

# Abstracts presented as posters

Saturday August 10th

## Contents

Neurophysiology and Ocular Physiology (posters 1-10) .....	2
Muscles and Training (posters 11-28) .....	14
Cardiac Physiology (posters 29-45) .....	33
Vascular Physiology and Blood Pressure (posters 46-63) .....	51
Respiratory Physiology (posters 64-67).....	70
Digestion and Metabolism (posters 68-77).....	75
Renal Physiology (posters 78-86) .....	87

# Neurophysiology and Ocular Physiology

## **Posters 1-10**

## Poster 1

### **Differences in effect of intermittent fasting on autonomic nervous system balance and antioxidant content in female and male Wistar rats**

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#### **Introduction**

Female and male rats response differently to stress and these differences should be taken into account then extrapolation on human population are made. However, the most animal studies are done on male rats and a few on female.

#### **The aim**

of our study was to compare effects of intermittent fasting (IF) on autonomic nervous system balance and antioxidant defense system.

#### **Materials and Methods**

The experiment was done on female (n=22) and male (n=27) Wistar rats. Animals from control group were fed *ad libitum*; rats from experimental group have been performing IF for 4 days (get food for 1 hour a day). ECG registration, blood and liver sampling were taken on 4<sup>th</sup> day of IF. The ECG registration were performed after recovery period (*ad libitum* conditions for 7 days).

#### **Results**

RMSSD increased significantly in female rats only by 55% ( $p=0.011$ ) in the end of IF; effect vanished after recovery; SI was decreased by 138% ( $p=0.025$ ) only in male rats in the end of IF. Catalase (by 24%,  $p=0.026$ ) and superoxide dismutase (by 24%,  $p=0.015$ ) in blood plasma were decreased in female. Ceruloplasmin in blood for both sexes were statistically increased (by 18.5%,  $p=0.02$  for female and by 32.5%  $p=0.003$  for male).

#### **Conclusions**

Parasympathetic nervous system in female (RMSSD increased) and sympathetic in male (SI decreased) change heart rate due to IF. Increased catalase and superoxide dismutase in females marks inflammation. Ceruloplasmin (important protein for iron exchange) was increased in both sexes, which mark iron metabolism changes.

## Poster 2

### **Insulin differentially modulates GABA<sub>A</sub>R-activated currents along the dorsoventral axis of the mouse hippocampus.**

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The hippocampus has a crucial role in learning and memory and it can be anatomically divided into the subfields of dentate gyrus (DG), CA1 and CA3. Increasing evidence suggests that a functional segregation exists along the hippocampal dorsoventral axis. While the dorsal hippocampus (DH) is important for cognition, the ventral hippocampus (VH) is linked to emotional and metabolic regulation (Fanselow.M, 2010). GABA ( $\gamma$ -Aminobutyric acid) modulates neuronal excitability by binding to synaptic and extrasynaptic GABA<sub>A</sub> receptors (GABA<sub>A</sub>R) that mediate phasic and tonic currents, respectively. Insulin has been shown to influence neuronal transmission and alterations in insulin signalling are reported in several neurodegenerative diseases (Tokarz.V, 2018).

We studied GABA<sub>A</sub>R-mediated currents recorded from DG granule cells and CA3 pyramidal neurons in the absence/presence of insulin using the whole-cell patch-clamp technique. In DG, the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) was higher in VH compared with DH. Similarly, under the application of tetrodotoxin, the frequency of miniature inhibitory postsynaptic currents (mIPSCs) recorded from DG of VH was higher compared with DH. In contrast, the frequency of only mIPSCs but not the sIPSCs in CA3 neurons was higher in VH than in DH. Acute application of insulin increased GABAergic synaptic frequency of both sIPSCs and mIPSCs in DG from both DH and VH. In CA3, insulin only significantly increased mIPSCs in DH but also enhanced the tonic GABAergic currents from both DH and VH.

The results demonstrate distinct modes of GABAergic inhibition along the hippocampal dorsoventral axis which can be differentially modulated by insulin.

### Poster 3

#### **Interferon- $\gamma$ enhances GABAergic transmission in the rat hippocampus**

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#### **Introduction**

The immune system can produce small signal molecules including cytokines that regulate various neuronal functions such as neural transmission in the central nervous system. The cytokine interferon- $\gamma$  (IFN- $\gamma$ ) activates its receptor and plays important roles under both physiological and neuroinflammatory conditions. However, the effect of IFN- $\gamma$  on GABAergic inhibition in the hippocampus has not been explored.

#### **Materials and Methods**

Hippocampal slices were prepared from male Wistar rats between postnatal days 16 and 22. Quantitative PCR was performed to detect the mRNA expression of receptors for interferon- $\gamma$  in the hippocampus. GABA-activated currents were recorded by the whole-cell patch-clamp assay on hippocampal slices pre-incubated with IFN- $\gamma$  (100ng/ml) or artificial cerebrospinal fluid (aCSF).

#### **Results**

Both IFN- $\gamma$  receptor subunit gene transcripts (*Ifngr1* and *Ifngr2*) were abundantly expressed in the rat hippocampus (n=6). In addition, pre-incubation with IFN- $\gamma$  (100 ng/ml) increased the frequency of both spontaneous and miniature inhibitory postsynaptic currents (sIPSC, n=17 and mIPSC, n=11) in hippocampal CA1 neurons.

#### **Conclusions**

IFN- $\gamma$  potentiates GABAergic inhibition in hippocampal neurons by both action potential-dependent and independent release of GABA.

Poster 4

**Pharmacological induction of serotonergic signalling mimics parasite-induced behavioural manipulation of California killifish (*Fundulus parvipinnis*)**

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Parasites are increasingly being recognised as important players in community dynamics. Some parasites change their host's behaviour to aid transmission to the next host in their life cycle. However, the mechanisms driving this manipulation in parasite-host systems remains poorly understood. Using the California killifish (*Fundulus parvipinnis*) and its brain-infecting trematode parasite *Euhaplorchis californiensis* (Euha) as a model system, we investigated the role of serotonin (5-HT) in parasite manipulation of vertebrate hosts. Euha-infected killifish exhibit up to four times greater conspicuous behaviours and are 10 times more likely to be eaten than uninfected conspecifics. The central monoamine neurotransmitter 5-HT is known for its role in regulating vertebrate behaviour and was previously found to be altered in an infection intensitydependent manner in Euha-infected killifish. Here, we hypothesized that behavioural manipulation caused by *E. californiensis* is mediated through serotonergic disruption. To test this hypothesis, we compared routine and exploratory behaviour in groups of uninfected and experimentally infected killifish with pharmacologically manipulated serotonergic activity (sea water sham, 5-HT receptor agonist, 5-HT receptor antagonist). The results showed that parasite infection increases the frequency of conspicuous behaviours, but reduces activity and exploration. Intriguingly, the data indicates that treatment with the 5-HT agonist mimics the effects of infection in uninfected killifish. These data point to disruption of serotonergic signalling as a proximate mechanism driving *E. californiensis* behavioural manipulation of killifish.

## Poster 5

### **Retinal degenerations with different time course in mice with two distinct mutations in the Microphthalmia-associated transcription factor (*Mitf*) gene**

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The microphthalmia-associated transcription factor (*Mitf*) gene is crucial for development of the retinal pigment epithelium (RPE) and photoreceptors. We examined changes over time in retinal function and structure in mice with two mutations in the *Mitf* gene, between 1-5 months of age. *Mitf*<sup>mi-wh/+</sup>, *Mitf*<sup>mi-wh</sup>/*Mitf*<sup>mi</sup> mice were examined and C5BL/6J mice as control. Mice were anesthetized by ketamine and xylazine. Fundus and optical coherence tomography (OCT) images were obtained with a Micron IV. Flash electroretinography (ERG) from mice with a corneal electrode was used to determine retinal function.

Fundus images from all mutant mice show hypopigmentation at 1 month, with larger nonpigmented areas later. *Mitf*<sup>mi-wh/+</sup> mice are either blind or not at 1 month, with the ERG normal in amplitude in some, and absent in others. The *Mitf*<sup>mi-wh/+</sup> mice with ERG responses at 1 month show slow retinal degeneration, with reduced responses at 5 months. *Mitf*<sup>mi-wh</sup>/*Mitf*<sup>mi</sup> mice show either abnormal ERG responses at 1 month, or a flat ERG. The ERG in some of the *Mitf*<sup>mi-wh</sup>/*Mitf*<sup>mi</sup> mice decrease with time, becoming flat at 4 months. OCT images reveal that retinal layers in animals with flat ERG are thinner than with reduced ERG, but abnormally thin in both.

We conclude that *Mitf*<sup>mi-wh</sup>/*Mitf*<sup>mi</sup> mutant mice show evidence of fast retinal degeneration. The *Mitf*<sup>mi-wh/+</sup> mutant mice are either blind at 1 month or show evidence of slow degeneration. Mice with these *Mitf* mutations may serve as models of retinal degenerations progressing at different rates, due to mutations in a gene expressed in the RPE.

## Poster 6

### Angiotensin Receptor Blockers in Cyclodextrin Nanoparticle Eye Drops: Ocular Pharmacokinetics and Pharmacologic Effect on Intraocular Pressure

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#### Introduction

Orally administered angiotensin II receptor blockers (ARBs) reduce intraocular pressure (IOP). Topical administration may reduce potential side-effects. The main purposes of this study are (1) to determine the pharmacokinetics of irbesartan and candesartan in  $\gamma$ -cyclodextrin nanoparticle eye drops in the anterior segment of the rabbit eye and (2) to test the hypothesis that irbesartan and candesartan eye drops lower IOP in rabbits.

#### Materials and methods

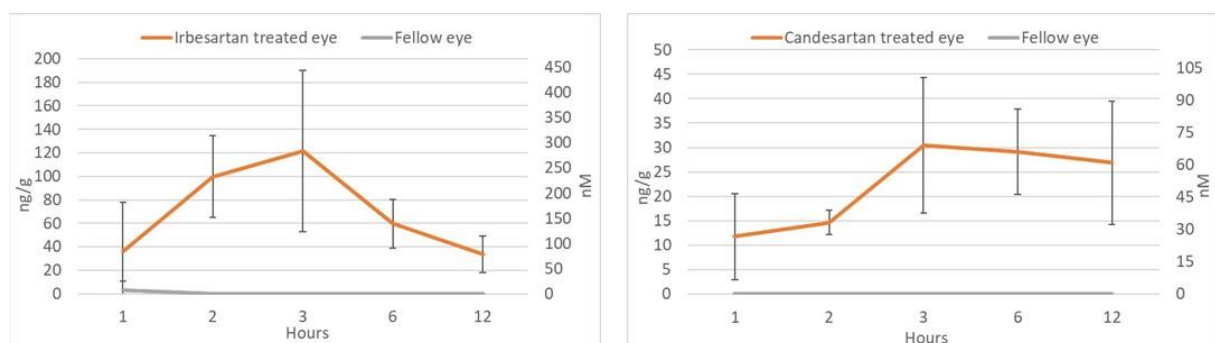
1.5% irbesartan and 0.15% candesartan eye drops were applied to rabbits. The pharmacokinetics in cornea and aqueous humor of single eye drop application were studied in 25 rabbits. The effect of the eye drops on IOP was studied in 10 rabbits using an iCare tonometer and compared with 0.5% Timolol eye drops.

#### Results

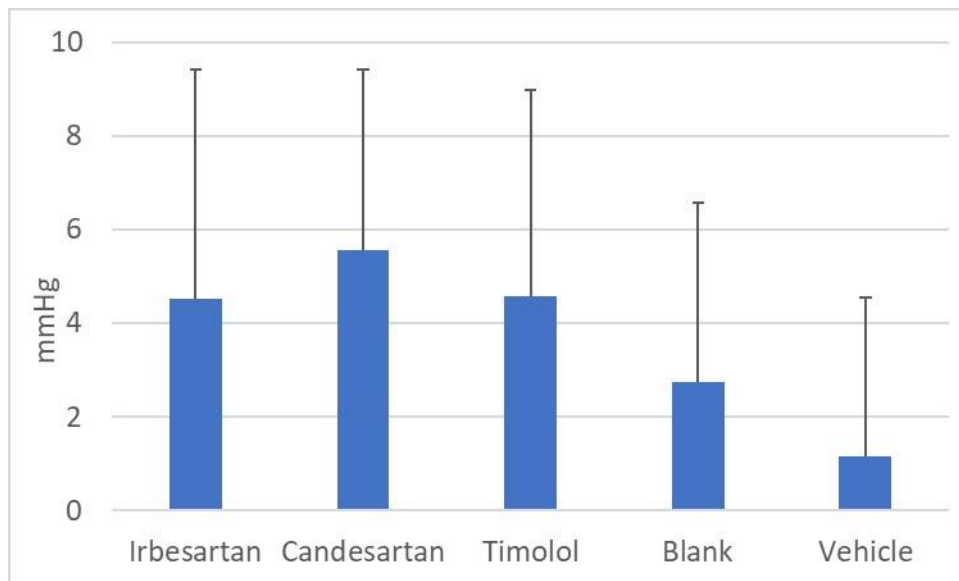
Candesartan lowered the IOP from  $24.6 \pm 5.1$  mmHg to  $19.0 \pm 2.9$  mmHg ( $p=0.030$ ,  $n=10$ ). Irbesartan lowered IOP from  $24.2 \pm 1.7$  mmHg to  $20.2 \pm 0.9$  mmHg ( $p=0.14$ ,  $n=10$ ). Timolol decreased the IOP from  $24.9 \pm 4.2$  mmHg (mean  $\pm$  SD) to  $20.4 \pm 4.8$  mmHg ( $p=0.036$ ,  $n=10$ ). The pharmacokinetics data show that both formulations deliver effective amounts of drug into the intraocular tissues, reaching a concentration of  $121.4 \pm 68.8$  ng/g (mean  $\pm$  SD) in the aqueous humor after a single dose administration.

#### Conclusions

Topical application of irbesartan and candesartan eye drops delivers effective drug concentrations to the anterior segment of the eye in rabbits. They reduce IOP in normotensive rabbits comparable to timolol. ARB eye drops have potential as a new class of glaucoma drugs.







*Figures 1 and 2. Drug concentration (mean  $\pm$  SD, n=25) in the aqueous humor after single application in the left eye and fellow untreated eye.*

Poster 7

**Age-dependent modulation of GABAergic signalling by insulin in hippocampal dentate gyrus granule cells in wild type and a mouse model of Alzheimer's disease.**

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Age and altered insulin signalling are risk factors for the development of cognitive decline and progression of Alzheimer's disease (AD) in humans. Also, insulin resistance has been reported in several mouse models of AD (Griffith CM et al., 2018). The dentate gyrus (DG) of the hippocampus plays an important role in memory formation and is one of the first regions where plaques are deposited in AD. As insulin and insulin sensitizers can improve cognitive function (Arnold SE et al., 2018), we examined the effect of insulin on GABAergic signalling in DG granule cells (GCs) in wild type (WT) and AD model animal, transgenic mice overexpressing betaAPP with Swedish mutation (tgSwe).

Adult (5-6 months old) and aged (10–12 months old) male and female tgSwe and C57BL6 WT mice were euthanized by cervical dislocation. Whole-cell patch-clamp recordings of inhibitory currents were performed on DG GCs from dorsal hippocampal brain slices.

The frequency and the amplitude of spontaneous inhibitory post-synaptic currents recorded from GCs were similar in WT and tgSwe littermates in both age-matched groups and were not significantly affected by age or disease. However, the GABA-evoked tonic current density was significantly increased in DG GCs of aged tgSwe mice compared with the WT littermates. Further, we pre-incubated slices with 1 nM insulin and showed that insulin normalized the increased GABA-activated tonic currents density in the aged tgSwe AD model mice to the control level. The data is consistent with that insulin at physiological concentrations modulates GABAergic signalling in aged AD mouse model.

## Poster 8

### **Multiscale biophysical investigation of a novel KCNA2 (KV1.2) variant associated with epilepsy**

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*De novo* mutation c.906T>G in *KCNA2* was detected in an infant with epilepsy by an exomebased panel. *KCNA2* encodes the pore-forming subunit of K<sub>v</sub>1.2 channels. The mutation results in amino acid substitution F302L.

F302L is located at transmembrane helix S4, an essential component of the K<sub>v</sub>1.2 voltagesensing domain (VSD). Whole-channel homology modeling and molecular dynamics simulations revealed that F302L causes the rotation of S4 and its differential exposure to membrane lipids. VSDs respond to membrane depolarization by undergoing conformational changes. We optically tracked K<sub>v</sub>1.2 VSD activation by voltage clamp fluorometry on K<sub>v</sub>1.2 channels heterologously expressed in *Xenopus* oocytes, voltage-clamped using the cut-open oocyte vaseline gap approach. F302L modestly enhanced activation of the K<sub>v</sub>1.2 VSD, shifting it to more negative membrane potential and increasing its effective valence.

Accordingly, channels opened at more negative membrane potentials (13mV shift) and at an accelerated rate (2-fold faster at -20mV). Longer depolarizations revealed that K<sub>v</sub>1.2-F302L channels also inactivate faster and at more negative potentials. That is, F302L produced both gain and loss of K<sub>v</sub>1.2 function.

Finally, a classical computational neuronal model (Hodgkin-Huxley neuron) endowed with K<sub>v</sub>1.2-F302L conductance exhibited diminished excitability, having 3-fold higher stimulus threshold for tonic firing. Since epilepsy is typically associated with aberrant excitability, we posit that the seizures arise due to preferential inhibitory neuron suppression, increasing overall circuit excitability.

This multidisciplinary and multiscale study underscores the value of thorough experimental and computational methods to assess the effects of genetic variants and facilitates the development of specific personalized treatments.

**Poster 9**

**Diversity of burst mechanisms in dopamine midbrain neurons**

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Dopamine (DA) midbrain neurons control fundamental brain functions like voluntary movement, reward-based learning and working memory. In addition to a single spike pacemaker-like discharge mode in the range of 1-8 Hz, they also fire in short high frequency bursts, which leads to a phasic boost of dopamine release. This burst is believed to have an essential computational function as representing a positive reward prediction error, which - as recent optogenetic studies have demonstrated - causally drives learning. However, bursting of some DA neurons might also be involved in action selection and the signalling of salient or even adverse events. In accordance with this diversity, we have previously identified molecularly and biophysically distinct burst mechanisms to be present in vivo in subpopulations of dopamine midbrain neurons (Schiemann et al. 2012 Nat. Neuroscience). We have now combined in vivo recording with retrograde tracing, which enabled us to record, label and identify DA neurons in vivo with defined axonal projections. Using this approach, we detected more diversity in in vivo burst pattern. We found that among those DA neurons in the substantia nigra (SN) that project to the dorsolateral striatum, only those located in the lateral - in contrast to the medial - SN displayed in vivo bursting. In contrast, all bursting DA cells in the medial SN were identified as projecting to ventral striatum - in particular to the lateral shell of the nucleus accumbens. We are currently using in vivo patchclamp recordings to directly study how burst discharge occur in vivo.

## Poster 10

### **Spinal TRPM3 is involved in mediating the hypersensitivity effect induced by sphingolipids in the rat**

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#### **Background and aims**

Earlier results have shown that endogenous sphingolipids in the spinal dorsal horn may contribute to pain hypersensitivity induced by peripheral nerve injury. Also, it has been shown in patch clamp recordings performed in cell cultures that sphingolipids can activate transient receptor potential melastatin-3 (TRPM3) that is a  $\text{Ca}^{2+}$ -permeable nonselective cation channel. Here we studied whether spinal TRPM3 is involved in mediating the pain hypersensitivity effect of sphingolipids.

#### **Methods**

Experiments were performed in healthy adult male Hannover-Wistar rats with a chronic intrathecal catheter for spinal drug administrations. Pain behavior was assessed with calibrated monofilaments (mechanical nociception) and with Hargreaves' test (heat nociception). N,N-dimethylsphingosine (DMS) was administered intrathecally (i.t.) to induce pain hypersensitivity. Ononetin, a TRPM3 channel antagonist, was used in attempts to block the effect of spinally administered DMS. Blocking of TRPM3 by ononetin was verified in a patch clamp study.

#### **Results**

DMS alone produced within 15 min a dose-related (0.05-0.5  $\mu\text{g}$ ) mechanical hypersensitivity that lasted at least 24 h. Spinally administered DMS did not influence heat nociception. Preemptive treatment with ononetin (TRPM3 antagonist; 100  $\mu\text{g}$  i.t.) delayed and attenuated development of hypersensitivity, while ononetin failed to reverse established hypersensitivity. Ononetin alone had no effect on pain behavior. Ononetin blocked dose-dependently intracellular calcium responses evoked by DMS in hTRPM3 expressing HEK cells.

