity lead to mitochondrial damage in brain. For this reason we aimed to investigate protective effects of γ-glutamyl cysteinyl ethyl ester (GCEE), GSH precursor, on mtDNA damage in KA-induced excitotoxicity.

Methods: In our study, Sprague-Dawley rats were used; divided into three groups as control (n=4), KA (10 mg/kg) (n=4) and KA+GCEE (10 mg/kg + 150 mg/kg) (n=4). Cerebellum and cortex samples were used for determination of mtDNA damage by Long PCR and diphenylamine (DPA) DNA fragmentation methods .

Results: KA treatment significantly increased the mtDNA damage in cerebellum assessed by DPA and long PCR methods when compared to control group($p<0.05$). In addition GCEE treatment against KA significantly decreased the mtDNA damage in cerebellum when compared to KA group($p<0.05$). No significant differences were found between groups in cortical samples.

Conclusion: It is concluded that KA leads to mtDNA damage in cerebellum and GCEE treatment may be helpful as a neuroprotective agent because of its decreasing effect on mtDNA damage against neurodegenerative processes triggered by KA. Our study provides an innovation by searching the effect of KA on cerebellum which is a different brain region from the previous studies about KA-induced excitotoxicity.

P6-16

The effect of 3', 4'-dihydroxyflavonol on lipid peroxidation in brain ischemia-reperfusion damage in rat

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Aim: The aim of present study was to determine the effect of 3',4'-dihydroxyflavonol on lipid peroxidation in experimental brain ischemia-reperfusion in rats.

Methods: Present study was performed on the 34 male Wistar –albino rat, weigth 350-400 gr Experiment groups were organised as follows: 1-Sham; 2-Ischemia-reperfusion; animal were anesthesized and carotid arteried were clemped for 20 minutes and reperfusion (7 days). 3-DiOHF + Ischemia-reperfusion; DiOHF was given to animals as 10 mg/kg by intraperitoneal. 4- Ischemia + DiOHF + Reperfusion; 5- Ischemia-reperfusion + DiOHF.
At the end of the study animals were anesthesized blood samples were taken and analysed for malondyaldehyde (MDA), NO (nitric oxide) , xanthine oxidase (XO), glutathione (GSH) and glutathione peroxidase (GPx).

Results: Blood MDA levels were significantly higher ischemia-reperfusion groups ($P<0.005$). However, these increased levels were inhibited by DiOHF that given just before ischemia and reperfusion. Ischemia-reperfusion led to increased XO and NO but DiOHF application reduced NO and XO. DiOHF increased GSH and GPx levels compared to ischemia-reperfusion group.

Conclusion: The results of present study show that intraperitoneal DiOHF supplementation has protective effect on brain ischaemi-reperfusion damage in rat stimulated by antioxidant system.

P6-17

The relationship between neuronal nitric oxide synthase genotype and prepulse inhibition in humans

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Aim: Evidence is growing that nitric oxide (NO) plays role in the pathogenesis of schizophrenia. Several studies have reported association between schizophrenia and the genetic variability of neuronal nitric oxide synthase (nNOS), the enzyme producing NO in neurons. Prepulse inhibition of the acoustic startle reflex (PPI) is an important endophenotype and translational biomarker of schizophrenia. We investigated the relationship between PPI and the genetic variability of nNOS.

Methods: Healthy adult humans volunteers (N=150) were genotyped for 3 nNOS polymorphisms associated with schizophrenia and cognition. PPI was assessed by recording EMG of the periocular muscles. Acoustic pulses (105 dB, 40 ms) and prepulses (75 dB, 20 ms) were presented upon a constant background noise (55 dB) via calibrated headphones. PPI was calculated as the percentage of startle reduction in prepulse-pulse trials (interstimulus intervals: 30, 60, and 120 ms) relatively to pulse alone trials.

Results: PPI was significantly weaker ($P<0.05$) in the carriers of risk alleles of nNOS.

Conclusions: These data provide support for a possible role of NO in schizophrenia pathogenesis.

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P6-18

The protective effect of selenite ions on catalase activity in the brain of aliuminium-treated mice

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Aim: The present study was conducted to investigate the protective effect of selenite (SeO_3^{2-}) ions on catalase activity in aluminium (Al) ion-treated mice brain.

Methods: Experiments were done on outbred white laboratory mice using intraperitoneal (i.p.) injections of $AlCl_3$ and/or Na_2SeO_3 solutions. Control mice were i.p. injected with the same volume of saline (0.9% NaCl). The exposure-time was 24 h or 14 days. Catalase activity in mice brain was determined by reaction with ammonium molybdate which give a yellow complex with hydrogen peroxide that absorbs 410 nm light wavelength.

Results: Following 24 h after i.p. injections of $AlCl_3$ (0.5 LD_{50}) and Na_2SeO_3 (0.025 LD_{50}) solutions alone or in their combination, catalase activities in mice brain remained at the control levels. After 14 days of daily injections of $AlCl_3$ solution (0.1, 0.15 and 0.25 LD_{50}), the activity of catalase increased by 22%, 22% and 32% respectively as compared to the control. In contrast to Al ions, SeO_3^{2-} did not cause considerable changes of brain catalase activity at this time-period of mice intoxication. Furthermore, Na_2SeO_3 pre-treatment revealed a tendency to reduce the effect of Al on catalase activity.

Conclusions: Our studies revealed that in the brain selenite ions could counteract the effect of Al on the activity of catalase after 14 days of intoxication.

P6-19

Changes in nitric oxide generation in rats reared in social isolation

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Aim: Neurodevelopmental disturbance due to chronic stress is considered to play a major role in the etiopathogenesis of severe mental disorders. Therefore, social isolation early in life is widely used to study the neurobiology of psychiatric diseases in rodent models. Recent findings indicate that brain nitric oxide (NO) is involved in mental disorders. Thus, we explored the effect of post-weaning social isolation on behavioral habituation and NO generation in the brain.

Methods: Male Wistar-Kyoto (WKY) rats were used in the study. At postnatal day 21 the animals were randomly divided into two groups. In the first group, rats were reared singly (isolation reared, IR) while in the second group, rats were reared 3 per cage (socially reared, SR). After 29 weeks of isolation, acoustic startle reflex (ASR) and its habituation was tested. The animals were sacrificed shortly thereafter. Activity of NOS was assessed in the cerebellum. Expressions of neuronal NOS (nNOS) and inducible NOS (iNOS) proteins were assessed in the cerebellum and the hippocampus. In addition, we assessed plasmatic activity of TNF-alpha, IFN-gamma and GM-CSF cytokines.

Results: Habituation of ASR was significantly decreased in IR rats. Cerebellar NOS activity and nNOS protein expression were significantly decreased in IR compared to SR rats. On the contrary, we found a significant up-regulation of nNOS and iNOS and isoforms in the hippocampus. In accordance with iNOS up-regulation, plasmatic level of GM-CSF cytokine was elevated.

Conclusion: Our findings indicate that post-weaning social isolation results in region-specific changes in NO production in the brain, which may play a role in the pathophysiology of mental disorders.

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P6-20

Hypoxia-induced toxicity mechanism in brain is age-dependent

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Aim: Mitochondrial permeability transition (MPT) is a key cell death inducer during myocardial infarction, but its role in brain ischemia is not quite clear. Recent evidence suggests MPT might be regulated by mitochondrial respiratory chain complex I activity. We tested cell sensitivity to hypoxia in brain slice cultures of young, adult and aged brain and examined the effect of complex I and MPT inhibitors on hypoxia-induced damage.

Methods: Slices were prepared from the cerebral hemispheres of young (7-days), adult (2-3 months) and aged (7-10 months) Wistar rats. After 2 weeks *ex vivo*, slice cultures were subjected to 24-hour hypoxia together with 10 mM of glycolysis inhibitor deoxyglucose, followed by 7-day reoxigenation. Cell death was evaluated by lactate dehydrogenase (LDH) level in incubation medium and by propidium iodide and Hoechst 33342 fluorescence. Microglial cells in the cultures were visualized with isolectin GS-IB_4.

Results: Hypoxia alone did not affect young and aged brains but together with deoxyglucose increased LDH release and caused significant amount of necrotic nuclei. This was abolished by mitochondrial respiratory complex I inhibitors rotenone and amytal and diminished by MPT inhibitor cyclosporin A. In adult brain slices hypoxia alone induced cell death that was insensitive to complex I inhibitors or cyclosporin A. Addition of deoxyglucose significantly reduced hypoxic toxicity but did not change the number of microglia. LDH levels did not increase during reoxigenation indicating no further necrosis.

Conclusions: Young and aged brains are sensitive to hypoxia together with deoxyglucose. Adult brains are sensitive to hypoxia, and deoxyglucose is protective. In young and aged brains, hypoxia and deoxyglucose induced damage is related with mitochondrial complex I mediated MPT but this is not the case in hypoxic damage of adults.

P6-21

Degradation of brain hyaluronic acid triggers homeostatic plasticity-like changes in the AMPA receptor pool of hippocampal neurons

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Aim: It has been identified that chronic fluctuation in the electric activity level of neurons can cause compensatory alterations in synaptic strength maintaining it within optimal range. This phenomena was called homeostatic plasticity, or synaptic scaling. It is determined by accumulation of calcium-permeable GluR1-containing postsynaptic AMPA receptors. Here we study whether partial degradation of hyaluronic acid as a component of the brain extracellular matrix have an effect on synaptic plasticity.

Methods: This study was performed on hippocampal cultures from C57BL/6J mice. Two models were used. The first were primary hippocampal cultures from embryonic day 18 and the second were organotypic hippocampal slice cultures from mice. Hyaluronic acid was removed by the hyaluronidase from *Streptomyces hyalurolyticus* (Sigma).

Results: Enzymatic degradation of hyaluronic acid as a component of the brain extracellular matrix results in significant increase of synaptic GluR1-containing AMPA-receptors concentration to total actin at 48 hours after hyaluronidase administration. Previously in our laboratory it was shown that degradation of hyaluronic acid trigger seizure-like activity in hippocampal neurons.

Conclusions: It was shown that enzymatic degradation of hyaluronic acid as a component of the brain extracellular matrix triggered homeostatic plasticity-like changes in hippocampal neurons by upregulation of GluR1-containing AMPA-receptors. This may cause the appearance of epileptiform activity because of excessive calcium influx into excitatory pyramidal neurons through the calcium-permeable GluR1-containing AMPA receptors.

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P6-22

Investigation of cognitive functions with Wechsler Adult Intelligence Scale's comprehension subtest and Mini-Mental State Examination in patients with end-stage renal diseases

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Aim: Chronic kidney diseases (CKD) are a condition characterized by the irreversible loss of kidney function which affects negatively all systems in the body. The present study was designed to reveal the changes in Cognitive function (CF) and explain the possible mechanisms in patients with CKD.

Materials and Methods: The present study was conducted with 52 peritoneal dialysis patients who admitted to the Turgut Ozal Medical Centre in the end-stage of CKD. Control group constituted with 52 healthy individuals with similar demographic features (age, race, gender, etc.). Cognitive status measured by Wechsler Adults Intelligence Scale (WAIS) comprehension subtest, and Mini-Mental State Examination (MMSE). Individuals with other chronic diseases and smoking/alcohol habits which may impair CF were not included in the study.

Results Test results analysed for normality of the distribution with Kolmogorov–Smirnov test. MMSE test results were not shows normal distribution thereby Mann-Whitney U test used to evaluate ($p<0.001$). WAIS test score was normal distributed so Student T test used to examine the data ($p=0.001$). The results obtained from intelligence tests were statistically significant between patient and control groups.

Conclusions: Test results which revealed the patients with end-stage renal failure has impaired CF were consistent with the literature but further studies are needed to clarify the physiological mechanisms.

Key Words:
End-stage renal diseases, Cognitive functions, Wechsler adult intelligence scale, Mini-mental state examination.

P6-23

Search for criteria that allow shortening of pupillography sleepiness test time

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Aim: To find the criteria that could allow shortening 11 minutes pupillographic measurement to half time using pupillary unrest index (PUI) and the pupil diameter (PD).

Methods: Pupillographic sleepiness test (PST) results of 946 persons obtained with F2D Fit-For-Duty test (AMTech GmbH, Germany) were analysed in respect to the PUI and the PD. Data obtained from persons that are using medication or with interpolation greater than 20% in any of 8 segments were excluded from further analysis. According the mean PUI in the test all persons were divided into alert, borderline and sleepy group.

Results: From all PST 65% of measurements were acceptable for analysis. There was found negative correlation between PD in each segment and mean PUI in the test ($p<0.05$). PD in the 4^{th} segment more than 8 mm was found only in alert group, but less than 3 mm – only in sleepy group. PD in the 4^{th} segment allowed predicting of mean PUI group in the test for 9.1 % of persons. There was strong association between mean PUI in the 2^{nd}, 3^{rd} and 4^{th} segment (PUI_{234}) and mean PUI in the test. PUI_{234} lower than 0.7 was found only in alert group, while greater than 2.5 – only in sleepy group. The PUI_{234} allowed predicting of mean PUI group in the test for 59.4 % of persons. Both indices – PD in the 4^{th} segment and PUI_{234} - allowed predicting of mean PUI group in the test for 60.4% of persons.

