# New Frontiers in Basic Cardiovascular Research

## France–New EU Members


**Organizers**
PhyMedExp
Inserm U1046 – University of Montpellier – CNRS UMR9214

# Program & Book of Abstracts

Sète, France
May 22 - 24, 2024

Supported by











# New Frontiers in Basic Cardiovascular Research: France – New EU members

*May 22 - 24, 2024*

Sète, France

# Program & Book of Abstracts

Edited by Dr. A. C. Meli and Dr. R. Andriantsihohaina

## *Welcome address*

*Dear Colleagues and Friends,*

*It is our pleasure to extend our warmest invitation to you for the upcoming 15th "New Frontiers in Basic Cardiovascular Research: France - New EU Members" conference, scheduled to take place in Sète, France, from May 22-24, 2024.*

*Originating from a rich legacy that began in Prague in 1994 under the guidance of Prof. B. Ošťádal (Czech Republic) and Prof. R. Fishmeister (France), this conference series has flourished over the years. Initially fostering collaboration among researchers from France, Czech Republic, and Slovakia, the conference has evolved to encompass all countries of the V4 region and New EU members since 2006. Spanning across various venues in the V4 countries and France, this conference has been pivotal in advancing cardiovascular research. This year, we are pleased to welcome researchers from Romania, Serbia and Turkey.*

*True to the spirit of the "Frontiers" meetings, our gathering will feature a dynamic blend of basic scientific and clinical sessions. Attendees can anticipate enlightening lectures from esteemed keynote speakers, alongside engaging discussions on the latest research findings during free oral communication sessions. For budding researchers, there will be ample opportunities to present their work through both oral and poster presentations, fostering an environment of knowledge exchange and collaboration.*

*Beyond the scientific program, we are committed to curating an enriching social agenda, ensuring ample opportunities for networking and camaraderie amongst participants.*

*Despite the rigor of our scientific agenda, we aim to carve out moments for fruitful discussions and opportunities to explore the captivating city of Sète.*

*We sincerely hope you will join us in making this conference a resounding success, contributing to the advancement of cardiovascular research and fostering lasting connections within our scientific community.*

*On behalf of the Organizing committee*

*Albano C. Meli, Ramaroson Andriantsitohaina, Jean-Luc Pasquié and Alain Lacampagne*

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## GENERAL INFORMATION

### Venue and date

The meeting will be held in the Domaine du Lazaret, 223 Rue du Pasteur Lucien Benoit, 34200 Sète on May 22-24, 2024.

Door access code: 2409A / Website: www.lazaretsete.com

### Registration

On the premises of the Domaine Lazaret from Wednesday May 22th, 2024 from 8:30 a.m.

### Accommodation

The accommodation has been arranged in the Domaine du Lazaret, 223 Rue du Pasteur Lucien Benoit, 34200 Sète on May 22-24, 2024.

### Information for presenters

Oral presentations of invited speakers - 20 min including discussion
Oral presentations from submitted abstracts - 15 min including discussion
Posters should be mounted before 11:00 A.M. on May 22th
The authors should be present during the Poster session.
Poster board size – 100 cm (height) x 80 cm (width). Clamps will be provided.

# NEW FRONTIERS IN BASIC CARDIOVASCULAR RESEARCH
## *A FRANCE-NEW EU MEMBERS SYMPOSIUM*

22-24 May 2024, Sète, France

Place: Domaine du Lazaret, Sète (link: https://www.lazaretsete.com/)

**PROGRAM**

**Day 1, May 22nd**

**8h30** Badge pick-up

**9h00: Introduction:** Presentation by Rodolphe Fischmeister and Bohuslav Ostadal (30th anniversary)

**9h15-10h40** **Session 1 "CARDIAC ARRHYTHMIAS AND SUDDEN CARDIAC DEATH"**

Chairs: Zoltan Papp, Marketa Bébarovà

9h15-9h35: **Zoltan Papp** (Debrecen, Hungary)

*Will direct myosin inhibitors prevent sudden cardiac death?*

9h35-9h55: **Marketa Bébarovà** (Brno, Czech Republic)

*Analysis of Ca2+ transients in hiPSC-derived cardiomyocytes with Y4734C-RYR2 variant: preliminary data*

9h55-10h10: **Belma Turan** (Ankara, Turkey)

*The electrotonic modulation of the electrical conduction by non-myocytes can be a novel approach for possible role of short QT-characterized cardiac remodeling in sudden cardiac arrest*

10h10-10h25: **Pietro Mesirca** (Montpellier, France)

*Genetic ablation of G protein-gated $K^+$ channels (GIRK4) rescues cardiac conduction defects and fibrosis in mouse model of Sinus Node Dysfunction*

10h25-10h40: **Albano C. Meli** (Montpellier, France)

*How can we effectively model RyR2 dysfunction in patients with inherited arrhythmias and autism?*

**10h40-11h00** coffee break

**11h00-12h25 Session 2 "EMERGING MECHANISMS AND SIGNALLING PATHWAYS IN CARDIOVASCULAR DISEASES"**

Chairs: Michaela Adamcova, Alain Lacampagne

11h00-11h20 : **Olga Lencova** (Hradec Kralove, Czech Republic)

*Pharmacological inhibition of ATM in the heart facilitates the development of chronic anthracycline cardiotoxicity*

11h20-11h40: **Danina Muntean** (Timisoara, Romania)

*Monoamine oxidase is a novel off-target target of the antidiabetic drugs in the cardiovascular system*

11h40-11h55: **Hana Mauer Sutovska** (Bratislava, Slovakia)

*Effects of artificial light at night and high-fat diet on cardiovascular system in hypertriglyceridemic rats*

11h55-12h10: **Tomas Chmelir** (Prague, Czech Republic)

*Type 2 Diabetes Mellitus and the Cardiac NPB/W Signaling System: Unraveling Molecular Mechanisms in ZDF Rats*

12h10-12h25: **Marta Novotova** (Bratislava, Slovakia)

*Growth related structural activity of sarcolemma in cardiac myocytes*

**12h35-14h00 Lunch**

**14h00-16h00 Poster session**

**16h00-17h25** **Session 3 " CARDIOVASCULAR METABOLISM AND HEART FAILURE"**

Chairs: Florence Pinet, Olga Pechanova

16h00-16h20: **Florence Pinet** (Lille, France)
*Nrf2-dependent antioxidant signalling pathways modulate metabolic stress-induced cardiac dysfunctions in mice*

16h20-16h40: **Frank Lezoual'ch** (Toulouse, France)
*Regulation of iron metabolism and ferroptosis in cardiomyocytes*

16h40-17h55: **Olga Gawrys** (Warsaw, Poland)
*Characterisation of a new model of doxorubicin-induced HFrEF in Ren-2 transgenic rats*

17h55-18h10: **Daphné Corboz** (Créteil, France)
*Angiopoietin-like 4 improves left ventricular function in a pig model of heart failure with preserved ejection fraction*

18h10-18h25: **Marie Kervella** (Montpellier, France)
*Three-dimensional Genome Architecture in Cardiac Muscle Cells: Pathophysiological Implications in DMD- and LMNA-Dilated Cardiomyopathy*

**19h30** Get together

**20h00** Diner

**Day 2, May 23rd**

**9h00-10h25 Session 4 "NOVEL STRATEGIES FOR CARDIOPROTECTION ASSOCIATED WITH MYOCARDIAL ISCHEMIA REPERFUSION"**

Chairs: François Roubille, Jitka Zurmanovà

9h00-9h20 : **Jitka Zurmanovà** (Prague, Czech Republic)

*Role of JAK/STAT pathway and mitochondria in infarct size limiting effect of cold acclimation*

9h20-9h40: **Ludovic Gomez** (Lyon, France)

*Phosphorylation of SERCA2, a physiological and clinical event in ischemic heart diseases that regulates intracellular $Ca^{2+}$ and cell death.*

9h40-9h55: **François Roubille** (Montpellier, France)

*Acute myocardial infarction: does cardioprotection remain a target?*

9h55-10h10: **Branislav Kura** (Bratislava, Slovakia)

*New prevention and treatment options for radiation-induced heart disease*

10h10-10h25: **Petra Alanova** (Prague, Czech Republic)

*HIF-1α limits myocardial infarction by promoting mitophagy in mouse hearts adapted to chronic hypoxia*

**10h30-11h00 coffee break**

**11h00-12h35 Session 5 "NUTRITIONAL APPROACHES IN CARDIOMETABOLIC PROTECTION"**

Chairs: Monika Bartekova, Ramaroson Andriantsitohaina

11h00-11h20: **Monika Bartekova** (Bratislava, Slovakia)

*Quercetin as a potential cardioprotective agent in ischemia-reperfusion injury*

11h20-11h40: **Mathias Mericskay** (Orsay, France)

*Vitamins B cocktail: a nutritional approach for the treatment of heart failure*

11h40-11h55: **Goran Koricanac** (Belgrade, Serbia)

*Quercetin reduces fructose drinking in a model of fructose-induced insulin resistance*

11h55-12h10: **Olga Pechanova** (Bratislava, Slovakia)

*Lipid profile and nitric oxide production in experimental metabolic syndrome: Effects of natural polyphenolic substances*

12h10-12h25: **Ramaroson Andriantsitohaina** (Montpellier, France)

*Carrot Genotypes Displayed Differential Pharmacological Profiles in Vascular and Metabolic Cells and Improve Blood Pressure and Reduces Aortic Root Lesions in an Atherosclerosis-Prone Genetic Mouse Model*