#### **Conclusions**

The results suggest that spinal TRPM3 is involved in mediating pain hypersensitivity induced by sphingolipids.

## Muscles and Training

**Posters 11-28**

## Poster 11

### **Diet induced vitamin D deficiency results in impaired skeletal muscle mitochondrial function in C57BL/6 mice**

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#### **Introduction**

40% of adults in Europe can be classified as vitamin D deficient, with severe deficiencies resulting in skeletal muscle myopathies. Previous reports show that the treatment of human skeletal muscle myoblasts with vitamin D results in an increase in mitochondrial respiration. Whilst these data highlight a potentially new role for vitamin D within skeletal muscle, it is unknown how vitamin D status influences skeletal muscle mitochondrial function *in vivo*. Therefore, the primary aim of the study was to determine whether vitamin D status influences mitochondrial function in the skeletal muscle of C57BL/6 mice.

#### **Material and Methods**

10 week old C57BL/6 mice were fed either a vitamin D replete (2,200 IU/kg) or deplete (0 IU/kg) diet for a period of one, two and three months. Mitochondrial function was assessed in permeabilised skeletal muscle fibres using high-resolution respirometry (Oroboros Oxygraph, O2k) at each time point. Data mean $\pm$ SD and analysed via *Two-Way ANOVA*.

#### **Results**

Three months of diet induced vitamin D deficiency resulted in a decrease in complex I and II supported mitochondrial respiration ( $451.37 \pm 196.40$  vs.  $291.76 \pm 48.66$  pmol $\cdot$ sec $^{-1}$  $\cdot$ mg $^{-1}$ dry wt,  $p < 0.05$ ,  $n = 6$ ), whilst a reduction in the maximal capacity of the electron transport chain ( $560.58 \pm 205.09$  vs.  $352.90 \pm 46.56$  pmol $\cdot$ sec $^{-1}$  $\cdot$ mg $^{-1}$ dry wt,  $p < 0.05$ ,  $n = 6$ ) was also observed.

#### **Conclusion**

Vitamin D deficiency results in the impaired mitochondrial function in the skeletal muscle of C57BL/6 mice. Given that vitamin D deficiency is widespread in the general population and even in athletes, these data highlight an important role for vitamin D in the maintenance of skeletal muscle function.

## Poster 12

### Activity-induced muscle growth requires myosin function

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#### Background

In human and animal models, high-force exercise results in muscle growth, but the mechanisms remain unknown. Mechanical force is suggested to be important for the effects of exercise on muscle, but the role of force *per se* has not been distinguished from differences in electrical activation, and precisely how force is transduced to promote growth is unclear. Understanding muscle growth is important for the development of therapies that target aging-related muscle weakness.

#### Methods

A zebrafish larva model was used to investigate activity-dependent growth. Muscle activity was induced by direct electrical stimulation, or by optogenetic stimulation using transgenic zebrafish expressing the light-sensitive channel Channelrhodopsin-2 (ChR2).

#### Results

Muscle that is made inactive by anaesthetic exhibits reduced growth, which can be fully recovered by brief electrically- or optogenetically-induced activity. Treatment with either of two inhibitors of myosin movement prevents the recovery of growth despite the maintenance of upstream electrical signals. RNA-sequencing analysis revealed that *forcin* expression correlates strongly with changes in muscle activity and myosin blockade. A drug mimic of Forcin activity rescues muscle growth in anaesthetised larvae in the absence of activity.

#### Conclusions

We conclude that myosin function is required for activity-dependent growth and hypothesise that force generated by myosin is driving the growth adaptation. Our data further suggest that Forcin is a molecule involved in the growth process downstream of myosin. Future work will explore the molecular mechanism(s) by which Forcin regulates growth.



## Poster 13

### **The Epigenetic landscape of two phenotypic extreme skeletal muscles - soleus and EDL**

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#### **Introduction**

Skeletal muscle tissue is composed of approximately 40 - 60 % myonuclei, while the remaining are non-muscle cells with a different origin such as stromal and hematopoietic. Therefore, it is important to isolate the subpopulation of interest from the whole tissue, as a mixed-cell population are prone to increase the number of false-positive results.

In this study, we present a novel method for isolating myonuclei from whole native muscle. We applied the method to explore the muscle-specific epigenetic landscape of the two phenotypic extremes the oxidative Soleus and the glycolytic Extensor Digitorum Longus (EDL) muscles.

#### **Methods**

Animals were sedated with isoflurane. Following deep anesthesia, the muscles were surgically removed and frozen in liquid nitrogen. The myonuclei population was biochemically isolated, followed by Chromatin Immunoprecipitation coupled with next-generation sequencing (ChIP-Seq) and bioinformatic analyses.

#### **Results**

Using our newly developed isolation method, we show that the epigenetic environment of myonuclei is significantly different compared to whole muscle tissue. Genome-wide analysis of the myo-specific epigenetic landscape in the Soleus and EDL identified large dynamic differences between the two muscle types and that the muscles seem to be controlled by two distinct regulatory networks.

#### **Conclusion**

We have developed a novel method to isolate the myo-specific nuclei from skeletal muscle. We use the method to generate a global map of the epigenetic landscape of the two extremes, the oxidative Soleus and the glycolytic EDL muscles and find that their physiological different properties are reflected in their epigenome.

## Poster 14

### **The role of $\alpha 2$ Isoform of Na,K-ATPase in *m. soleus* adaptation to chronic motor dysfunction.**

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#### **Introduction**

Na,K-ATPase abundance in skeletal muscles is affected by motor activity. The isoform specificity of these changes was mainly studied under conditions of chronic motor dysfunction in traumas and during aging. We used Bla/J mice, a model for deficiency of dysferlin - the key protein responsible for the maintenance of sarcolemma integrity during contractile activity.

#### **Methods**

The localization of nicotinic acetylcholine receptors (nAChRs) and  $\alpha 2$  isoform of Na,KATPase in the motor endplate membrane of isolated *m. soleus* was determined using  $\alpha$ -bungarotoxin ( $\alpha$ -BTX) and ouabain labeled with BODIPY. The level of mRNA encoding  $\alpha 1$  and  $\alpha 2$  isoforms of Na,K-ATPase and their protein content were estimated by q-PCR and Western blot methods. Muscles were also stained with fluorescent 22-NBD-cholesterol (NBD) which distribution in bilayer is opposite to that of cholesterol. C57Bl/6 mice were used as control.

#### **Results**

In Bla/J mice, the total endplate area was greater by 15% than in C57Bl/6 mice. Both BODIPY ouabain ( $\alpha 2$  Na,K-ATPase) and  $\alpha$ -BTX (nAChRs) relative fluorescence were reduced in Bla/J mice suggesting decreased density of membrane distribution of these proteins. In addition, increase in NBD fluorescence intensity indicates loss of membrane cholesterol. The  $\alpha 1$  and  $\alpha 2$  Na,K-ATPase mRNA expression was similar in both mice. The  $\alpha 2$  Na,K-ATPase protein content also was not changed in Bla/J mice; in contrast, the  $\alpha 1$  Na,K-ATPase protein content was strongly decreased.

#### **Conclusions**

Demonstrated isoform-specific Na,K-ATPase changes can be a result of *m. soleus* adaptation to chronic motor dysfunction in mice model of dysferlinopathy.

Supported by Russian Science Foundation grant no. 18-15-00043.

**Poster 15**

**Myosin relaxed states are altered in MYH7-related skeletal myopathies**

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Mutations in the MYH7 gene are associated with skeletal muscle disorders known as Myosin Storage Myopathy and Laing Distal Myopathy. These mutations are relatively subtle, typically missense mutations modifying one DNA nucleotide, in turn resulting in the substitution of just one residue in the beta/slow myosin heavy chain molecule. The aim of the present study was to unravel the primary biophysical defects by which these subtle point mutations alter myosin structure and function. To achieve this, we isolated skeletal myofibres from a wide range of patients diagnosed with either Myosin Storage Myopathy or Laing Distal Myopathy as well as from age-matched control individuals. We then performed a series of experiments on these fibres including myosin filament length calculation and the Mant-ATP chase approach. So far, we have found that in the presence of MYH7 mutations, despite a relative preservation of the filament length, relaxed myosin molecules are unusually disordered consuming more ATP than normal. Such phenomena are likely to contribute to the development of the muscle disorders. We plan to verify this by running further studies in our newly designed zebrafish models of Myosin Storage Myopathy and Laing Distal Myopathy.

## Poster 16

### **The time-course of muscle glycogen utilization at different subcellular localizations and fiber types during endurance exercise in lean males.**

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#### **Introduction**

While the depletion of muscle glycogen during exercise is linked to fatigue, the underlying mechanisms are poorly understood. We aimed to investigate the time-course of muscle glycogen utilization in three subcellular compartments during exhaustive endurance exercise.

#### **Methods**

11 lean males (25±4 years) cycled (75% VO<sub>2</sub>max) until exhaustion (112±22 min). Muscle biopsies (VL) were obtained before, after one hour and at exhaustion. The volumetric content of glycogen in the intermyofibrillar ( $\mu\text{m}^3 \cdot \mu\text{m}^{-3} \cdot 10^3$ ), intramyofibrillar ( $\mu\text{m}^3 \cdot \mu\text{m}^{-3} \cdot 10^3$ ) and subsarcolemmal ( $\mu\text{m}^3 \cdot \mu\text{m}^{-2} \cdot 10^3$ ) compartments were quantified from transmission electron microscopy. Values in the results are presented as medians and interquartile range.

#### **Results**

In type 1 fibers, we found a 44% decrease in intermyofibrillar glycogen after 1 hour (19.8 (17.4:25.2) vs 11.0 (7.5:13.0),  $P < 0.001$ ), but no or only a small change from 1 hour until exhaustion (8.4 (1.8:12.3),  $P = 0.70$ ). Intramyofibrillar glycogen decreased by 70% after 1 hour (2.8 (1.7:4.0) vs 0.8 (0.6:2.1),  $P < 0.001$ ), but no or only a small change from 1 hour until exhaustion (0.6 (0.2:1.9),  $P = 0.32$ ). Subsarcolemmal glycogen decreased by 65% after 1 hour (66.6 (39.2:73.7) vs 23.3 (18.3:30.1),  $P < 0.001$ ), and further by an additional 12% from 1 hour until exhaustion (15.4 (2.3:19.7),  $P = 0.034$ ). The utilization of glycogen from these compartments in type 2 fibers was less, but the time-course was comparable to type 1 fibers.

#### **Conclusion**

During the first hour, intramyofibrillar and subsarcolemmal glycogen are relatively more depleted than intermyofibrillar glycogen, while subsarcolemmal glycogen show the relatively largest reduction during the last part of exercise. Thus, during exhaustive exercise the utilization of muscle glycogen depends on the subcellular localization and time-course.

## Poster 17

### **The inhibitory effects of two distinct inhibitors on glycogen phosphorylase activity and contractile function in chemically skinned single fibers**

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#### **Introduction**

Glycogen breakdown, with glycogen phosphorylase (GP) being the rate-limiting enzyme, is key for maintaining muscle function especially during physical activity. Novel enzymatic inhibitors of GP (1,4-dideoxy-1,4-imino-D-arabinitol (DAB) and CP-316,819 (CP)) allow for acute manipulation of GP activity, but little is known about their effects on skeletal muscle. We examined the effects of DAB and CP on GP activity and contractile function in skeletal muscle.

#### **Materials and methods**

In a coupled assay, the inhibitory effects of DAB or CP on GP activity was investigated in rat skeletal muscle homogenate. Further, the effect of 2 mM DAB or 10  $\mu$ M CP on contractile function was examined in chemically skinned single rat EDL fibers. Maximal  $\text{Ca}^{2+}$  induced force and the force-pCa relation was established using solutions with various intracellular  $[\text{Ca}^{2+}]$  (pCa=6.7 to 4.7 (maximal)).

#### **Results**

50 % GP inhibition was achieved with 2.0  $\mu$ M DAB or 0.8  $\mu$ M CP led to 50 % inhibition of GP, while 3.8 mM DAB and 3.4  $\mu$ M CP led to 95 % inhibition. CP reduced maximal  $\text{Ca}^{2+}$  induced force by  $11 \pm 9$  % compared to controls ( $p < 0.001$ ).  $\text{Ca}^{2+}$  sensitivity was reduced to  $5.65 \pm 0.03$  with CP ( $p < 0.001$ ) compared to  $5.83 \pm 0.12$  in controls. Both changes were partly reversed after washout. We observed no effects of DAB ( $p = 0.85$  and  $0.65$ , respectively).

#### **Conclusion**

Both DAB and CP can almost completely block GP activity with CP being the most potent. However, CP, but not DAB, has adverse side effects on maximal  $\text{Ca}^{2+}$  induced force and  $\text{Ca}^{2+}$  sensitivity, which should be accounted for.

## Poster 18

### **A cellular memory mechanism in skeletal muscle**

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#### **Introduction**

Muscle memory describes how previous exercise enhances the effects of subsequent exercise even with long-term intervening inactivity. It has previously been shown that myonuclei acquired through a period of testosterone treatment reside in the muscle cell during long-term detraining, crystallizing as a potential cellular memory mechanism in skeletal muscle, as these mice had an increased hypertrophic response in a synergist ablation retraining phase. The aim of this study was to investigate whether a similar mechanism exists in rats subjected to more physiological relevant training and retraining, such as exercise by climbing.

#### **Materials and Methods**

Rats were subjected to a period of training by climbing, followed by a period of detraining and subsequently a period of retraining by climbing. The trained rats were compared to age matched control rats that only underwent the retraining period. Cross sections of m. soleus and m. tibialis anterior were histologically analyzed.

#### **Results**

After training by climbing the muscle cells of trained rats had an increased myonuclear number and cross-sectional area compared to control rats. The myonuclear number was still elevated in the trained rats after detraining but there was no longer a difference in cross-sectional area compared to controls. After retraining the myonuclear number and cross-sectional area were increased in the trained rats compared to control rats.

#### **Conclusions**

These results support the previously described cellular memory hypothesis, since myonuclei acquired through exercise by climbing was not lost during atrophy and thus might be a part of the mechanism that aids hypertrophy when previously trained muscles are retrained.

## Poster 19

### **The time-course of intramuscular lipid droplet utilization at different subcellular localizations and fiber types during endurance exercise in lean males**

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#### **Introduction**

Skeletal muscles contain lipid droplets (LDs) in the intermyofibrillar and subsarcolemmal regions. Whether lipids from one or both regions are utilized during endurance exercise is still unclear. This study aimed to investigate the time-course of intermyofibrillar and subsarcolemmal LD utilization during exhaustive endurance exercise.

#### **Materials and Methods**

11 healthy lean males ( $25 \pm 4$  years) performed an endurance test at  $75 \pm 4\%$   $\text{VO}_2\text{max}$  to exhaustion on a controlled mixed diet. Muscle biopsies (VL) were obtained before, at 60 minutes and at exhaustion ( $112 \pm 22$  min). The volumetric content of intermyofibrillar and subsarcolemmal LDs was assessed by transmission electron microscopy. Values in the results are presented as medians and interquartile range.

#### **Results**

From before to exhaustion, a decrease in LD volumetric content was found in both the IMF (80%, 2.88 (0.82-5.72) to 0.59 (0.40-1.52),  $P < 0.001$ ) and the SS region (55%, 3.92 (2.48-5.26) to 1.75 (0.47-3.13),  $P = 0.001$ ). Within the first hour a decrease of 70% and 38% was seen in the intermyofibrillar and subsarcolemmal region respectively, whereas the decrease was 10% and 18% of pre values in the last  $52 \pm 22$  minutes. A discrimination between fiber types revealed no large difference between type 1 and 2 fibers in the time-course of intermyofibrillar ( $P = 0.88$ ) and subsarcolemmal ( $P = 0.47$ ) LD utilization.

#### **Conclusions**

During exhaustive endurance exercise at  $75\%$   $\text{VO}_2\text{max}$  LDs are utilized from both the intermyofibrillar and subsarcolemmal region in healthy lean males. Our results suggest that LDs from both regions are important energy sources during exercise within the conditions of the present study.

Poster 20

**Skeletal muscle sarcoplasmic reticulum Ca<sup>2+</sup> uptake preferentially use glycogenolytic derived ATP**

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**Introduction**

Muscle glycogen represents a primary fuel source. Studies on both rodent single fibers and humans have pointed to a modulating role of glycogen availability on SR Ca<sup>2+</sup> release rate. However, little is known about the role of glycogenolytic derived ATP on SR Ca<sup>2+</sup> uptake.

**Materials and Methods**

In order to test the role of glycogenolytic derived ATP on SR Ca-ATPase function, single fibers from rat fast-twitch muscles were mechanically skinned and SR Ca<sup>2+</sup> content were estimated in the control situations, followed by inhibition of glycogen phosphorylase (GP) inhibitors (DAB (2 mM) or CP-316,819 (10 μM)) or treatment with glucoamylase (GA). The SR Ca<sup>2+</sup> content was estimated by repeatedly exposure of the fibre to a release solution (caffeine 30 mM) and load solutions for different load times (15 to 180 s) before being emptied again. The time-integral (i.e. area) of the force response being indicative of the amount of Ca<sup>2+</sup> in the SR.

**Results**

There was a clear and pronounced treatment effect of inhibiting GP and GA treatment, decreasing maximal SR Ca<sup>2+</sup> contents (46%, CP, n=7); 25% (DAB, n=9) and 24% (GA, n=8) (P<0.001). With the lower maximal SR Ca<sup>2+</sup> content, the time to reach half maximal content was significantly shorter for all treatments (P=0.008). However, total Ca<sup>2+</sup> loaded for all load times were significantly decreased.

**Conclusions**

This study provides direct evidence that glycogenolytic derived ATP is vital for normal SR Ca<sup>2+</sup> uptake rate and maximal content, even in the presence of high global muscle ATP and aerobic metabolism.



## Poster 21

### **Characterizing a calcium- dependent ‘warm-up effect’ in fast twitch mouse skeletal muscle fibres undergoing repeated contractions**

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#### **Introduction**

Cytoplasmic free  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) is tightly regulated in the skeletal muscle fibre and plays an essential role in contractile force generation. Fast twitch muscle fibres display a progressive potentiation of tetanic  $[\text{Ca}^{2+}]_i$  during the initial phase of repeated contractions. This initial  $[\text{Ca}^{2+}]_i$  potentiation may act as a “warm-up effect” to enhance skeletal muscle contractile function. The aim is to investigate the characteristics of the potentiation of tetanic  $[\text{Ca}^{2+}]_i$  observed in the early onset of repeated contractions.

#### **Materials and Methods**

$[\text{Ca}^{2+}]_i$  was measured using indo-1 in single intact enzymatically dissociated mouse flexor digitorum brevis (FDB) muscle fibres that were repeatedly stimulated at different frequencies and time intervals.

#### **Results**

Potentiation of tetanic  $[\text{Ca}^{2+}]_i$  was seen at high (120Hz and 70Hz) but not at low (30Hz) frequency stimulation. Moreover, tetanic  $[\text{Ca}^{2+}]_i$  potentiation was also observed when the muscle was stimulated at short (1 and 2s) but not at longer (5 and 10s) intervals. The tetanic  $[\text{Ca}^{2+}]_i$  potentiation was seen in conditions when resting  $[\text{Ca}^{2+}]_i$  was elevated, either during the high frequency tetanic stimulation and during short stimulation intervals. Furthermore, low frequency 30Hz tetanic  $[\text{Ca}^{2+}]_i$  could be potentiated when it was immediately proceeded a priming bout involving repeated stimulation at high frequency (70 Hz).

#### **Conclusion**

Intense activation with high-frequency stimulation or activation with short rest intervals appears essential in inducing the potentiation of  $[\text{Ca}^{2+}]_i$  during repeated contractions.

## Poster 22

### Running reverses tumor-induced muscle weakness in mice with breast cancer

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#### Introduction

Patients with breast cancer experience muscle dysfunction, which is a clinical challenge that is not restricted to advanced stage patients, but also observed in newly diagnosed weight-stable patients with low tumor burden. Recent data indicate that physical activity can reduce breast cancer-associated mortality, suggesting that improved muscle performance *per se* can have positive impact on survival. Here, the transgenic PyMT mouse model of breast cancer was used to elucidate molecular mechanisms underlying breast cancer-induced muscle impairments.