Conclusions: PD in the 4th segment and mean PUI in 2nd, 3rd and 4th segment indices allow shortening measurement time to 5.5 minutes in about 60.4% of acceptable PST.

P6-24

Dysregulated gene expression network of oligodendrocyte lineage markers in the cerebellum of autistic patients

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Aim: Autism is a neurodevelopmental disorder manifested by impaired social interaction, deficits in communication skills, restricted interests, and repetitive behavior. In psychiatric disorders, glial cells undergo morphological, biochemical, and functional rearrangements, which are critical for neuronal development, neurotransmission, and synaptic connectivity. Cerebellar function also contributes to cognition and may be affected in autism. Here, we searched for evidence for developmental oligodendropathy in autism.

Methods: STRING 9.1 was used to create an *in silico* network model of interactions of oligodendrocyte markers. Publicly available microarray data were obtained from the Geo DataSets database. Differential gene expression from diseased cerebellar samples vs. healthy controls of "OLIGO" network members was elucidated by using the limma package from R and false discovery rate (FDR), and relative values were plotted over the network by utilizing the ViaComplex software.

Results: The *in silico* model (OLIGO) showed the network of interactions between oligodendrocyte markers and demonstrated that more than 50% (16 out of 30) of the genes within this model displayed significant changes of expression (corrected *p*-value <0.05) in the cerebellum of autistic patients. In particular, we found up-regulation of OLIG2, MBP, OLIG1, and MAG specific oligodendrocyte markers.

Conclusions: Aberrant expression of oligodendrocyte-specific genes, potentially related to changes in oligodendrogenesis, may contribute to abnormal cerebellar development, impaired myelination, and anomalous synaptic connectivity in autism spectrum disorders (ASD).

P6-25

Neurotoxic effects of biological fluids of Alzheimer's disease patients' on cerebellar granule cell cultures

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Aim: Alzheimer's disease (AD) is the most common form of dementia that affects millions people worldwide. Molecular mechanisms of Alzheimer's disease as well as biomarkers for early diagnostics are unclear. The aim of this study was to investigate effects of patients' biological fluids on primary cerebellar granule cell cultures (CGC).

Methods: Primary mixed neuronal-glial cultures from rat cerebella (CGC) were prepared from 7-8 days old Wistar rats. Blood serum and cerebrospinal fluid (CSF) were donated by healthy, age-matched individuals (with zero degree of dementia; control group) and patients with Alzheimer's disease (diagnosed with Alzheimer's type dementia DAT). CGCs were incubated with serum and CSF for 24 hours. The viability of neurons in cultures was assessed by fluorescence microscopy.

Results: We found that neither control or patients' sera had any effect on viability of neurons and glial cells. CSF from control group did not affect neuronal viability while patients' CSF reduced neuronal viability by about 20%. CSF from both groups had no effect on viability of glial cells. Interestgly, patients' CSFs significantly reduced density of neurons in CGC cultures, while control CSF had no effect on neuronal density. Patients' CSF also stimulated microglial proliferation, whereas control CSF (and control and patients sera) had no effect on microglial proliferation.

Conclusions: our study revealed that CSF from patients with Alzheimer's disease but not healthy subjects exhibit neurotoxic effects on CGCs cultures. This can be used as a basis for development of diagnostic methods for early diagnostics of Alzheimer's disease.

P6-26

Effect of alcohol on the rat visual cortex visually evoked potentials

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Aim: Visual system is one of the most investigated sensory systems. However, there are many unanswered questions, particularly related to effect of alcohol on this system. Therefore our aim was to investigate effect of alcohol on the rat visual cortex visually evoked potentials to visual stimulus onset and offset.

Methods: We investigated effect of acute alcohol administration on visually evoked potentials (VEPs) in anesthetized adult rats (n=10). The effect of alcohol on VEPs latency and amplitude was observed for one hour after intraperitoneal injections of two ethanol doses - 1g/kg and 2g/kg. Three VEPs components - N59, P96 and N143 – elicited after stimulus onset and offset were analyzed.

Results: Alcohol increases latency (except for OFF responses component N_{144}) and reduces amplitudes (except for ON and OFF responses component N_{144}) of early components in ON and OFF responses. Independent of dose, alcohol differently affects early components of ON and OFF responses: causes latency differences of N_{144} component and reduces amplitude differences of P_{98} and N_{144} components between ON and OFF responses. Alcohol's effect on latency of measured components is stable within 1 hour, but effect on amplitude varies and depends on the type of response and alcohol dose.

Conclusions: Our results show that both doses of alcohol increase latencies of all three VEP components during an hour, but there are differences between "on" and "off" responses. The effect of alcohol on VEPs amplitudes is diverse and dose dependent. Thus, our results indicate that alcohol's influence on visual system is complex and deserves further investigation.

P6-27

Sema3A and NGF interplay in regeneration of DRG axons

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Aim: Successful regeneration of the injured peripheral nervous system is complicated process involving neuron survival and correct neurite pathfinding. Dorsal root ganglions (DRGs) axon guidance is mainly governed by repulsive growth cone-collapsing factor semaphorin3A (Sema3A). On the other hand, increasing evidence shows that DRG axon development in both pathological and physiological conditions can be stimulated by nerve growth factor (NGF). In this study, we aimed to evaluate whether increased NGF concentrations can counterweight Sema3A-induced inhibitory responses.

Methods: Fifteen days old mouse embryo (E15) DRGs were maintained in Neurobasal-based media supplemented with NGF (0-100 ng/mL) concentrations. The development on NGF-dependent axons was evaluated under constant Sema3A (10 ng/mL) pressure. The effect of these signaling molecules was evaluated at neuron survival, axon elongation and growth cone guidance levels, by evaluating axon number, measuring axon outgrowth and assessing growth cone morphology. The measurements were performed after 16 hours of explants incubation in the presence or absence of Sema3A at different NGF concentrations. Growth cone collapse rate was determined after 1 hour of Sema3A treatment following 23 hours growth *in vitro* in presence of different NGF concentrations.

Results: Our results showed that increase of NGF concentration increased survival of DRG neurons (axon number) independently of Sema3A. Moreover, Sema3A-induced axon outgrowth inhibition was abolished by increased NGF concentration whereas NGF concentration had no effect on Sema3A-induced collapse rate.

Conclusions: Overall our results support hypothesis that NGF has therapeutic properties in nervous system regeneration promoting axon growth while having only limited effect on impeding Sema3A-depended guidance.

P6-28

Effect of selenite ions against aluminium-induced oxidative stress in mice tissues

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Aim: The present study was carried out investigating a possible protective effect of selenite ions (SeO_3^{2-}) on the redox status in mice blood and brain under short-term exposure to aluminium ions (Al^{3+}).

Methods: Experiments were done on 4-6 weeks old Balb C mice intraperitoneally treated with $AlCl_3$ (0.15 LD50) and/or Na_2SeO_3 solution/s (0.025 LD50) for 14 days. Control mice received injections of the same volume of saline solution. Reduced glutathione (GSH) was determined by reaction with 5,5'-dithiobis (2-nitrobenzoic acid), absorbtion was measured at 412 nm. Lipid peroxides were estimated by a content of malondialdehyde (MDA).

Results: Al^{3+} reduced GSH concentration in blood by 9% ($p>0.05$). The exposure to SeO_3^{2-} had no effect on blood GSH concentration. However, treatment with Al^{3+}+$SeO_3$2- decreased blood GSH by 24% as compared to control. After 14 days exposure to Al3+ and/or SeO_3^{2-} , there were no statistically significant changes in brain GSH concentration in any experimental group. The treatment with Al^{3+} or SeO_3^{2-} did not cause any significant changes in blood MDA concentration. However, the treatment with both effectors induced significant increase in blood MDA concentration by 26%. Al^{3+} as well as SeO_3^{2-} increased brain MDA concentration by 17% and 13% respectively, as compared to control. Coexposure to SeO_3^{2-} and Al^{3+} resulted in the increase in brain MDA concentration by 16% as compared to control. Thus, the pretreatment with Na_2SeO_3 20 min before $AlCl_3$ injections, could not reduce Al^{3+}-induced brain lipid peroxidation. Moreover, SeO_3^{2-} itself induced increase in brain MDA concentration.

Conclusions: The pretreatment with SeO_3^{2-} did not protect mice brain from Al^{3+}-induced oxidative damage. Increase in blood MDA concentration after coexposure to SeO_3^{2-} and Al^{3+} may be due to GSH depletion.

P6-29

Kisspeptin elicits cytosolic calcium responses through protein kinase C-dependent mechanism in immortalized RFamide-related peptide-3 neurons

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Aim: Kisspeptins are reported to be the most potent activators of hypothalamus–pituitary–gonadal (HPG) axis known to date. Kisspeptin and its receptor (GPR54) are expressed in GnRH neurons and regulate their function. rHypoE-7 cell line was developed as a model to explore the effects of neuroendocrine factors on RFRP-3 neurons. RFRP-3 neurons have been shown to express kisspeptin receptors. For this aim, the effects of kisspeptin on intracellular free Ca^{2+} levels ($[Ca^{2+}]_i$) in RFRP-3 neurons were investigated by using *in vitro* calcium imaging system.

Methods: rHypoE-7 cells were plated on glass coverslip and loaded with 1 μM Fura-2 AM. After loading the cells were washed 3-4 times for 20 min with standard recording medium to remove the extracellular fura-2 AM. $[Ca^{2+}]_i$ responses were quantified by the changes in 340/380 ratio by using fluorescence imaging system. We tested the effects of different concentration of kisspeptin treatment after recording of basal $[Ca^{2+}]_i$.

Results: Kisspeptin caused a significant increase in basal levels of $[Ca^{2+}]_i$ after application at the doses of 100 nM ($n=42$, $p<0.01$) and 1 μM ($n=34$, $p<0.001$). The changes in $[Ca^{2+}]_i$ were significantly attenuated by pre-treatment with protein kinase C inhibitor ($n=19$, $p<0.001$). The stimulatory effect of kisspeptin (1 μM) on $[Ca^{2+}]_i$ was persistent in Ca^{2+} free conditions ($n=33$, $p<0.001$).

Conclusions: We demonstrated that kisspeptin activates intracellular calcium signaling in RFRP-3 neurons in a dose dependent manner. This effect of increased of $[Ca^{2+}]_i$ levels of kisspeptin is dependent of PKC pathway.

P6-30

Inhibition of mitochondrial respiratory chain complex I is protective against ischemia induced rat cortex and cerebellum mitochondrial damage

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Aim: To investigate the effect of mitochondrial respiratory chain complex I inhibitor rotenone (Rot) on respiration, calcium induced- mitochondrial permeability transition pore (mPTP) opening of isolated rat brain cortex and cerebellum mitochondria and necrosis after 120 min. ischemia.

Methods: Brains of adult Wistar male rats were exposed to 120 min. ischemia with/without Rot. Respiration of isolated cortical and cerebellar mitochondria was measured oxygraphically using a Oroboros instrument. Ca^{2+}-induced mPTP opening determined as calcium retention capacity (CRC) was measured using fluorescent dye Calcium Green 5N. Necrosis was detected as the release of lactate dehydrogenase (LDH) into perfusate which activity was measured spectrophotometrically following oxidation of NADH in the presence of pyruvate.

Results: Brain ischemia significantly decreased resistance of isolated cortex and cerebellum mitochondria to calcium-induced mPTP opening and inhibited respiration rate in state 3 with substrates pyruvate plus malate and succinate. However, Rot infusion to *vena cava* before ischemia re-established CRC of both, cortex and cerebellum mitochondria to control level. Rot infusion also protected against ischemia-induced inhibition of state 3 respiration with succinate of cerebellum mitochondria, while state 3 respiration of cortex mitochondria with succinate as well as respiration of cortex and cerebellum mitochondria with pyruvate and malate was not changed with Rot after ischemia. LDH activity as necrosis marker was increased in perfusate of both cortex and cerebellum after ischemia comparing to control, however, Rot infusion did not prevent necrosis in both brain regions.

Conclusions: These results demonstrate that Rot protects against 120 min ischemia-induced mPTP opening but had no effect on necrotic cell death.

P6-31

Effect of kisspeptin on calcium signalling in cultured rHypoE-8 cell line

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Aim: Kisspeptin neurones in hypothalamus has crucial role in activation of GnRH neurones. rHypoE-8 cell line is used as a model of kisspeptin neurons. The aim of this study was to investigate the effect of kisspeptin on kispeptin concentration and intracellular calcium level ($[Ca^{2+}]_i$) by using *in vitro* calcium imaging system in cultured rHypoE-8 cells.