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|---|---|
| **12h35-14h00** | **Lunch** |
| **14h00-16h00** | **Posters** |
| **16h00-19h00** | **Photo followed by free time** |
| **19h00** | **Bus departure to Paillotte M at Palavas-les-Flots** |
| **19h30-2h00** | **Gala dinner and entertainment at Paillotte M** |

**Day 3, May 24th:**

**9h00-10h35** **Session 6 "ADVANCES IN ATRIAL FIBRILLATION"**

Chairs: Jean-Luc Pasquié, Maria Holicka

9h00-9h20: **Jean-Luc Pasquié** (Montpellier, France)

*Atrial fibrillation and ultra-endurance*

9h20-9h40: **Maria Holicka** (Brno, Czech Republic)

*Atrial fibrillation from clinical perspective, new approaches in treatment*

9h40-9h55: **Angelo Torrente** (Montpellier, France)

*Age impairs the autonomic modulation of cardiac activity in Bottlenose Dolphin (Tursiops truncatus) in a way similar to humans*

9h55-10h10: **Francisco Jaque-Fernandez** (Orsay, France)

*Role of RyR2-S2030 phosphorylation in the calcium clock during the beta-adrenergic response*

10h10-10h25: **Hugo Benoit** (Montpellier, France)

*Heart and Mind at Risk: Decoding Channel Disruptions in the Food Poisoning Enigma*

**10h35-11h00** **coffee**

**11h00-12h35** **Session 7 "NOVEL TARGETS FOR VASCULAR DISEASES"**

Chairs: Carmen Martinez, Michal Maczewski

11h00-11h20: **Carmen Martinez** (Montpellier, France)

*Extracellular vesicles as novel targets against vascular diseases associated to metabolic syndrome*

11h20-11h40: **Michal Maczewski** (Warsaw, Poland)

*Impairment of coronary flow reserve at the capillary level underlies cardiotoxicity related to multiple myeloma*

11h40-11h55: **Takiy Berrandou** (Paris, France)

*GWAS meta-analysis in SCAD, a women predominant ischemic heart disease, reveals common variants and genes related to artery integrity and tissue-mediated coagulation*

11h55-12h10: **Anna Labedz** (Krakow, Poland)

*Angiogenic and lymphangiogenic effects of mesenchymal stem cell extracellular vesicles through transfer of microRNAs and proteins, including ITGα5 and NRP1*

12h10-12h25: **Jaroslav HRDLICKA** (Prague, Czech Republic)
*Sex differences in cardiac function and remodeling induced by early abdominal aortic constriction in Wistar rats.*

**12h35-14h00 Lunch**

Departure – End of meeting

## Committee of organization

Albano Meli
Ramaroson Andriantsitohaina
Alain Lacampagne
Jean-Luc Pasquié

**Session 1 "CARDIAC ARRHYTHMIAS AND SUDDEN CARDIAC DEATH"**

**S1-**18
**Title: Variants in the hERG channel associated with idiopathic ventricular fibrillation: clinical, genetic, and functional analysis**

Markéta BÉBAROVÁ (1,2), Natálie JANKOVÁ (1), Olga ŠVECOVÁ (1), Martin KRÁL (1), Jiří PACHERNÍK (3), Samuel LIETAVA (2), Jana ZÍDKOVÁ (4) Tomáš NOVOTNÝ (2)

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4. Center of Molecular Biology and Genetics, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

**Abstract:**
Objectives: In some patients suffering from idiopathic ventricular fibrillation (VF), variants in genes encoding cardiac ionic channels may be identified. A complex analysis has to be used to consider the possible pathogenic character of the identified variant. This study is focused on clinical, genetic, and functional analysis of a proband suffering from idiopathic VF and carrying two variants in the hERG (KCNH2) gene. Methods: After clinical and genetic investigation of the proband and family members, we have recently started electrophysiological analysis using whole-cell patch clamp technique at 37°C on Chinese hamster ovary cells transiently expressing human hERG channels, both wild type (WT) and carrying S1021Qfs*98 variant. Results: The proband, a female, was diagnosed at the age of 22 when she experienced her first attack of VF and was successfully resuscitated. QT interval corrected to the heart rate by the Bazett formula (QTc,B) and Fridericia formula (QTc,F) were 460 ms at rest. Qtc,B and QTc,F were 480 and 400 ms, respectively, during the exercise test and 460 and 420 ms, respectively, during the following recovery. No arrhythmia was detected during the exercise test as well as Holter monitoring. Echocardiography did not reveal any pathology as well. Hence, she was diagnosed as idiopathic VF, ICD was implanted, and she was treated using betaxolol. Repeated episodes of VF were reported during the following years (16 episodes per 7 years). Two hERG variants in trans, p. A228V and p.S1021Qfs*98, were identified in the proband, thus, functional analysis has been started. We have not identified any clear deviation in activation and deactivation of S1021Qfs*98 channels, but the tail current was significantly decreased by about 70 % in S1021Qfs*98 channels vs. WT channels. Currently, analysis of the channel inactivation proceeds. Conclusions: A proband suffering from idiopathic VF and carrying two hERG variants was identified and studied. Functional analysis of S1021Qfs*98 channels revealed a significantly decreased tail current with unaltered activation and deactivation gating. The functional analysis of A228V and co-expressed A228V/S1021Qfs*98 channels as well as analysis of channel expression and trafficking will follow.

**Keywords:** fibrillation, hERG, A228V, S1021Qfs*98, function
**Funding:** Supported by NU22-02-00348 (Ministry of Health of the Czech Republic) and MUNI/A/1547/2023 (Ministry of Education, Youth and Sports of the Czech Republic).
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**S1-**64

**Cardiac safety evaluation of a novel recombinant bispecific antibody targeting the ether-à-go-go related gene 1 (hERG1)-β1 integrin macromolecular complex.**

Monica MUSUMECI (1), Lorenzo SANTINI (1), Claudia DURANTI (2), Chiara PALANDRI (1), Lucrezia GIAMMARINO (1), Lucia CARLUCCI (3), Chiara CAPITANI (2,4), Rossella COLASURDO (2,4), Fabio RECCHIA (3), Elisabetta CERBAI (1), Raffaele COPPINI (1) and Annarosa ARCANGELI (2)

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3 Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy
4 Department of Medical Biotechnologies, University of Siena, 53100 Siena, Italy

**Abstract:**
In recent decades ion channels became an interesting anti-tumoral target. The human *ether-á-go-go–related gene* (hERG1) potassium channel regulates the cardiac repolarization through $I_{Kr}$ and plays a key role in different cancers where hERG1 binds to the β1 subunit of integrin adhesion receptors; instead, in the heart this does not occur. Based on these evidence, hERG1-b*1* could represent an ideal target for anti-cancer therapy. To this aim, different antibodies have been developed: bispecific antibody *scDb-hERG1*-b*1*, the full length anti-hERG1 monoclonal antibody (mAb-hERG1) and the anti-hERG1 single chain fragment variable. The main goal of this work to evaluate their effect on the action potential duration (APD).

Viable cardiomyocytes were obtained from surgical septal and atrial samples after enzymatic and mechanical digestion. The cells were incubated at 37°C for 1h with each antibodies (10μg/ml) and patch-clamp experiments were performed to measure APD with and without the specific hERG1-blocker E4031.

hERG1-mAb and scFv-hERG1 significantly prolonged APD of left ventricular and atrial cardiomyocytes compared to vehicle, at all frequencies tested. Instead, this did not occur with scDb-hERG1-β1, where APD was shorter than hERG1-mAb or scFv-hERG1and comparable to the vehicle. Moreover, the specific hERG1 blocker E4031 (5μM) confirmed that the prolongation of APD was the direct consequence of hERG1 block.

We tested the cardiac safety of a novel recombinant bispecific antibody targeting hERG1-β1 integrin complex on human atrial and ventricular cardiomyocytes, evaluating its effect on the electrical properties of the cells. No changes in APD kinetics were observed in cells incubated with the scDb-hERG1-b1 compared to the vehicle. Conversely, both the hERG1-mAb and the scFv-hERG1 produced a prolongation of APD. This evidence suggests that scDb-hERG1-β1 could be applicated to anti-cancer therapy preventing a pathological prolongation of repolarization that could lead to the long QT type 2 (LQT2) syndrome and the related fatal arrhythmia.

**S1-**39

**Title: Analysis of Ca2+ transients in hiPSC-derived cardiomyocytes with Y4734C-RYR2 variant: preliminary data**

Olga SVECOVA (1), Martin KRAL (1), Stefan ZELENAK (2), Jiri PACHERNIK (2), Tomas BARTA (3), Samuel LIETAVA (4), Iva SYNKOVA (5), Jana ZIDKOVA (5), Tomas NOVOTNY (4), Marketa BEBAROVA (1,4)

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(5) Center of Molecular Biology and Genetics, Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