#### Materials and Methods

PyMT mice and wildtype (WT) littermates w/wo access to an in-cage running wheel for four weeks (week 8-12). Functional readouts included  $\text{Ca}^{2+}$  imaging; isometric force measurement on single fibers and intact fast- and slow-twitch muscles. Intramuscular signaling was assessed using immunofluorescence, immunoblotting and enzymatic assays.

#### Results

The specific force (i.e. force/cross-sectional area) was significantly decreased by ~ 35% in slow-twitch soleus muscles from breast cancer mice as compared to WT muscles, which was the result of reduced  $\text{Ca}^{2+}$  release and impaired myofibrillar function. There were no difference in muscle size or fiber type between the two groups. However, higher intramuscular stress (e.g. p38 activation and carbonylation (DNP)) was observed in PyMT than in WT. Intriguingly, voluntary running for four weeks reversed the weakness and PyMT soleus muscles generated similar forces as muscles of exercised WT mice. The running induced higher SOD2 expression and normalized levels of p38 and DNP.

#### Conclusion

Intrinsic contractile dysfunction and higher intramuscular stress was present in mice with breast cancer, which was counteracted with voluntary running.

**Poster 23**

**Longitudinal changes in fitness in children 6 to 9 yr old. The Health Oriented Pedagogical Project (HOPP)**

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Measuring the development of fitness in children is important for health professionals. Cross sectional studies are useful as they provide valuable insight in the situation at data collection. Even more interesting are longitudinal studies that can track the individual child's development. Such studies are not so common, as they are more complicated to do. We present here data from the Health Oriented Pedagogical Project (HOPP), a seven-year longitudinal project started in 2015. This study aims at recording several anthropometric and fitness variables in a group of young children, and to follow the children for several years. One-hundred-and-fourteen children six-years old had their stature, body mass, waist circumference (WC), body composition and aerobic fitness (as maximal oxygen uptake with treadmill running and performance in the Andersen interval shuttle run test) measured in 2015, and again in 2018. Those belonging to the 75<sup>th</sup> quartile of Ponderal Index ( $\text{kg}/\text{m}^3$ ) in 2015 were compared with the rest of the group on body composition and aerobic fitness in 2018. In 2018, the heaviest children had poorer aerobic fitness (48.5 vs. 41.7  $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ,  $p<0.001$ ), poorer aerobic performance (943 vs. 843 m,  $p<0.001$ ), more body fat (18.5 vs. 24.2, %,  $p<0.001$ ) and larger WC (56.0 vs. 62.9 cm,  $p<0.001$ ) than the rest. The results indicate that the group with high PI develop in a more negative way than the rest of the participants. Simple measures of fitness, such as Body Mass Index, PI, WC, may possibly be used to select children for extended care and early interventions.

## Poster 24

### **Acid neutralisation in blood during and after 2 min cycling to exhaustion**

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#### **Introduction**

Intense exercise requires both aerobic and anaerobic energy release. Consequently,  $\text{CO}_2$  and lactic acid are released to blood in large amounts, changing its acid-base status. Aim: To examine how the acid load is handled in blood.

#### **Materials and methods**

Seven healthy young men cycled for 2 min to exhaustion. Blood was drawn from catheters in the femoral artery and vein during exercise and at intervals for 1 h after. Blood samples were analysed for pH, blood gases, lactate, haemoglobin, and plasma proteins and electrolytes; blood acid-base status, chloride shift, and Bohr-Haldane-effect were calculated thereof.

#### **Results**

Arterial blood lactate concentration ( $\text{cLa}$ ) rose to  $13.8 \text{ mmol L}^{-1}$ ,  $p_a\text{CO}_2$  fell by 21%, plasma pH fell to 7.18, blood bicarbonate concentration ( $\text{cHCO}_{3,\text{B}}$ ) fell by 53%, and base deficit ( $\text{cBD}$ ) rose 28% more than blood lactate concentration did. Femoral-venous minus arterial differences ( $\text{fv-a}$  difference) peaked at  $1.8 \text{ mmol L}^{-1}$  ( $\text{cLa}$ ), 68 hPa ( $p\text{CO}_2$ ),  $-0.23$  (pH),  $5.5 \text{ mmol L}^{-1}$  ( $\text{cHCO}_{3,\text{B}}$ ),  $4.4 \text{ mmol L}^{-1}$  ( $\text{cBD}$ ), and the total peripheral acid load (carbonic and non-carbonic origin) was  $9.4 \text{ mmol L}^{-1}$  at exhaustion. During exercise the Bohr-Haldane effect neutralised  $2.5 \text{ mmol L}^{-1}$  acid. Chloride shift amounted to 50% of the total acid load throughout, and the red blood cells swelled in proportion to the chloride shift.

#### **Conclusions**

Hydrogen ions added to the blood were largely transferred to and neutralised in the red blood cells, and the chloride shift was important for this transfer.

## Poster 25

### **NFATs regulatory potential and role in skeletal muscle during voluntary exercise**

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#### **Introduction**

Nuclear factor of activated T cells (NFAT) is a family of transcription factors, named NFATc1-4, they are activated in response to calcium signals. Linking change in the intercellular environment with gene expression. The NFATs are important for the development and phenotype of skeletal muscles, and the activity of the factors is enhanced during exercise. To better understand the role of NFAT, we have explored the transcriptional activity of the family members and how they contribute to muscle plasticity during exercise.

#### **Methods**

Cell culture experiments were conducted in cells with a chromatinized reporter construct. For the in vivo exercise experiments a NFAT-luciferase reporter mouse line was used. After voluntary running for 5 days, animals were sedated with isoflurane and the muscles of interest were surgically removed. Animals were sacrificed by cervical dislocation while under deep anaesthesia.

#### **Results**

Investigation of the transcriptional potential of the NFATs shows that the family members have individual activation potentials and are able to recruit a specific subset of co-regulators and chromatin modifying enzymes to target sites.

Analysis of the NFAT activity in vivo shows that the activity increases in response to endurance training. The findings are observed for both the soleus and the plantaris muscles, with the highest activity in the plantaris.

#### **Conclusion**

The results presented gives a novel insight into the difference between the NFAT family members and how they exert their regulatory functions.

**Poster 26**

**Myosin dysregulation in nemaline myopathy**

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Nemaline myopathy (NM) is a genetic muscle disease mainly caused by mutations in the gene encoding nebulin (NEB). Recent studies have shown that, in the presence of such nebulin mutations, one of the main pathophysiological mechanisms involves impairments in actin filament structure and/or function ultimately depressing the muscle force production. As current experimental therapies try to target the motor protein, myosin, to compensate for the actin dysregulation, in the current study, we aimed to investigate whether myosin molecules are also disrupted in nebulin-related NM. For that, we isolated muscle fibres from five patients with nebulin mutations and five age-matched controls. We then membrane-permeabilised them and applied the Mant-ATP chase protocol. Interestingly, we observed that myofibres from the patients had more disordered-relaxed myosin molecules than the muscle fibres from the controls. We are now in the process of exploring the potential underlying molecular mechanisms by which the primary actin filament dysregulation leads to such unexpected finding at the myosin filament level.

## Poster 27

### **Blood flow restricted resistance exercise, but not remote ischemic conditioning improves muscle functional capacity and quality of life despite extensive anabolic resistance in heart failure patients**

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#### **Introduction**

Chronic heart failure patients exhibit impaired functional capacity and quality of life which are associated to structural and functional changes within skeletal muscle. Current rehabilitation strategies include traditional high-load resistance exercise which may not be optimal for heart failure patients characterized by exercise intolerance and increased fatigability. We investigated if remote ischemic conditioning (RIC) and low-load blood flow restricted resistance exercise (BFRRE) could stimulate skeletal muscle adaptations and improve functional capacity and quality of life in heart failure patients.

#### **Methods**

36 heart failure patients ( $64 \pm 9$  yr.) were randomized to BFRRE, RIC, or non-treatment control (CON). BFRRE and RIC were performed 3 times per week for 6 weeks. Before and after the intervention, muscle biopsies, tests of physical function, and quality of life assessments were performed. Throughout, deuterium oxide was administered to measure cumulative protein and RNA synthesis. Changes in muscle fiber morphology and mitochondrial respiratory function, were also assessed.

#### **Results**

Only BFRRE improved 6-minute walk test ( $p < 0.05$ ), strength-endurance capacity ( $p < 0.05$ ), isometric strength ( $p < 0.01$ ), dynamic strength ( $p < 0.01$ ), and quality of life ( $p < 0.05$ ). Likewise, only BFRRE stimulated muscle mitochondrial function ( $p < 0.01$ ) and ribosomal biogenesis ( $p < 0.05$ ). Neither BFRRE nor RIC stimulated myofibrillar and mitochondrial protein synthesis rate, muscle hypertrophy, muscle stem cell content.

#### **Discussion**

Our results suggest that BFRRE can stimulate functional capacity and improve quality of life despite anabolic resistance in heart failure patients. Oppositely, RIC cannot promote functional capacity and quality of life in heart failure patients. The results have important clinical implications for rehabilitation of heart failure patients.

**Poster 28**

**The effects of antioxidant supplementation (Vitamin C&E) in sprint interval training in young and older adults**

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Sprint interval training (SIT) is a time-efficient alternative to continuous endurance training at lower intensity. Here, we studied whether SIT is as effective in improving endurance in elderly as in young subjects. Recreationally active elderly and young (mean age 65 and 24 years) men participated in the study. Training consisted of 3 sessions/week for 3 weeks of 4-6 repetitions of 30-s all-out cycling bouts interposed by 4 min rest. After three weeks of SIT, the power at exhaustion during an incremental cycling test was increased in both young and elderly subjects, whereas maximum oxygen uptake ( $\text{VO}_{2\text{max}}$ ) and SERCA and mitochondrial protein expression in muscle were increased only in young subjects following training. The sarcoplasmic reticulum  $\text{Ca}^{2+}$  channel, the ryanodine receptor 1, was fragmented in some subjects 24 hours after a SIT session both in the untrained and trained state. In conclusion, 27 min of sprint exercise over three weeks can improve physical performance in young and elderly human subjects, but the mechanisms underlying the differences in adaptability between the two age groups remains to be established.



## Cardiac Physiology

**Posters 29-45**

Poster 29

**Electrophysiology of sinus venosus – the “fourth chamber” of the snake heart**

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**Introduction**

In reptiles, the large sinus venosus (SV) consists of myocardial cells, contracts prior to the atria and appears to contribute significantly to atrial filling. We have previously shown that the electrical activation of the SV occurs in retrograde direction of blood flow and that the driving pacemaker seems to be located at the sinoatrial junction, similar to mammals.

**Materials and methods**

Here we used sharp glass microelectrodes and standard patch-clamp technique to study the SV and the pacemaker in Burmese pythons (*Python molurus*).

**Results**

We found a discrete group of pacemaker cells in proximity of the sinoatrial valve, whereas the myocytes in the SV had a stable membrane potential of  $-82.3 \pm 2.6$  mV ( $n=9$ ) with little pacemaker activity. The amplitude and duration of the action potentials (APs) of the SV resembled the atrial cells and were considerably shorter than APs in the ventricle. Patch clamp of isolated myocytes revealed large differences in the densities of the major ionic currents ( $I_{Na}$ ,  $I_{CaL}$ ,  $I_{Kr}$ ,  $I_{K1}$ ,  $I_{KACH}$ ) between atrial and sinus cells. The latter had densities of  $I_{Na}$ ,  $I_{CaL}$  and  $I_{Kr}$  much lower than in atrial and ventricular myocytes. In the region adjacent to sinoatrial valve, pacemaker APs with marked diastolic depolarization were observed, while hyperpolarization-activated  $I_f$  current could be recorded in spontaneously-beating cells from that region.

**Conclusion**

The snake SV contains mainly working-type myocytes with atrial-like electrical activity, while pacemaking myocytes are concentrated around sinoatrial valve.

Supported by RFBR 18-315-20049 and Danish Council for Independent Research, Natural Sciences (FNU).

## Poster 30

### **Migraine-associated mutation in the $\alpha 2$ isoform Na,K-ATPase is associated with cardiomyopathylike changes in the mouse heart**

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#### **Background**

Epidemiological studies have identified migraine with aura as a significant risk factor for developing heart disease. The underlying mechanism is unknown. Familial hemiplegic migraine type 2 (FHM2) is an autosomal dominant classical migraine with aura associated with pointmutations in the  $\alpha 2$  Na,K-ATPase. Heterozygote mice bearing FHM2-associated mutation ( $\alpha 2^{+/G301R}$ ) showed ~50% reduction of the  $\alpha 2$  Na,K-ATPase in the heart. We hypothesized that the FHM2-associated mutation leads to structural and functional abnormalities in the heart.

#### **Methods**

Young (~4 months old) and elderly (~8 months old)  $\alpha 2^{+/G301R}$  mice were compared with age-matching wild type (WT) mice. Left ventricle protein expression was analyzed using iTRAQ LC-MSMS proteomics. Left ventricle cross-sections were used for stereological analyses and ventricle wall ultrastructure. Cardiac structure and performance was assessed with *in vivo* echocardiography. Blood pressure (BP) was assessed with 24h telemetry.

#### **Results**

The heart-weight-to-body-weight ratio was similar for young mice of both genotypes but was increased in elderly  $\alpha 2^{+/G301R}$  mice. Elderly  $\alpha 2^{+/G301R}$  mouse hearts showed protein profile characteristic for cardiomyopathies. There was a tendency for increase in ventricular wall thickness of elderly  $\alpha 2^{+/G301R}$  mice vs. WT. However, no difference in cardiomyocyte morphology was found, but a tendency for cardiomyocyte hyperplasia was observed. Furthermore, there was a tendency towards a decreased ejection fraction in the  $\alpha 2^{+/G301R}$  mice that was associated with reduced blood pressure in elderly  $\alpha 2^{+/G301R}$  mice.

#### **Conclusion**

FHM2-associated mutation in the  $\alpha 2$  Na,K-ATPase leads to cardiomyopathy-like abnormalities suggesting an association between migraine and cardiovascular disease.

## Poster 31

### Acquired LQTS in horses

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### Introduction

The voltage-gated K<sup>+</sup>-channel Kv11.1 (hERG) potassium channel plays a central role in repolarization of the cardiac action potential. Human Kv11.1 is susceptible to unspecific drug interactions due to the presence of two aromatic amino acids residing in the inner vestibule of the pore. Blockage of Kv11.1 has been linked to acquired long QT syndrome (LQTS), torsade de pointes arrhythmia and sudden cardiac death (SCD) in humans. The aromatic residues are also present in the inner vestibule of the equine orthologue of Kv11.1, suggesting that equine Kv11.1 may also be prone to high-affinity block by a range of different chemical entities, which potentially could cause acquired LQTS and SCD in horses.

### Methods and Results

Kv11.1 cDNA was cloned from equine hearts and exhibited 93% homology with the human orthologue. High-throughput screening of selected compounds on human Kv11.1 expressed in a mammalian cell line was performed using an automated patch clamp system, the SyncroPatch 384PE (Nanion Technologies, Munich, Germany). Results were validated on equine Kv11.1 expressed in CHO-K1 cells by manual patch clamp. Acepromazine maleate ( $IC_{50} = 0.5 \mu M$ ), trimethoprim ( $IC_{50} = 100 \mu M$ ), diphenhydramine hydrochloride ( $IC_{50} = 2 \mu M$ ) and cyproheptadine hydrochloride ( $IC_{50} = 1.84 \mu M$ ) inhibited equine Kv11.1 current at clinically relevant drug concentrations.

### Conclusion

The results suggest that drug interaction with Kv11.1 can occur in horses and that some drugs potentially may induce repolarization disorders in horses.

## Poster 32

### Integration of contractile element in an *in silico* model of hiPSC cardiomyocyte

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#### Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) represent a promising *in vitro* model to test drug cardiac safety. Recently, also the assessment of contractility was involved in this kind of assays. Therefore, reliable hiPSC-CMs *in silico* models should feature also mechanisms as force generation. To this aim, we integrated a contractile element (CE) model into one existing hiPSC-CM action potential model.

#### Methods

The hiPSC-CM Paci model served as starting point and the Negrone model of myofilament contraction was then integrated into it. We challenged this enhanced model (hiPSC-CM-CE) simulating hiPSC-CM developed isometric force ( $F_m$ ) in response to different levels of intracellular calcium, sarcomere length ( $L_m$ ) and Ouabain administration.

#### Results

The model developed a peak  $F_m$  of 1.95mN/mm<sup>2</sup> ( $L_m$  1.05μm), i.e. the same order of magnitude reported in adult rabbit ventricular cardiomyocytes. Changes in  $L_m$  between 0.9μm and 2.1μm corresponded to changes in  $F_m$  ranging from 1.69mN/mm<sup>2</sup> to 6.00mN/mm<sup>2</sup>, following a power function as in cat trabecular cells. The hiPSC-CM-CE sensitivity to  $[Ca]_i$  variations was assessed by increasing  $I_{CaL}$  or  $I_{bCa}$  or  $I_{NCX}$  (systolic  $[Ca]_i$  between 15μM and 45μM), leading to  $F_m$  increments similar to *in vitro* observations. Furthermore, we qualitatively reproduced changes in  $F_m$  resulting from administration of [0.1,3,10,30,100,300]nM Ouabain, showing  $F_m$  increasing up to 30nM and then decreasing for higher concentrations.

#### Conclusions

Our work represents the first attempt to integrate a CE model in *in silico* hiPSC-CMs. Future developments will include optimization of the model parameters to improve the accuracy of simulating the experiments.

Poster 33

**Endothelial cell activation by pro-inflammatory cytokines exerts novel paracrine effects on cocultured cardiomyocytes**

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Microvascular dysfunction has a pivotal role in the progression of myocardial inflammation into heart failure. In addition to barrier disruptions and a pro-angiogenic activity, this involves changes in the soluble factors released by endothelial cells (EC), including cardioactive molecules. Yet paracrine effects of microvascular dysfunction on cardiomyocytes (CM) have never been confirmed. We hypothesised that pro-inflammatory activation of EC would disrupt CM functions. Rat myocardial slices from left ventricles were cultured for 24h with *Cytomix* (TNF- $\alpha$ , IL-1 $\beta$ , IL-6R $\alpha$ /IL-6 chimera). *In vitro*, human cardiac microvascular EC were pre-conditioned for 24h with *Cytomix* and co-cultured in transwell inserts above adult rat ventricular CM. Calcium transient recordings with Fluo4 were the primary readout of CM function. Finally, co-culture supernatants were analysed using a Cytokine Array and ELISAs.

Myocardial slices treated with *Cytomix* produced stronger and significantly shorter contractions when stretched at 23% (>2  $\mu$ m sarcomere length). In co-cultures, *Cytomix* pre-conditioning of EC induced a shortening of calcium transients in CM, compared to co-cultures with untreated EC. This effect correlated with a release of CC/CXC motifs chemokines and G-/GM-CSF. According to published RNAseq datasets however, CM do not constitutively express the receptors for these markers, so it is likely that other soluble factors are involved.

The lusitropic effect of *Cytomix* on myocardial slices contrasts with published works on isolated cardiomyocytes or individual cytokines. We were able to replicate this lusitropic effect by preconditioning EC with *Cytomix* prior co-culture above CM. This suggests EC may contribute to regulating CM function during inflammation using paracrine factors.

Poster 34

**T-tubule disruption is a prominent feature of HFrEF but not HFpEF**

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Heart failure comprises two main entities; heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF). HFpEF is associated with concentric hypertrophy, stiffening of the ventricular wall, and a predominant impairment of diastolic function. HFrEF, by comparison, is associated with left ventricular dilation, wall thinning and markedly reduced systolic function. Impaired cardiomyocyte contractility in HFrEF has been linked to disruption of membrane invaginations called t-tubules, but it is unknown if such changes occur in HFpEF.

We aimed to compare t-tubule remodeling in HFpEF and HFrEF patients to determine whether the differing *in vivo* functional phenotypes of these conditions reflect distinct cardiomyocyte structural pathophysiology.

Cardiomyocytes examined from HFpEF patients exhibited *increased* t-tubule density in comparison with non-failing controls. Super-resolution imaging revealed that higher t-tubule density resulted from both tubule dilation and proliferation. In contrast, t-tubule density was reduced in patients with HFrEF, as tubules maintained their widths and did not proliferate to match the cellular hypertrophy. Augmented collagen deposition was observed within the t-tubules of HFrEF but not HFpEF hearts. In agreement with recent data linking mechanical stress to t-tubule disruption, we observed markedly elevated wall stress in HFrEF patients, whereas the robust ttubule presence in HFpEF patients was associated with maintained stress across the concentrically remodeled ventricle.