Methods: These cells were incubated under normal conditions (95% O_2, 5% CO_2, at 37± 1°C) until plates were 80–90% confluent for kisspeptin hormone secretion studies. The medium was then removed and plates were rinsed three times with serum-free media, and replaced with serum-free DMEM 24h prior to experiments. Cells were challenged with DMEM (vehicle control), 100 nmol/L kisspeptin for 15, 30 and 60 minutes. Media (50 µL) was collected at each time point to monitor changes in kisspeptin secretion. Kisspeptin ELISA kit was used for determining kisspeptin concentrations. In the other protocol, rHypoE-8 cells were plated on glass coverslip and loaded with 1µM Fura-2 AM. $[Ca^{2+}]_i$ responses were quantified by the changes in 340/380 ratio.

Results: Kisspeptin caused a significant increase in basal levels of $[Ca^{2+}]_i$ after application at doses of 10 nM ($n=33$, $p<0.01$) and 100 nM ($n=42$, $p<0.001$). The stimulatory effect of kisspeptin (100 nM) on $[Ca^{2+}]_i$ was persistent in Ca^{2+} free conditions ($n=45$, $p<0.01$). Kisspeptin (100 nmol/L) increased kisspeptin concentration at 15^{th} min ($n=7$, $p<0.01$), 30^{th} min ($n=7$, $p<0.02$) and 60^{th} min ($n=7$, $p<0.05$) compared to control.

Conclusions: We demonstrated that kisspeptin activates intracellular calcium signalling in kisspeptin neurons in a dose dependent manner and extracellular Ca^{+2}- free condition.

P6-32

Elaboration of the method to determine brain tumor boundaries

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Aim: Brain tumors (gliomas) represent an important target for medical treatments. The present study was designed to find the electrophysiological differences between normal and pathological tissues to develop a method for intraoperative diagnostics of gliomas' boundaries.

Methods: Dissociated hippocampal cultures were prepared from C57BL/6J mouse embryos (E18); astrocytic cultures were prepared from C57BL/6J mice (P2); gliomas cell cultures were prepared from rat's cell line 2211. Field potentials were induced in response to electrical stimulation (single or 5 stimuli at 50Hz frequency), excitatory amino-acid transporters currents were induced by application of L-glutamate. *In vivo* field recordings were held on rats with transferred brain tumors.

Results: Absence of the field potentials in astrocytic and glioma cultures is due to lack of the neuronal cells and processes, which are capable to conduct electrical signals. However, transporter currents recorded in the gliomas cells represents the same stoichiometry as in the normal tissue. Notably, *in vivo* studies reveal an absence of electrical conductivity among gliomas tissue, whenever the intact tissue indicate a clear change in the electrical potential. Moreover, it demonstrates the propagation of stimulus from tumor region to non-damaged area and absence of propagation in the opposite case.

Conclusions: The electrophysiological differences in normal and pathological tissues give us a key to detect the boundaries of brain tumors during the operative resection.

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P6-33

Improved abnormal brain electrical activity via HMG-CoA reductase inhibitor rosuvastatin and the role of PI3K/Akt pathway

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Aim: Epilepsy is a common and chronic neurological disorder worldwide. Statins are competitive inhibitors of HMG-CoA reductase. Recently several experimental and clinical studies have shown that statins have anti-inflammatory properties and exert a number of neuroprotective actions. Among HMG-CoA reductase inhibitors, rosuvastatin was found the most effective statin. In this study, the effects of rosuvastatin on penicillin-induced seizures and the involvement of the PI3K/Akt pathway were electrophysiologically examined.

Methods: Wortmannin, a PI3K inhibitor, was administrated alone and together with rosuvastatin. Electrocorticogram (ECoG) was recorded for monitoring seizure activity then Fast Fourier Transform (FFT) analysis was performed. Changes in total spectral power density (PSD) were calculated. Adult male Sprague Dawley rats were divided into following groups: 1. vehicle (100%DMSO, i.c.v.); 2. wortmannin (0.1 mM dissolved in 100%DMSO, i.c.v.); 3. rosuvastatin (20mg/kg, oral gavage) 4. wortmannin and rosuvastatin. Rosuvastatin was given 3 days before; wortmannin was administered 30 min prior to penicillin injection.

Results: PSD analysis demonstrated increased spectral power at higher frequencies during seizures (5-10 Hz). Rosuvastatin, when applied alone, decreased seizure activity 76±9.0% within the first hour ($n=5-6$ per group, $p<0.05$) in comparison with all groups. Especially, the spectral power in the 4-6 Hz range was significantly lower in rosuvastatin group compared to vehicle and wortmannin groups ($p<0.05$). On the other hand wortmannin effect has become significant 30 min after rosuvastatin effect. There was a time lag between the effect of rosuvastatin and wortmannin.

Conclusions: In conclusion, rosuvastatin revealed early and sustained seizure attenuation. Although the PI3K/Akt pathway suggests a logical candidate for mediating seizure activity, early seizure

attenuation in rosuvastatin groups suggests that this effect may not be related to PI3K/Akt pathway.

P6-34

Midkine, TNF-α and cytochrome C expressions in kainic acid-induced epileptic seizures in rats

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Aim: Midkine (MK) is a neurotrophic factor, which is extensively expressed, in embryonic nervous system. It has been shown that MK expression is increased after brain insult; however it is not known if it is an endogenous repairing mechanism preventing further damage. The aim of the project is to investigate the possible role of MK and evaluate MK, TNF-α and cytochrome C expression levels during acute and subacute phases of epileptic seizures induced by Kainic Acid (KA) in cortex and hippocampus.

Methods: Adult male Sprague/Dawley rats were used in this study. 0.4mg/0.2µl KA was injected to hippocampus to induce seizures. The seizures were observed by EEG and behaviorally. Single dose injection of kainate receptor agonist KA in hippocampus induces pathologies similar to human temporal lobe epilepsy. The project included 3 groups: 1. Vehicle group, 2. KA-1 day (decapitation 1 day after KA injection), 3. KA-3 days (decapitation 3 days after KA injection). ELISA was used to determine MK, TNF-α, cytochrome C expressions; TUNEL and cresyl violet staining were used to determine damaged neurons.

Results: We found that MK expressions increased in cortex of KA-1 day group (1.1 ng/dl to 1.5 ng/dl, $p<0.05$) however turned back to its control level after 3 days (1.0 ng/dl, KA-3 group). The expression level of TNF-α level elevated in both cortex regions of KA-1 and KA-3 days groups. However, cytochrome C expression elevated both hippocampus and cortex in the left hemisphere of KA-1 and KA-3 days groups.

Conclusions: This project is suggested that the expression of MK may play an important role in the acute phase of physiopathological mechanism of KA induced epileptic seizures.

7. Renal physiology

P7-1

Effects of silymarin on methotrexate-induced nephrotoxicity in rats

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Aim: Methotrexate (MTX) is widely used in the treatment of various malignancies and nononcological diseases but its use has been limited by its nephrotoxicity. Silymarin (SLY), a natural flavonoid, has been reported to have antioxidant, anti-inflammatory and anti-apoptotic effects. This study was carried out to determine whether SLY exerts a protective effect against MTX-induced nephrotoxicity.

Methods: 36 Rats were divided into six groups: Group 1 (saline, i.p., single injection), Group 2 (0.5% carboxymethyl cellulose (CMC), by gavage once daily for 5 consecutive days), Group 3 (SLY, 300 mg/kg per day,p.o., for 5 consecutive days) Group 4 (MTX, 20 mg/kg, i.p., single injection), Group 5 (MTX+CMC similarly as groups 2 and 4) and Group 6 (MTX+CMC+SLY similarly as groups 2, 3 and 4). Histopathologic alterations including apoptotic changes in the kidney were evaluated.

Results: MTX injection exhibited dilated Bowman's space, inflammatory cell infiltration, glomerular and peritubular vascular congestion and swelling of renal tubular epithelium cells. Apoptotic cell death was also markedly increased in renal tubules after MTX administration. SLY treatment resulted in statistically significant amelioration in the histological alterations and reduced the number of TUNEL-positive cells as compared with the MTX treated rats ($P<0.05$).

Conclusions: SLY treatment leads to a reduction on MTX-induced renal damage in rats. Since SLY is safe and acceptable for human consumption, further studies to define the exact mechanism of the protecting effect of SLY on MTX-induced nephrotoxicity and the optimum dosage of this compound would be useful.

P7-2

Expression of ghrelin and GHS-R1a in long term diabetic rat's kidney

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Aim: Ghrelin, a hormone with 28 amino acids, binds to the growth hormone secretagogue receptor (GHS-R) and has multiple functions. Diabetes mellitus affects the ghrelin levels in different tissues. In this study, we focused on relative ghrelin and GHS-R1a gene expression in the kidney of long-term diabetic rats.

Material and Methods: A total of 40 male Wistar albino rats were divided into four groups: C- control group, DI- one month diabetic rats group, DII- two month diabetic rats group and DIII- three month diabetic rats group. Diabetes was induced by streptozotocin STZ (40mg/kg i.p). The rats were decapitated under ketamine anesthesia and their kidney tissues were removed. Tissue GHS-R mRNA levels, ghrelin expression, and histopathological damage scores were then compared.

Results: At the end of the experiment, dilatation in the distal tubules, epithelial desquamation into the lumen of the tubules and transparent tubules showing glycogen vacuolation were observed in all diabetic groups. Ghrelin immunoreactivity was significantly greater in group DI compared to group C, whereas in group DII and group DIII ghrelin immunoreactivity was similar with group C. GHSR-1a mRNA level in group DIII was significantly lower than in group C.

Conclusions: As a result, ghrelin immunoreactivity is increased at the beginning of diabetes; however with increase in duration of diabetes ghrelin immunoreactivity approaches to control values. In addition, expression of GHSR-1a mRNA is decreased with increase in diabetes duration. It seems that down-regulation of GHSR-1a contributes to the renal damage induced by long-term diabetes.

P7-3

The effects of erdosteine on experimental myoglobinuric acute kidney injury in rats

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Aim: In our study, we aimed to investigate the effects of erdostein on myoglobinuric acute kidney injury that has been reported to have antioxidant, free radical scavenger properties.

Methods: In our study, 28 male Spraque Dawley rats, 145-185 grams in weight, were used. The rats were deprived of water for 24 hours before injection. 1st and 2nd groups were injected with physiological serum, 3rd and 4th groups were injected with intramuscular 50 % glycerol 8 ml/kg. One hour later, 1st and 3rd groups received ip FS and 2nd and 4th groups were given 10 mg/kg erdosteine orally. 24 hours after the glycerol injection, the blood samples and kidneys of the rats were taken under the anesthesia.

Results: We found that the levels of urea, creatinine, potassium, ALT, AST and CK in serum samples, and MDA in renal tissue samples, and fractional excretion of sodium were increased in the 3rd group when it's compared with the 1st group ($p<0.05$). When the parameters of 3rd and 4th groups compared, in group 4, a significant increase in serum sodium and creatinine clearence were observed, however, there were a significant decrease in the levels of kidney NO, and serum potassium and creatin kinase, and fractional sodium excretion ($p<0.05$).

Conclusions: In this study, measurable protective effects of erdostein were detected. Given these results, to determine the protective effect of erdostein on myoglobinuric acute kidney injury in this model, we concluded that after determining the effective dose of erdostein more comprehensive study should be planned targeting the treatment time modality.

P7-4

Erythrocyte rheological properties in peritoneal dialysis patients with chronic renal disease

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Aim: In this study, we focused on the influence of peritoneal dialysis on two of the hemorheological parameters, blood viscosity and erythrocyte aggregation, in peritoneal dialysis patients.

Methods: This study included 35 volunteers (20 patients/15 controls). For hemorheological measurements, 10 ml venous blood from each patient was used. Blood viscosity was measured using a cone/plate viscometer. LORCA system was used to determine erythrocyte aggregation. Erythrocyte aggregation was measured in autologous plasma and dextran70 solution. Three indices of RBC aggregation were measured: (i) total change in aggregation signal (AMP); (ii) time required for half maximal change in aggregation signal (aggregation half time; $t_{1/2}$); (iii) the extent of RBC aggregation (aggregation index; AI).

Results: Hematocrit was significantly lower in peritoneal dialysis patients than the control group. Blood viscosity at native hematocrit was significantly decreased at all shear rates in the dialysis patients compared to the control. However, although viscosity was higher at each of the shear rates in the patients than the control at corrected haematocrit (45%), difference was statistically significant only at the shear rates of 20 Pa and 40 Pa. Plasma viscosity was found significantly higher in the patients than control. The AI and $t_{1/2}$, were found significantly different in the peritoneal dialysis patients in plasma. AMP was found significantly different in the peritoneal dialysis patients in Dextran solution.