**Abstract:**
Objectives: Changes in intracellular Ca2+ concentrations are crucial for the excitation-contraction coupling and regulation of inotropy. The release of Ca2+ into the cytosolic space is controlled by the ryanodine receptor type 2 (RYR2) located on the sarcoplasmic reticulum. Dysfunction of RYR2 is involved in the pathogenesis of inherited and non-inherited diseases such as cardiac arrhythmias including ventricular fibrillation. In a patient with idiopathic ventricular fibrillation, the variant p. Y4734C in RYR2 was found. Besides investigation of electrical properties using multielectrode array, a pilot study has been recently started analyzing changes in Ca2+ transient of patient-specific cardiomyocytes derived from human-induced pluripotent stem cells (hiPSC-CM). Methods: Ca2+ transients of patient-specific hiPSC-CM (Y4734C) and hiPSC-CM unrelated healthy control (WT) were measured using the Myocyte Calcium and Contractility System (IonOptix LLC). Cell clusters were loaded with 1 μmol/L Fura-2-AM (Molecular Probes, Invitrogen) during 15-min incubation in Tyrode solution at 37 °C and then washed repeatedly with Tyrode solution followed by incubation for 10 min in Tyrode solution at 37 °C. Measurements were performed in Tyrode solution at 37 °C; cells were not stimulated. Analysis of Ca2+ transients was performed using CytoSolver software (IonOptix LLC). Since data were not normally distributed, median is stated and Mann-Whitney test was used. Results: Several parameters describing basic Ca2+ transient characteristics and frequency of beating of Y4734C and WT were evaluated. Time to peak and decay time constant were significantly longer in Y4734C (time to peak: 0.24 s in Y4734C vs. 0.09 s in WT, $P < 0.01$; decay time constant: 0.20 s in Y4734C vs. 0.11 s in WT, $P < 0.05$; $n = 8$ in Y4734C and 4 in WT). No change was observed in the amplitude of Ca2+ transient (0.22 a.u. in Y4734C and 0.15 a.u. in WT, $n = 8$ and 4, respectively). Cell clusters with the Y4734C variant had higher frequency of beating than WT (40 bpm in Y4734C and 28 bpm in WT, $n = 8$ and 4, respectively). Conclusions: The preliminary data showed a delayed release of Ca2+ from the sarcoplasmic reticulum in the Y4734C variant and prolonged reabsorption of Ca2+ back into the sarcoplasmic reticulum. Further analysis is needed to finally describe changes in Ca2+ cycling in hiPSC-derived cardiomyocytes with the Y4734C-RYR2 variant.

**Keywords:** RYR2, Ca2+ transient, ventricular fibrillation
**Funding:** Supported by NU22-02-00348 (Ministry of Health of the Czech Republic) and MUNI/A/1547/2023 (Ministry of Education, Youth and Sports of the Czech Republic).
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**S1-**40
**Title: Allosteric mechanism of ryanodine receptor inactivation by calcium and magnesium ions**

Alexandra ZAHRADNÍKOVÁ, Bogdan IAPAROV, Ivan ZAHRADNÍK

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**Abstract:**
Ryanodine receptors of skeletal and cardiac muscle cells are inactivated by Ca2+ and Mg2+ ions by a mechanism that is not yet clear. The putative inhibitory divalent ion binding site residing in the EF-hand region has similar ion affinity in both the skeletal (RyR1) and the cardiac (RyR2) isoform when observed in ion binding experiments; however, the ion sensitivity of the open probability to inactivation observed in single-channel experiments is by an order of magnitude smaller in RyR2 than in RyR1. The aim of this work was to test allosteric models of RyR inactivation for conforming with both experimental findings. We used statistical thermodynamics to construct allosteric models of RyR gating based on the Monod-Wyman-Changeux principles and fitted the resulting equations of Ca2+- and Mg2+-dependence of open probability to published data on single-channel activity of RyR1 and RyR2 channels under a variety of conditions. In the models, each RyR monomer contained an activation site, at which Ca2+ binding was a positive and Mg2+ binding a negative allosteric effector of channel opening, and an inhibition site, at which Ca2+ and Mg2+ were not distinguished and both were positive allosteric effectors of channel inactivation. Two gating models were compared: the first model had an activation gate and an independent inactivation gate, while in the second model, the channel makes transitions between the closed, open, and inactivated macrostate, controlled by the allosteric pathways from the activation and inhibition binding sites to the single channel gate. The independent model could describe the experimental data only if the ion affinity of the inhibitory site was higher in RyR1 than in RyR2, at odds with experimental findings. The three-macrostate model could describe the experimental data when the ion affinities of RyR1 and RyR2 and the strength of interaction between the activation and the inhibition site were identical but the allosteric strength of the inactivation pathways was different. The efficiency of the activating Ca2+ binding was allosterically increased by the presence of ATP and weakly depended on species and RyR isoform. Considering structural and biochemical data together with single-channel data allowed building the model of RyR operation for both RyR1 and RyR2 isoforms on the grounds of the same complex allosteric mechanism.

**Keywords:** ryanodine receptor, gating model, inactivation
**Funding:** Supported by Slovak Research and Development Agency grants APVV-21-0473 and APVV-23-0545.
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**S1-**46

**Title: Age impairs the autonomic modulation of cardiac activity in Bottlenose Dolphin (Tursiops truncatus) in a way similar to humans.**

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**Abstract:**
Heart rate variability (HRV) is the physiological oscillation of heart beating, induced by the autonomic nervous system, to adapt cardiac activity to body needs. Such heart rate (HR) modulation could be impaired by aging. Indeed, regardless of lifestyle, aged people are subjected to a decrease of maximal HR and HRV, whose reasons remain unclear. To clarify them we investigated HR and HRV in a colony of common dolphins (Tursiops truncatus) hosted at the Oceanographic of Valencia. We studied these animals because contrary to terrestrial mammals their intermittent breathing and underwater life oblige them to important variation of HR at each cycle of respiration, according to the mechanism of respiratory sinus arrhythmia. Such physiological arrhythmia allows them to significantly slow down HR during apnea, to spare oxygen during their underwater life. Thus, during apnea, dolphins allow to study HRV, independently from the fast and constant respiration cycles typical of terrestrial mammals. To study HRV in bottlenose dolphins we developed a submersible electrocardiogram-accelerator logger based on a suction cup attachment technique. With this method we recorded high resolution electrocardiograms that allowed us to study the effect of aging on the autonomic modulation of HR. Similarly to humans, at rest common dolphins have an average HR of 75±3 bpm. However, their HR could oscillate between 52±2 and 92±4 bpm (n=6). As expected, short apnea of 1 min decreased HR to 50±5 bpm (**p<0.01 by paired T-test, n=6). To test the effect of aging on the autonomic modulation of cardiac activity, we correlated HR and two HRV indexes to the animal age. Specifically, we used the coefficient of variability of RR intervals (CVRR) and RMSSD, respectively as indexes of sympathetic and parasympathetic modulation. We observed that although the average HR under rest did not correlate with age, CVRR decreased with age. As well, during apnea CVRR tended to decrease and RMSSD significantly decreased with age. Moreover, max HR measured under short fasting significantly decreased with age. We thus highlighted that age affects dolphins' HR in a similar way to what is known in humans. This suggests that also the impairment of the autonomic modulation should be taken in consideration as a detrimental consequence of aging, opening the way to possible therapies that could overcome the age-related impairment of cardiac modulation by direct pharmacological stimulation of cardiac activity.

**Keywords:** Heart, aging, respiration, dolphins, apnea.
**Funding:** ANR DicWoc and University of Montpellier
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**S1-**51
**Title: Modelling the neurocardiac junction in Long QT syndrome type 2**

Lamia GOUAL (1), Elisa BOUNASRI (1,2), Sophie NICOLE (3), Etienne JACOTOT (4), Fabien Brette (1), Philippe LORY (3), Alain LACAMPAGNE (1), Jean-Luc PASQUIÉ (1), Albano C. MELI (1), Jérôme THIREAU (1)

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**Abstract:**
Long QT syndrome (LQT, 1:2000 of live birth) is a potentially life-threatening cardiac arrhythmia characterized by delayed myocardial repolarization that produces QT prolongation and increased risk of Torsades de Pointes (TdP). This syndrome triggers syncopes, seizures, and sudden cardiac death in otherwise healthy young individuals with structurally normal hearts. LQT type 2 (LQT2) is a rare condition (30-40% of LQTS) caused by pathogenic variants in the HERG gene. HERG encodes the α-subunit of the human ether-à-go-go (hERG) channel, thus affecting the rapid component of the delayed rectifier K+ current (IKr) of the action potential. Associations between HERG, LQT2 and SUDEP (Sudden Unexpected Death in Epilepsy) suggest that HERG mutations confer a susceptibility to primary neuronal excitability defects. The main objective of our study is to model the neurocardiac axis using a human-derived neuro-cardiac junction from both a healthy individual and a LQT2 patient, aiming to elucidate the potential involvement of neurocardiac defects in LQT2. Using a 2D sandwich-based protocol, we differentiated hiPSC-derived ventricular cardiomyocytes (hiPSC-CM) and sympathetic neurons (hiPSC-NR), which we respectively characterized by sarcomeric (α-actinin and cardiac troponin I) and by sympathetic neuronal markers (β3-tubulin and tyrosine hydroxylase). We confirmed the expression of hERG in both cell types regardless of genotypes and investigated the functional properties of hiPSC-CM alone or innervated by hiPSC-NR, focusing on the intracellular Ca2+ handling and contractile properties. Our preliminary data show a retention of hERG in the perinuclear area in LQT2 hiPSC-CM. Moreover, LQT2 mutation seems to affect intracellular Ca2+ dynamics in the two cell types. Additionally, we developed an LQT2 organ-on-chip in microfluidic compartmentalized chambers enabling the neuronal axons to project towards cardiomyocytes. Innervated LQT2 hiPSC-CM exhibit a higher incidence of aberrant Ca2+ transients and contractions compared to LQT2 hiPSC-CM alone. We also confirmed that nicotinic activation of hiPSC-NR modulates hiPSC-CM contractile functions through ‘synaptic junction’. Overall, our data indicate a loss of function of hERG in the LQT2 line due to impaired trafficking to the membrane, potentially compromising neurocardiac function. These technological and conceptual advancements should facilitate our understanding of the neurocardiac dysfunctions underlying the LQT2 syndrome.