While t-tubule disruption is a hallmark feature of HFrEF, t-tubule growth likely helps preserve systolic function in HFpEF. These findings support the notion that HFpEF and HFrEF are distinct entities with divergent underlying remodeling.

Poster 35

**Implantable loop recorders for arrhythmia detection – a new promising diagnostic tool for longterm arrhythmia monitoring**

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Atrial fibrillation (AF) is the most common cardiac arrhythmia. Large animal models such as goats, pigs and horses are used for longitudinal AF studies where AF burden over time is crucial information. Loop recorders (LRs) are implantable ECG devices, capable of automatically detecting and storing arrhythmic events for up to three years. The LRs are increasingly used to detect paroxysmal AF in human patients. The objectives were to test if LRs could identify AF episodes in horses with both induced AF and horses with paroxysmal AF.

First, thirteen LRs were implanted subcutaneously in up to four different locations (pectoral, sixth and ninth left intercostal space (ICS-6 and ICS-9), and xiphoid region) in five Standardbred horses. The R and T wave amplitudes were measured in all positions at two different time points. AF burden was registered by the LRs over a 2-month period of induced AF.

Second, LRs were implanted in three horses with suspected paroxysmal AF. All positions showed ECG signals stable over time and the LRs could automatically detect the induced AF. ICS-6 showed higher R and T wave amplitudes compared to the other positions ( $P < 0.0001$ ). Movement artefacts at rest were rare, and none of them led to a false positive AF diagnosis. In addition, the LR found two AF episodes in the horses with suspected paroxysmal AF. Implantable loop recorders, designed for humans can detect AF occurrence in horses. For in-vivo longitudinal studies using large animal models, the LRs may be a valuable tool for long-term arrhythmia monitoring.



## Poster 36

### **Acetylcholine affects the electrical properties of neonatal caval vein myocardium via noncanonical mechanism**

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#### **Introduction**

The parasympathetic innervation and cholinergic regulation of the heart is already established before the birth. However, it is unknown how acetylcholine regulates the bioelectrical activity of the superior vena cava's (SVC) myocardium which demonstrates both pacemaker and working myocardium characteristics during early postnatal period.

#### **Materials and Methods**

Electrically evoked (EAP), spontaneous action potentials (SAP) and resting membrane potential (RPM) were recorded in multicellular SVC preparations of neonatal male Wistar rats using standard microelectrode technique under control conditions and application of acetylcholine (ACh, 1  $\mu$ M) or a gap junction uncoupling agent octanol (1.2 mM). The data is presented as mean $\pm$ SD.

#### **Results**

The application of ACh had no effect on RMP of both EAP and SAP, did not alter the slow diastolic depolarization phase of SAP, but induced a significant decrease of the fast sodiumcurrent-dependent upstroke velocity (by  $39.8\pm26.5$  V/s,  $p<0.05$ ) in SAP and prolonged the time delay for EAP excitation (by  $0.8\pm0.5$  ms,  $p<0.05$ ). These ACh effects can be associated with gap junctions uncoupling in SVC. However, the octanol effects were only partially similar with ACh, since octanol induced a decrease of upstroke velocity both in SAP and EAP (by  $60.9\pm30.4$  V/s,  $p<0.05$ ), depolarized the RMP of SAP (by  $3\pm2$  ms,  $p<0.05$ ) and prolonged the EAP activation time delay (by  $3.5\pm2.6$  ms,  $p<0.05$ ).

#### **Conclusions**

The comparison of ACh and octanol effects in neonatal SVC myocardium allow us to suppose that gap junctions and fast sodium current channels are the non-canonical targets for the cholinergic regulation of supraventricular myocardium.

## Poster 37

### Heart rate variability mechanisms dissected by multiscale information decomposition

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#### Introduction

Heart rate variability (HRV; variability of the RR interval of ECG) results from the activity of several coexisting control mechanisms, involving the influence of respiration (RESP) and systolic blood pressure (SBP) oscillations operating across multiple temporal scales and changing in different physiological states. The aim of this study was to dissect the physiological mechanisms related to HRV origin on faster and slower time scales separately using multiscale information decomposition.

#### Materials and methods

In 78 young volunteers, we monitored RR, SBP and RESP noninvasively at rest and during postural and mental stress evoked by head-up tilt (HUT) and mental arithmetics (MA). After representing RR, RESP, and SBP at different time scales through a recently proposed method based on multivariate state space models, the joint information transfer was decomposed into unique, redundant and synergistic components, describing respectively the strength of baroreflex modulation independent of respiration, nonbaroreflex and baroreflex-mediated respiratory influences, and simultaneous presence of baroreflex and nonbaroreflex respiratory influences.

#### Results

We found that fast HRV oscillations – respiratory sinus arrhythmia – originate from the coexistence of baroreflex and non-baroreflex (central) mechanisms at rest with the stronger involvement of baroreflex during HUT. Focusing on slower HRV oscillations, the baroreflex mechanism becomes dominant and MA leads to its higher involvement. Respiration influences on slow time scales independent on baroreflex are present and further enhanced during HUT.

#### Conclusions

Multiscale information decomposition demonstrated the changes in the involvement of baroreflex, respiration and other mechanisms in the origin of HRV in relation to analysed time scale.

Poster 38

**Effect of Selective  $I_{K,ACh}$  Inhibition by XAF-1407 in an Equine Model of Tachypacing-induced Persistent Atrial Fibrillation (AF)**

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**Background and Purpose**

Inhibition of the G-protein gated acetylcholine-activated inward rectifier potassium ( $K^+$ ) current,  $I_{K,ACh}$ , may be an effective atrial selective treatment strategy for atrial fibrillation. Therefore, the anti-arrhythmic and electrophysiological properties of a novel potent and highly specific  $I_{K,ACh}$  inhibitor, XAF-1407 (3-methyl-1-[5-phenyl-4-[4-(2-pyrrolidin-1-ylethoxymethyl)-1-piperidyl]thieno[2,3-d]pyrimidin-6-yl]azetidin-3-ol), were investigated in an equine, atrial-tachypacing-induced model of persistent AF.

**Experimental Approach**

In this model 11 horses were equipped with implantable cardioverter defibrillators (ICD) enabling atrial-tachypacing into self-sustained AF. The electrophysiological effects of XAF-1407 were investigated at different time points over a period of one month. Cardioversion success, drug induced changes of atrial tissue refractoriness and ventricular electrophysiology were assessed at baseline (day 0) and at day 3, 5, 11, 17 and 29 after AF induction.

**Key Results**

XAF-1407 is inhibiting  $K_{ir}3.1/3.4$  hetero- and  $K_{ir}3.4$  homotetramers, underlying the  $I_{K,ACh}$  current, with the same potency and at high specificity. XAF-1407 treatment in horses prolongs atrial effective refractory period (aERP), decreases atrial fibrillatory rate (AFR) and successfully cardioverts AF, although with a decreasing efficacy over time (100 % up to day 5 and 46% at day 29). XAF-1407 shortens AV-nodal refractoriness, and provides a minor QT prolongation, but does not affect QRS duration. Ventricular pro-arrhythmicity was not observed.

**Conclusion and Implications**

Treatment with XAF-1407 was found to efficiently cardiovert shorter duration AF of tachypacing induced AF in horses and did not induce any safety compromising side effects. The study thereby suggests that  $I_{K,ACh}$  inhibition may constitute a potentially potent and safe treatment modality of paroxysmal AF.

**Poster 39**

**Cardiac repolarization in horses**

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**Introduction**

In horses, sudden, unexplained deaths occur relatively often. Spontaneous arrhythmias have been detected in horses, but studies coupling specific arrhythmias to sudden cardiac death (SCD) are sparse. The voltage-gated K<sup>+</sup>-channels Kv7.1 (KCNQ1) and Kv11.1 (hERG) play central roles in repolarization of the cardiac action potential. In humans, inherited loss-of-function mutations in Kv7.1 and Kv11.1 have been linked to congenital long QT syndrome (LQTS), torsade de pointes arrhythmia and SCD.

**Methods and Results**

Kv7.1 and its subunit KCNE1 as well as Kv11.1 cDNAs were cloned from equine hearts. All subunits exhibit a high degree of homology with their human orthologues. Expression of equine Kv7.1 + KCNE1 and Kv11.1 in CHO-K cells resulted in currents with properties similar to the human Kv7.1/KCNE1 and Kv11.1 under voltage-clamp conditions. The functional roles of Kv11.1 in equine hearts were tested in arterially-perfused wedge preparations from the right ventricular wall. Action potentials were recorded at different pacing rates in the absence and presence of specific blockers. In the presence of 10  $\mu$ M of the Kv11.1 blocker terfenadine a marked prolongation of the action potential duration (from 420 ms to 495 ms at 90 % of repolarization (APD<sub>90</sub>) at a basic cycle length of 2000 ms) was observed.

**Conclusion**

Kv7.1/KCNE1 and Kv11.2 channels are important for cardiac repolarization in equine hearts. This could indicate that loss-of-function mutations in Kv7.1/KCNE1 and Kv11.1 could cause LQTS. Presently it is, however, not clear whether LQTS in horses is associated with increased risk of SCD.

Poster 40

**Late sodium current in canine, guinea pig and human ventricular myocardium**

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Although late sodium current ( $I_{Na-late}$ ) has long been known to contribute to plateau formation of mammalian cardiac action potentials, lately it was considered as possible target for antiarrhythmic drugs. However, many aspects of this current is still poorly understood. The present work was designed to study the true profile of  $I_{Na-late}$  in canine and guinea pig ventricular cells and compare them to  $I_{Na-late}$  recorded in undiseased human hearts.  $I_{Na-late}$  was defined as a tetrodotoxin-sensitive current, recorded under action potential voltage clamp conditions using either canonic- or self-action potentials as command signals.  $I_{Na-late}$  was also recorded using conventional voltage clamp. Action potentials were recorded from multicellular preparations with sharp microelectrodes. Under action potential voltage clamp conditions the amplitude of canine and human  $I_{Na-late}$  monotonically decreased during the plateau (decrecendo-profile), in contrast to guinea pig, where its amplitude increased during the plateau (crescendo profile). The decrecendo-profile of canine  $I_{Na-late}$  could not be converted to a crescendo-morphology by application of isoproterenol, calmodulin, ramp-like command voltages or command action potentials recorded from guinea pig cells. Conventional voltage clamp experiments revealed that the crescendo  $I_{Na-late}$  profile in guinea pig is due to the slow decay of  $I_{Na-late}$  in this species.  $I_{Na-late}$  was increased by isoproterenol but not by calmodulin in canine myocytes. Tetrodotoxin decreased APD in a reverse rate-dependent manner in multicellular ventricular preparations, which effect was the largest in human, while smaller in canine and the smallest in guinea pig preparations. It can be concluded that important interspecies differences exist in the expression and behavior of  $I_{Na-late}$ . Canine myocytes represent a better model of human ventricular cells than those of the guinea pig regarding the properties of  $I_{Na-late}$ . Present results should be taken into account when the results of pharmacological studies are interpreted and extrapolated to human. Accordingly, canine ventricular tissues or myocytes are suggested for pharmacological studies with  $I_{Na-late}$  inhibitors or modifiers.

## Poster 41

### **NLRP3 inflammasome is activated in the atrium of an ovine model of sustained obesity**

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#### **Introduction**

NLRP3-inflammasome activation contributes to the pathogenesis of atrial fibrillation (AF) and requires two steps: 1st) a priming event including an NFkB-activating stimuli, and 2nd) a triggering event including the assembly of the complex and activation of caspase-1 which promotes the production of pro-inflammatory cytokines like IL-1 $\beta$ . We used a sheep model of sustained obesity to characterize the association between atrial myocardial fat infiltration, atrial activation of the NLRP3-inflammasome and development of an atrial arrhythmogenic substrate for AF.

#### **Material and Methods**

Eight sheep were fed calorie-dense diet over 40 weeks and were maintained in this state of sustained obesity for another 40 weeks. Eight lean and aged-matched sheep served as control. Atrial fat infiltration was determined by oil-red-O staining and NLRP3- inflammasome activation was assessed by immunoblot in atrial whole-tissue lysate. Atrial effective refractory periods (aERPs) were evaluated (twice diastolic threshold, cycle length of 400 ms, S<sub>1</sub>S<sub>2</sub> -protocol).

#### **Results**

Sustained obesity was associated with increased atrial fat infiltration ( $p=0.1$ ) and shorter aERP ( $p=0.03$ ). Protein levels of caspase-1 and mature IL-1 $\beta$  were significantly enhanced ( $p=0.04$  and  $p=0.01$ , respectively). Further, shortening of aERP correlated with increasing atrial protein levels of caspase-1 ( $r=0.59$ ,  $p=0.02$ ). Levels of TNF $\alpha$  and NFkB were not changed.

#### **Conclusions**

Sustained obesity is associated with increased expression of NLRP3-inflammasomerelated proteins and the development of an arrhythmogenic substrate for AF. Our study suggest that the increased activity is due to increased triggering, rather than increased gene transcription. Whether NLRP3-inflammasome activation represents a modifiable target to prevent AF in obesity warrants further study.

**Poster 42**

**VEGF-B overexpression do not counteract heart failure in DOCA-salt hypertensive rats**

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**Background**

Cardiac hypertrophy is an adaptive response to physiological and pathological overload. Increased oxygen demand promote myocardial angiogenesis to maintain cardiac contractile function. VEGF-B (angiogenic growth factor) has shown protective against ischemic heart disease. However, its role in heart failure remains unknown. In this study, the effect of VEGF-B on cardiac function was investigated in deoxycorticosterone acetate (DOCA)-salt treated rats.

**Material and Methods**

VEGFB overexpressing ( $\alpha$ MHC-VEGFB-TG) and WT male Wistar rats were assigned to DOCA (150mg) pellets sc. and sodium chloride (1%) drinking water. After 6 weeks of treatment, blood pressure (BP) was measured with radio-telemetry (DSI) and LV dimension/function with echocardiography (Vevo 2100). Cardiac cryosections (8 $\mu$ m) were immunolabeled with PECAM (1:100) and Masson's trichrome as vascular, fibrotic and scar marker. Resident cardiac immune cells were isolated and identified using flow cytometry.

**Results**

VEGFB-TG rats demonstrated cardiac hypertrophy and increased BP, whereas cardiac function parameters were not affected prior to treatment. After DOCA-salt, systolic BP increased more in WT ( $\Delta 25 \pm 3$  mmHg, n=6) than VEGFB-TG ( $\Delta 10 \pm 1$  mmHg, n=6); however, VEGFB-TG had greater ventricular hypertrophy, vascularization as well as interstitial fibrosis and inflammation. After DOCA, VEGFB-TG developed dilated cardiomyopathy with impaired LV systolic function, as shown by decreased EF (VEGFB,  $51 \pm 2\%$  versus WT,  $70 \pm 2\%$ ,  $p < 0.001$ , n=6) and reduced LV posterior wall thickness (PWd, VEGFB,  $2.46 \pm 0.02$  mm versus WT,  $3.23 \pm 0.07$ ,  $p < 0.05$ , n=6).

**Conclusion**

DOCA-salt in VEGFB-TG recapitulates the heart failure phenotype in rats, independent of systemic arterial blood pressure. This rat model may provide insight into the mechanisms of human cardiovascular remodeling.

## Poster 43

### **In vivo Small Conductance Ca<sup>2+</sup>-activated K<sub>3</sub> (SK<sub>3</sub>) RNA silencing in rats using LNA-GapmeRs: a new engineered large molecule protects against Atrial Fibrillation (AF)**

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#### **Introduction**

Antisense Locked Nucleic Acids (LNA) GapmeRs are stable small oligonucleotides able to bind their mRNA target with high affinity. We designed novel SK<sub>3</sub> specific LNA-GapmeRs which *in vitro* demonstrated specific SK<sub>3</sub>-knockdown. Here, we show the effect of transient SK<sub>3</sub> gene knockdown *in vivo* to investigate the potential of LNA-GapmeRs in AF treatment.

#### **Material and methods**

At day one and seven, 23 randomized male Wistar rats (150 g) received subcutaneous injections of either vehicle or LNA-GapmeR (50 mg/Kg). At day fourteen, rats were sacrificed, organs removed and hearts mounted on a Langendorff system. By pacing hearts at 7 and 10 Hz, action potential duration (APD<sub>90</sub>) and effective refractory period (ERP) were investigated. To study AF likelihood, AF events were induced by high frequency atrial electrical pacing. To test SK<sub>3</sub> channel activity, SK blocker ICAGEN (1.0  $\mu$ M) was perfused for 15 minutes and refractoriness was measured. SK<sub>3</sub> protein expression was assessed through western blot assay in the atrium and hypothalamus.

#### **Results**

LNA-GapmeR downregulated SK<sub>3</sub> protein expression level in the atrium ( $p < 0.01$ ) but did not alter its expression in the hypothalamus. SK<sub>3</sub>-silencing reduced duration of induced and spontaneous AF episodes ( $p < 0.05$ ). GapmeR treatment showed a tendency in APD<sub>90</sub> prolongation at 7 Hz and no effect at 10 Hz. Although refractoriness was not altered at baseline, ICAGEN did not profoundly prolong ERP in SK<sub>3</sub>-knockdown group.

#### **Conclusion**

The designed SK<sub>3</sub>-LNA-GapmeR silenced the cardiac SK<sub>3</sub> channels, preventing AF in rats. Thus, GapmeR technology offers a platform for generation of specific drug development.



## Poster 44

### Physical Activity and Sudden Cardiac Death in Patients with Coronary Artery Disease

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#### Purpose

The association between leisure time physical activity (LTPA) and a risk for sudden cardiac death (SCD) is not known in coronary artery disease (CAD) patients at modern treatment era. We investigated the association between LTPA and the risk for SCD and non-SCD in CAD patients.

#### Methods

Patients with angiographically documented CAD (n=1,946) underwent a clinical examination including LTPA questionnaire and extensive risk profiling at the baseline. LTPA groups were divided: 1) Inactive; 2) Irregularly active; 3) Active, exercise regularly 2-3 times weekly; 4) Highly active, moderate or high intensity exercise regularly four times or more weekly. Age, sex, body mass index, left ventricular ejection fraction, type 2 diabetes, Syntax-score, CCS-class, depression and exercise capacity were used as covariates in multivariate Cox regression analysis.

#### Results

During follow up (median 6.3 years) 52 SCDs and 49 non-SCDs were observed. Inactive and highly active patients had a higher risk for SCD than active patients (HR: 2.57, 95 % CI: 1.05-6.29;  $p<0.05$  and HR: 2.04, 95 % CI: 0.85-4.87;  $p=0.10$ , respectively). Among patients with CCS-class 2 or higher, highly active patients had the highest risk of SCD (HR: 7.35, 95 % CI: 2.29-23.6;  $p<0.001$ ) followed by inactive patients (HR: 3.67, 95 % CI: 1.17-11.6;  $p<0.05$ ) compared to active patients. A linear association was observed between LTPA and non-SCD, those with high LTPA had the lowest risk for non-SCD.

#### Conclusion

A U-shaped association was identified between LTPA and risk for SCD but a linear association between LTPA and risk for non-SCD. The risk for SCD is particularly high among highly active symptomatic patients.

Poster 45

**Temperature-dependent changes in electrical excitation of rainbow trout ventricular myocytes**

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Under acute temperature changes cardiac output in fish is mainly regulated by changes in heart rate. According to the hypothesis of temperature-dependent deterioration of electrical excitation (TDEE) heat-dependent depression of heart rate in fish is due to the mismatch between depolarizing Na current ( $I_{Na}$ ) and repolarizing K current ( $I_{K1}$ ) of ventricular myocytes. To elucidate the molecular mechanism of the TDEE hypothesis, we examined temperature-dependence of action potential generation, and density and kinetics of  $I_{Na}$  in ventricular myocytes of the rainbow trout (*Oncorhynchus mykiss*). Patch-clamp recordings in ventricular myocytes of 12°C-acclimated trout were conducted at 12°C and 25°C. When temperature was increased from 12°C to 25°C there was small increase in the density of the peak  $I_{Na}$  and a small shift in voltage-dependence of steady-state activation to more positive values. In contrast, charge transfer via  $I_{Na}$  at 25°C was only about 50% of that at 12°C due to much faster inactivation kinetics of  $I_{Na}$  at 25°C. Simultaneously the resting ion leakage of sarcolemma was doubled (input resistance was halved) at 25°C. These changes generated a mismatch between depolarizing current flow (Na influx) and repolarizing current flow (K efflux), which appeared as elevated threshold of action potential generation at 25°C. This temperature-dependent mismatch may be the cause for missed beats, ventricular bradycardia and asystole of the ventricle at high temperatures.