Conclusions: Our results suggest that although blood in peritoneal dialysis patients does not get exposed to any blood-contacting equipment, these patients still have a circulatory risk due to deteriorated plasma constituents.

P7-5

The protective effect of melatonin on carbon tetrachloride (CCl_4) – induced nephrotoxicity in rats

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Aim: To evaluate the protective effect of melatonin on CCl_4-induced nephrotoxicity in rats.

Methods: Adult male Wistar albino rats weighing 200-220 g were used. The rats were divided into 4 groups (control group, CCl_4 group, 10 week CCl_4 + melatonin and 12 week CCl_4 + melatonin). CCl_4 (1.5 ml/kg, twice a week) and melatonin (10mg/kg, daily) were administered subcutaneously. The duration of the experiment was 10-12 weeks. Kidney tissues were removed and routine histological procedures were applied. Sections were stained by Hematoxylin and Eosin (H&E), Masson's trichrome and Periodic Acid Shiff (PAS) and were examined by light microscopy.

Results: In the **CCl_4** treated rats, basement membrane thickening and glomerular congestion in the Bowman's capsule were observed. Additionally, loss of proximal tubule brush borders, swelling and detachment of tubular epithelial cells were noted. Collagen fiber accumulation in the tubulointerstitial area of cortex and corticomedullary region was found in some sections. In the melatonin treated CCl_4 groups, renal damage was reduced. There was no noticeable difference between 10 and 12 week melatonin treatments.

Conclusion: Melatonin might ameliorate **CCl_4** induced nephrotoxicity in rats.

Keywords:
Rat, Kidney, Carbon tetrachloride, Nephrotoxicity, Melatonin.

P7-6

Cyclosporine and MMF use in combination in patients after renal transplantation

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Aim: Cyclosporine (CsA) inhibits the enterohepatic (re)circulation of mycophenolic acid (MPA) and its

inactive mycophenolic acid glucuronide (MPAG) metabolite resulting in significantly lower dose-corrected MPA concentrations in CsA-treated patients and early clinical MPA under exposure in 50%. The need to double the dose of mycophenolate mofetil (MMF) in case of CsA co-administration to achieve the same MPA levels has been attributed. Aim of this study was to assess cyclosporine influence on MMF pharmacokinetics and dosing.

Methods: 12 hour total serum concentration-time profiles of MPA and 12 hour total blood concentration-time profiles of CsA were obtained after an oral administration. MPA concentration was determined by a validated HPLC method and CsA - by using a LC-MS method. The AUC $_{(0\text{-}12)}$ was calculated using a Bayesian estimator and a 3-point limited sampling strategy.

Results: The study included 3 groups of patients (post transplantation time >1 year): receiving CsA and MMF (n=21 patient; CsA 200 mg/day and MMF 2000 mg/day) (i), receiving only CsA (n=9 patients; 200 mg/day) (ii) and receiving only MMF (n=7 patients; 1000 - 3000 mg/day) (iii). C_0, AUC $_{(0\text{-}12)}$, C_{max} were compared between the groups. CsA pharmacokinetic parameters were compared between the first and the second group and the MMF pharmacokinetic parameters were compared between the first and the third group. There were no significant differences. Only MMF dose was 28.6% higher in MMF monotherapy group (1428.57 mg (±731.93 SD) and 2000.00 mg (±0.00 SD), $p<0.05$ ($p=0.01$)).

Conclusions: The survey results show that cyclosporine affects the pharmacokinetics of MMF. Cyclosporine used in combination with MMF increases the need of MMF dose for 28.6%. No other significant differences between pharmacokinetic parameters were observed.

8. Cellular and molecular physiology

P8-1

Calcium signaling evoked by beractant in normal human lung fibroblasts

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Aim: Beractant, an exogenous lung surfactant, induces an antifibrotic phenotype in normal human lung fibroblasts (NHLF) and promote fibroblast apoptosis. Because Ca^{2+} signaling is implicated in the onset of apoptosis as well as in phenotypic switch, we aimed to assess the effect of Beractant on $[Ca^{2+}]i$ in primary cultures of NHLF.

Methods: NHLF were obtained from kidney donors with brain death and no history of smoking or lung disease and previous signed consent of the family. Cultured NHLF were loaded with 3 μM Fura-2 and Ca^{2+} signals were recorded by microfluorimetric techniques.

Results: Beractant causes a concentration-dependent increase in $[Ca^{2+}]i$. The Ca^{2+} signal evoked by Beractant consist in an initial spike which could be followed by Ca^{2+} oscillations or a sustained plateau. The initial Ca^{2+} increase evoked by Beractant was abolished by store depletion by cyclopiazonic acid (CPA), blockage of phospholipase C (PLC) activity with U73122, inhibition of inositol 1,4,5-trisphosphate receptors (IP3Rs) with 2APB, but not by the inactive U73122 analog, U73343, and genisteine. Ca^{2+} entry contributes to the initial Ca^{2+} spike evoked by Beractant. The plateau and Ca^{2+} oscillations were blocked by removal of extracellular Ca^{2+} and by inhibitors of store-operated channels, such as 2APB and low concentrations of La^{3+} and Gd^{3+}, but not by Ni^{2+} or nifedipine.

Conclusions: Beractant activates a Ca^{2+} signal through Ca^{2+} release from intracellular stores mediated by PLC, Ca^{2+} release from IP3Rs and Ca^{2+} influx via a store-operated pathway.

P8-2

Ceranib-2 induces apoptosis in prostate cancer cells

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Aim: Ceramidase enzymes decrease the level of ceramide and this reduction causes resistance to apoptosis in cancer cells. We hypothysized that increase in the amount of ceramide by ceranib-2, a ceramidase inhibitor, may affect the survival and/or induce apoptosis of prostate cancer cells (LNCaP and DU145) *in vitro*.

Methods:
Cell number was determined with spectrophotometer and apoptosis with flow cytometry, and structural changes were examined both with confocal and transmission electron microscopies.

Results:
Comparing to the control, 25 and 50 µM ceranib-2 reduced the percentage of viable LNCaP cells down to 40 and 15 after 24 hr, and to 27 and 11 after 48 hr applications, respectively. When DU145 cells were cultured with the same doses of ceranib-2, numbers of living cell were about 41 and 18 % after 24 hr, and 8 and 5 % after 48 hr, respectively. Observed early apoptotic rates of LNCaP cells were 5 and 36 % after 24 hr, and 15 and 60 % after 48 hr treatments with 25 and 50 µM ceranib-2, respectively. The signs of apoptosis were detected as chromatin condensations, fragmented nuclei and cytoskeleton lacerations in the cells.

Conclusions:
Ceranib-2 posseses a dose and time dependent survival decreasing effect on both prostate cancer cell lines. Although most of the toxic function of ceranib-2 on LNCaP cells was due to apoptosis, it was not due to only apoptosis on DU145 cells.

P8-3

Inflammatory expression profiles after targeted treatments of experimental meconium-induced respiratory failure

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Aim: Meconium aspiration syndrome (MAS) is a serious life-threatening condition of the neonates. Therapy of MAS is based on the support ventilation and administration of exogenous surfactant into the lungs of patients. However, aspirated meconium initiates local inflammation processes, which may inactivate surfactant and reduce the effect of therapy. Adding of anti-inflammatory agents – budesonide (B) or N-acetylcysteine (NAC) into the surfactant therapy should suppress development of inflammation and contribute to better efficiency of the combined therapies in experimental MAS.

Methods: New Zealand rabbits with meconium-induced respiratory failure were divided into the groups according to the therapy (n=6 each): non-treated (M), surfactant therapy (M+S), anti-inflammatory therapies (M+B, M+NAC) and combined therapies (M+S+B, M+S+NAC), or controls with saline instead of meconium (C). After sacrificing the animals, lung oedema (lung tissue wet/dry weight ratio) and inflammatory expression profiles (IL-2, -6, -10, -13, TNFα) in lung homogenates using RT-PCR were determined.

Results: Combined therapies M+S+B and M+S+NAC reduced W/D ratio ($p<0.05$ *vs*. M, M+B, M+S) and decreased expression of monitored cytokines compared to untreated group ($p<0.01$ for M+S+B, M+S+NAC *vs*. M), and this significant change was seen at TNFα, IL-2, IL-10 compared to M+S ($p<0.05$ for M+S+B).

Conclusions: Adding of anti-inflammatory agent to surfactant therapy may have beneficial effect on formation of lung oedema and expression of inflammatory markers, as reflected also by better respiratory functions and outcome of patients. The results indicate that use of anti-inflammatory therapy may prevent surfactant inactivation and contribute to enhanced therapy effectiveness of MAS.

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P8-4

Ceranib-2 inhibits the proliferation and induces apoptosis of glioma cells in vitro

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Aim: We questioned whether increased level of ceramide by ceranib-2, a novel ceramidase inhibitor, affects the survival and/or promotes apoptosis of glioma cell lines (T98, U87 and C6) *in vitro*.

Methods: Cells were treated with 0.1, 1, 5, 10, 25 and 50 µM ceranib-2 doses for 24 and 48 hr. Cell viability and apoptosis were determined with MTT test and flow cytometry.

Results: Application of respective ceranib-2 doses for 24 hr inhibited the number of alive T98 cells by 9, 38, 48, 53, 54 and 61 %; U87 cells by 2, 4, 11, 59, 80 and 82 % , and C6 cells by 7, 15, 27, 30, 31 and 40 %. Culturing of these cells for 48 hr reduced further the numbers of cells by 10, 53, 60, 62, 64 and 70 %; 7, 10, 15, 62, 83 and 96 %, and by 7, 15, 30, 41, 40 and 64 %, respectively. After treatments with more toxic doses, 25 and 50 µM, the early apoptotic rates of T98 and C6 cells were 6 and 8 %; 8 and 23 % for 24 hr, 15 and 13 % and 19 and 52 % for 48 hr, respectively. Early apoptotic rates of U87 cells were 11 and 37 % after 24 hr and 18 and 7 % for 48 hr fallowing 10 and 25 µM ceranib-2 applications.

Conclusions: Differing with the type of cell, ceranib-2 posseses a strong dose and time dependent survival decreasing effect on glioma cells via apoptosis.

P8-5

Effect of s-nitroso-n-acetyl-penicillamine (SNAP) on the inflammation and oxidative stress in experimental model of acute lung injury

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Aim: To evaluate effect of treatment with soluble donor of nitric oxide (SNAP) on lung inflammation and oxidative stress in experimental acute lung injury (ALI).

Methods: ALI was induced by repetitive saline lung lavage (30 ml/kg) in New Zealand rabbits. Animals were randomly divided into 3 groups: healthy controls (Control); animals with ALI non-treated (ALI) and treated with SNAP (ALI+SNAP) (7 mg/kg). After 5 hours of ventilation animals were sacrificed. Total and differential counts of cells in blood and bronchoalveolar lavage fluid (BAL) were measured. Concentrations of markers of oxidative stress (TBARS), inflammation (IL-1, -6, and -8), epithelial (esRAGE) and endothelial (S1PR3) damage in the lung tissue, nitrites/nitrates in plasma, and mRNA expresion of iNOS were analysed.

Results: In ALI group, higher counts of cells, mainly neutrophils, in BAL fluid and overproduction of pro-inflammatory substances were observed compared to controls. In SNAP-treated group, reduced leak of cells into the lung was observed and markers of oxidative stress, inflammatory and tissue injury and mRNA expression of iNOS decreased.

Conclusions: SNAP treatment had beneficial effect on inflammation and oxidative stress in ALI model.

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P8-6

Determination of proliferation effect of deguelin on glioma cell lines

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Aim: Glioblastoma multiforme is the most common and very aggressive type of brain tumors. Isolated from several plant species deguelin is a natural plant rotenoid. Several studies have shown that it is a promising cancer-preventive and therapeutic agent. Since the function of deguelin on glioma cells has not been defined yet, we decided to test its possible proliferative effect on rat (C6) and human (T98G) glioma cell lines.

Methods: The cells were inoculated at a concentration of $2x10^4$ cells/well. The next day, they were treated with 1, 10, 25, 50, 75, 100, 150, and 200 µM deguelin for 24 or 48 hr. At the end of incubation periods, cell survival was measured with 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Mossman.
Results: Deguelin decreased the survival of both cell lines and IC_{50} value of C6 was calculated as 132 µM, but IC_{50} of T98G cells could not be detected after 24 hr incubation. IC_{50} values after 48 hr incubations were found to be 46 µM and 38 µM for C6 and T98G cells, respectively.

Conclusions: Deguelin is dose and time dependently cytotoxic to C6 and T98G glioma cells *in vitro* and these effects did not clearly differ between the two cell lines.