**Keywords:** hiPSC, cardiomyocytes, neurons, LQT2, microfluidics
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**S1-**54
**Title: Role of RyR2-S2030 phosphorylation in the calcium clock during the beta-adrenergic response**

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**Abstract:**
Normal automaticity of Sino-atrial node (SAN) pacemaker cells is regulated by an integrated two coupled clocks system: the "voltage clock", and the "calcium (Ca2+) clock". RyR2 Ca2+ release is a key component of the "Ca2+ clock". Under stress, the pacemaker accelerates its rate (positive chronotropic effect) due to β-adrenergic stimulation, which results in elevating cAMP levels and PKA activation. While cAMP regulation of the voltage clock is well known, it is not clear whether the PKA RyR2 phosphorylation has any effect on the SAN response to stress. We focused on the S2030 site. Experiments were performed in homozygous RyR2-S2030A, RyR2-S2030D knock in mice and wild-type (WT) controls. ECG recorded in mice showed that the heart rate response to isoproterenol injection was blunted in phosphomutant mice. Consequently, we studied the electrical activity and the Ca2+ handling in the isolated SAN tissue associated with the β-adrenergic response. SAN tissue was chirurgically isolated and electrical mapping was performed using a 64-electrode system (mapping lab). For Ca2+ measurements, the tissue was loaded with Fluo-4 AM and Intracellular calcium variations were recorded using confocal microscopy (Leica). We also enzymatically isolated the SAN cells and incubated it with Fluo3-AM. In both, SAN tissue and isolated cells we measured spontaneous Ca2+ transients, waves and sparks. Finally we studied SR Ca2+ content on isolated SAN cells. Spontaneous Ca2+ transients rate in pacemaker cells was slower in S2030D compared to WT and S2030A at basal conditions, but perfusion with 20 nM isoproterenol failed to accelerate SAN rate in S2030A compared to WT and S2030D. However, SAN Ca2+ transient's duration at 50% are not accelerated in S2030A after Iso compared to S2030D and WT. Moreover, S2030A and S2030D show higher Ca2+ spark frequency in stress conditions than WT. We also observed the lost of the shift in the pacemaker leading site associated to β-adrenergic response in both phosphomutants. In conclusion, altering RyR2 phosphorylation at S2030 modifies Ca2+ spark frequency, Iso response, associated with a loss of pacemaker leading site adaptation. Thus, our results show that phosphorylation of the RyR2 at S2030 participates in the β-adrenergic response of the pacemaker.

**Keywords:** calcium, pacemaker, RyR2, phosphorylation, arrhythmia
**Funding:** INSERM ANR IFOR and NIH Grant (2R01HL055438-22)
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**S1-**55
**Title: Toward a Predictive Assay for Torsadogenic Effects of Drugs Using Neuro-Cardiac Organ-on-Chip**

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**Abstract:**
Long QT syndrome (LQT) is a primary arrhythmia syndrome with high risk of sudden cardiac death with genetic or iatrogenic etiology. For the drugs known to induce LQT, the consequence is the blocking of the IKr current, encoded by the human ether-a-go-go-related gene (hERG), inducing an abnormal lengthening of ventricular repolarization (VR) and finally Torsades de Pointes (TdPs). Consequently, regulatory evaluations assessing the potential hERG channel-blocking effects of new drugs are imperative for ensuring their safety. Although the literature showed that hERG blockers may also have to affect the activity of the autonomic nervous system to trigger TdPs, current regulatory tests do not consider possible autonomic effects of drugs that might interfere with the cardiac responses. Therefore, the objective is to pioneer a novel predictive assay capable of detecting torsadogenic effects of drugs, which involves leveraging a neurocardiac organ-on-chip composed of patient-derived autonomic neurons (NRs) innervating cardiomyocytes (CMs) all derived from human induced pluripotent stem cells (hiPSC). To achieve this, hiPSC-CMs and NRs were differentiated and characterized by the expression of sarcomeric cardiac markers (α-actinin, cardiac-troponin-I), neuronal structure markers (β3-tubulin), and sympathetic markers (tyrosine-hydroxylase, dopamine-β-hydroxylase). Expression of hERG is also monitored. We investigated the cellular functional properties (intracellular Ca2+ cycling, contractile and electrical properties). Our data show that CMs respond to adrenergic stimulation by increasing intracellular Ca2+ transient (CaT) and contraction frequency. CMs with hERG blockers exhibit aberrant CaT, longer decay time and aberrant contractility. Preliminary data were obtained using microelectrode-array, suggesting modulation of the field potential duration (FPD) in presence of hERG modulators. Concerning NR, our data show a response to nicotinic stimulation and hERG modulators, by modulating CaT properties. Ongoing experiments involve combining hiPSC-CM and NR on microfluidic device to elucidate the neurocardiac dysfunctions underlying TdPs and to determine the conditions that promote the onset of TdPs induced by hERG-blocking compounds. We aim to comprehensively investigate the neurocardiac dysregulations associated with TdPs and establish a patient-specific testing platform to enhance the evaluation of new pharmacological compounds for safety and efficacy.

**Keywords:** LQT, hiPSC, neurocardiac-organ-on-chip, drugs, microfluidic
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**S1-**56
**Title: Type I Cyclic AMP-dependent Protein Kinase Regulates Cardiac Automatism**

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**Abstract:**
**Background**
The heart expresses two main subtypes of PKA (type I and II) differing in their regulatory subunits, RIα and RIIα. The respective contribution of these PKA subtypes to β-adrenergic (β-AR) stimulation of the heart is not well understood. Recently, our team showed that ablation of the *Prkar1A* gene encoding RIα in adult mouse ventricular myocytes increases PKA activity and cardiac contraction. These results raise the question of the role of *Prkar1A* in the regulation of cardiac automatism.

**Aim**
Define the role of RIα in regulating cardiac automatism.

**Methods**
Expression of RIα in pacemaker cells was assessed by fluorescent *in situ* RNA hybridization in intact sino-atrial node (SAN) tissue. Mouse model with conditional ablation of *Prkar1A* (KO) in cardiac automatic centers was generated using tamoxifen-inducible recombinase (CreERT2) under the control of the *Hcn4* gene promoter. The construct includes a *Tomato* reporter gene enabling specific validation of Cre in pacemaker cells. Transgenic line with *Tomato* expression under the control of Hcn4-CreERT2 but carrying wild-type *Prkar1A* alleles served as a control (WT). Heart rate was recorded *in vivo* by telemetry EKG in conscious WT and KO mice. *Ex vivo* spontaneous beating rate was recorded on WT and KO Langendorff-perfused hearts using EMapRecord system.

**Results**
RIα transcript were abundantly expressed in SAN and in cells expressing HCN4 transcripts. In basal, daytime and nighttime HR was similar in WT and KO mice. However, HR was higher in KO mice upon inhibition of the autonomous nervous system (atropine 1 mg/kg + propranolol 2 mg/kg) and the difference was abolished after injection of the HCN blocker ivabradine (5 mg/kg). Dose-response curves to carbachol, a muscarinic agonist, as well as to isoproterenol, a β-AR agonist, were similar between WT and KO mice. Preliminary data show that *ex vivo* spontaneous HR was higher in KO than WT hearts.

**Conclusion**
Our preliminary data clearly demonstrate an involvement of PKA type I in regulating cardiac pacemaker activity.

**Keywords:** Pacemaker, PKA, electrocardiogram
**Funding:** ANR-21-CE14-0082 (to GV)
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**S1-**43
**Title: Will direct myosin inhibitors prevent sudden cardiac death?**

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**Abstract:**
Objective: Mutations in sarcomere protein genes result in hypercontractility of the left ventricle (LV) and are thought to drive pro-hypertrophic signalling in hypertrophic cardiomyopathy (HCM). Accordingly, targeting the myosin heavy chain-β by small molecules appears to be an effective approach to treat HCM and consequently to prevent sudden cardiac death (SCD). Indeed, clinical evidence now supports that modulation of the myosin motor can be an effective treatment option to well-defined HCM patient groups. Moreover, the potential benefit of myosin inhibitors has been also implicated for heart failure patients with preserved ejection fraction (HFpEF) in the absence of known genetic alterations. However, it is unclear if cardiomyocytes with distinct genetic backgrounds respond identically to myosin inhibitors or not. Methods: Here, we tested the contractile effects of aficamten, a second generation myosin inhibitor agent, in cardiac preparations of healthy rodents, dogs, and humans. Moreover, these effects were also contrasted to those obtained in cardiomyocytes isolated from HCM patients following myectomy. Results: Aficamten reduced maximal Ca2+-activated tensions (Fmax) and evoked robust rightward shifts in the Ca2+-sensitivities of isometric force production of permeabilized cardiomyocytes of all sources. Nevertheless, aficamten-induced Ca2+-desensitisations were higher in HCM cardiomyocytes than those in cardiomyocytes of healthy animals or humans. Conclusion: Our results illustrate largely comparable contractile effects upon myosin inhibitor applications irrespective of the myosin isoform composition or the presence of mutated sarcomeric proteins in cardiomyocytes of different species. These data also implicate that pro-hypertrophic signalling (predisposing for SCD) can be also limited to similar levels by aficamten in cardiomyocytes with different genetic backgrounds.

**Keywords:** sudden cardiac death, hypertrophic cardiomyopathy
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**S1-**66
**Title: How can we effectively model RyR2 dysfunction in patients with inherited arrhythmias and autism?**

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**Abstract :**
Introduction: Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a severe genetic arrhythmia linked to dysfunction in over 80% of cardiac ryanodine receptor type 2 (RyR2). It is characterized by ventricular tachycardia induced by catecholamines and sympathetic nervous system (SNS) stimulation, often resulting in syncope or sudden death during childhood. Additionally, CPVT patients may develop neuro-psychological disorders, including epilepsy and autism. Autism is a neurodevelopmental disorder that typically emerges early in life. It is marked by challenges in social interaction and communication, as well as restricted and repetitive behaviors. Research indicates that autism arises from a combination of genetic and environmental factors. The behavioral and cognitive aspects of autism are linked to widespread abnormalities within the central nervous system (CNS).