## Vascular Physiology and Blood Pressure

**Posters 46-63**

Poster 46

**The effect of uric acid on the endothelial function of small mesenteric arteries in old Wistar-Kyoto rats during *in vitro* conditions.**

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The endothelial dysfunction is an early stage of many cardiovascular diseases (CVD), significantly contributing to global deaths and chronic disabilities. One of many factors that are associated with an increased risk of hypertension and CVD generally is a long-term elevated uric acid (UA) level in plasma – hyperuricemia. Nevertheless, the role of UA in the pathogenesis of endothelial dysfunction is still unclear and is subject to wide discussion.

In our *in vitro* studies, we observed acute effects of high concentrations of UA on the endothelial function. Experiments were performed in isolated small mesenteric arteries from aged (68-week-old or 57-week-old) Wistar-Kyoto male rats. Vascular reactivity was investigated at isometric conditions using the wire myograph. The arterial segments were pre-contracted with noradrenaline ( $10^{-5}$  mol/l) and then subjected to endothelium-dependent relaxation, as response to acetylcholine. Then, the UA was added to the myograph chamber at 600  $\mu$ mol/l (arteries from 68-week-old rats) or 1200  $\mu$ mol/l (arteries from 57-week-old rats) and incubated for 1 hour, followed by the acetylcholine-concentration-response curve in the presence of UA.

UA had no significant effect on acetylcholine-induced vasorelaxation. Likewise, no significant differences in noradrenaline-induced vasoconstriction were observed after uric acid pre-incubation in all investigated groups. Acute high concentrations of UA failed to affect endothelial functions of aged rats.

In conclusion, the acute incubation with high concentration of UA did not provoke endothelial dysfunction in the resistant mesenteric arteries from aged rats and had no effect on overall bioavailability of endothelium-derived factors.

*Supported by the grants VEGA 2/0190/17, APVV-16-0263 and APVV-15-0565.*

**Poster 47**

**The vasomotoric effect of exogenous and endogenous hydrogen sulphide in normotensive and spontaneously hypertensive rats**

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Cardiovascular studies confirmed that hydrogen sulphide ( $H_2S$ ) is involved in signaling pathways in physiological and pathological conditions, including hypertension. In the vessels  $H_2S$  is produced mainly by cystathionine  $\gamma$ -lyase (CSE) which converts L-cysteine to  $H_2S$ . The aim of study was to describe the vasoactive effect of exogenous and endogenous  $H_2S$  (after a CSE inhibition) in normotensive and spontaneously hypertensive rats (SHR).

In the experiments 17-20-weeks-old normotensive Wistar rats and SHR were included. Systolic blood pressure (sBP) was measured by plethysmographic method and vasoactivity of isolated thoracic aorta was recorded. The expression of CSE was determined by Western blotting. We observed an increased sBP and hypertrophy of myocardium in SHR. Dual effect of  $H_2S$  donor ( $Na_2S$ ) was showed in both strains; however an increased maximal vasorelaxation was proved in SHR. While in normotensive rats an acute inhibition of CSE had no effect on endotheliumdependent relaxation, in SHR reduced the sensitivity of smooth muscle cells to endogenous NO. CSE inhibition induced a moderated increase in the basal arterial tone of both strains. Moreover, in normotensive rats, in contrast to SHR, the increased sensitivity of adrenergic receptors to exogenous noradrenaline, was confirmed after CSE inhibition. There was no difference in the expression of CSE in normotensive rats and SHR.

The data confirmed that  $H_2S$  has a crucial role in the regulation of the vascular tone and vasoactive responses. It seems that in essential hypertension the sulfid signal pathway could regulate the arterial tone in favor of vasorelaxation.

*Supported: VEGA 2/0111/19, VEGA 2/0103/18, APVV-15-0565.*

## Poster 48

### **The effect of superparamagnetic iron oxide nanoparticles and acute stress co-exposure on pressor reactivity and nitric oxide production in normotensive rats**

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#### **Introduction**

Superparamagnetic iron oxide nanoparticles (SPIONs) can be used in various biomedical applications. However, the effect of SPIONs on mean arterial pressure (MAP) reactivity and vascular function during acute stress is unclear. This study investigated if SPIONs modify pressor reactivity, vascular function and nitric oxide (NO) production in Wistar-Kyoto rats.

#### **Material and Methods**

Acute stress was induced by 5-sec air-jet 10 min before and 20 min after SPIONs administration. MAP reactivity was determined in the carotid artery in conscious rats and expressed as the air-jet-induced  $\Delta$ MAP (%) vs. MAP preceding air-jet. SPIONs (30 nm, polyethylene glycol-coated magnetite) were administered i.v. (1mg Fe/kg). NO production was determined by conversion of 3H-L-arginine. Endothelial function was determined in the femoral artery by wire myography in isometric conditions.

#### **Results**

SPIONs and saline, respectively, had no effect on MAP. Air-jet, after saline infusion, resulted in the ~30% increase of MAP vs. pre-stress value. Air-jet after SPIONs increased MAP by ~45%. No alterations in the endothelium-dependent acetylcholine-induced relaxation were found after saline, air-jet, SPIONs or their co-exposure. NO production was reduced significantly in the hypothalamus and brainstem but elevated in the kidneys of rats co-exposed to SPIONs plus air-jet vs. SPIONs per se.

#### **Conclusion**

Results showed that SPIONs elevated acute stress-induced MAP responses in rats without changes in endothelial function. Elevated pressor response may result from reduced NO bioavailability in the brainstem and/or hypothalamus, suggesting the effect of acute stress on incorporation of SPIONs to the abovementioned brain structures.

Supported by APVV-16-0263, VEGA 2/0160/17 and SAV-BAV-18-11.

## Poster 49

### **ATP induces contraction of brain capillary pericytes in vitro, indicating that pericytes may play a role in regulation of capillary blood flow in the brain.**

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#### **Introduction**

The neurovascular unit (NVU) is composed of endothelial cells, surrounded by pericytes and astrocytes. Purinergic signalling has been implicated to play a role in the intercellular communication in the NVU and it has been suggested that ATP cause a contraction of pericytes. The aim of the study was to investigate ATP-induced purinergic signalling and influence on contractility in brain capillary pericytes.

#### **Materials & Methods**

Primary cultures of pericytes were cultured from bovine brain capillaries and expression of cell markers was investigated, using immunocytochemical staining in combination with CLSM imaging and realtime PCR. Cytosolic Ca<sup>2+</sup> was measured with indicator dyes, changes in pericyte cross-sectional area were estimated using CLSM.

#### **Results**

Primary cultures of bovine pericytes were cultured for 5-7 days. The pericytes displayed an irregular cell morphology, expression of alpha-smooth muscle actin ( $\alpha$ -SMA). PDGFR-B was highly expressed at the mRNA level. Von Willebrands factor (VWF) and the astrocyte marker glial fibrillary acidic protein (GFAP) were not present, indicating pure cultures. Addition of ATP to cultured pericytes, caused increases in cytosolic calcium levels, which could be inhibited by the purinergic antagonist PPADS. ATP caused the cultured pericytes to contract, as shown by CLSM imaging of dye-loaded cells.

#### **Conclusions**

We established a protocol for a primary culture of brain pericytes, and observed that ATP induced cytosolic calcium increases via purinergic receptors, concomitant with a pericyte contraction. Our data indicate that ATP may regulate microvascular tone *in vivo*.

## Poster 50

### Arterial stiffness index CAVI in obese adolescents – an association with autonomic function

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### Introduction

Index CAVI (Cardio-ankle vascular index), non-invasively estimating arterial stiffness, is increasingly used for the early atherosclerotic changes assessment. In previous studies obesity was found to be associated with paradoxically decreased CAVI values. The VaSera device enables the measurement of CAVI (heart-*a.tibialis*) and kCAVI (knee CAVI – heart-*a. poplitea*).  $\Delta$ CAVI (= kCAVI - CAVI) reflects the effect of muscular part of circulation on CAVI. The aim of our study was to find out if changed sympathetic activity can contribute to differences in CAVI between young obese patients and control volunteers.

### Methods

We examined 29 obese (14f, age  $16.44 \pm 2.7$  y., BMI:  $33.31 \pm 4.4$  kg.m<sup>-2</sup>) and 29 non-obese gender and age matched adolescents (BMI:  $21.01 \pm 2.3$  kg.m<sup>-2</sup>). Arterial stiffness indices CAVI and kCAVI were measured using VaSera VS-1500 (Fukuda Denshi, Japan). Sympathetic activity index LF<sub>SAP</sub> (spectral power of systolic blood pressure oscillations in low frequency band – 0.04-0.15 Hz) was calculated from 300 beats long recordings of continuously measured blood pressure (Finometer, Netherlands).

### Results

In non-obese volunteers we found no significant difference between CAVI<sub>cont</sub> and kCAVI<sub>cont</sub> ( $p=0.914$ ), while in obese group kCAVI<sub>ob</sub> was significantly higher than CAVI<sub>ob</sub> ( $p0.001$ ). LF<sub>SAP</sub> was significantly lower in obese group ( $p0.001$ ) and was negatively correlated with  $\Delta$ CAVI in obese group ( $\rho=-0.397$ ,  $p=0.033$ ).

### Conclusion

Decreased sympathetic activity in young obese participants can be responsible for significant difference between kCAVI and CAVI in this group of participants. Thus, peripheral vasodilation present in obesity can influence the results of this investigation method.

Grants: VEGA 1/0117/17, VEGA 1/0200/19, ITMS project “BioMed Martin” no.26220220187.



## Poster 51

### **Browning of perivascular adipose tissue prevents vascular dysfunction and reduces hypertension in Angiotensin-II infused mice**

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#### **Introduction**

Hypertension is a world-leading cause of cardiovascular disease and premature deaths. Vascular tone is in part regulated by perivascular adipose tissue (PVAT) that releases pro and anti-contractile factors. In hypertension, dysfunctional PVAT is observed and studies have indicated a causal relationship between dysfunctional PVAT and vascular damage in hypertension. The phenotype of PVAT on resistance vessels is primarily white adipose tissue. The present study investigates the impact of a changed phenotype i.e. browning of PVAT, on vascular function and the development of hypertension.

#### **Material and methods**

Browning was induced by  $\beta_3$ -adrenergic agonist in control and Angiotensin II-induced hypertensive mice. Studied parameters included blood pressure by tail cuff plethysmography and vascular function by wire myography. Browning was confirmed through an immunohistochemical and gene analysis approach.

#### **Results**

The anticontractile effect of PVAT is lost in untreated hypertensive mice and vascular tone and blood pressure is increased. Browning of PVAT resulted in maintained anticontractile effect, improved endothelial function and reduced development of hypertension.

#### **Conclusions**

The phenotype of PVAT is a major determinant of PVAT health during hypertensive conditions. Our data clearly demonstrates that browning of PVAT, i.e. changing the phenotype of PVAT, protects the vascular function and counteract the development of hypertension. This study provides novel insights into how PVAT can be protected in pathologies and thus limit the development of hypertension.

Poster 52

**The role of perivascular adipose tissue and H<sub>2</sub>S in the regulation of vascular tone isolated artery in normotensive and spontaneously hypertensive rats**

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Perivascular adipose tissue (PVAT) can play important role in pathophysiology of cardiovascular diseases. Hydrogen sulfide (H<sub>2</sub>S) can be one of vasoactive factors produced by PVAT and/or arterial wall.

The aim of study was to compare the effect of PVAT and endogenous H<sub>2</sub>S on contractile and relaxation responses of isolated mesenteric artery (MA) in adult normotensive Wistar rats and spontaneously hypertensive rats (SHR). The changes in isometric tension were evaluated after transmural nerve stimulation (TNS) or after application of exogenous noradrenaline (NA) in MA with preserved or denuded PVAT. To inhibit the endogenous H<sub>2</sub>S production the inhibitor of cystathionine  $\gamma$ -lyase, propargylglycine, was used.

In SHR, unlike in Wistar rats, PVAT induced pro-contractile effect on responses induced by endogenous NA after TNS. Surprisingly, PVAT revealed a more pronounced anti-contractile effect on responses induced by exogenous NA in SHR compared to Wistar rats. In Wistar rats, H<sub>2</sub>S produced by arterial wall and PVAT revealed anti-contractile effect on contractile responses induced by exogenous NA. In SHR, H<sub>2</sub>S produced by arterial wall revealed pro-contractile effect, however, this effect was counter-balanced by anti-contractile effect of H<sub>2</sub>S produced by PVAT. The results indicated that the pro-contractile effect of PVAT was closely associated with perivascular nerve stimulation, and besides the pro-contractile action of H<sub>2</sub>S in the arterial wall, could represent the pathologic features of SHR. On the other hand, we confirmed that the anticontractile action of PVAT associated with participation of H<sub>2</sub>S could probably be part of compensatory vascular mechanisms triggered in SHR.

Supported: VEGA 2/0103/18, APVV-15-0565.

Poster 53

**Selective inhibition of tumour necrosis factor signalling blunts resistance artery myogenic tone in diabetic hypertensive mice**

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Diabetes is related with microvascular complications that promote organ failure. Augmented myogenic responsiveness alters tissue perfusion and exacerbates hypertension. Tumor necrosis factor (TNF) is proposed as a mechanoreceptor in resistance arteries and regulates peripheral resistance affecting blood pressure. Studies demonstrate that TNF augments myogenic responses in pathological conditions. TNF exists as a membrane-bound (tmTNF) and a soluble form (solTNF). SolTNF activates TNFR1 inducing inflammation, while tmTNF stimulates TNFR2 promoting immune regulation. We hypothesize that in a diabetic state elevated TNF levels augment myogenic response and can be reversed by inhibition of solTNF signalling. To examine this, 2<sup>nd</sup> order mesenteric arteries were isolated from streptozotocin-induced mice and treated with angiotensin II (60 ng/kg/min) for 8 days. The arteries were mounted in a pressure-myograph subjected to step-wise increase in pressure (20-160 mmHg) in the presence of selective inhibitor of solTNF, Xpro1595, non-selective inhibitor of both TNF forms, etanercept (ETN), or a physiological saline solution. We observe that interference of TNF activity with a selective inhibitor and non-selective inhibitor of TNF abolishes the augmented myogenic tone in WT mice measured as percentage of the maximal diameter of a vessel at a given pressure (PSS vs. Xpro1595(male): $17.28 \pm 2.41$  vs.  $9.74 \pm 3.01$ ,  $n=7$ ,  $p=0.008$ ), (PSS vs. Xpro1595(female): $16.47 \pm 2.85$  vs.  $4.34 \pm 3.57$ ),  $n=7$ ,  $p=0.0278$ ) and STZ-ANGII mice (PSS vs. Xpro1595: $8.1 \pm 3.15$  vs.  $2.78 \pm 2.38$ ,  $n=7$ ,  $p=0.03$ ), (PSS vs. ETN: $8.09 \pm 2.57$  vs.  $2.38 \pm 1.52$ ,  $n=7$ ,  $p=0.04$ ). Mechanistically, the present investigation identifies TNF as a central pathological mediator that augments mesenteric artery tone in our mice models. Suggesting that solTNF is a major contributor to augmented myogenic tone in diabetic hypertensive mice.

Poster 54

**The effect of selective inhibition of soluble tumour necrosis factor on abdominal aortic aneurysm expansion and on macrophage function in vitro**

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Abdominal aortic aneurysm (AAA) is a chronic dilatation of the abdominal aorta that associates with high mortality. No medical treatment exists. Tumour necrosis factor (TNF) is elevated in AAA and exists as a membrane-bound form, involved in immune regulation, and a soluble form, involved in inflammation and apoptosis.

We hypothesize that selective inhibition of solTNF, by Xpro1595 will inhibit AAA expansion and more effectively than non-selective anti-TNF therapy, by etanercept(ETN). Using the elastase induced AAA model, mice were treated with XPro1595, ETN, or vehicle for 7 or 14 days. In primary bone marrow derived macrophages treated with XPro1595, ETN or vehicle and TNF and interleukin 10 secretion was examined.

XPro1595 treatment let to significantly smaller AAA than in controls ( $125 \pm 15$  vs.  $217.9 \pm 20\%$ ,  $n=14-16$ ,  $p=0.009$ ). A similar trend was observed after ETN-treatment, though non-significantly ( $156 \pm 23\%$ ,  $n=17$ ). TNF aneurysmal-protein levels showed a trend to be elevated after ETNtreatment, which was significantly lowered by XPro1595-treatment ( $V:18.1 \pm 2.1$  vs.  $E:26.7 \pm 8.0$ , vs.  $X:11.6 \pm 1.3$  pg/mg,  $n=5-8$ ,  $p<0.05$ ). Neither ETN nor XPro1595 affected a series of high abundant extracellular matrix- and cellular-components 7 days after AAA initiation when compared to vehicle( $n=4-7$ ). In age matched controls treated with ETN fibulin2, Myosin9, collagenIV, and integrin1beta was all significantly affected in the aortic wall ( $n=4-7$ ,  $p<0.05$ ). In M0 macrophages TNF and IL10 protein-levels increased after ETN-treatment, but was unaffected after Xpro1595-treatment. In M1 macrophages TNF levels increased 2000 times and IL10 increased 400 times compared to M0, and both were unaffected by anti-TNF-treatment ( $n=5-6$ ,  $p<0.05$ ).

In conclusion, selective inhibition of solTNF, by XPro1595, limits AAA expansion without increasing TNF levels as seen by ETN.

Poster 55

**The effect of superparamagnetic iron oxide nanoparticles on vascular reactivity of isolated femoral artery of normotensive and spontaneously hypertensive rats**

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Superparamagnetic iron oxide nanoparticles (SPIONs) have attracted an immense attention for drug delivery applications, but the effect of SPIONs on vascular function is unclear. This study was design to investigate whether the SPIONs modify vascular function of isolated femoral artery of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR).

SPIONs (30 nm, polyethylene glycol-coated) were administered i.v. (1 mg Fe/kg) into conscious WKY rats (WKY-SPIONs) and SHR (SHR-SPIONs). Endothelium-dependent and endothelium-independent relaxation was determined in the femoral artery by wire myography in isometric conditions.

The endothelium-dependent acetylcholine-induced relaxation was not altered in isolated femoral arteries of WKY rats or WKY-SPIONs. We did not observed any difference in endotheliumdependent acetylcholine-induced relaxation of femoral arteries of SHR and SHR-SPIONs. The administration of L-NAME into the organ chamber reduced acetylcholine-dependent relaxation of all studied groups. Femoral arteries of WKY-SPIONs showed impaired response to acetylcholineinduced relaxation after NO synthase inhibition by L-NAME in comparison to femoral arteries of WKY. The endothelium-independent relaxation measured by sodium nitroprussid administration was not significantly different in any group, but we observed a rightward shift in vessels of WKYSPIONs to the level seen in SHR.

In conclusion, our results showed that the administration of SPIONs does not change the acetylcholine-induced endothelium-dependent relaxation of femoral arteries of WKY rats and SHR, but alter the sensitivity of vascular smooth muscle cells to NO.

Study was supported by APVV-16-0263, VEGA 2/0160/17 and SAV-BAV-18-11.

Poster 56

**Multifaceted sides of resistance arteries from patients with cardiovascular disease by bradykinin induced endothelial-dependent vasorelaxation**

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In resistance-sized arteries from rats and mice, endothelium-dependent vasodilatation involves endothelium-dependent hyperpolarization (EDH) and NO synthase (NOS) generating NO stimulating soluble guanylyl cyclase (sGC) in smooth muscle. In nitrergic neurotransmission, the transmitter is proposed to be nitroxyl (HNO) instead of NO and to resist inactivation by superoxide anions ( $O_2^-$ ). Here we evaluated in pericardial resistance arteries from patients undergoing cardiothoracic surgery, the roles of the NOS/NO/sGC pathway and of EDH in responses to bradykinin (BK). Isolated vessel segments were first contracted with  $K^+$  to mimic myogenic tone and attenuate hyperpolarization. Relaxing responses to BK and Na-nitroprusside (SNP) averaged  $62 \pm 20$  and  $58 \pm 19\%$ , respectively. Those to SNP were not modified by endothelium-removal, LNAME or N-acetylcysteine, but were abolished by cPTIO, DETCA or ODQ. Those to BK were abolished by endothelium-removal, L-NAME or ODQ, but were not modified by cPTIO, N-acetylcysteine or DETCA. Next, BK-induced relaxations were recorded during contraction stimulated by a thromboxane  $A_2$  analogue or by endothelin-1 (ET1). In presence of ET1, BK-induced relaxations were not attenuated by any pharmacological inhibitors mentioned and not by three inhibitors of NADPH oxidases.