P8-7
High efficacy but low potency of delta-opioid receptor-G protein coupling in Brij58-treated low-density membrane domains

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Aim: Membrane domains (plasma membrane compartments resistant to solubilisation by non-ionic detergents and enriched in cholesterol and glycosphingolipids) play important role in processes of signal transduction. In this study we tried to improve the methodological conditions for preparation of functional membrane domains with preserved coupling between delta-opioid receptors (DOR) and G proteins.

Methods: HEK293 cells stably expressing PTX-insensitive DOR-Gi1alpha (C^{351}-I^{351}) fusion protein were homogenized, exposed to increasing concentrations of non-ionic detergent Brij58 and fractionated by flotation in sucrose density gradient. Isolated membrane domains were analysed by radioligand binding of [^{3}H]DADLE (DOR agonist), G protein activity was determined by [^{35}S]GTPgammaS binding assay. Direct effect of Brij58 on the hydrophobic interior of plasma membrane was studied by measurement of steady-state fluorescence anisotropy of DPH.

Results: In optimum range of detergent concentrations (about 0.025% w/v), Brij58-treated, low-density membrane domains exhibited 2-3-fold higher efficacy of DADLE-stimulated [^{35}S]GTPgammaS binding and 2-fold lower level of DOR than membrane domains prepared in the absence of detergent. The affinity of agonist response (potency) was decreased by one order of magnitude in Brij58-treated membrane domains. High detergent concentration resulted in drastic diminution of [^{35}S]GTPgammaS binding and [^{3}H]DADLE binding. The measurement of steady-state DPH anisotropy indicated that Brij58 exhibited a strong "fluidization" effect of hydrophobic plasma membrane interior.

Conclusions: Limited perturbation of plasma membrane structure by exposure to low concentrations of non-ionic detergent Brij58 results in increase of the efficacy but decrease of the potency of DOR-G protein coupling. The total degradation of plasma membrane structure at high detergent concentrations results in diminution of functional coupling between DOR and G proteins.

P8-8
The effects of ghrelin on oxidative stress parameters in lipopolysaccharide induced sepsis of rats

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Aim: There were demonstrated that ghrelin was to protect organ injury during sepsis. It was shown in different studies that ghrelin treatment was decreased levels of thiobarbituric acid reactive substances (TBARS) and increased the levels of antioxidant enzymes such as Superoxide dismutase (SOD) by reducing Reactive Oxygen Species (ROS). We aimed to investigate the effects of exogenously given ghrelin on TBARS and SOD levels, as known indicators of oxidative stress, Lipopolisaccharide (LPS) treated of rats in liver tissue.

Material and Methods: In our study, male *Wistar albino* rats were separated into four groups including; Control (n=10), LPS (E.coli O127:B8, 5 mg/kg). Ghrelin (10 nmol/kg, n=10), LPS + Ghrelin (n=10). Rats were decapitated 24 hours after first enjection.

Results: In our study, liver SOD activity in LPS and LPS + ghrelin groups were significantly increased compared to that of control group ($p<0.001$). Also, we observed that both of the levels of Ghrelin and

TBARS were higher in LPS group than the other experimental groups ($p<0.01$).

Conclusion: We suggested that exogenously given ghrelin may be shown antioxidant activity by increasing SOD and TBARS levels on liver tissue depending on Ghrelin's dosage and duration in rats with treated LPS.

P8-9

Neuroprotective effect of the combined treatment with resveratrol and hypothermia in preventing apoptosis in a neonatal rat model of hypoxic-ischemic encephalopathy

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Aims: The main objective of this study was to test the protective effect of resveratrol and mild hypothermia in HI encephalopathy at new-born rats. The changes in histology and apoptosis were determined in different brain regions to assess the local damages.

Materials and methods: The experiment was performed on 32 new-born Wistar rats pre-treated with resveratrol in a dose of 20 mg/kg/day for seven days. At the end of this period the animals were exposed to hypoxia (9% O_2, 90 minutes) and ischemia (ligation of right carotid artery). To test the effect of combined therapy of resveratrol with hypothermia, several animals were exposed after HI injury to hypothermia (with 4 °C) for 3 h.

Results: In global HI encephalopathy resveratrol offers neuroprotection by reducing the number of cells expressing apoptosis in CA1, CA2, CA3 and dentate gyrus of the hippocampus and cerebral cortex under the conditions of conjugation with post-injury hypothermia.

Conclusion: The results of this study prove that resveratrol offers neuroprotection in HI brain injuries, but the protection is conditioned in most of the brain regions by conjugation of the protective therapy with post-injury hypothermia treatment.

P8-10

Hereditary spherocytosis associated with defects of human red blood cell membrane

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Aim: Hereditary spherocytosis is characterized by the morphologic transformation of erythrocytes into a spherical shape due to hereditary defect of cell membrane proteins associated with disruption of the skeletal structures of erythrocyte membrane proteins (ghost). Contrary to the literature, pores were detected in the erythrocytes of a patient with hereditary spherocytosis. The aim of our current study was to determine the deficient proteins that were responsible for the pores.

Methods: Ghost isolation was performed to determine the proteins responsible for pores observed on the patient's erythrocytes. Erythrocyte membrane proteins were visualized using SDS-PAGE. Quantitative protein assessments were conducted using matrix-assisted laser desorption/ionization mass spectrometry (Maldi Tof MS) to determine the patient's membrane protein deficiencies. A difference was detected in the comparative analysis of the erythrocyte membrane proteins following SDS-PAGE. Deficient proteins in the erythrocytes were identified as result of proteomic analyses.

Results: Ghost obtained from the patient's blood was visualized using an SEM smear preparation. Maldi Tof MS analysis was done in order to interpret the pathophysiologic mechanism of the erythrocytes. It was found that Band 3 and protein 4.2, which play a particular role in membrane structure, were decreased 4.573 and 4.106 fold, respectively, compared with controls.

Conclusions: We believe that the pores seen in the morphology of the erythrocytes developed due to diminishment of these proteins, which reside in the erythrocyte membrane structure.

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P8-11

Cytotoxic effect of flavonoids combined with imatinib on human chronic myeloid leukemia cell lines

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Aim: Flavonoids are present in the human diet and isolated from various plant extracts.Some flavonoids show great potential as cancer chemopreventive agents in cell culture studies. The present study was designed to evaluate possible chemopreventive effects of apigenin, luteolin and the recently synthesized N-desmethyl sinensetin (6-hydroxyluteolin 6,7,3',4'-tetramethyl ether) cytotoxicity on human chronic myeloid leukemia cells K562 and its combination with imatinib.

Methods: Cell proliferation was detected by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphentyltetrazolium bromide) assay.The cell viability was assayed based on the ability of live cells to reduce MTT.The concentrations of the apigenin and luteolin at which 50% of the cell die (IC50) were calculated.
K562 cells were treated with apigenin, luteolin, sinensetin and imatinib mesylate (anticancer drug).

Results: Concentrations of apigenin, luteolin and sinensetin ranging from 25 to 200 µM and imatinib 5 to 50 µM for 72 h was studied. The results indicated significant cytotoxic activities with apigenin IC_{50} 140 µM, luteolin IC_{50}: 100 µM, imatinib IC_{50}:5 µM for the same incubation period, against K562 leukemia cells. The cytotoxic potency of sinensetin at >200 µM concentration was lower than those of apigenin and luteolin.

Conclusions: Finally, the combination of these flavonoids and imatinib mesylate were able to enhance cytotoxic effect on K562 cells**.** Our results suggest that the flavones could be considered as chemotherapeutic agents which may help to prevent human chronic myeloid leukemia.

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P8-12

The role of different connexins in formation of tunneling tubes and transfer of siRNA between remote HeLa cells

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Aim: Membranous tunneling tubes (TTs) are a form of intercellular communication between remote cells allowing their electrical synchronization, migration and even transfer of cytosolic components. The aim of our study was to determine the role of different connexins (Cxs) in cell mobility, formation and properties of TTs between remote HeLa cells and permeability of TTs to siRNA.

Methods: Experiments were performed on HeLa cells stable transfected with Cxs fused with green or cyan fluorescent proteins (Cx36-EGFP, Cx43-EGFP, Cx40-CFP) or Cxs without fluorescent marker (Cx45, Cx47). The role of different Cxs was investigated using time-lapse and fluorescence imaging, wound healing assay and dual whole-cell patch clamp methods.

Results: The geometry and morphology of TTs were not influenced by expression of Cxs; however, Cx36-EGFP-expressing cells formed more TTs and Cx43-EGFP-, Cx45- or Cx47-expressing cells less TTs compared with HeLa wt cells. Wound healing was faster in Cx36-EGFP- and Cx40-CFP-expressing cells compared with wt or Cx43-EGFP-, Cx45- and Cx47-expressing ones. TTs containing Cx40-CFP, Cx43-EGFP and Cx47 gap junctions were permeable to small interfering RNA (siRNA) while containing Cx36-EGFP and Cx45 were not. Human mesenchymal stem cells were capable of coupling to cancer cells through TTs containing Cx43 gap junctions.

Conclusions: Our results suggest that different Cxs may modulate the mobility of cells and formation of TTs in opposite manner. SiRNA transfer through the GJs-containing TTs is Cx isoform-dependent. These results contribute to the knowledge about mechanisms of cancer invasion and metastasis and suggest new approaches for targeted gene-based cancer treatment.

P8-13

The role of N-terminal glutamates in modulation of connexin36 gap junction channel conductance by intracellular pH and magnesium ions

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Aim: Gap junctions (GJ) formed of connexin 36 (Cx36) are strongly regulated by pH_i and $[Mg^{2+}]_i$. Due to the active carboxyl group, the glutamates may participate in Mg^{2+} and H^+ binding. Therefore, our aim was to examine the role of glutamates in the regulation of Cx36 channels by $[Mg^{2+}]_i$ and pH_i.

Methods: Glutamates E8 and E12 in the N-terminal domain of Cx36EGFP protein were replaced with glutamines (Q) and respective cell lines expressing Cx36EGFP mutants were prepared. The dependence of electrical conductance (g_j) of *wt* and mutant Cx36EGFP channels on pH_i was measured at different $[Mg^{2+}]_i$ using dual whole-cell patch-clamp and fluorescence microscopy techniques.

Results: Earlier we have shown that high $[Mg^{2+}]_i$ (5 mM) decreased and low $[Mg^{2+}]_i$ (0.01 mM) - increased the g_j of Cx36EGFP *wt* channels. In this study we found that the dependence of g_j of Cx36EGFP *wt* channels on pH_i was non-monotonic with $g_{j,max}$ at pH_i=7.2. Moreover, this dependence was shifted to the left at high $[Mg^{2+}]_i$ and to the right – at low $[Mg^{2+}]_i$. Under control $[Mg^{2+}]_i$ (1 mM), E8Q mutation reduced and E12Q mutation increased the inhibitory effect of alkalization, while either mutation potentiated the inhibitory effect of acidification on g_j. At high $[Mg^{2+}]_i$ (5 mM), either mutation reversed the stimulatory effect of acidification to the inhibitory one but did not modify the effect of alkalization. The experiments with low $[Mg^{2+}]_i$ (0.01 mM) are in progress.

Conclusions: E8 and E12, both are involved in regulation of the non-monotonic dependence of Cx36EGFP g_j on pH_i by intracellular magnesium ions.

P8-14

The effects of CAPE on the oxidant status of the liver and the serum in rat model of acute methanol intoxication

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Aim: We aimed to investigate the effects of caffeic acid phenethyl ester (CAPE) on the total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) parameters of the liver and the serum in a rat model of acute methanol intoxication.

Methods: Rats were treated with intraperitoneal (i.p.) Methotrexate (MTX) for 7 days. On the 8th day, i.p. methanol was administered in the methanol, ethanol and CAPE groups. Four hours after methanol treatment, Ethanol was injected i.p. in the ethanol group; CAPE (i.p.) in the CAPE group; serum physiologic i.p. in other groups. After eight hours, rats were sacrificed and the serum and the liver samples were obtained for biochemical analyses.

Results: The OSI value was significantly higher in the methanol group compared to the ethanol and CAPE groups. Serum TAS levels of the methanol group were significantly different compared to the control group, but not compared to the MTX group. The amelioration of oxidative stress was greater in the CAPE group compared to the ethanol group but was not statistically significant.

Conclusion: This study demonstrates that CAPE treatment ameliorates oxidative stress in the serum and liver in a rat model of acute methanol intoxication.

P8-15

Curcumin decreases HO-1 and COX-2 expression and sensitizes pancreatic cancer cells to gemcitabine via CUGBP2 mediated post-transcriptional regulation pathway

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Aim: Pancreatic adenocarcinoma is highly resistant to conventional chemotherapy, thus the aim of this study was to assess the effects of curcumin on CUGBP2 mediated post-transcriptional regulation of cyclooxygenase2 (COX-2) and hemooxygenase1 (HO-1) in human pancreatic cancer cells, response to gemcitabine (GEM) and cell viability.