Objective: Given that RyR2 is expressed in both the heart and the brain, and building upon our previous research, we propose that dysfunctional RyR2 in CPVT patients affects both cardiomyocytes and neurons.

Materials and Methods: We utilize patient-specific induced pluripotent stem cells (hiPSCs) and differentiate them into cardiomyocytes and neurons. We conduct morphological, structural, and functional analyses of both control and patient-derived cardiomyocytes and neurons using techniques such as immunofluorescence, confocal microscopy, IonOptix, video-edge capture, immunoblotting, and mass spectrometry.

Results and Discussion: Our differentiation protocols yield ventricular cardiomyocytes and CNS neurons from hiPSCs. Patient-derived cardiomyocytes display structural abnormalities, calcium handling issues, contractile defects, and leaky RyR2 channels typical of CPVT. Similarly, patient-derived neurons exhibit altered size, structural remodeling, dysregulated intracellular calcium dynamics, leaky RyR2 channels, and abnormal neurotransmitter secretion. Some of these neuronal defects are consistent with characteristics observed in autism. Furthermore, alterations in neurotransmitter levels accompany these changes at the neuronal level. Treatment with S107, a Rycal agent that stabilizes the closed conformation of RyR2, partially reverses many of these abnormalities.

Conclusion: Our findings demonstrate that both patient-derived ventricular cardiomyocytes and CNS neurons exhibit defects, including RyR2 leakage and neuronal impairment reminiscent of autism. This suggests, for the first time, a potential link between RyR2 mutation and autism, highlighting a promising therapeutic target.

**Keywords:** hiPSC-CMs, calcium, ryanodine receptor, CPVT, autism, neurons, cardiomyocytes
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**S1-**27

**The electrotonic modulation of the electrical conduction by non-myocytes can be a novel approach for possible role of short QT-characterized cardiac remodeling in sudden cardiac arrest**

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**Abstract:**
Short QT-interval induction causes irregular heart rhythms further leading to cardiac arrest. Considering the contributions of macrophage to myofibroblast transition, together with macrophage coupling with cardiomyocytes via Cx43, by hypothesizing the involvement of non-myocyte-associated passive electrical contributions in cardiac remodeling, we examined whether they can contribute to cardiac remodeling of early-stage MetS rats characterized by a short QT-interval. Action potentials (APs) of isolated papillary muscles, and L-type $Ca^{2+}$-channel currents (LTCC) and $K^+$-channel currents ($I_K$) in ventricular cardiomyocytes were studied in the MetS rat hearts fed with high-sucrose for 14-16 weeks (named as early-stage) compared with age-matched controls. The distributions of $Na_V1.5$ $Na^+$-channels and $K^+$-channesl as well as the distributions of non-myocytes such as myofibroblasts and macrophages in the intact hearts were evaluated by using immunohistochemistry and immunofluorescence techniques by light and confocal microscopy. The increased heart rate, shorten QT- and PR-intervals, and significantly high response to sympathetic stimulation were determined in the MetS rats. In this group, there was a slightly dense distribution of collagen I-III in the extracellular matrix and increased numbers of positive cells stained with either α-SMA or CD68 in interfibrillar spaces of the hearts with slightly developed fibrosis. Furthermore, the phosphorylated Cx43 was prominently localized on the longitudinal cell membrane while the Cx43 was on the intercalated discs of the MetS hearts. Moreover, we determined a significantly shortened AP duration with depolarized resting membrane potential in the papillary muscle strips of the MetS rats. These data were further supported by a significantly increased density of $Na_V1.5$ on the membranes and depressed LTCC with no change in total $I_K$. Considering the electrotonic contribution to intercellular propagation of APs in the heart via increases in the numbers of non-myocytes together with changes in phosphorylation and localization of Cx43, we, for the first time, demonstrated that electrical remodeling in early-stage MetS heart is characterized by a short QT-interval with contributions of electrotonic coupling of excitable and non-excitable cells as part of the causal pathway, leading to activation of ephaptic-coupling by mixed-mode conduction in the heart. Overall, our data suggest that this type preconditioning stimulus further leading to the development of long QT-interval in chronic conditions may underlie the appearance of a sudden cardiac arrest or both with various concomitant events, via one firing the others.

**S1-**20

**Title: Genetic ablation of G protein-gated $K^+$ channels (GIRK4) rescues cardiac conduction defects and fibrosis in mouse model of Sinus Node Dysfunction**

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**Abstract:**
Introduction In everyday life, cardiac automaticity is generated in the sino-atrial node (SAN) by specialised myocytes called pacemaker cells. Failure to generate a normal SAN impulse causes Sinus node dysfunction (SND), also referred to as 'sick sinus syndrome'. In SND, decline of pacemaker activity is attributed to intrinsic remodelling of the SAN structure and expression of ion channels involved in automaticity. A decrease in intrinsic electrical coupling due to progressive tissue fibrosis have been commonly observed in this dysfunction.
Objective: To test if genetic inhibition of G-protein-gated inwardly rectifying potassium 4 (Girk4) channel rescues cardiac conduction defects and fibrosis in $Cav1.3^{-/-}$ mouse model of SND.
Methods: We performed telemetric electrocardiogram recordings in control, $GIRK4^{-/-}$ knockout (KO), $Cav1.3^{-/-}$ KO and $GIRK4^{-/-}/Cav1.3^{-/-}$ double-KO mice. Animals were then euthanized and SAN tissues were isolated for optical and electrical mapping investigations. Immunohistochemistry (IHC) staining was performed to study fibrosis and inflammation in SAN tissues.
Results: P-wave duration, PR and RR intervals were significantly longer in $Cav1.3^{-/-}$ than in WT and $GIRK^{-/-}$ mice, suggesting conduction troubles and enhanced fibrotic tissue in $Cav1.3^{-/-}$ animals. Genetic ablation of Girk4 channels in $Cav1.3^{-/-}$ animals significantly shorten P-wave duration and both PR and RR intervals to WT values. Shift of the leading pacemaker site and defects of conduction recorded in $Cav1.3^{-/-}$ SAN isolated tissue was restored to control condition by genetic inhibition of Girk4 in SND tissue. IHC experiments showed significantly less fibrotic tissue in $GIRK4^{-/-}/Cav1.3^{-/-}$ than $Cav1.3^{-/-}$ SAN tissue.
Conclusion: In the context of SND generated by Cav1.3 ablation, concomitant inhibition of GIRK4 normalises heart rate by restoring normal cardiac conduction pathway by limiting fibrosis in SAN tissue

**Session 2 "EMERGING MECHANISMS AND SIGNALLING PATHWAYS IN CARDIOVASCULAR DISEASES"**

**S2-**38
**Title:** Key role of JAK/STAT pathway and mitochondria in infart size limiting effect of cold acclimation

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**Abstract:**
Objective: We have shown that continuous exposure of rats to moderate cold (CE; 9˚C) increases cardiac resilience to ischemia after as early as 3 days and remains for another 4 weeks (wks) of CE. CE induces desensitization of adrenergic system and decreases sensitivity of mitochondria to calcium overload in left myocardium. Alteration in cytokine levels during the 10 days of CE revealed immunosuppressive and anti-inflammatory effect of the acclimation. Based on these results, we selected the JAK/STAT pathway as a promising candidate for cardioprotection. We aimed to investigate the role of JAK/STAT pathway, antioxidant system, and sensitivity of mitochondrial permeability transition pore during the course of CE. Methods: Male Wistar rats were exposed to moderate cold (9˚C) for 1, 3, 10 days, and for 4 wks with 2 wks of recovery at the control room temperature (24˚C). The effects of the short-term or long-term exposure to moderate cold on myocardial sensitivity to ischemia in vivo, mitochondrial function, incidence of ischemia and reperfusion arrhythmias, profile of polyunsaturated fatty acids (PUFA) in membrane phospholipids, and expression of selected enzymatic antioxidants, and STAT3 related cytokine levels were assessed. Finally, the specific inhibition by AG490 of the JAK/STAT3 pathway was applied in the most protective chronic regimen of 4-wks CE. Results: While the infarct size limiting effect appeared already after 3 days and the BAT matured after 10 days of CE, a cold-elicited anti-arrhythmic phenotype was detected after 4 wks of CE. Interestingly, after 2 wks of the recovery infarct-size limiting effect persisted, however the anti-arrhythmic phenotype was lost. The anti-arrhythmic n-3/n-6 PUFAs ratio has changed accordingly. Mitochondrial antioxidants in the left ventricle increased only after the first day of exposure and then remained unchanged. While inducible HMOX1, which participates in mitochondrial quality control, was significantly elevated also after 4 wks of CE. The profile of cytokine levels manifested significant anti-inflammatory shift. The inhibition of the JAK/STAT3 pathway diminished cold-induced cardioprotective phenotype as well as mitochondrial resistance to calcium overload.