Thus, in resistance arteries from cardiovascular disease patients, the endothelium-dependent vasodilator BK stimulates NOS, sGC and EDH. The link between NOS and sGC does not involve NO or HNO and is resistant to  $O_2^-$ . During agonist-induced contraction, EDH accounts for a part or for the entire endothelium-dependent relaxation. In presence of ET1, the role of NOS/sGC is taken over by  $H_2O_2$  that is not derived from NADPH oxidases or Cu/Zn superoxide dismutase.

**Poster 57**

**Preeclampsia is associated with reduced nitric oxide homeostasis and signaling compared with healthy pregnant women: emerging role of erythrocyte-derived arginase**

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**Background**

Preeclampsia (PE) is characterized by hypertension and proteinuria. It is a serious complication of pregnancy with key underlying cardiovascular pathophysiology. Nitric oxide (NO) deficiency, due to compromised function of NO synthase (NOS), has been linked with development and progression of cardiovascular disease. Both arginase and NO synthase (NOS) compete for L-Arginine. Studies have highlighted a crucial role of arginase-dependent regulation of NO homeostasis in erythrocytes in control of cardiac function (Yang et al. *PNAS*, 2013) and type 2 diabetes (Zhou et al. *JACC*, 2018). The interaction between erythrocyte-derived arginase and NO homeostasis in PE has not previously been investigated.

**Methods**

Arginase activity in plasma and erythrocytes from healthy and PE pregnancies was examined. In separate experiments, plasma and RBC samples were incubated with murine aortas for assessment of vascular reactivity.

**Preliminary results**

Levels of nitrate and nitrite, as well as cGMP levels in PE vs healthy plasma were significantly decreased (indirect markers of NOS activity and NO signaling, respectively). Reduced NO bioavailability was detected in the plasma of newborn offspring from these mothers. We observed significantly increased arginase activity in erythrocytes, but not plasma, from PE vs healthy pregnancies. Incubation of erythrocytes from PE pregnancies, but not healthy pregnancies, with wild-type murine aortas significantly impaired endothelial-dependent vasorelaxation.

**Conclusion**

Our data suggest a functional interaction between erythrocyte-derived arginase with circulating NO-bioavailability and endothelial dysfunction in PE. Ongoing studies investigate the underlying mechanism(s) and value of therapeutic strategies to alleviate the impact of upregulated arginase activity and boost NO formation.

Poster 58

**The effect of selective inhibition of soluble tumor necrosis factor on abdominal aortic aneurysm development in mice**

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Increased levels of tumor necrosis factor (TNF) have been reported in abdominal aortic aneurysm (AAA) tissue. TNF exists in two forms; a transmembrane form (tmTNF) and a soluble form (sTNF). Non-selective pharmacological inhibition of both forms reduces AAA formation in mice. However, non-selective inhibition of TNF has been associated with severe side effects in other inflammatory disorders, and is presumed to be linked with inhibition of tmTNF and its receptor mediated immunoregulating functions.

We hypothesized that selective inhibition of sTNF by a mutated TNF drug (XPro1595) will prevent AAA expansion. To examine this, AAAs were induced in ApoE<sup>-/-</sup> mice by chronic angiotensin-II infusion (60 g/kg/hour) via subcutaneous minipumps. Mice were injected i.p. with XPro1595 (20 mg/kg) or vehicle every third day for 28 days. At day 28 mice were sacrificed and aortic tissue was harvested. The outer abdominal aortic diameter shows a tendency towards decreased aortic diameter in XPro1595-treated mice compared to vehicle-treated control mice (median=1.132 mm vs. 1.893 mm, n=9, p=0.077), though not significantly. Surface area of AAAs were significantly smaller in XPro1595-treated mice (mean±SEM = 8.419 mm<sup>2</sup> ± 0.584 vs. 11.490 mm<sup>2</sup> ± 1.110, n=9, p<0.05), while wet weights of the abdominal aorta showed no difference between groups (mean±SEM = 0.553 ± 0.061 mg/g body weight vs. 0.781± 0.179 mg/g body weight, n=9, p=0.246).

These findings suggest that inhibition of sTNF by Xpro1595 in an angiotensin-II-induced AAA mouse model reduces surface area of AAAs, but not maximal outer abdominal aortic diameter or wet weights.



## Poster 59

**Timing of food intake controls diurnal blood pressure rhythm in mice**

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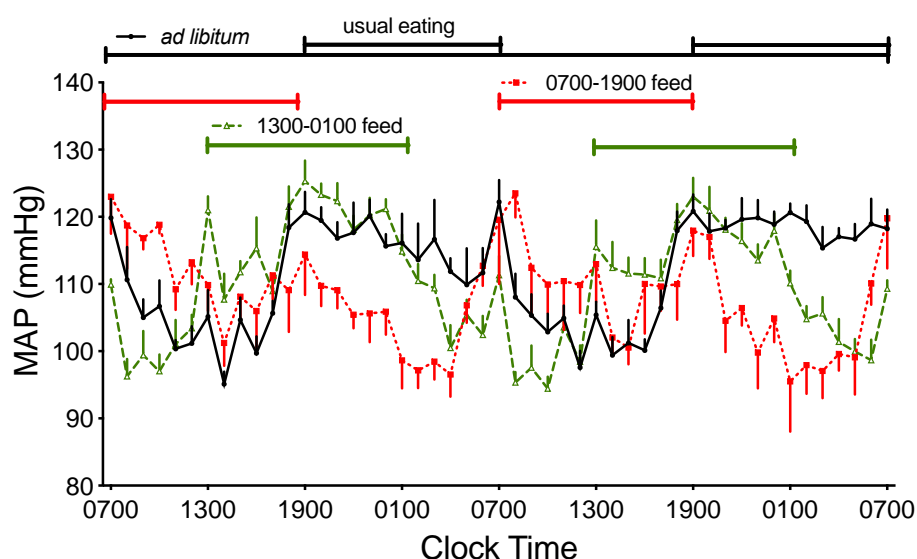
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Recent studies suggest that timing of food intake plays pivotal roles in regulating many physiological processes, such as blood pressure (BP), but the impact on BP rhythms remains unknown. Here we tested the hypothesis that feeding during the inactive period (reverse feeding or RF) disrupts BP rhythms in mice. Male C57Bl/6J mice (WT, 12-16 wk old, n=11) and Bmal1 knockout mice (Bmal1KO, 12-16 wk old, n=7) were maintained in 12 hr light/dark periods. Mice had free access to food and water before 6-days of RF. Food and water intake were monitored twice per day at the beginning of active (lights off, 7pm-7am) and inactive (lights off, 7pm-7am) periods (AP and IP). As expected, mean arterial pressure (MAP, telemetry) was significantly higher in AP compared to IP when WT mice were fed ad libitum. MAP in Bmal1KO was comparable between AP and IP. WT mice consumed the majority of their food during their active period; however, Bmal1KO mice consumed the same amount of food throughout the day. RF led to an inversion of diurnal MAP in WT. Furthermore, RF induced a significant diurnal MAP rhythm in Bmal1KO. In mice maintained in constant darkness to eliminate light cues, normal MAP rhythms were observed with ad lib feeding, while MAP increased only during food availability during RF. An additional group were fed from 1pm-1am, which again increased MAP during the feeding period. These data demonstrate that timing of food intake controls diurnal blood pressure rhythm in mice.

*Mean arterial pressure in wildtype mice maintained in constant darkness. Food was consumed during the times indicated by horizontal bars.*



Funded by an AHA pre-doctoral fellowship (18PRE33990345) to DZ and NHLBI P01 HL136267 to DMP.

Poster 60

**Endothelium and Rho-kinase are not essential for nongenomic relaxatory effects of thyroxine in rat skeletal muscle arteries**

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**Introduction**

Thyroid hormones (TH) regulate the circulatory system by genomic and nongenomic mechanisms. Fast nongenomic vasorelaxatory effects of TH were observed in several arteries, but underlying mechanisms are still unclear and may differ among the vascular beds. In this study, we explored the mechanisms of TH nongenomic effects in rat skeletal muscle arteries.

**Materials and methods**

The experiments were performed on Wistar rats (m=250-350 g). Gastrocnemius feed (sural) arteries (d=260-380 micron) were isolated and studied in a wire myograph. In some experiments, endothelium was removed with rat whisker. Arterial responses were compared using 2-way ANOVA, n was 6-12 for each group.

**Results**

T4, but not T3 applied in the concentrations from 0.02 to 10 microM induced concentration-dependent relaxation of the arteries precontracted by methoxamine ( $\alpha_1$ -agonist), the minimal effective concentration was 2 microM. Preincubation with T4 (3-10 microM) depressed the contractile responses to methoxamine (both maximum force and pD2 were reduced). This effect was absent in the presence of tetrac (3 microM), the competitive inhibitor of integrin  $\alpha v \beta 3$ . T4 reduced contractile responses in the presence of L-NNA (100 microM) as well as after endothelium removal. Moreover, the relaxatory effect of T4 on methoxamine-induced contractions was not attenuated by Y27632 (Rho-kinase inhibitor, 3 microM).

**Conclusions**

T4 induces nongenomic endothelium-independent relaxation of the sural artery through integrin  $\alpha v \beta 3$ . The vasorelaxation is not associated with NO production or Rho-kinase inhibition. Fast T4-induced vasorelaxation may contribute to the decrease of total peripheral resistance associated with hyperthyroidism. Supported by the RFBR (Grant N19-015-00482).

## Poster 61

### **The impact of TASK-1 channels in tone regulation of rat peripheral vasculature decreases with maturation**

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#### **Introduction**

The TASK-1 channels conduct background  $K^+$ -current in arterial smooth muscle and, consequently, can stabilize the membrane potential and suppress vasoconstriction. Noteworthy, anticontractile influence of several  $K^+$ -channels ( $K_{ir}$  and  $K_{v7}$ ) decreases with maturation. However, the developmental alterations of TASK-1 vasomotor role have not been studied yet. We hypothesized that the impact of TASK-1 channels on peripheral vascular tone and systemic arterial pressure (AP) decreases during postnatal development.

#### **Materials and Methods**

Isometric force and membrane potential were recorded simultaneously in endothelium-denuded saphenous arteries using wire myography and the sharp microelectrode technique. mRNA expression level was determined in endothelium-denuded arterial samples by qPCR. AP was recorded in the carotid artery under urethane anesthesia.

#### **Results**

In arteries from young (10-15-days) male rats, TASK-1 blocker AVE1231 (1  $\mu$ M, kindly provided by Sanofi) induced strong basal depolarization (23 mV) and tone development (35% of max active force) and potentiated the effects of  $\alpha$ 1-adrenoceptor agonist methoxamine on membrane potential and active force. In contrast, in arteries of adult (2-3-months) male rats, AVE1231 caused only moderate depolarization (10 mV), not associated with either tone development or changes in methoxamine-induced contractions. mRNA content of TASK-1 poreforming subunit was significantly higher in samples of young rats compared to adults. Importantly, the increase of AP by AVE1231 (4 mg/kg, i.v.) was stronger in young than in adult rats.

#### **Conclusions**

The anticontractile role of TASK-1 channels in rat saphenous artery and its influence on AP level is particularly strong in early postnatal period, in contrast to mature organism.

*Supported by the Russian Science Foundation (grant N19-15-00210) and DAAD (Short-Term Grants, 2018)*

## Poster 62

### Upregulation of Endothelial Kir2.1 Channel Disturbs Neurovascular Coupling in Familial Hemiplegic Migraine

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#### Background

It has been shown that neurovascular coupling (NVC) is primarily mediated through  $K^+$  signaling and that endothelial  $K^+$  inward rectifying 2.1 (Kir2.1) channels are central in NVC. We have previously shown that cerebral arteries from heterozygous mice bearing a familial hemiplegic migraine type 2 associated mutation (G301R) of the Na,K-ATPase  $\alpha 2$  isoform, i.e.  $\alpha 2^{+/G301R}$  mice, were hypercontractile leading to a consequent hypoperfusion of the brain tissue. Interestingly, hypoxia is known to strongly upregulate endothelial Kir2.1 channels. In this study we hypothesized that NVC was disturbed in  $\alpha 2^{+/G301R}$  mice.

#### Methods

NVC was assessed noninvasively in-vivo using laser speckle imaging and ex-vivo using confocal microscopy in brain slices. Arterial dilation to increased bath concentrations of  $K^+$  was assessed in isometric myograph and smooth muscle cell membrane potential was measured simultaneously. Kir2.1 channel expression was assessed by whole-mount immunostaining of middle cerebral artery.

#### Results

Whiskers stimulation increased perfusion of the corresponding sensory cortex and this response was larger in  $\alpha 2^{+/G301R}$  mice. Neuronal excitation *ex-vivo* led to increased dilation of arterioles from  $\alpha 2^{+/G301R}$  mice. Pre-constricted cerebral arteries from  $\alpha 2^{+/G301R}$  mice dilated stronger to increased  $K^+$  concentrations. Accordingly,  $K^+$ -induced hyperpolarization of smooth muscle cells from  $\alpha 2^{+/G301R}$  mice was increased. Endothelial denudation abolished the difference in  $K^+$ -induced dilations between genotypes. Endothelial Kir2.1 channel expression was increased in middle cerebral arteries from  $\alpha 2^{+/G301R}$  mice whereas smooth muscle expression was unchanged.

#### Conclusion

$\alpha 2^{+/G301R}$  mice showed significantly exaggerated increase in blood flow in response to neuronal activity. This was associated with an increased expression of endothelial Kir2.1 channels.

## Poster 63

### The hemodynamic effects of skin blood flow in human thermoneutral zone

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#### Introduction

Acral skin blood flow fluctuates within human thermoneutral zone (TNZ) due to arterio-venous anastomoses' vasculature resistance. Resistance changes may affect cardiovascular variables. We assessed the human hemodynamic responses within TNZ.

#### Methods

Eight young females were exposed for three ambient temperatures each lasting 40 min (20°C, 26°C and 32°C) in a climate chamber. Simultaneous recordings of beat-by-beat laser Doppler flux from index fingers and left forearm, together with heart rate (HR, ECG) and noninvasive finger arterial blood pressure (Finometer), providing mean arterial pressure (MAP) and cardiac stroke volume (SV). Medians and 95% confidence intervals calculated by Hodges-Lehmann's estimates. Wilcoxon signed rank sum test for paired samples tested differences between conditions.

#### Preliminary results

Temperature rise from 20°C to 32°C, increased blood flow to both fingertips and forearm ( $p=0.007$ ). Right fingertip flux increased five-fold from 26 AU (95%CI: 12, 33) to 133 AU (49, 192), while forearm flux doubled from 12 AU (4, 18) to 27 AU (7,37). Correlation between flux signals was highest during 26°C (not significant). Neither MAP (90 mmHg at 20°C, 86 mmHg at 32°C,  $p>0.25$ ), nor SV (84 ml at 20°C, 85 ml at 26°C, 84 ml at 32°C,  $p>0.5$ ,  $n=6$ ) did change. HR increased minimally from 26°C (61 bpm (55, 64) to 32°C (63 bpm (57, 66) ( $p=0.02$ ), but did not change between 20°C and 26°C.

#### Conclusions

The marked changes in skin blood flow at different ambient temperatures were not reflected in the hemodynamic variables. The human temperature control mechanisms affect the central hemodynamics minimally within TNZ.

## Respiratory Physiology

**Posters 64-67**

**Poster 64**

**Pulmonary surfactant and polymyxin B in the treatment of lipopolysaccharide-induced lung injury in rats**

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After inhalation, lipopolysaccharide (LPS) interfere with a pulmonary surfactant (PSUR), a mixture of phospholipids (PLs) and associated proteins that are required for formation and stability of surfactant film in the alveolus. An antibiotic polymyxin B (PxB) is able to bind to PSUR membranes and increase its resistance to inactivation. The aim was to evaluate the effect of combined therapy PSUR+PxB against LPS-induced lung injury in rats.

Adult Wistar rats were used. Lung injury was induced by instillation of LPS (500µg/kg; 2.2ml/kg). Animals were subsequently treated by exogenous PSUR (Curosurf®, 50mg PL/kg b.w.) or PSUR with PxB 1% w.w. (PSUR+PxB). Controls received saline. After the experiment, left lung was lavaged, right lung was homogenized. The markers were determined in homogenized lung (HL) tissue and bronchoalveolar lavage fluid (BALF). Lung oedema was expressed as wet/dry weight ratio.

In comparison to control, LPS increased lung oedema formation, oxidative stress and levels of inflammatory markers in HL and BALF. PSUR reduced lung oedema, oxidative stress in HL and IL-6 ( $p<0.05$ ) in BALF. With exception of oedema, the effect was potentiated by PxB added to PSUR. PSUR+PxB also reduced IL-1 $\beta$ , MCP-1 ( $p<0.05$ ) in BALF and TNF- $\alpha$ , MCP-1 ( $p<0.01$ ) in HL.

Enrichment of exogenous surfactant with PxB potentiates the effect of surfactant therapy in LPS induced lung injury by mitigating inflammation and oxidative stress. The results indicate the potential of surfactant preparations to carry the drugs directly to the site of its action.

Acknowledgements: APVV-17-0250, APVV-15-0075, VEGA 1/0055/19, BioMed 26220220187.

## Poster 65

### Epithelial $\alpha 3\beta 4$ nAChR stimulate ciliary activity in the mouse trachea

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### Introduction

We previously identified non-neuronal acetylcholine in the airway lining fluid where it contributes to regulation of epithelial ion transport and cilia-driven transport, a critical parameter of mucociliary clearance. The contribution of nicotinic receptors (nAChR) to ciliary activity is unknown and was addressed in the present study.

### Materials and Methods

Expression of nAChR subtypes mRNA was examined in the murine tracheal epithelium by RT-PCR. Cilia-driven particle transport speed (PTS) on the mucosal surface was analyzed.

### Results

RT-PCR revealed constant expression of nAChR subunits  $\alpha 3$ ,  $\alpha 5$ ,  $\alpha 7$ ,  $\alpha 9$ ,  $\alpha 10$ ,  $\beta 2$  and  $\beta 4$  in abraded murine tracheal epithelium. Nicotine evoked an increase in PTS with a maximum effect (84% increase) at  $10^{-4}$  M. Desensitization was observed upon repetitive stimulation. Nicotine-induced increase in PTS was insensitive to atropine ( $10^{-6}$  M, muscarinic antagonist), dihydro-betaerythroidine ( $10^{-5}$  M, antagonist addressing  $\alpha 4\beta^*$ ),  $\alpha$ -bungarotoxin ( $10^{-5}$ - $10^{-7}$  M,  $\alpha 7/\alpha 9$ -antagonist) and  $\alpha$ -conotoxin MII ( $10^{-3}$  M,  $\alpha 3\beta 2$ -antagonist). Strychnine ( $\alpha 9$ -antagonist) was ineffective at  $10^{-5}$ - $10^{-6}$  M. Mecamylamine ( $10^{-4}$  M) and d-tubocurarine ( $10^{-5}$  M) fully inhibited,  $\alpha$ -Conotoxin AulB ( $10^{-3}$  M,  $\alpha 3\beta 4$ -antagonist) largely reduced the effect. Cytisine ( $10^{-4}$  M,  $\alpha 3\beta^*$ ) and epibatidine ( $10^{-4}$  M,  $\alpha 3\beta^*$ ), but not cotinine ( $10^{-4}$  M,  $\alpha 7$ ) exhibited agonist activities. Supportively, the nicotine-induced PTS-increase persisted in mice deficient for nAChR subunits  $\alpha 5$ ,  $\alpha 7$ ,  $\alpha 9$ ,  $\alpha 10$ ,  $\alpha 9+\alpha 10$  and  $\beta 2$ , but was lost in  $\beta 4$  subunit deficient mice.

### Conclusion

Activation of  $\alpha 3\beta 4$  nAChR, expressed by the tracheal epithelium acutely stimulates ciliary activity. Due to its rapid desensitization, however, this effect is probably not translated into enhanced clearance upon chronic stimulation.



## Poster 66

### Does breathing pattern consciously applied during exercise affect exercise performance?

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#### Introduction

Enhanced breathing during exercise is a physiological adaptation to increased energy consumption. On the other hand, intentional breathing patterns can affect the physiological response to exercise and, perhaps, its energy efficiency. The aim of our study was to find the effect of consciously manipulated breathing frequency and airway resistance on exercise performance during short-term low-intensity trunk stabilization exercise (SLSE).