Methods: Expression of CUGBP2, COX-2 and HO-1 were evaluated using QRT-PCR and Western blot methods. Cell viability after treatment with GEM and/or curcumin was evaluated using MTT and crystal violet tests.

Results: CUGBP2 expression at mRNA level was lower 2.2 folds (p=0.007) but HO-1 expression was increased 6.9 (p=0.023) and COX-2 was increased 2.3 (p=0.046) folds in cancer tissues, compared to heath donor pancreas tissues. Curcumin increased CUGBP2 in MiaPaca2 pancreatic cancer cells, downregulated HO-1 and COX-2 expression, and strongly sensitized cancer cells to GEM.

Conclusions: Decreased activity of CUGBP2 could be associated with high chemoresistance and early dissemination of pancreatic cancer through the HO-1 and COX-2 mediated cytoprotective and carcinogenesis pathways. Curcumin significantly increases the effectiveness of GEM treatment *in vitro* via CUGBP2 mediated post-transcriptional regulation pathway.

P8-16

The protective effects of roflumilast in rat acute pancreatitis model

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Aim: The systemic damage in acute pancreatitis (AP) can be characterized by oxidative stress (OS) and the release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). Roflumilast has been shown to be a potent anti-inflammatory and anti-oxidant agent. In the present study, we aimed to investigate the effect of roflumilast in cerulein induced acute pancreatitis.

Methods: Thirty-two male Wistar rats were randomly divided into four equal groups; group 1 (sham-operated), group 2 (roflumilast), group 3 (AP), and group 4 (AP+ roflumilast). AP was induced in by injecting 4x75 µg/kg-body weight of cerulein intraperitoneally at an interval of 1 hour. Rats were sacrified at 12 hours following last cerulein administration. AP was confirmed by a serum amylase level elevation and typical inflammatory features observed histopathologically.

Results: Morphological changes consistent with tissue damage were observed in the pancreas of rats. Amilase levels were higher in Group 3 (2007.5±575.6) and 4 (2097±469.5) than Group 1 (566.1±196.9) and 2 (630.63±40.6) ($p<0.05$). Serum levels of TNF-α, IL-1β, and IL-6 significantly increased in the AP group (113.9±24, 837.7±244.9, 66.1±23.7, respectively) ($p<0.05$) and roflumilast decreased all these levels significantly (44.7±1.8, 420.1±24.2, 26.8±2, respectively) ($p<0.05$). Total oxidant status (TOS) was significantly higher and Total anti-oxidant capacity (TAC) was lower in the AP group in comparison with others ($p<0.05$). While roflumilast significantly decreased TOS, and increased TAC in comparison with AP group ($p<0.05$).

Conclusion: Roflumilast significantly decreased OS and inflammatory mediators in the cerulein induced pancreatitis in rats.

P8-17

Effects of ellagic acid on experimental acute pancreatitis in rats

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Aim: Acute pancreatitis (AP) is an inflammatory disease with high morbidity and mortality. Reactive oxygen species may play a pivotal role in the pathogenesis of AP. We aimed to investigate the possible ameliorating effects of ellagic acid (EA) against the harmful effects resulting from acute pancreatitis.

Methods: A total of thirty two Wistar-Albino rats were divided into four groups as control, control+EA, AP, and AP+EA. Acute pacreatitis was induced by L-arginin. After induction of AP, EA was applied via oral gavage at a dose of 85 mg/kg. Twelve hours later, experiment terminated and blood samples, remote organ tissue samples were obtained by laparotomy. In all groups, serum total antioxidant capacity (TAC), total oxidative status (TOS), TNF-α, IL-1β, IL-6, amylase levels were determined. Pancreas, liver, lung and kidney tissues were evaluated for histopathological changes.

Results: Acute pancreatitis were provided with high amylase level and histopathological eveluation of pancreas both AP and AP+EA groups. Compared to the AP group (0.69±0.10), serum TAC levels increased significantly in the AP-EA group (0.83±0.079) (p=0.003).
TOS levels were highest in AP group (1478.29±446.32). Compared to the AP group, serum TOS levels decreased significantly in the AP-EA group (804.14±140.54) (p=0.001). Serum levels of TNF- , IL-1β and IL-6 increased in the AP group and EA decreased all these levels significantly (p=0.009,p=0.002,p=0.004; respectively). Histopathological changes were supported our result.

Conclusions: These results suggest that ellagic acid reduced inflammation and oxidative stress induced by acute pancreatitis.

P8-18

Effects of seranib-2 on cell survival and tnf-alpha in colon cancer cell line

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Aim: Previous studies showed that anti-cancer drugs causes apoptosis by increasing the Ceramide level in cells. Ceramide level increases when the intracellular enzyme acid Ceramidase level decreases. Depression of the Ceramidase induced apoptosis and prevents progression of cancer in prostate, liver, lung, intestine and melanoma cancer cells. In this study we aimed to investigate the effects of a novel drug seranib-2 which is an anti-cancer drugs targeting the acid Ceramidase in colon cancer line.

Method: We detected the cell survival effects in 24 and 48 hours of 0, 1, 2, 5, 10, 25, 50 µM concentrations of seranib-2 colon cancer cells (Caco-2) via MTT technique. Effects of 25 and 50 µM concentrations on mRNA expression of TNF-alpha, TNFR1 and ASAH (acid seramidase) was detected via RT-PCR.

Results: Compared to that of control, the effect of Seranib-2 on survival decreased from the lowest doses and this decrease was significant in 50 µM concentration group in both 24 and 48 hours ($p<0.001$). mRNA amount of TNF-alpha showed no difference between groups, however TNFR1 significantly decreased in both concentrations in 24 hours compared to that of control ($p<0.05$) and we didn't see this decrease in 48 hours. mRNA amount of ASAH was significantly high in 50 µM concentration group in 48 hours compared to all groups ($p<0.001$).

Conclusion: Our study showed that Seranib-2 has a strong anti-cancer effect in a dose and time dependent manner.

P8-19

Caffeic acid phenethyl ester prevents fluoxetine-induced hepatotoxicity in rats

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Aim: Fluoxetine is a potent serotonin reuptake inhibitor that is commonly used and prescribed for neurological disorders as depression and anxiety disorder. Caffeic acid phenethyl ester (CAPE) is an active constituent of honey bee propolis resembling flavonoids in structure. In this study, we purpose the preventive effects of CAPE in hepatic failure due to long term use of fluoxetine that is to control of superoxide radicals in rats.

Methods: The effects of one week of Fluoxetine treatment on lipid and protein peroxidation, the activity of transaminases and transferases in the serum and tissue were investigated in rats Forty eight Wistar rats were divided into six equal groups of eight animals each: (1) controls (2) animals that received 10 µmol CAPE /kg/day intraperitoneal (i.p.) (3) 10 mg Fluoxetine/kg/day intragastrically (4) 100 mg Fluoxetine/kg/day intragastrically. (5) 10 mg Fluoxetine/kg/day intragastrically + 10 µmol CAPE /kg/day i.p. (6) 100 mg Fluoxetine/kg/day intragastrically + 10 µmol CAPE /kg/day i.p.

Results: Fluoxetine treatment increased the levels of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase and total oxidant status (TOS) in the serum of the study groups .We observed no changes in the level of the concentration of total antioxidant stress (TAS) and paraoxonase in either the study or of treated rats compared to the control group

Conclusion: Our study indicates that Fluoxetine induces liver damage and mediates free radical reactions. CAPE prevents effect of the toxic capacity of this process as partly.

P8-20

Reduced expression of citrate synthase is associated with increased anaerobic glycolysis in C2C12 muscle cells

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Aim: Citrate synthase (CS) is is a key enzyme of mitochondrial tricarboxylic acid cycle. CS plays an important role in regulation of substrate oxidation by mitochondria (Rudderman et al., 1999). The aim of this study is to compare cellular metabolism and adaptations in C2C12 muscle cells treated with Cs mRNA targeting shRNA (Cs shRNA cells) and control shRNA (Con shRNA cells).

Methods: Oxygen consumption rate (OCR) and proton production rate (PPR) was measured in C2C12 cells after incubation with 10 mM glucose using Seahorse XF24-3 analyzer. Western blotting was used to assess levels of proteins associated with energy metabolism.

Results: Cs shRNA cells had 50% lower levels of CS protein ($p < 0.01$) and enzyme activity ($p < 0.001$) compared to Con shRNA cells. Compared to Con shRNA cells, Cs shRNA cells tended to have lower OCR (76.4 vs 89.2 umol/O2/sec/mg protein), but higher PPR (15.8 vs 14.2 umole/H+/sec/mg protein). PPR to OCR ratio was higher ($P < 0.0001$) in Cs shRNA compared to Con shRNA cells. Cs shRNA cells had higher Glut4 protein expression ($p < 0.01$) compared to Con shRNA cells.

Conclusions: Our result suggests that ~ 50% reductions in CS associated with an increased reliance on anaerobic glycolysis in C2C12 muscles cells.

P8-21

Changes of mitochondrial function and energy transfer enzymes in muscles of mice with deleted wolframin (wfs1) gene

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Aim: To assess changes of mitochondrial function and activities of enzymes involved in the transport of energy in different muscles of wfs1 deficient mice.

Methods: Mitochondrial function was assayed by high resolution oxygraphy of permeabilized muscle fibers. Respiration related to oxidative phosphorylation was calculated by subtracting initial basal respiration in the presence of pyruvate and malate from respiration with ADP. Proton leak related respiration was found by subtracting residual oxygen consumption with rotenone from basal respiration. The difference between the respiration rates in the presence of ADP+pyruvate+malate and rotenone was considered to represent the activity of Compex I in the electron transfer chain, and the difference between ADP+succinate and rotenone represented the activity of Complex II. Activities of enzymes were measured by spectroscopy of muscle homogenates.

Results: Compared to controls, there was no change of proton leak and hexokinase activity in the wfs1 deficient heart and *m. soleus*, but in *m. rectus femoris* 16.1-fold ($p<0.002$) and 1.7-fold ($p<0.01$) increases were found respectively. However, oxidative phosphorylation was not changed in any muscle group. The Complex I / Complex II ratio was decreased in heart by 15% ($p<0.03$) and in *musculus rectus femoris* by 21% ($p<0.01$). Activities of creatine and adenylate kinase were decreased in *m. rectus femoris* by 34% ($p<0.01$) and 48% ($p<0.02$) respectively.

Conclusions: In heart and *m. rectus femoris* of wfs1 deficient mice the ratio of Complex I and Complex II activities was decreased. In *m. rectus femoris* of wfs1 deficient mice proton leak and hexokinase activity were increased, but activity of other energy tranfer enzymes was decreased.

P8-22

Lipopolysaccharide-induced inactivation of pulmonary surfactant and its reversal

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Aim: Possible role of pulmonary surfactant as a drug carrier in acute lung injury is of clinical significance. The aim of the study was to investigate the effect of lipopolysaccharide (LPS) on the surface properties of pulmonary surfactant *in vitro* and whether it is possible to reverse the inhibition by polymyxin B (PxB), and to develop the model for further *in vivo* testing.

Methods: Curosurf® was diluted to 1.75 mg/ml of phospholipids in 0.9% NaCl. LPS was added at 1, 5, 10 and 20% (w/w) and PxB at 1, 2 and 3% (w/w). Surface tension was evaluated with a pulsating bubble surfactometer. In adult rats acute lung injury was induced by intratracheal instillation of LPS (100 and 500 µg/kg of body weight), control group received saline. Respiratory parameters were registered and inflammatory markers were evaluated in plasma, tissues and bronchoalveolar lavage fluid.

Results: Minimum surface tension (γ_{min}) of surfactant was significantly increased after addition of 5, 10 and 20% LPS ($p<0.05$ and $p<0.01$, respectively). PxB reduced the γ_{min} of Curosurf mixed with LPS at 5, 10 and 20% when added at 3 % (for all $p<0.01$). Instillation of LPS 500 µg/kg b.w. led to acute lung injury demonstrated by increased oedema formation expressed as a wet/dry lung weight ratio compared to control group ($p<0.05$) and redistribution of inflammatory cells between lungs and systemic circulation.

Conclusions: LPS inactivates pulmonary surfactant *in vivo* and *in vitro*. Addition of 3% PxB increases resistance of surfactant to inactivation by LPS.

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P8-23

Effect of ischemia-reperfusion *in vivo* on rat kidney mitochondrial functions

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Aim: Mitochondria (the producers of cellular energy) play an important role in pathophysiology of ischemia-reperfusion induced kidney injury. Ischemia affects renal function in kidney transplant and nephron sparing surgery. Duration of ischemia is very important factor which influences postoperative kidney function. The aim of this study was to test the effect of renal ischemia *in vivo* on mitochondrial functions.