**Keywords:** Cardioprotection, Cold Acclimation, JAK/STAT, Mitochondria
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**S2-**14

**Title: Type 2 Diabetes Mellitus and the Cardiac NPB/W Signaling System: Unraveling Molecular Mechanisms in ZDF Rats**

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**Abstract:**
Introduction Neuropeptide B (NPB) and neuropeptide W (NPW) constitute NPB/W signalling system together with their receptors. The location and function of this signalling system have been predominantly detected and mapped within the CNS including its role in modulation of inflammatory pain, neuroendocrine functions and autonomic nervous system. Altered serum levels of both neuropeptides have been previously shown in patients with DM, suggesting its potential role in pathophysiology of diabetes mellitus (DM). The aim of this study is to investigate the impact of diabetes on NPB/W signalling system in different heart compartments and neurons, which innervates it, in animal model of type 2 DM. Methods Zucker diabetic fatty (ZDF) rats were sacrificed by decapitation at week 40 of age. The heart, stellate ganglia and upper thoracic dorsal root ganglia were rapidly excised and directly frozen. In addition, samples containing coronary arteries walls from left ventricle tissues were collected via laser capture microdissection. Total RNA or protein was isolated. Obtained RNA was reverse-transcripted and subsequently quantitative PCR analysis was done. Relative expression of mRNA of NPB, NPW and NPBWR1 was expressed as a ratio of target gene concentration to control gene. In Western Blot analysis, the mean of all the technical replicates was used to analyse the expression of protein for each analyte. Additionally, sections of tissues were examined using the methods of immunofluorescence. Results In RT-qPCR analysis, we observed the upregulation of mRNA for preproNPB in RV (3.0-fold, $p = 0.018$), for preproNPW in LA (3.2-fold, $p = 0.022$) and for NPBWR1 in DRG in diabetic rats. On the contrary, expression of mRNA for NPBWR1 was downregulated in the LV (0.56-fold, $p = 0.029$) in diabetic rats. In WB analysis, significant downregulation of NPBWR1 in LV (0.54-fold, $p= 0.046$) in diabetic rats was observed at proteomic level. Moreover, specific NPB and NPW immunoreactivity was detected in nerve fibers innervating intracardiac ganglia, as well as nerve cell bodies of these ganglia, nerve fibers in between cardiomyocytes and smooth muscle cells of coronary vessels. Conclusions NPB/W signalling system is involved in regulation of heart functions and long-term diabetes leads to changes in the expression of individual members of this signalling system differently in each cardiac compartment, which is related to the different morphology and function of these cardiac chambers.

**Keywords:** NPB, NPW, RT-qPCR, WB, ZDF
**Funding:** This work was supported by the Charles University Research Fund [Progres Q39].
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**S2-**49

**Title: Dyad remodeling, calcium signalling and contractility in left ventricular myocytes of female Zucker Diabetic Fatty rats after treadmill running**

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**Abstract:**
Objective: Cardiovascular diseases represent the most common cause of death worldwide, and sexual dimorphism seems to play an important role in their specific forms. In this regard, the Zucker Diabetic Fatty (ZDF) rat model represents a suitable model of obesity-induced cardiomyopathy. However, the vast majority of existing research has been performed in males. Independently, physical exercise is known to improve cardiac performance. It has been previously demonstrated in male ZDF rats that treadmill running increases the calcium sensitivity of myofilaments. Therefore, the aim was to analyze calcium transients and contractility in left ventricular myocytes isolated from ZDF female rats after six weeks of forced running. Methods: Lean and obese female ZDF rats aged 12 weeks were divided into lean sedentary (LS), lean running (LR), obese sedentary (OS), and obese running (OR) groups. Treadmill exercise was performed 5 days/week, gradually increasing from 15 minutes at 9m/min to 30 minutes at 18m/min during the first four weeks and then maintained. For ultrastructural analysis, papillary muscle from the left ventricle was isolated and fixated with 2.5% glutaraldehyde, 2% paraformaldehyde, and post-fixed with osmium tetroxide. Random ultrathin (60-nm) longitudinal sections were studied by transmission electron microscopy at 80 kV. Dyads were classified into two groups, compact and loose, counted, and expressed as their number per area of myocyte Ns. For analysis of calcium transients and contractility, myocytes were isolated from the left ventricle, loaded with Fluo-3/AM, perfused, and electrically stimulated. Fluorescence was recorded by confocal microscopy. Line-scan data were further transferred to in-house developed automatic software for the determination of parameters of calcium transients and contraction. Significance was evaluated using the Linear Mixed-Effects Model. Results: After training, Ns of both compact and loose dyads was increased in the LR compared to the LS group; Ns of compact dyads was decreased while Ns of loose dyads was increased in the OS compared to the LS group. Ns of compact dyads was increased but that of loose dyads was unchanged in the OR compared to the OS group. Neither exercise nor obesity affected calcium transients and contractility of myocytes. Conclusion: Both obesity and endurance training had a significant impact on cardiac myocyte dyad organization while calcium transients and contractility have not been affected.

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**S2-**23

**Title: Effects of long-term activation of NRF2 function by DMF under normal and long-term stress conditions in an experimental model of prehypertension**

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**Abstract:**
Objective: Our study investigated the effects of long-term activation of nuclear factor erythroid 2-related factor 2 (NRF2, encoded by Nfe2l2) by dimethyl fumarate (DMF) under normal and chronic stress in 10-week-old male borderline hypertensive rats (BHR), which were exposed to crowding stress for 6 weeks. We hypothesized that long-term NRF2 activation would prevent the development of stress-induced hypertension in association with NRF2-mediated modulation in expressions of antioxidant, anti-inflammatory and iron-regulatory genes and may improve vascular functions in BHR. Methods: Systolic blood pressure (BP) was measured by tail-cuff plethysmography. Samples of the left heart ventricle (LHV) were taken for gene expression studies, nitric oxide synthase (NOS) activity, level of conjugated dienes (marker of oxidative damage) and magnetic parameters measured by SQUID magnetometry. Vascular reactivity was determined in the rings of femoral arteries using wire myograph. Results: Stress increased BP and reduced body weight gain, while DMF treatment suppressed the stress-induced BP increase. Interestingly, DMF induced LHV hypertrophy, however, there were no changes in magnetic properties in any experimental group. On the other hand, the adrenal glands hypotrophy occurred in all experimental groups vs. control group. Regarding NO production, stress significantly decreased NOS activity while DMF increased it vs. control group. Interestingly, administration of DMF during stress reduced oxidative damage in line with upregulated gene expressions of Nfe2l2, antioxidants (Hmox1, Sod1, Gpx4), NO-producing enzyme (Nos2) and iron-regulating proteins (Trfr1, Dmt1, Hamp), while stress itself additionally upregulated expression of pro-inflammatory factors (Tnf, Il1b). In the femoral arteries, stress significantly elevated noradrenaline-induced contractions, which were attenuated by simultaneous DMF treatment, without changes in endothelium-dependent and endothelium-independent relaxations. Conclusions: Our study showed that DMF may prevent the development of chronic social stress-induced hypertension via the reduction of oxidative stress in LHV and attenuation of contractile function in the arteries. However, it also pointed out to the possible development of hyper-reductive state in the heart, when a higher dose of DMF is administered for a long time.

**Keywords:** NRF2, DMF, prehypertenion, gene expression
**Funding:** Study was supported by the grants VEGA 2/0157/21 and APVV-22-0296.
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**S2-**30
**Title: Growth related structural activity of sarcolemma in cardiac myocytes.**

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**Abstract:**
The sarcolemma of cardiac myocytes participates in the adaptation of myocardium to physiological and pathological conditions. The recent understanding of the structural dynamics of sarcolemma came from studies on laboratory rodents. Nevertheless, little is known about the structure of sarcolemma of human myocytes. In this study, we compare the dynamics of sarcolemma in myocytes of the postnatal rat and of the diseased adult human myocardia. We focus on the transmission electron microscopy of the surface of myocytes undergoing cellular hypertrophy. Samples of rat myocardium were taken from left ventricles of 3 – 9 day old animals. The dissected hearts were relaxed in the Ca-free Tyrode solution (0 Ca, 10 mM EGTA), and fixed in 2% glutaraldehyde cacodylate buffer. Samples of human myocardium were obtained from patients with acute systolic dysfunction undergoing examination according to the procedure approved by the Ethical committee of the National Institute of Cardio-Vascular Diseases, Bratislava. Human heart biopsies were taken from the right ventricle, washed off blood in the relaxing Tyrode solution, and fixed in the modified Karnovsky solution (2% paraformaldehyde, 2.5 % glutaraldehyde in cacodylate buffer). Neonatal rat myocytes had a form of thin spindle like cells that grow radially by formation of new sarcomeres in the cytoplasm under the sarcolemma. At these regions, the sarcolemma forms short bending protrusions to the extracellular space, which interact at their tips with the nearby sarcolemma of the same or the neighbor myocyte. Upon fusion of the interacting membranes of the same cell, the protrusions enclose and internalize the sarcolemma. The emerging vesicular inclusions contain some material of the basal membrane and small dyadic complexes originally present at the surface sarcolemma. These inclusions interact with the newly formed sarcomeres, form radial t-tubules, and contribute to the radial growth of myocytes. We observed principally the same process in myocytes of adult human heart, except that the sarcolemmal inclusions contained also fibrous material common at the myocyte surface. We conclude that the internalization of the sarcolemma is the first step in the formation of the t-tubule network and dyads near myofibrils of growing postnatal myocytes, and which is also a part of the mechanism leading to the structural adaptation of adult myocytes to pathologically stressed myocardium.

**Keywords:** Cardiomyocyte, ultrastructure, growth, sarcolemma
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**S2-**29

**Title: Ultrastructure of cardiomyocytes of patient diagnosed with systolic dysfunction associated with SARS-CoV-2 infection**.