#### Materials and Methods

After baseline measurements at rest, fifteen healthy young adults performed SLSE at three consecutive days, different breathing pattern (a, b, c) but the same SLSE were applied each day: breathing with locomotor-respiratory coupling (LRC) 1:1 at low airway resistance (a), LRC1:1 with increased airway resistance (b) and LRC1:7 with increased airway resistance (c). Oxygen consumption ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), respiratory quotient (R), heart rate (HR) and ventilatory equivalent were measured with Cosmed Quark PFT. Data were analysed using SigmaStat with the significance level  $P < 0.05$ .

#### Results

Analysis showed statistically relevant decrease in  $\text{VO}_2$  ( $568,30 \pm 30,16 \text{ mL/min}$ ),  $\text{VCO}_2$  ( $460,50 \pm 33,07 \text{ mL/min}$ ), R ( $0,80 \pm 0,02$ ) and HR ( $103,85 \pm 2,69 \text{ /min}$ ) during exercise at breathing pattern b compared to a ( $\text{VO}_2 = 671,76 \pm 25,97 \text{ mL/min}$ ;  $\text{VCO}_2 = 694,13 \pm 37,10 \text{ mL/min}$ ;  $R = 1,03 \pm 0,03$ ,  $\text{HR} = 110,11 \pm 4,13 \text{ /min}$ ) and c ( $\text{VO}_2 = 689,17 \pm 25,89 \text{ mL/min}$ ;  $\text{VCO}_2 = 627,43 \pm 41,87 \text{ mL/min}$ ;  $R = 0,90 \pm 0,04$ ;  $\text{HR} = 114,24 \pm 3,56 \text{ /min}$ ). Statistically significant difference in ventilatory equivalent among breathing patterns ( $56,70 \pm 2,73$  (a),  $4,28 \pm 1,38$  (b) and  $32,47 \pm 1,55$  (c)) was found.

#### Conclusions

Breathing pattern applied during exercise may affect the exercise performance. LRC1:1 with increased airway resistance was found as energetically, but not respiratory, most efficient for SLSE performed. The best respiratory efficiency was associated with prolonged expiration pattern (c), which was, however, energy consuming.

Poster 67

**Implication of RAGE Signaling in establishing models of COPD pathogenesis**

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Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the United States and it is characterized by inflammation and respiratory tissue loss. Tobacco smoke is the major cause of COPD. Studies identified receptors for advanced glycation end-products (RAGE) as a smoke-induced pattern recognition receptor with potent pro-inflammatory characteristics and we've seen increased pulmonary RAGE following first and secondhand smoke (SHS) exposure and that RAGE transgenic mice that conditionally increase RAGE are possible smokeless models of a smoker's lung. We evaluated inflammatory effects of SHS with and without semi-synthetic glycosaminoglycan ethers (SAGEs), a family of anionic, partially lipophilic sulfated polysaccharide derivatives known to inhibit RAGE signaling. The current research evaluated the *in vivo* effects of short-term smoke exposure in RAGE null, conditional RAGE over-expressing, and control mice via a nose-only exposure platform for 4 weeks (Sireq Scientific) and compared them to animals exposed to room air only. Groups of mice were also co-treated with SAGEs via weekly ip injections (a 30mg/kg body weight). Molecular characterization of smoke exposure revealed significant pulmonary inflammation and apoptosis mediated in part by RAGE. Inflammatory cell behaviors were assessed by determining the activation of Ras, intracellular signaling kinases, and cellularity/cytokine secretion in bronchoalveolar lavage fluid (BALF). Inflammatory signaling intermediates and downstream responses induced by exposure were influenced by the availability of RAGE, as evidenced by RAGE nulls and SAGE treatment. These data reveal captivating information suggesting a role for RAGE signaling in lungs exposed to tobacco smoke and implicates plausible therapeutic modalities.

## Digestion and Metabolism

**Posters 68-77**

Poster 68

**Gathering pieces on the stomach diversity puzzle: The case of *Histrio histrio* and *Cephaloscyllium ventriosum* “multi-tasking” stomachs**

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The stomach is characterized by the occurrence of acid (HCl) and pepsin-producing glands, presenting a great variability across vertebrates. As evolutionary pressures fluctuated over time, some organisms appear to have lost the gastric phenotype.

Examples can be found in multiple lineages of vertebrates, such as mammals (e.g. monotremes), chimaeras (Holocephali), lungfishes (Dipnoi) and a great number of teleosts. This loss of function is intriguing, as the stomach's development is amongst the major innovations that arose within the early gnathostomes. In the Tetraodontiform puffer and porcupine fishes (Diodontidae and Tetraodontidae), stomach inflation for defence correlates with the lack of a stomach and acidpeptic genes, representing a potential driver for the loss of the gastric phenotype. The sargassum fish (*Histrio histrio*; Lophiiformes) and the swell shark (*Cephaloscyllium ventriosum*; Carcharhiniformes) display a similar inflation response. This work aimed to clarify if the inflation capacity is accompanied by a gastric function loss in these species. The stomach phenotype was confirmed by histology and immunohistochemistry through the detection of gastric glands, zymogen granules, and the gastric proton pump  $H^+/K^+ATPase$  immunoreactivity. In addition, genes associated with the gastric function: *atp4a* (the  $\alpha$  subunit of the  $H^+/K^+$  proton pump), and two pepsinogens *pgc* (pepsinogen C) and *pga*: (pepsinogen A) were also detected in the stomach. Taken together, these results indicate a fully functional stomach in the two studied species, demonstrating the possibility of coexistence of both acid-peptic digestion and the inflation phenotypes, pointing to other evolutionary pressures as potential drivers of stomach loss.

Poster 69

**Localisation and Regulation of Plasma Membrane  $\text{Ca}^{2+}$ -ATPase 1 in Renal and Intestinal Epithelia**

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Calcium ( $\text{Ca}^{2+}$ ) is essential for a wide range of physiological processes, such as neurological function and intracellular signalling. As such, the systemic and intracellular  $\text{Ca}^{2+}$  concentration must be tightly regulated through exchange from the bone and by modulation of  $\text{Ca}^{2+}$  transport in the kidney and intestinal epithelia.

Plasma Membrane Calcium ATPases (PMCA) are known to play an important role in  $\text{Ca}^{2+}$  (re)absorption. In the kidney, previous research found high PMCA4 expression in the distal nephron. However, the exact distribution of the PMCA1 isoform in renal and intestinal epithelia remains to be clarified. As such, we have raised PMCA1 monoclonal antibodies in mice. In the kidney we have observed PMCA1 immunoreactivity in the sub-apical membrane domains of all segments of the nephron.

In the duodenum, PMCA1 immunoreactivity was clear in the basolateral membrane of the enterocyte. To fully understand PMCA1 and PMCA4 contribution to duodenal calcium handling, we have compared the expression of these two transporters in epithelium and smooth muscle. Here we found PMCA1 expression to be higher in the epithelial cells whilst PMCA4 is expressed in the smooth muscle layer.

To further unveil the mechanisms of  $\text{Ca}^{2+}$  regulation, we have challenged mice with a diet enriched with Vitamin D and examined transcriptomic changes in both duodenum's epithelial and smooth muscle cells.

Our study finds that PMCA1 is, most likely, the main PMCA in the intestine, driving transcellular  $\text{Ca}^{2+}$  transport while maintaining a housekeeping function in renal epithelial cells.

## Poster 70

### **Does streptozotocin model of diabetes induce ongoing pain in the rat?**

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#### **Introduction**

Streptozotocin (STZ) is used to induce diabetes mellitus in experimental animal studies on diabetic neuropathy. Animals with STZ model of diabetes commonly develop changes in test stimulus-evoked pain behavior, but it is still unclear whether rats with STZ model of diabetes have ongoing pain. Here we assessed whether rats with STZ-induced diabetes have ongoing pain-like behavior using conditioned place-preference (CPP) paradigm.

#### **Methods**

Diabetes was induced by STZ (50-60 mg/kg i.v.). CPP was tested in the fourth week of diabetes by pairing for two days one chamber of the CPP device with vehicle and another chamber with either pregabalin (an established analgesic; 30 mg/kg i.p.) or with Chembridge-5861528 (a TRPA1 receptor antagonist; 30 mg/kg i.p.). After the drug-pairing days, the animals were allowed to move freely and to choose which chamber they preferred. Mechanical sensitivity was assessed with monofilaments and function of cutaneous nociceptors by assessing mustard oil-induced pain behavior. For comparison, CPP was determined also in rats with a spared nerve injury (SNI) model of neuropathy.

#### **Results**

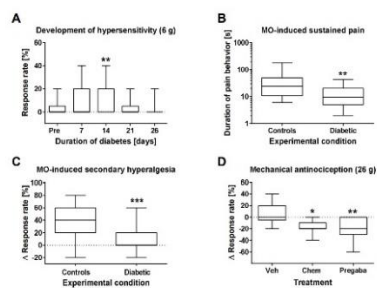
Diabetic animals developed during the first two weeks mechanical hypersensitivity that disappeared by third week. Mustard oil-induced pain behavior was reduced during 4th week of diabetes. Neither pregabalin nor the TRPA1 antagonist produced CPP in diabetic animals, although mechanically evoked pain behavior was acutely attenuated by treatments. In contrast, SNI animals preferred the chamber associated with antinociception.

#### **Conclusions**

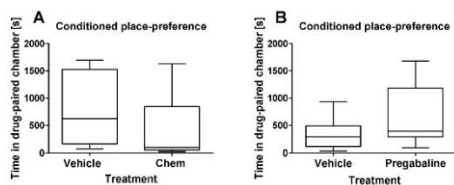
STZ model of diabetes failed to induce ongoing pain-like behavior in rats when the animals had significant changes in peripheral nerve-evoked pain behavior.

### Attempt to induced conditioned place-preference in SZT model of diabetes

Chem / pregabalin i.p.

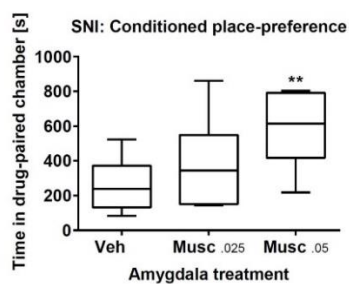


A. Mechanical hypersensitivity was maximal at D14 and disappeared by D26. At the end of the observation period (1 month), sustained pain behavior (B) and secondary hypersensitivity (C) induced by topical mustard oil were reduced. At the currently used doses, the studied drugs had a significant mechanical antinociceptive effect at the time when drug-pairing of the CPP test was done (D).



Chem or pregabalin failed to produce conditioned place-preference one month after induction of diabetes (at a time point, when mechanical hypersensitivity had disappeared and topical mustard oil-induced responses were reduced, and at doses that produced significant mechanical antinociception during drug pairing experiments).  $n=9/\text{group}$

We are working to get a positive control for the CPP test by assessing the effect of i.t. Chem in SNI animals. In the meanwhile, the below data (effect of muscimol in amygdala in SNI animals) provides a positive control (in addition to our previously published i.t. clonidine data in SNI animals).



## Poster 71

### **Low dose of superparamagnetic iron oxide nanoparticles induces superoxide production and alterations in gene expression in the liver of normotensive rats**

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#### **Introduction**

Superparamagnetic iron oxide nanoparticles (SPIONs) are being widely used in magnetic resonance imaging. However, various studies showed that SPIONs may trigger oxidative stress. We investigated if a single administration of a low dose of SPIONs can alter redox balance and genes involved in nitric oxide (NO) formation and iron metabolism in the liver of adult Wistar-Kyoto rats.

#### **Material and Methods**

Biocompatible SPIONs [30 nm, polyethylene glycol (PEG)-coated magnetite] were administered i.v. in a low dose (1 mg Fe/kg body weight, 10 min infusion). Superoxide production was determined by lucigenin-enhanced chemiluminescence. The gene expressions of genes involved in redox balance [superoxide dismutase 1 (superoxide dismutase 1(SOD1), peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2)] NO production (endothelial and inducible NO synthase, eNOS, iNOS) and iron homeostasis [(hepcidin, divalent metal transporter 1 (DMT1), ferritin heavy chain (FTH1)] were determined by qRT-PCR in the liver 90 min after SPIONs infusion.

#### **Results**

SPIONs significantly elevated superoxide production by 110% vs. control. Only non-significant tendencies in increasing of expression of DMT1, SOD1, PPAR $\gamma$  and Nrf2 were determined. No changes were found in eNOS while iNOS, FTH1 and hepcidin were significantly increased by 106%, 56% and 90% vs. control.

#### **Conclusion**

Results showed that even the single administration of the low dose of biocompatible SPIONs induces oxidative stress, may activate NO release and immune responses via induction of iNOS and lead to iron-overload similar changes in the genes involved in iron homeostasis in the liver. Supported by APVV-16-0263, VEGA 2/0160/17 and SAV-BAV-18-11.



## Poster 72

### **An abundance of the Na,K-ATPase modulates homeostasis of blood glucose.**

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#### **Background**

Glucose intolerance is a result of impaired insulin-stimulated glucose disposal. Decrease in  $\alpha 2$  Na,K-ATPase abundance in skeletal muscles of elderly individuals is associated with glucose intolerance but the underlying mechanism is unknown. *We hypothesized* that heterozygous mice bearing a mutation (G301R) in the  $\alpha 2$  Na<sup>+</sup>,K<sup>+</sup>-ATPase, i.e.  $\alpha 2^{+/G301R}$  mice, have impaired glucose control that worsens with age.

#### **Methods**

Young (3–4 months old) and elderly (~8 months old) male and female  $\alpha 2^{+/G301R}$  mice were compared with wild type mice. Glucose tolerance tests with oral (oGTT) and intraperitoneal (iGTT) glucose administration were performed and blood plasma was collected for insulin measurements. The Na,K-ATPase, insulin receptor and facilitated glucose transporter member 4 (GLUT4) expression was analyzed with Western blot.

#### **Results**

$\alpha 2^{+/G301R}$  mice showed reduced abundance (by ~20%) of both  $\alpha 1$  and  $\alpha 2$  Na,K-ATPase in skeletal muscles. No changes in insulin receptor and GLUT4 was detected. The magnitude and duration of glucose elevation in iGTT was significantly larger in elderly  $\alpha 2^{+/G301R}$  mice in comparison with wild type. Both sexes demonstrated this glucose intolerance. There was no difference in insulin concentration between elderly genotypes during GTT suggesting reduced glucose sensitivity of the insulin release pathway. No difference in glucose concentration was observed during oGTT in elderly mice. In younger mice there was no difference in glucose concentration between genotypes during iGTT nor oGTT.

#### **Conclusion**

Reduced abundance of the Na,K-ATPase in skeletal muscles of  $\alpha 2^{+/G301R}$  mice is associated with impaired control of blood glucose and this possibly aggravates due to age-dependent decline in insulin signaling.

Poster 73

**The obligatory role of host microbiota in the bioconversion of dietary nitrate**

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Nitric oxide (NO) is a key signalling molecule in the regulation of cardiometabolic function and its impaired bioactivity and bioavailability play an important role in the onset of cardiovascular disease. Research during the last two decades has demonstrated an alternative NO-generating pathway in which the inorganic anions nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) are serially reduced to form NO, independently of NO synthases. This work specifically aimed at investigating the role of commensal bacteria in mediating the favourable cardiometabolic effects of dietary nitrate supplementation.

In this study, 20 germ-free (GF) and conventional male mice (C57/BL6) were fed a Western-diet and received a NOS inhibitor (L-NAME, 1g/l) in combination with either nitrate ( $\text{NaNO}_3$ , 10mM) or placebo (NaCl, 10mM) in the drinking water. Weekly body weigh monitoring was followed by blood pressure (tail cuff), glucose tolerance (IPGTT) and body composition (DEXA) measurements. A group of GF and conventional mice (n=6 each) was fed a regular chow.

During the six weeks of treatment, both conventional and GF mice within the placebo group gained weight with increased fat and reduced lean mass, showed increased blood pressure, impaired glucose tolerance and fatty liver. As expected, nitrate was found to protect conventional mice from the deleterious effects of a Western-diet and L-NAME. However, no nitrate-like effect was detected in the absence of host bacteria.

In conclusion, here we show that the host-microbiota is indispensable to the favourable cardiometabolic effects of dietary nitrate, due to its obligatory role in bioconversion of nitrate to nitrite and then NO.

**Poster 74**

**Vitamin D and hypoxia in human mesenchymal stem cells adipogenesis**

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**Introduction**

The enlargement of adipose tissue associated with local tissue hypoxia, elevated hypoxia inducible factor 1-alpha (HIF1 $\alpha$ ) and chronic inflammation. Hypoxia is a major trigger for adipose tissue remodeling of the hypertrophic or/and the hyperplasic growth and associated metabolic disturbances in human obesity. We hypothesize that anti-inflammatory vitamin D (VitD) play an important role in regulating adipocyte inflammation, HIF1 $\alpha$  and modulate obesity associated adipocyte dysfunction.

**Materials and methods**

Human mesenchymal stem cells (hMSCs) differentiated into adipocytes  $\pm$  VitD (1-100nM) containing adipogenic induction and maintenance medium under either hypoxic (1%O<sub>2</sub>) or normoxic (21%O<sub>2</sub>) conditions. At the end of differentiation, adipogenesis were assessed using oil red O staining. Differentiated adipocytes were stimulated with lipopolysaccharide (LPS 10ng/ml)  $\pm$  VitD (1-100nM) for 24H and the inflammatory marker measured by ELISA and the insulin-stimulated cellular glucose uptake were measured using fluorescent glucose analogue 6NBDG. Gene and protein expressions were analyzed.

**Results**

hMSCs adipogenesis was inhibited under hypoxia while VitD enhanced adipogenic differentiations. VitD (100nM) inhibited basal (83% vs 66%) and LPS induced (100nM:10nM:1nM) 66%:40%:19% vs 56%:44%:2%) IL6 secretion in hypoxic and in normoxic adipocytes respectively ( $p < 0.05$  vs LPS). In addition, VitD significantly ( $p < 0.05$ ) upregulated insulin-stimulated glucose uptake in hypoxic ( $144 \pm 16.6$  vs  $333 \pm 61.1$ ) and normoxic ( $401 \pm 69.9$  vs  $803 \pm 142.6$ ) condition vs control. VitD reduced the hypoxic HIF1 $\alpha$  and increased peroxisome proliferator activated receptorgamma (PPAR $\gamma$ ) expressions in both normoxic and hypoxic condition.

**Conclusion**

Our data demonstrates that, sufficient VitD levels would be able to modulate adipose tissue hypoxia induced inflammation, remodel adipogenic differentiation potential and increase the insulin sensitivity.

**Poster 75**

**Effects of Simvastatin and Sesame Oil on Nitric Oxide Generation in Obese Zucker Rats.**

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Dayar, Ezgi.

Cebova, Martina.

Current treatments for cardiovascular and obesity-associated diseases, such as statin therapy, may be associated with several side effects. We aimed to study effects of sesame oil, rich in polyphenolic compounds, and simvastatin on plasma lipid profile, nitric oxide generation, and oxidative load in obese Zucker rats. 12-week-old male Zucker rats were divided into the control and sesame oil- (1.25ml/kg/day) treated Zucker lean groups, the control and sesame oil (1.25ml/kg/day), or simvastatin (15mg/kg/day) together with sesame oil-treated Zucker fa/fa groups, n = 6 in each group. Treatment lasted for 6 weeks. Plasma lipid profile were analyzed. Nitric oxide synthase (NOS) activity, eNOS, phosphorylated eNOS, and iNOS protein expressions were determined in the left ventricle and aorta. Oxidative load, measured as conjugated diene (CD) and TBARS concentrations, was detected in the liver. Neither sesame oil nor cotreatment with simvastatin affected plasma lipid profile in Zucker fa/fa rats. Sesame oil and similarly cotreatment with simvastatin markedly increased NOS activity and phosphorylated eNOS expressions in the left ventricle and aorta of Zucker fa/fa rats. There were no changes in eNOS and iNOS expressions within the groups. Hepatic CD concentration was higher in Zucker fa/fa comparing Zucker lean rats, and sesame oil decreased it significantly. Interestingly, this decrease was not seen after cotreatment with simvastatin. In conclusion, phosphorylation of eNOS and decreased oxidative load may significantly contribute to increase in total NOS activity with potential beneficial properties. Interestingly, simvastatin did not affect NO generation already increased by sesame oil in obese Zucker rats.