Methods: Vascular clips were placed over rat's renal artery to induce renal ischemia. After ischemia (20 min, 30 min or 40 min) the clips were removed and kidney reperfusion was performed for

30 minutes. Mitochondria from rat's kidneys was isolated by differential centrifugation. The mitochondrial respiration (oxygen consumption) rates were measured using an Oxygraph-2k (OROBOROS).

Results: It was determined, that ischemia/reperfusion (20 min/30 min) did not affect oxygen consumption in kidney mitochondria but slightly increased (by 20%) permeability of inner membrane. Ischemia/reperfusion longer than 20 minutes leads to renal mitochondrial injury. Mitochondrial respiration rate in state 3 with glutamate + malate and the respiratory control index (RCI) was decreased by 38% and 68% after of 30 and 40 min ischemia and 30 min of reperfusion, respectively, as compared to control. Succinate oxidation was also affected. After 40 min of ischemia respiration rate was decreased by about 50%. Mitochondrial State 3 respiration rate after addition of cytochrome c increased by 1.23 and 2.37-fold after 30 min and 40 min of ischemia/reperfusion whereas control mitochondria had only minor effect.

Conclusions: Ischemia *in vivo* longer than 20 minutes leads to rat kidney mitochondrial injury which increases progressively with the increased duration of ischemia.

P8-24

The characterization of diffusion obstacles in rat cardiomyocytes

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Aim: Mitochondria are very important, providing cells with energy in the form of ATP and controlling cell death. Molecules that participate in regulation of cellular environment need to be transported between organelles efficiently to take their action in the right time and place. Kinetic measurements on permeabilized cardiomyocytes have shown rather high diffusion restrictions for ATP and ADP molecules that reduce significantly in some pathological conditions. These restrictions may be caused by intracellular structures or VDAC on the mitochondria outer membrane.
The aim of this work is to evaluate the contribution of obstacles in cytoplasm to the overall intracellular diffusion restriction, and distinguish it from the restriction resulting from the mitochondrial outer membrane.

Methods: Rat cardiomyocytes were permeabilized and put into flow chamber under the fluorescence microscope that enabled continuous solution changes. Different ADP concentrations were used to stimulate respiration. NADH autofluorescence signal changes were recorded with fluorescent microscope to follow redox state of the cell and characterize its respiration. High resolution images of rat cardiac cell NADH autofluorescence response due to ADP titration were taken and analyzed. Experimental data was analyzed by a mathematical model that enabled to distinguish the contribution of diffusion obstacles in cytoplasm and the reduction of mitochondrial outer permeability induced by closure of VDAC.

Results: Analysis shows that apparent oxidative respiration Km for ADP in the vicinity mitochondrial outer membrane is about 0.15 mM, corresponding to 150 open VDACs per μm2. The apparent diffusion constant in cytoplasm for ADP is 25 μm2/s.μ

Conclusions: Our results suggest that VDAC and diffusion obstacles both contribute to the processes of energy transfer, signaling and apoptosis in the heart.

P8-25

Surfactant proteins B and C are more than lipid membranes stabilizers

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Background: Specific proteins B and C have been for a long time considered to be engaged solely in surface properties of pulmonary surfactant. Within last years, there is increasing evidence on their anti-inflammatory function in respiratory system.

Aim: To prove the hypothesis that SP-B and SP-C have an immune role in LPS-induced fever and in patients with chronic rhinosinusitis.

Methods: In adult rats fever was induced by intraperitoneal administration of LPS at a dose 100 μg/kg of b.w.; control group received saline. SP-B and SP-C were evaluated in bronchoalveolar lavage fluid (BALF) and lung tissue (LT). The prospective study was conducted in 30 patients with chronic rhinosinusitis (CRS) and healthy controls. SP-B and SP-C were detected in nasal

lavage fluid (NALF). Bacterial culture from the middle nasal meatus and inflammatory markers were evaluated.

Results: SP-B increased in LT and SP-C in BALF of LPS-instilled animals (both P<0.05 vs. controls). SP-B and SP-C were identified in in NALF in significantly higher concentration in patients with CRS compared to healthy controls (P<0.01). Identification of pathogenic bacteria in the middle nasal meatus was associated with higher levels of SP-B and SP-C in NALF in patients with CRS and control group.

Conclusions: Alterations of SP-B and C may be related to general inflammatory response to pyrogen beside their main role in stabilizing the lungs during changed breathing pattern. Surfactant proteins B and C play an important role in innate host defence of upper respiratory system.

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P8-26

Experimental investigation of immunostimulating effect of *Propolis* ethanolic extract

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Aim: The aim of this experimental investigation was to reveal immunostimulating effect of an ethanolic extract derived from Lithuanian *Propolis* on cell-mediated immunity.

Methods: Material of investigation - the 10% ethanolic extract from *Propolis* was provided by Lithuanian manufactuter "Medicata Filia". The immunostimulating potency of *Propolis* preparation was experimentally investigated using laboratory mice and analysis of certain immunological and clinical laboratory techniques. The amount of leucocytes, thymocytes and splenocytes (10^9/ l) was counted by laboratory analysis. Blood smears had been made, it was fixed and coloured using Giemsa method. Forms of leucocytes in the leukogram were counted by using immersion system.

Results: The investigation was based on evaluation of immunostimulating properties of Lithuanian *Propolis* extract. The usage of the preparation produced from Lithuanian *Propolis* has increased general amount of leucocytes and lymphocytes, thymocytes and splenocytes, as immunocompetent cells in experimental mice blood. The results showed, that usage of the *Propolis* extract increased general amount of leucocytes, especially stimulated the increase of lymphocytes ($p<0.05$). *Propolis* preparation activates T and B lymphocytes production ($p<0.05$) in central (thymus gland) and peripheral (spleen) immune system organs.

Conclusions: The results of the study demonstrate that the ethanolic extract from Lithuanian *Propolis* activates immune system by affecting immunocompetent cells production. From these particular immuno-pharmacological effects of Lithuanian *Propolis,* investigated preparation possesses immunostimulating potency.

P8-27

Histochemical observations on protective effects of melatonin against carbon tetrachloride (CCl_4) - induced hepatotoxicity

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Aim: To investigate the curative and protective effects of melatonin (MEL) against liver injury induced by CCl_4.

Methods: Wistar albino rats were divided into 4 groups: Control (G1), CCl_4 (G2), 10 weeks CCl_4+MEL (G3) and 12 weeks CCl_4+MEL (G4). CCl_4 was administered (1.5 ml/kg) for 10 weeks subcutaneously (s.c.) twice a week. At the beginning of CCl_4 injection, MEL (10mg/kg s.c.) was applied to G3 and G4. Following the cessation of CCl_4 injection, MEL application to G4 was continued for two weeks. At the end of the study liver tissues were removed and fixed in formalin.The tissues were dehydrated and embedded into paraffin. 5µm thin sections were taken. The sections were stained with Hematoxylin & Eosin (H&E) for general structure, with Masson's trichrome for collagen fibers, with Periodic-acid Schiff (PAS) for glycogen content and with Reticulin for reticular fibers.

Results: In G1, hepatocytes were ordered in plates one to two cells wide. However in G2, the plates were disorganized and some of the cells were swollen. The sinusoids were slightly to moderately dilated. There was perivenular fibrosis due to accumulation of collagen. PAS negative focal necrotic areas and reticular fiber thickening were observed. In G3, liver damage and hepatic fibrosis was reduced. G4 displayed somewhat improved results in comparison to G3.

Conclusions: It can be proposed that melatonin reduces liver damage and plays anti-fibrotic role against CCl_4 induced hepatotoxicity.

Keywords:
CCl_4, Hepatic damage, fibrosis, melatonin, liver histopathology

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P8-28

Thermoregulation in the raccoon dog *(Nyctereutes procyonoides)*

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Aim: Aim of the study was to investigate wintering of the raccoon dog (RD) and how it maintains appropriate body temperature via thermoregulation at different seasonal phases. We hypothesize that thermogenic brown adipose tissue (BAT) and/or the recently discovered phenomenon of white adipose tissue (WAT) "browning" into BAT-like beige cells could aid RDs to maintain body temperature at challenging environmental conditions that require extra heat production.

Methods: Expression of BAT in RD was analyzed in vivo with a clinical 18F-FDG PET/CT scanning and with histological analysis. The "browning" capacity of WAT was determined by treating RD primary adipocytes with Irisin/FNDC5 fragments that have a "browning" effect in rodent and human primary adipocytes. Primary adipocytes were treated with 50 nM Irisin/FNDC5 peptides for six days to induce "browning". UCP1 was used as an established marker for BAT and beige cells and TBX1 as a specific marker for beige cells in mRNA expression level analysis.

Results: BAT was not visualized by PET/CT imaging and the histological staining confirmed the absence of BAT. Treating the RD adipocytes with Irisin/FNDC5 peptides did not induce changes in cell structure or relative mRNA expression of UCP1 and TBX1.

Conclusions: Our results suggest that adult RDs do not have active BAT and WAT is not recruited into beige cells in response to Irisin/FNDC5 peptide treatments. Thus, the thick fur, insulating WAT and muscle shivering are likely to provide adequate heat for maintaining body temperature in the RD.

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9. Biochemistry and metabolism

P9-1

Selected haematological and biochemical parameters in healthy Damascus goats

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Aim: The objective of the present study was to determine the reference values of haematological and biochemical blood parameters in young and adult Damascus goats.

Materials and Methods: A total of 150 blood samples were taken from clinically healthy 75 young (6 months old) and 75 adult (3 years old) Damascus goats. The collected samples were analysed for white blood cells (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRA), red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). In the serum, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), gamma glutamyl transferase (GGT) enyzme activities, and urea, creatinine, albumin, glucose, total protein, and total bilirubin levels were observed. The animal care and use protocol was approved by Local Ethical Committee of Kirikkale University.

Results: In young goats, WBC, LYM, RBC, Hb, PCV, MCHC, total protein levels were lower, MCV, MCH, glucose values, and ALT, AST, ALP, CK,

GGT enzyme activities were statistically higher in comparison to the adult goats ($P<0.05$). However, It was not observed statistically significant difference depending on age in MON, GRA, albumin, creatinine, urea, and total bilirubin levels ($P>0.05$).

Conclusions: As a result of this study, the haematological and biochemical blood values were determined in healthy young and adult Damascus goats. The results obtained from this study can be useful as a reference for the veterinary practitioners and further researches on this breed.

Keywords:
Age, Biochemistry, Damascus goats, Haematology

P9-2

Is there any relationship between irisin and thyroid hormones?

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Aim: It has been thought that irisin could regulate whole body energy expenditure led to the expectation that irisin may have therapeutic potential in some metabolic diseases.
We showed that intracerebroventricular (icv) irisin infusion caused to increases in food consumption and body temperature but not change body weight in rat [1]. The presence study was designed to define relationship between irisin and thyroid hormones.

Methods: In this study, 40 male Wistar-Albino rats were randomly divided into four groups (n=10). Brain infusion kits were implanted to right lateral ventricle in all rats, expect control group.
The artificial cerebrospinal fluid (vehicle) and 10 and 100 nM concentration of irisin were infused via Alzet osmotic pumps (10 µl/h) for 7 days. After icv irisin infusion, animals were decapitated and blood samples were collected. Serum TSH, T3 and T4 levels were measured by using commercial ELISA kits.

Results: The all concentrations of irisin caused to statistical reduction in serum TSH, T3 (expect 10 µM) and T4 levels ($p<0.05$).

Conclusions: Our results indicate that irisin may alter serum TSH, T3 and T4 hormone and thus it can undertake considerable physiologic roles on hypothalamus-pituitary-thyroid axis. Determination of the levels of TRH is fairly important to be completed to knowledge about hypothalamus-pituitary-thyroid axis.

Acknowledgments:
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Reference:
[1] Tekin S, Erden Y, Colak C, Sandal S. (2014). “Effects of chronic central administration of irisin on food intake, body weight and body temperature in the rats” Acta Physiologica, 211, s697:138.

10. Cell physiology and regeneration

P10-1

New synthesized benzofuran substituted chalcones on human prostate cancer cell lines: an *in vitro* study

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Aim: Cancer is one of the most clinical problem in the world. Among the wide range of compounds approved as potential anticancer agents, derivatives bearing functionalities as α,β-unsaturated Michael acceptor have attracted great interest. Chalcones, the compounds having 1,3-diaryl-2-propen-1-on system, also showed a broad spectrum of biological activities including anti-inflammatory, antimalarial, anti-invasive, antibacterial and anticancer. Besides chalcones are capable of inducing apoptosis. Consequently, these compounds are regarded as encouraging anticancer agents. Due to interaction with the amino groups of nucleic acids, a number of clinically useful anticancer drugs have genotoxic effects. However, chalcones have not been found to show such undesired side effects.