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**Abstract:**
Recent outburst of COVID-19 led to increased death toll also due to myocardial infections by SARS-CoV-2. In contrast to the myocardial function, information at the level of cardiomyocyte is scarce. Here we present results of single case study obtained in patient diagnosed with acute systolic dysfunction and undergoing examination according to the guidelines approved by the Ethical committee of the National Institute of Cardio-Vascular Diseases, Bratislava. The male patient, 52 years old, experienced a history of mild COVID-19 treated as influenza 3 months before the admission. The biopsy of myocardium was taken from the right ventricle, immediately washed of blood and conditioned for 10 minutes by the relaxing Tyrode solution (0 Ca, 10 mM EGTA), fixed in the modified Karnovsky solution (2% paraformaldehyde, 2.5 % glutaraldehyde in cacodylate buffer), and processed for the transmission electron microscopy observation. In ultrathin 60 nm sections, the cytoarchitecture of many myocytes displayed typical features as known from normal working myocardium, while many other myocytes displayed clear signs of structural perturbations. These included the smeared z-lines, irregular intercalated discs, presence of very thin myofibrils, widened T-tubules with fibrous material, damaged and splitting mitochondria, ceroids, numerous vesicular bodies and vacuoles of various size and shape, phagolysosomes, fragmented endoplasmic reticulum, phagophores, and empty cytosol. In these myocytes, we observed virus particles resembling SARS-CoV-2 at various stages of proliferation as we described previously in the VERO E6 cells model. Similar virus particles were observed also in the nearby endothelial and interstitial cells. We conclude that the acute myocardial dysfunction in this patient might have been related to the past COVID-19 infection with infiltration and proliferation of SARS-CoV-2 in myocardium.

**Keywords:** Cardiomyocyte, ultrastructure, systolic dysfunction, SARS-CoV-2
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**S2-**26

**Title: Pharmacological inhibition of ATM in the heart facilitates the development of chronic anthracycline cardiotoxicity**

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**Abstract:**
Anthracycline anticancer drugs (e.g., daunorubicin or doxorubicin) are associated with a risk of cardiac damage, potentially resulting in cardiomyopathy and heart failure. The pathogenesis of anthracycline cardiotoxicity has been connected with topoisomerase II beta (TOP2B)-mediated DNA damage in the heart. However, the downstream events remain unclear. Notably, the specific role of DNA damage response (DDR) signaling downstream of TOP2B remains elusive. This question has practical implications because pharmacological inhibitors of DDR are being introduced into oncology practice in combination with conventional DNA-damaging cytostatics, including anthracyclines. This study aimed to examine DDR signaling in anthracycline-exposed hearts and to elucidate the safety of combined administration of anthracyclines with pharmacological inhibitors of key DDR molecules. Daunorubicin was administered at a clinically relevant dose (DAU 3 mg/kg i.v.), and DNA damage and DDR were studied in the heart 1.5 and 6 hours after drug administration to rabbits. The effect of the cardioprotectant dexrazoxane (30 mg/kg i.v.), which acts as a catalytic TOP2B inhibitor, and AZD0156 (0.5 mg/kg i.v.), a selective ATM inhibitor investigated in a clinical trial as a chemosensitizer, were studied. Chronic cardiotoxicity was induced by daunorubicin administered weekly for 10 weeks, and the effect of combinations with both dexrazoxane and AZD0156 in this chronic setting was compared. A single dose of DAU induced a significant increase in double-strand breaks (determined as γH2AX) in rabbit hearts and triggered ATM/p53-dependent activation of DDR signaling. Both dexrazoxane and the ATM inhibitor AZD0156 effectively inhibited all these events. However, the chronic combination of DAU with AZD0156 or dexrazoxane led to opposite results. AZD0156 significantly increased the incidence of DAU-induced blood congestion, end-stage heart failure, and heart failure-related death (60% versus 20% in the DAU-alone group), whereas dexrazoxane effectively prevented these changes. The validity of these results was further confirmed using a different pharmacological ATM inhibitor and an animal model (chronic doxorubicin cardiotoxicity in mice). In summary, anthracycline-induced DDR signaling in the heart appears ATM-dependent, and pharmacological inhibition of ATM sensitizes the heart to anthracycline-induced chronic cardiotoxicity, contrasting with the protecting outcomes obtained with dexrazoxane.

**Keywords:** Anthracycline, cardiotoxicity, DNA damage, ATM
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**S2-**13

**Title: Impact of Post-Weaning Social Isolation on Left Ventricular Function in a Rat Model of Pressure-Volume Overload**

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**Abstract:**
Objective: Post-weaning social isolation (PWSI) in rats has been shown to result in anxiety- and depressive-like behaviour. Anxiety and depression, prevalent in humans, are recognized risk factors for cardiovascular disorders, particularly heart failure. This study aims to investigate the impact of PWSI-induced behavioural alterations on functional consequences of myocardial remodelling in rat model of pressure-volume overload. Methods: Animal experiment adhered to the European Community: Guide for the Care and Use of Laboratory Animals and to the national regulations. Sixteen male Sprague Dawley rats (3 weeks old) were randomly divided into two groups: socially isolated rats (group I; n=8) were individually housed in T3 containers; group-housed rats (group G; n=8) were housed in social groups of 4 animals in T4 containers. All rats were kept in one room with controlled atmospheric pressure, humidity, temperature, and 12/12 light-dark cycle. The socially isolated rats had no visual contact with other rats. These housing conditions remained consistent throughout the whole experiment. Rats were fed with standard diet; water was accessible ad libitum. After 7 weeks, rats from both groups underwent unilateral nephrectomy in deep isoflurane anaesthesia. Subsequently, deoxycorticosterone acetate (DOCA) was administered once a week in depot dose (20 mg, s.c.) to induce pressure-volume overload. After 3 weeks, left ventricular functions were assessed using pressure-volume catheter (Millar). Data analysis was performed using LabChart 8 Pro (ADI) and Prism 10 (GraphPad), $p<0.05$ was considered as statistically significant. Results are reported as mean ± SD. Results: The index dP/dtmax, reflecting left ventricular contractility, was significantly lower in group I than in group G (6035±1300 mmHg/s vs. 7957±577 mmHg/s; p=0.008). The index dP/dtmin, indicative of relaxation ability, was significantly higher in group I compared to group G (-5840±797 mmHg/s vs. -8091±1066 mmHg/s; p=0.002). Left ventricular developed pressure was significantly reduced in group I compared to group G (93.3±3.1 mmHg vs. 110.7±7.7 mmHg; p=0.001). Conclusion: PWSI-induced behavioural changes led to decreased contractility and worsened relaxation ability of the left ventricle in a rat model of pressure-volume overload.

**Keywords:** heart remodelling; rat; social isolation
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**S2-**62

**Monoamine oxidase is a novel off-target target of the antidiabetic drugs in the cardiovascular system**

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**Abstract:**
Cardiovascular diseases are a major cause of concern worldwide because of their rising prevalence and severe consequences. Oxidative stress is a the major pathomechanisms that underlies these pathologies and promotes both disease progression and the occurrence of complications but the sources of reactive oxygen species (ROS) are far from being completely elucidated. Monoamine oxidase (MAO) with two isoforms A and B are mitochondrial enzymes that have emerged in the past decades as significant contributors to oxidative stress via the constant generation of ROS in the cardiovascular system. Moreover, its expression has been reported to increase in conditions associated with inflammation and ageing.
There is unequivocal evidence for the role of both classic and novel antidiabetics in improving the outcome of non-diabetic and diabetic patients with cardiovascular diseases yet the pathophysiological mechanisms remain elusive. Metformin is the central pillar of type 2 diabetes therapy with antioxidant effect. The sodium-glucose-cotransporter 2 inhibitors (SGLT-2i) are the novel antidiabetics, which exert cardiovascular protection via direct, partially elucidated effects in patients with heart failure, even in the absence of diabetes.
Metformin and the SGLT-2i, empagliflozin and dapagliflozin, reduced ROS production and MAO expression in vascular and cardiac preparations from murine models and humans with cardio-metabolic diseases. MAO is a novel cardiovascular off-target of both classic and new antidiabetic drugs.

**Key words:** monoamine oxidase, antidiabetic drugs, off-target effect, cardiovascular protection

**S2-**1

**Title: Characterisation of a new model of doxorubicin-induced HFrEF with nephrotic syndrome in Ren-2 transgenic rats**

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**Abstract:**
Objective: Anthracyclines, including doxorubicin (DOXO), are still one of the most effective anticancer drugs, however they exhibit cardiotoxicity with inherent risk of irreversible cardiomyopathy leading to heart failure with reduced ejection fraction (HFrEF). Present pharmacological strategies are less effective for this type of HFrEF, hence there is an urgent need for novel therapeutic strategies. The prerequisite for success is a thorough understanding of the pathophysiology of this HFrEF form, which obviously requires application of an appropriate animal model of the disease. Methods: The aim of this study was to comprehensively characterise a novel model of cardiorenal syndrome, i.e. DOXO-induced HFrEF with nephrotic syndrome, in which DOXO was administered to Ren-2 transgenic rats (TGR) via five intravenous injections in a cumulative dose of 10 mg/kg of body weight. Results: We have shown that the new model adequately mimics the cardiac remodelling described as “eccentric chamber atrophy” and myocardial damage typical for DOXO-related late cardiotoxicity and obvious sings of nephrotic syndrome, without major impairment of the peritoneum, lungs and liver. This pattern corresponds well to a clinical situation of cancer patients receiving anthracyclines, where HF develops with some delay after the anticancer therapy. Conclusion: The new model is optimal for studying the pathophysiology of chemotherapy-induced HFrEF and evaluation of long-term effectiveness of new therapeutic strategies.