## Poster 76

### **Does Aldosterone via mineralocorticoid receptors and ENaC promote IL-17 production in lymphocytes from hypertensive Type 2 diabetic patients?**

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#### **Introduction**

Approximately, 50% of diabetes mellitus (DM) patients have hypertension and are in high risk of developing cardiovascular diseases. Treatment goals for hypertension in these patients are more difficult to reach than in non-diabetic patients. Studies have shown that interleukin 17 (IL-17) may be an important factor in the pathophysiology of hypertension. IL-17 is increased in hypertensive T2DM patients compared to non-diabetic patients. In hypertension models in IL-17-deficient mice, hypertension is blunted. *In vitro* studies have shown the epithelial sodium channel ENaC to be expressed in lymphocytes. Also, activation of mineralocorticoid receptor shows to promote pro-inflammatory Th-17 lymphocyte differentiation in a spironolactonesensitive way. We therefore hypothesized aldosterone and ENaC may promote IL-17 production in lymphocytes from hypertensive T2DM patients.

#### **Methods**

In a randomized double blinded study, T2DM patients were allocated to receive either placebo or spironolactone for 16 weeks. From each patient (n=119) paired plasma samples before/after spironolactone/placebo were obtained. Moreover, in an open label non-controlled follow-up study, patients received amiloride (5 and 10 mg) for 8 weeks. Paired plasma samples from both cohorts are being analyzed measuring IL-17, IL-6, IL-1 $\beta$ , IL-10, IFN- $\gamma$ , and TNF- $\alpha$  using a multiplex ELISA.

#### **Results**

Currently measurements of key cytokines in plasma samples are ongoing. Preliminary data suggest no difference in IL-17 levels between baseline and intervention groups.

#### **Conclusion**

Remaining samples must be analyzed to conclude on differences in cytokine levels after spironolactone/amiloride interventions compared to placebo/baseline levels. Future analyses include transcriptional analyses of “buffy-coats” after placebo/spironolactone intervention of key transcripts involved in Th-17 differentiation.

**Poster 77**

**Late gestational testosterone exposure causes glucose deregulation and elevated cardiac VCAM-1 and DPP-4 activity in rats**

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Gestational testosterone exposure has been associated with development of cardiometabolic disorder in offsprings in later life. Increased vascular cell adhesion molecule-1 (VCAM-1) expression has been reported to be a critical link between obesity and atherosclerotic cardiovascular diseases while increased dipeptidyl peptidase-4 (DPP-4) activity has also been implicated in the development of disrupted glucose regulation and tissue inflammation. This study aimed to investigate the effect of gestational testosterone exposure on glucose metabolism, atherogenic dyslipidemia, as well as circulating and cardiac VCAM-1, oxidative stress biomarkers and DPP-4 activity in pregnant rats. Pregnant Wistar rats received either vehicle (olive oil) or testosterone (0.5 mg/kg; sc) between gestational days 14 and 19. Gestational testosterone exposure resulted in impaired glucose homeostasis that was accompanied with atherogenic dyslipidemia (TG/HDL-C & TC/HDL-C ratios), elevated circulating and cardiac levels of VCAM-1, uric acid, malondialdehyde as well as increased DPP-4 activity. Gestational testosterone exposure however led to a decrease in circulating and cardiac nitric oxide levels. This study shows that gestational testosterone exposure causes glucose deregulation and atherogenic dyslipidemia that is accompanied by increased circulating and cardiac VCAM-1, a marker of vascular inflammation, and DPP-4 activity.

## Renal Physiology

**Posters 78-86**

**Poster 78**

**PET CT Evaluation of Glomerular Filtration Rate in Rats**

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Glomerular Filtration Rate (GFR) is a pivotal parameter for kidney function. Because of their noninvasive and repeatable nature, imaging modalities are ideal to evaluate the small animal kidney function. Positron emission tomography CT (PET-CT) can give high quality of the total activity acquisition by the small animal. In this study we used PET/CT and F-18 labeling of a small molecular tracer that is cleared exclusively by glomerular filtration to evaluate GFR in male Wistar rats (n=6). After anesthesia with isoflurane, a CT scan was performed, followed by injection of 5-10 MBq of the F-18 labeled tracer in the tail vein. The rats were scanned by dynamic PET for 10 minutes. The GFR was calculated by total signal intensity on the kidney / area under the blood vessel signal curve. Standard GFR measurement by iohexol clearance were also tested on all the experiment rats. The PET CT and iohexol GFR (mean  $\pm$  SD) was  $3.0 \pm 0.4$  and  $3.0 \pm 0.63$  ml/min, respectively. The  $R^2$  for the correlation between PET CT and iohexol GFR was 0.78 ( $P < 0.05$ ). PET CT left and right kidney GFR were  $1.53 \pm 0.19$  ml/min and  $1.47 \pm 0.18$  ml/min, respectively. We have established new method for GFR measurement, enabling single kidney function. More experiments should be done to test the sensitivity of the method in both physiological and pathophysiological conditions.



## Poster 79

### **Intratubular Complement Activation in Kidney Transplant Recipients with Proteinuria**

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#### **Introduction**

Proteinuria accelerates chronic kidney disease and predicts negative outcome in kidney transplant recipients. The underlying mechanisms remain to be elucidated. We hypothesized that circulating complement factors are aberrantly filtered to the tubular fluid where they are activated by all 3 pathways to promote inflammation, tissue injury and fibrosis.

#### **Materials and Methods**

In a cross-sectional design, spot urine samples from kidney transplant recipients with (n=18) and without proteinuria (n=19) were analyzed by ELISA using neo-epitope monoclonal antibodies against complement split products C4c (classical and lectin pathways), C3c and C3d (alternative pathway) and C9 (soluble terminal complement complex, C5b-9). To compensate for difference in degree of urine concentration, complement factor concentration values were normalized by creatinine concentration.

#### **Results**

There was no significant difference in eGFR or graft age between groups. Approximated mean arterial blood pressure was higher in proteinuric recipients (mean difference 11 mmHg,  $p<0.001$ ). Urine excretion of C4c and C3d was significantly increased ( $p<0.001$ ) in proteinuria but there was no difference in urinary C3c levels. Urine soluble C5b-9 was increased 10-fold in kidney transplant recipients with proteinuria compared to recipients without proteinuria. A significant correlation in urine between complement factors and albumin and plasminogen was observed.

#### **Conclusion**

The correlation with albumin and plasmin(ogen) suggests that complement factors are filtered from the blood to the urine and the presence of C4c, C3c, C3d and soluble C5b-9 in urine samples indicates that complement activation and generation of split-products occurs in the luminal compartment of the tubular system.

Poster 80

**Trimethylamine N-oxide enhanced Angiotensin II contraction of renal afferent arterioles Via AT1 receptor and intracellular calcium in hypertensive mice**

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Trimethylamine N-oxide (TMAO) is a product of gut microbiota metabolism of dietary phosphatidylcholine and implicated in the pathogenesis of diseases such as obesity and atherosclerosis. However, the role of TMAO in hypertension has not been investigated. We hypothesized that TMAO augments angiotensin (Ang) II-induced vasoconstriction and hence promotes Ang II-induced hypertension. C57Bl mice implanted with subcutaneous osmotic minipump for continuous delivery of 0.9% NaCl, TMAO (40 $\mu$ g/kg/min), Ang II (400ng/kg/min) or Ang II+TMAO. Blood pressure (BP) was measured by tail cuff. Vasoconstriction of mesenteric arteries (MR) was examined by myography. Chronic NaCl and TMAO infusion did not affect BP or vasoconstriction of MR. Chronic Ang II infusion significantly increased BP and vasoconstriction of MR, responses that were exacerbated in Ang II+TMAO treated mice. Pretreatment with 0.3 mg/kg TMAO significantly increased the pressor responses to acute administration of Ang II (0.25 $\mu$ g/kg) via the jugular vein (20.6 $\pm$ 1.4 versus 12.2 $\pm$ 1.9 mmHg;  $P < 0.05$ ). Microperfused afferent arteriole (Af) responses to 0.1  $\mu$ M Ang II was significantly increased (55.9 $\pm$ 7.0 versus 32.3 $\pm$ 2.6 %,  $P < 0.001$ ) when pretreated with TMAO (200  $\mu$ M). The effects of TMAO on Ang II induced constriction of Af was blocked by Ang II type1 receptor (AT1), phospholipase c (PLC) and inositol 1, 4, 5-trisphosphate (IP3) inhibitors. TMAO enhanced Ang II (0.1  $\mu$ M)-triggered rise in Af intracellular calcium (219 $\pm$ 31 versus 122 $\pm$ 9%;  $P < 0.001$ ) that was blocked by PLC inhibitor. These results demonstrate that TMAO facilitates Ang II-induced vasoconstriction, thereby increasing the BP responses to acute and chronic Ang II infusion that may be mediated by AT1/PLC/IP3/Ca<sup>2+</sup> pathway.

## Poster 81

### Pyeloureter in context of an integrative physiology

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### Introduction

Model counteracting ultra-specialization is given by observations of motor activity from pyeloureter in context of an integrative physiology and genitourology [1,2].

### Method

Isolated pyelon/P & ureter/U of guinea-pig/GP & human/H: surgical tissue (n=242) ( $p < 0.05 < 0.01$ ). Spontaneous phasic contractions (SPC): isotonic/isometric recording, neurogenic contractions to electrostimulation (nCES 10+100Hz, 0.3ms, 3s, 5-30V) [2], abolished by TTX (1µg/ml) & hypothermia (25,15°C).

### Results

Various SPC-patterns (1-2/min) were observed in P&U of GP&H: Irregular (frequency), non-&uniform (amplitudes), burst-like, others were evident. After nCES appear in P-GP strong increase of SPC for 1-3 min, abolished by TTX and T (25,15°C). Cholinergic and alpha adrenergic blocking agents – atropin and RegitinR (1-10µmol/l) have no effect. nCES induced only single contractions in U, abolished by TTX. H P&U react similar to nCES.

*Open questions:* Factors of essential differences in rhythmogenesis for P&U. Role of various patterns for the motor function. Participation of excitatory & inhibitory non-cholinergic & non-adrenergic (co-) transmitters in neuroregulation of P&U.

### Conclusion

Information A-D demonstrates necessity for holistic and multidimensional research, leading to better prophylaxis & therapy in nephropathology, supporting **UNO-Agenda21** for better health, education, ecology, economy on global level [3].

**Ref.: 1-SIU-2016-B-Aires** (IntSocUrol) Abstr-Book/AB:136&223; **2011-Berlin-CongrUrol** 78/S3A:S188/S385; **2007-Paris** 70/S3A:232-3; **2004-Honolulu-BrJUrol** 94:24-5/258-9/305. **2-IUPS-2017-Rio-de-Janeiro**, AB:No. 997/999/1001/1003; **2009-Kyoto**, J.Physiol.Sci., 59/S1:168&214&447-8. **FEPS-2018-London-Europhysiol** AB:p334P-337P; **2014-Budapest**, Acta Physiol. Scand. 211/s697:p62-64; **1999-Prague**, PhysiolRes48/S5:138. **DPG-2002-Tübingen**, EurJPhysiol443/S1:S334. **3-FISP-2018-Beijing** (Philos) No.1348-50/1374-5; **2013-Athens** AB:464-5&503-4&766. **IAB-2018-India** (Bioethics) AB:392&393; **2016-Edinburgh** Proc93-95; **2008-Istria** 290&307.

## Poster 82

### Approaches to electrical activity of pyeloureter

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### Introduction

Renal normal and pathophysiology is essentially dependent on pyeloureter (P&U) motor function, which is related to electrical activity. Despite enormous information up today are fundamental open questions evident.

### Method

Electrical activity of P&U (intracellular -recording) in isolated preparations of guineapig/GP and some of human/H: surgical tissue (n=180,  $p < 0.05 < 0.01$ ) (*ref.* see Michailov-et-al. this congress).

### Results

Systematic observations on electrical activity demonstrate: Presence of very different electrical patterns – spikes/S, burst/B, burst-plateaus/BP, etc. These are dependent on ratio  $KCl:CaCl_2$  (5.6mmol/l:2.16mmol/l=1:1), e.g. S are after 8:2 transformed into BP. After  $BaCl_2$  2.16mmol/l=1x S are also transformed into BP, its duration is prolonged by higher concentration (2-3x).  $MgCl_2$  (1.2mmol/l=1x) has only negative chronotropic effect (upto 3x – independently from S and BP activities). Human P&U: Recording of electrical activity is very difficult: Myocytes generate similar electrical patterns as GP-cells

### Open questions:

Correlation of various motor with electrical patterns, esp. for spontaneous phasic (SPC 1-5/min) and tonic contractions (STC 0.1-0.2/min) which appear after after  $BaCl_2$  (cystotonometry in vitro). Importance of mechanosensitive channels for electrical and motor activities of P&U. Importance of renin-angiotensin system, esp. high sensitivity on H renal veins to angiotensin II incl. SPC-effects as well as STC – spontaneous or induced by vasopressin and prostaglandin F2alpha (*ref.*).

### Conclusion

Clarification of these questions could be of essential importance for pathology of urinary tract, i.e. also of angiocardiac system (neurohormonal regulation), supporting UNO Agenda 21 for better health, ecology, economy, etc. on global level.

## Poster 83

### On approaches to physiology in context of globalization

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### Introduction

*Kant* described firstly *physiological&pragmatic* anthropology, also medical philosophy. Physiology (Nobel-Price discipline) has moral&scientific responsibility to support new future conceptions for better regulation of science on international level leading to better healtheducation-medicine-psychology-ecology, also counteracting misuse of discoveries leading to disastrous situation of humanity.

Acc. to I.Kant has the human incl. physiologist obligations to himself, other humans, under-(animals,etc.), over-human beings. Future physiology can start discussions, beginning with human-zoon *logon echon* and *zoon politikon* (Aristoteles), *homo neurobiologicus* (Roth, Singer), *homo geneticus* (Dawkins), *homo oeconomicus* (Kirchgässner), actually *homo informaticus*. Creation of international institutes for physiology, beginning with some selected countries in context of international universities via network of national ones (proposed by *B.Russell*/Nobel Laureate and *G.Mensching*) could support new dimension in physiology.

### Conception

Scandinavian physiological societies could start discussions:

1-Enlargement of leading scientific-boards of physiological societies in context of meritocratic and triumvirate principles by permanent *honorary-presidents* (3) (moral-support, control continuity), *presidents (3:fixed-term)*, *interdisciplinary board*: Scientists from physiology, philosophy-law-logic (incl. informatics), psychology-medicine,etc. incl. also

2-Implication of educational (post-graduate) and *congress-programmes* topics about physiology, philosophy (epistemology-ethics-aesthetics), psychology, theology,etc.

3-Replacement of congress/conference abstract-books by *proceedings* or proper *scientific journals*, similar to other societies (*ActaPhysiol.Scand.*, *Int.J.Psychol.*,etc.).

4-Organization of *common interdisciplinary sessions* to physiological congresses/conferences with other societies.

5-Co-operation with *int. academies*, such as in USA: World Acad. of Arts&Science, Germany: ICSD/Int.Acad.Sci., San-Marino: Int.Acad.Sci., also Eur.Acad.Sci., others.

6-Possibility for *whole-life-working*.

7-Creation of *Int.Acad. for Physiology*.

**Conclusion**

Realization of proposals 1.-7. could increase scientific-political authority of physiology, supporting **UNO-Agenda21** for *better health-education-ecology-economy in all countries*.

**Poster 84**

**Mitochondrial angiotensin II receptors regulates oxygen consumption in kidney mitochondria from healthy and type 1 diabetic rats.**

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**Introduction**

Exaggerated activation of the renin angiotensin aldosterone system (RAAS) is a key feature in diseases such as hypertension, diabetes and chronic kidney disease. Recently, an intracellular RAAS was demonstrated with Ang II type 1 and type 2 receptors (AT<sub>1</sub>R and AT<sub>2</sub>R) expressed in nucleus and mitochondria. Diabetes is associated with both mitochondrial dysfunction and increased intracellular Ang II concentration in the kidney cortex. Therefore, the present study investigated the role of Ang II in kidney cortex mitochondria isolated from control and streptozotocin-induced diabetic rats.

**Material and methods**

Presence of mitochondria Ang II receptors was evaluated by binding assay. Mitochondrial oxygen consumption was evaluated after addition of Ang II, candesartan (AT<sub>1</sub>R antagonist), Ang II + candesartan, Ang II + PD-123319 (AT<sub>2</sub>R antagonist) or in combination.

**Results**

Ang II binds to mitochondrial AT<sub>2</sub>R in control rats and both AT<sub>1</sub>R and AT<sub>2</sub>R in diabetic rats. Ang II decreased oxygen consumption in mitochondria from both control and diabetic rats. AT<sub>1</sub>R inhibition did not affect the response to Ang II, whereas AT<sub>2</sub>R inhibition abolished the response in mitochondria from control animals. In diabetic rats, AT<sub>2</sub>R inhibition resulted in increased oxygen consumption through superoxide-induced mitochondrial uncoupling. Ang II in combination with both AT<sub>1</sub>R and AT<sub>2</sub>R inhibition did not exert any effect in either of the groups.

**Conclusions**

Ang II regulates mitochondrial respiration via AT<sub>2</sub>R-mediated nitric oxide release in both control and diabetic rats. AT<sub>1</sub>R does not regulate mitochondria function in control rats, but can regulate mitochondria function in diabetic animals in the absence of AT<sub>2</sub>R.

Poster 85

**Urinary Extracellular Vesicles contain renal transport proteins but are less suitable for measuring proteolytic activation of the Epithelial Sodium Channel, ENaC**

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Diabetic nephropathy, characterized by impaired kidney function, albuminuria and hypertension, is a common complication in diabetic patients. In animals, aberrant glomerular filtration of plasma proteases activates the aldosterone-sensitive Epithelial Sodium Channel (ENaC) proteolytically, leading to impaired sodium excretion and hypertension. Kidney biopsies are not an option for quantitative investigations in humans. Urinary Extracellular Vesicles (uEVs) are cell-derived nanovesicles released to urine potentially reflecting the cell of origin. We aim to test the hypothesis that uEVs can be used as non-invasive readout of intrarenal ENaC cleavage.

Ten males were given low (70 mmol/day) and high (250 mmol/day) salt diet for five days. Urine and plasma were collected. Isolated uEVs were analyzed by western blotting.

Participants gave written informed consent (VEK S-20150208) and the study was reported at [clinicaltrials.org](https://clinicaltrials.org) (NCT02823613). Participants were compliant with the diet and in response to low salt diet renin and aldosterone were significantly elevated. 2 uEV markers, ALIX and CD9, were significantly reduced in abundance with high salt intake. By contrast, with antibodies specific for cleavage, products of the ENaC  $\gamma$ -subunit were hardly detectable while a tissue control was positive in all blots while the  $\alpha$ -subunit appeared more consistently but not regulated by salt intake. Prostaticin, a principal cell-attached protease, with potential to cleave ENaC, was significantly up regulated with low salt.

The results indicate that uEVs are not suitable for measuring intrarenal ENaC subunit abundance or physiological proteolysis. The method may be appropriate for other transporters. Post release proteolysis is a likely challenge when studying uEVs.



**Poster 86**

**Effects of inorganic nitrate on cardio-renal functions following ischemia-reperfusion of the kidney**

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**Introduction**

New strategies that dampen oxidative stress and restore nitric oxide (NO) homeostasis may have therapeutic implications during acute kidney injury (AKI) and associated complications. Supplementation with inorganic nitrate has been associated with favorable effects in models of cardiorenal disease. However, there is less knowledge regarding the potential value of boosting the nitrate-nitrite-NO pathway following renal ischemia/reperfusion (I/R). Our aim was to investigate the therapeutic value of nitrate treatment during the development of AKI-induced renal and cardiovascular dysfunction.

**Material and Methods**

Mice underwent unilateral ischemia (40 min) of the left kidney and the right kidney was inspected but left intact. Sham operations were performed in the same way, but without clamping the renal artery/vein. After surgery, mice were treated with sodium nitrate (1 mmol/kg/day) or placebo (NaCl) for 2 weeks and thereafter euthanized. Blood pressure was monitored by tail-cuff technique and glomerular filtration rate was estimated by creatinine clearance. Function of interlobular and mesenteric arteries were assessed by myograph technique. Tissue samples were processed for later analyses.

**Results**

I/R mice given placebo displayed higher blood pressure, reduced GFR, renal endothelial dysfunction and increased angiotensin II responses, as well as tubular/glomerular injuries compared with the sham group. These adverse complications following I/R were partly prevented by nitrate treatment.

**Conclusions**

Renal oxidative stress coupled with NO deficiency have been implicated in AKI-induced complications. Our study suggests that treatment with inorganic nitrate might be a novel strategy to ameliorate renal and cardiovascular dysfunction following renal ischemia/reperfusion.