Methods: In this study, we aimed to design and synthesize new compounds (chemical structure; compound 1; (2*E*)-1-(5-bromo-1-benzofuran-2-yl)-3-phenylprop-2-en-1-one ($C_{17}H_{11}BrO_2$), compound 2; (2*E*)-1-(5-bromo-1-benzofuran-2-yl)-3-(2-furyl)prop-2-en-1-one ($C_{15}H_9BrO_3$), compound 3; (2*E*)-1-(5-bromo-1-benzofuran-2-yl)-3-(2-thienyl)prop-2-en-1-one ($C_{15}H_9BrO_2S$) with both benzofuran and chalcone units in one molecule and then anti-carcinogenic activity of this new synthesized chalcones no bearing substituent in the benzofuran ring as a different series against human prostate cancer cells (PC-3) were examined. Varying

concentrations of compound 1, compound 2 and compound 3 (1, 5, 25, 50 and 100 µM) for 24 h. Antitumor activities of these compounds were evaluated by 3-(4,5-dimethylthiazol–2-il)- diphenyl tetrasolium bromid (MTT) assay.

Results: The benzofuran substituted chalcones have anti-carcinogenic activity on PC-3 cells lines ($p<0.05$). At 100 µM concentrations of all the compounds significantly reduced the percentage of viability of PC-3 cells ($p<0.001$).
Conclusions: New synthesized these compounds (1-3) displayed potential antitumor activity towards on human prostate cancer cell lines (PC-3).

P10-2
Benzimidazole based a new therapeutic agent effective on ovarian cancer cells

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Aim: Ovarian cancer has the highest the mortality rate among all gynecologic cancers of the female reproductive system. Despite the contiounus advances in ovarian cancer research, diagnosis and clinical treatment, the exact solution for the treament of the cancer has not been fully elucidated.

Methods: In this study, a variety benzimidazole compounds including (phthalimide-2-yl)methyl group (chemical structures: compound 1; 1-phenylethyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide ($C_{24}H_{24}BrN_3O_4$), compound 2; 1-(4-nitrobenzyl)-3-(phthalimide-2-yl)methylbenzimidazolium chloride ($C_{23}H_{17}ClN_4O_4$), compound 3; 1-penthyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide ($C_{21}H_{24}BrN_3O_3$) compound 4; 1-allyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide ($C_{19}H_{18}BrN_3O_3$) were synthesized through nucleophlic substitution reaction of 1-substituted benzimidazole with appropriate alkyl halides. The structure of the synthesized new compounds were identified by spectroscopic techniques and micro analysis The synthesized new benzimidazole derivatives were investigated in terms of their mechanism of action and antitumor properties by using human ovarian cancer cell lines (A2780). Antitumor activities of the benzimidazole compounds (1-4) were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: Varying concentrations of benzimidazole compounds (1, 5, 25, 50 and 100 µM) were treated with A2780 for 24 h. All concentrations of compound **1** and **4** reduced cell viability of A2780 cells ($p<0.05$). Similarly, compounds **2** and **3** reduced cell viability of A2780 cells ($p<0.05$), except dose of 1µM.

Conclusions: This results suggest that, the new benzimidazole compounds have an antitumor activity on human ovarian cancer cell.

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P10-3
Anti-tumor properties of microwave-assisted synthesized 6,7-dihydroxycoumarins: An in vitro study

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Aim: Breast cancer is the one of the most common type of cancer in females. The study was aimed to determine to anti-tumour activity of the new synthesized coumarine compounds on human breast cancer cell lines (MCF-7).

Methods:In this study, 6,7-dihydroxy-1-(3-methylphenyl)coumarin **(1),** 6,7-dihydroxy-1-(4-methylphenyl)coumarin **(2)** and 6,7-dihydroxy-1-(3-chlorophenyl)coumarin **(2)**were synthesized from the reaction of 2-(2,4,5-trimethoxyphenyl)-1-(3-methylphenyl)acrylonitrile, 2-(2,4,5-trimethoxyphenyl)-1-(4-methylphenyl)acrylonitrile and 2-(2,4,5-trimethoxyphenyl)-1-(3-chlorophenyl)acrylonitrile with pyridinium hydrochloride by using microwave, respectively.Anti-tumor activities of these compounds (**1, 2** and **3**) were investigated in terms of their mechanism of action and antitumor properties by using MCF-7 cell lines. The cancer cell lines were treated with differant concentrations of compound **1 ,2** and **3** (1, 5, 25, 50 and 100µM) for 24 h. Anti-tumor activities of these compounds were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: 100 µM doses of compound **1** and **2** reduced the viability of MCF-7 cells ($p <0.05$). 25, 50 and 100 µM doses of compound **3** reduced the viability of MCF-7 cells ($p <0.05$).

Conclusions: Our results indicate that 6,7-dihydroxycoumarins have anti-tumor activity on human breast cancer cell lines.

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P10-4

Concentration effects of some new benzimidazole derivatives on Human Breast Cancer Cell Viability

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Aim: Benzimidazoles including imidazole functional group are studied widely due to the fact that they exist in the structure of natural products and different drugs. Benzimidazoles and their derivatives have shown a various pharmacological activities such as antibacterial, antifungal, antihelmintic, antiallergic, antineoplastic, local analgesic, antihistaminic.

Methods: In this study, a variety benzimidazole compounds (chemical structures: compound **1**; 1-[2-(4-methoxyphenyl)ethyl]-3-phenylethylbenzimidazolium bromide ($C_{24}H_{27}BrN_2O_2$), compound **2**; 1-[2-(4-methoxyphenyl)ethyl]-3-penthylbenzimidazolium bromide ($C_{21}H_{29}BrN_2O_2$), compound **3**; 1-allyl-3-[2-(4-methoxyphenyl)ethyl]benzimidazolium bromide ($C_{19}H_{23}BrN_2O_2$) were synthesized through nucleophilic substitution reaction of 1-substituted benzimidazole with appropriate alkyl halides. The structures of the synthesized new compounds were identified by NMR spectroscopy, FT-IR spectroscopy and element analysis techniques. Antitumor properties of synthesized new benzimidazole compounds were investigated by using human breast cancer cell lines (MCF-7). Varying concentrations of benzimidazole compounds (1, 5, 25, 50 and 100 µM) was treated with MCF-7 for 24 h. Antitumor activities of the benzimidazole compounds (**1**, **2** and **3**) were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: All studied benzimidazole compounds (**1**, **2** and **3**) reduced cell viability of MCF-7 cells (only 25, 50 and 100 µM doses; $p<0.05$).

Conclusions: These results suggest that, the newly synthesized benzimidazole compounds show considerable antitumor activity.

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P10-5

Determination of antitumor properties of cyclophosphazene derivatives bearing chalcone-groups against PC-3 cell lines

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Aim: Cyclotriphosphazene compounds have anti-tumor activity on different cancer cells [1-3]. The substituted chalcone and their analogues are an important class of flavonoid family. Chalcones display numerous biological properties, such as anti-cancer or anti-tuberculosis activity.

Methods: In the present study, cyclotriphosphazene compounds bearing chalcone groups (chemical structures; compound **1**; 2,2,4,4-(4'-oxychalcone)-6,6-bis[spiro(2',2''''-dioxy-1',1''''-biphenyl)] cyclotriphosphazene ($C_{72}H_{52}O_{10}N_3P_3$), compound **2**: 2,2,4,4-(4'-oxy-4-methylchalcone)-6,6-bis[spiro(2',2''''-dioxy-1',1''''-biphenyl)cyclotriphosphazene ($C_{76}H_{60}O_{10}N_3P_3$) and compound **3**: 2,2,4,4-(4'-oxy-4-methoxychalcone)-6,6-bis[spiro(2',2''''-dioxy-1',1''''-biphenyl)cyclotriphosphazene ($C_{76}H_{60}O_{11}N_3P_3$) were synthesized. And then *In vitro* anti-cancer activities of these chalcone-phosphazene compounds were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Anti-cancer activity of chalcone-cyclotriphosphazene compounds against independent human prostate cancer cell lines (PC-3) was investigated.

Results: Different concentrations of compound **1, 2** and **3** (1, 5, 25, 50 and 100 µM) were treated with PC-3 cell lines for 24 h. Compound **1, 2** and **3** (50 and 100 µM doses) reduced cell viability of PC-3 cells ($p < 0.05$).

Conclusions: These compounds (**1, 2** and **3**) displayed potential antitumor activity towards on human prostate cancer cell line.

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P10-6

Synthesis and evaluation of benzofuran substituted chalcones on human breast cancer cell lines: An *in vitro* study

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Aim: Benzofuran derivatives are an interesting class of heterocyclic compounds. Benzofuran derivatives are great interest in medicinal chemistry and have drawn remarkable attention due to their biological activities chemotherapeutic properties. Some benzofuran bearing various substituents at the C-2 position are greatly distributed in nature, e.g., ailanthoidol a neolignan derivative, has been reported to have antiviral, antioxidant and antifungal activities. Furthermore, most of the compounds prepared from 2-acetylbenzofuran have antimicrobial, anticancer, antitumor, antiinflammatory activity, antitubulin and also used for treatment of cardiac arrhythmias.

Methods: In this study, we aimed to design and synthesize new compounds (chemical structure; compound 1; 1-(1-benzofuran-2-yl)-3-phenylprop-2-en-1-one ($C_{17}H_{12}O_2$), compound 2; 1-(1-benzofuran-2-yl)-3-(2-furyl)prop-2-en-1-one ($C_{15}H_{10}O_3$), compound 3; 1-(1-benzofuran-2-yl)-3-(2-thienyl)prop-2-en-1-one ($C_{15}H_{10}O_2S$) with both benzofuran and chalcone units in one molecule and then anti-carcinogenic activity of this new synthesized chalcones no bearing substituent in the benzofuran ring as a different series against human breast cancer cells (MCF-7) were examined. Varying concentrations of compound 1, compound 2 and compound 3 (1, 5, 25, 50 and 100 µM) for 24 h. Antitumor activities of these compounds were evaluated by 3-(4,5-dimethylthiazol–2-il)- diphenyl tetrasolium bromid (MTT) assay.

Results: The benzofuran substituted chalcones have anti-carcinogenic activity on MCF-7 cells lines ($p<0.05$). At 100 µM concentrations of all the

compounds significantly reduced the percentage of viability of PC-3 cells ($p<0.001$).

Conclusions: New benzofuran compounds (**1, 2** and **3**) have antitumor activities on human breast cancer cell line.

P10-7

Application of polyimide films for cell scaffold in tissue engineering

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Aim: Employing of synthetic biocompatible scaffolds for incorporation of pluripotent stem cells is a rapidly developing field of artificial tissue engineering and regenerative medicine. The aim of the current study was to design and optimize the topography of scaffold structures femtosecond laser micro machined in polyimide (PI) films for application in the engineering of MSC-based scaffold for towards biological cardiac pacemaker.

Methods: bone marrow mesenchymal stem cells (hMSC) were cultured at 37 ºC in humidified 5 % CO2 atmosphere using complete MSCGM human Mesenchymal Stem Cell Growth BulletKit™ Medium (Lonza). Laser patterned PI film was cut into pieces, disinfected with 70% ethanol and treated with UV during 30 min before use. After sterilization, pieces were transferred onto bottom of culture dish. Cells were seeded on the film using previously described cultivation media, specimens were analyzed after 24 h.

Results: We verified biocompatibility of PI film using hMSCs. Migration, proliferation and monolayer formation properties did not differ compare to standard surfaces. The impact of PI film architecture on hMSCs behaviour was examined. Cells exhibited the best adhesion and typical spindle shape on the film with array of holes situated at 45 µm intervals. Our results suggest that the intercellular communication between two sides of PI film could be established through 3.83±0.45 µm diameter holes by TTs.

Conclusions: Scaffolds with different density and diameter holes demonstrated expressed dependency on the holes spacing and amount of cells attached. Successful formation of the tunnelling junction between cells through the scaffold structures was demonstrated.

P10-8

Fabrication of scaffolds for stem cells via ultrafast laser micro machining of polymer films and electrospun webs

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Aim: Scaffolds, i.e. structures that allow controlling the behaviour of the cells, require advanced fabrication techniques that enable realization of complicated 3D micro structures. Ultrafast laser processing methods are widely applied, especially multi-photon polymerization techniques. The main drawback of the mentioned method is the limited range of materials and involved processes that are not always biocompatible with live organism.

Methods: In the current work different approach was applied where top down lithography method - laser drilling of biocompatible material together with bottom-up method: electrospinning technique which allows obtaining a fibrous structure mimicking the extracellular matrix, was demonstrated.

Results: Using ultrafast 1030 nm wavelength Yb:KGW femto-second laser and micro-fabrication workstation thin biocompatible polymer films were pre-structured with micrometer and tens or micrometer ranges membrane and grid structures. 3D scaffolds, i.e. layer of micro-/nano-fibers, were obtained employing electrospinning of different polymer composition solutions.

Conclusions: Fabricated scaffolds provides suitable environment for the stem cell adhesion, migration and proliferation.