**Keywords:** doxorubicin; cardiotoxicity; HFrEF; TGR
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**S2-**9

**Title: Acute prenatal hypoxia alters autonomic function and attenuates stress response in adult female rat offspring**

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**Abstract:**
Prenatal hypoxia (PH), characterised by reduced foetal oxygen supply, is one of the most common pregnancy complications. PH significantly interferes with the development of the foetal heart, kidneys, and brain, predisposing offspring to hypertension and cardiovascular diseases. While previous studies in male rodents showed altered blood pressure (BP) regulation after acute PH, data on the effects in female offspring remain limited. Therefore, our objective was to analyse the long-term impact of acute PH on the cardiovascular system of adult female rat offspring. In experiment 1, pregnant Wistar rats were exposed to 12-h PH (10.5% O2) at gestational day (GD) 20. Female offspring (four-month-old) were sacrificed, left ventricle and kidney weights were measured, and plasma and left ventricles were collected for further analyses. Plasma corticosterone levels were determined using radioimmunoassay. Gene and protein expression were analysed in the left ventricle using PCR and western blotting. In experiment 2, pregnant Wistar rats were exposed to 4-h PH (10.5% O2) at GD19 and GD20. Female offspring (four-month-old) were implanted with telemetry sensors for heart rate (HR) and BP measurement under basal and stress (1-h restraint) conditions. Compared to the control, PH female offspring had lower kidney weight ($p = 0.012$; t-test), while left ventricular weight was similar between groups. Basal HR, systolic and diastolic BP, pulse pressure and locomotor activity did not differ between groups. However, PH females exhibited lower absolute high frequency band (parasympathetic activity; $p = 0.035$, Fisher's LSD) and higher low-to-high frequency ratio (sympathovagal balance; $p = 0.047$, Fisher's LSD) than the control females in the light phase of the day. During restraint stress, HR was lower in PH than control females ($p = 0.004$ for area under the curve; two-way ANOVA). Additionally, PH females exhibited lower plasma corticosterone levels than control offspring ($p = 0.055$; t-test). Moreover, the cardiac expression of beta-1 adrenergic receptor protein was significantly lower ($p = 0.042$; t-test), while the gene expression of transcription factor Nfe2l2 was higher ($p = 0.034$; t-test) in PH than in control female offspring. In conclusion, females exposed to PH exhibit altered autonomic drive and attenuated HR response to stressor. These findings demonstrate that acute PH during late pregnancy has a long-lasting impact on female offspring's cardiovascular regulation.

**Keywords:** hypoxia, female, telemetry, heart, restraint
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**S2-**8

**Title: Plasma levels of miR-34a-5p as a biomarker of cardiac injury during the development of chronic anthracycline-induced cardiomyopathy in rabbits**

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**Abstract:**
Anthracycline cardiotoxicity is a well-known complication of cancer treatment. This study investigates plasma miRNAs as markers of cardiac injury in the early and late stages of chronic anthracycline cardiotoxicity. Cardiotoxicity was induced in rabbits via daunorubicin administration (daunorubicin, 3 mg/kg/week; for 5 and 10 weeks), while the control group received saline solution. Myocardial miRNA expression was first screened using TaqMan Advanced miRNA microfluidic card assays, after which 32 miRNAs were selected for targeted analysis using qRT-PCR. Only one of them (miR-34a-5p) was detected in plasma. MiR-34a-5p was upregulated in the myocardium, with a fold-change relative to the control group of 4.49 ($p = 0.001$) and 33.16 ($p = 0.016$) after 5 and 10 weeks of DAU treatment, respectively. The expression levels of miR-34a-5p were strongly correlated with cTnT and the parameters of LV function. Furthermore, miR-34a-5p, unlike the other analyzed miRNAs, showed clear expression differences between the control and treatment groups based on a cluster analysis. In the plasma samples, only miR-34a-5p showed a significant ($p<0.001$) increase after 10 weeks of treatment (4.15-fold change). Interestingly, the plasma levels of miR-34a-5p correlated with miR-34a-5p changes in the myocardium ($R=0.821$, $p<0.001$). The correlations between plasma levels of miR-34a-5p and cTnT ($R=0.634$; $p<0.05$), FS ($R=-0.75$; $p<0.001$), and dP/dtmax ($R=-0.61$; $p<0.05$) were weaker when compared to the correlations calculated based on myocardial expression, yet still significant. This study suggests that miR-34-a could be used as a non-invasive biomarker of cardiac damage, although the sensitivity of this approach could be low for subclinical cardiotoxicity.

**Keywords:** Anthracyclines, cardiomyopathy, cardiotoxicity, miRNA, biomarker
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**S2-**6

**Title: Effect of Neuropeptide B and Neuropeptide W on Ventricular Myocardium Function in Zucker Diabetic Fatty Rats**

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**Abstract:**
Cardiovascular autonomic neuropathy is a serious complication of diabetes mellitus (DM) that negatively affects the quality of life and survival of individuals with DM. In addition to the signaling systems of classical neurotransmitters, the signaling systems of various neuropeptides are also more or less affected in the diabetic heart, including the NPB/W signaling system, which consists of neuropeptide B (NPB), neuropeptide W (NPW), and their receptors NPBWR1 (human, rat, mouse) and NPBWR2 (human). Altered serum levels of NPB and NPW have been observed in both human diabetic patients and Zucker Diabetic Fatty (ZDF) rats, a type 2 diabetes animal model, indicating a potential role of NPB/W signaling system in the pathophysiology of DM. Male Zucker Diabetic Fatty (ZDF) rats aged 40 weeks (584 ± 28 g) were fed a diabetogenic diet (Purina 5008: 56% carbohydrate, 27% protein, and 17% fat) from 8 weeks of age. Their plasma glucose levels were measured to be 13.6 ± 2.55 mmol/L. Male lean Zucker (control) rats of the same age (440 ± 11 g) served as controls with plasma glucose levels measured at 5.85 ± 0.41 mmol/L. Measurements were conducted in the left ventricle of 40 weeks old animals. RNA isolation was performed from whole tissues, followed by RT-qPCR analysis. Laser capture microdissection was employed to isolate cardiomyocytes, on which western blot analysis and immunofluorescence were carried out subsequently. To asses NPB/W effect on myocardial contractility, calcium transients and sarcomeric contractions in enzymatically isolated cardiomyocytes were measured in control solution, as well as in solutions containing either neuropeptide B (NPB) or neuropeptide W (NPW) both at concentration 0.1 □M. In our study using an animal model of type 2 diabetes mellitus, we found reduced expression of the NPBWR1 receptor, a component of the NPB/W signaling system, in the left ventricle of the heart. Surprisingly, despite this downregulation, we observed a positive inotropic effect of NPW on cardiac function. This discovery implies that NPW holds promise as a potential therapeutic target for addressing the contractile dysfunction observed in diabetic hearts.

**Keywords:** Diabetic rat, contractility, NPB, NPW
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**S3-**32

**Title: Effects of quercetin on cardiac physiological functions, hypertrophic remodeling and ischemia-reperfusion injury in aged ZDF rats**

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**Abstract:**
Recently, significant progress has been made in the research of mechanisms involved in cardioprotection. The demand to develop new tools for cardioprotection is highly relevant as the prevalence of serious cardiovascular diseases (CVD) continues to grow. Most often they occur in combination with higher age or with comorbidities, such as diabetes type 2. To date, several cardioprotective effects of natural polyphenol QCT have been documented, but they were mainly confirmed in animal models in young healthy individuals. The aim of our work was to reveal the effects of QCT on heart in elderly individuals who also suffer from an associated comorbidity - type 2 diabetes. In the study, QCT was orally administered for 6 weeks at a dose of 20 mg/kg/day to 6-month-old/1-year-old lean (fa/+) and obese (fa/fa) ZDF (Zucker diabetic fatty) rats. Blood pressure measurement and echocardiography was performed before the start and at the end of QCT administration. Oxidative stress markers were analyzed from blood plasma and the expression of the selected proteins were monitored in myocardium. Isolated hearts of ZDF rats were subjected to I/R (30 min/120 min). The total collagen content was determined in the left ventricle. Our data showed that administration of QCT significantly reduced blood pressure in 6-month-old rats, while it had no effect in 1-year-old rats. QCT significantly increased the antioxidant activity parameter in blood plasma of obese 1-year-old rats. Administration of QCT did not have cardioprotective effects against I/R damage in any group. QCT even negatively affected some parameters of post-ischemic functional recovery of hearts in 1-year-old rats. Complex activation of the RISK pathway wasn´t induced by QCT. In 1-year-old obese ZDF rats, administration of QCT normalized the E/A ratio (marker of diastolic dysfunction), reduced the thickness of the left ventricular wall and significantly decreased collagen content. QCT has been shown to have beneficial effects on blood pressure in a model of type 2 diabetes, but increasing age and/or development of diabetes impair its antihypertensive effects. In the prevention of I/R damage, QCT was not effective in both ages. Potential explanation of failing of QCT cardioprotection in increasing age and diabetes may be the failure to activate the RISK pathway. On the other hand, QCT suppressed diabetes-induced diastolic dysfunction and reduced the collagen content in the left ventricle in elderly individuals.

**Keywords:** quercetin, cardioprotection, diabetes mellitus, ageing
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**S3-**35

**Title: The effect of molecular hydrogen in comparison with effect of vitamin E on radiation-induced heart damage in older rats**

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**Abstract:**
Radiotherapy is a commonly used approach in cancer treatment. Alongside its therapeutic impacts, it can also induce damage to healthy tissues, particularly affecting the heart and surrounding blood vessels when targeting the chest area. Radiation, on the one hand, damages tumor cell compartments and, on the other hand, creates oxidative stress, which is also toxic and causes undesired side effects of radiotherapy. Mitigation of post-irradiation oxidative stress has potential for enhancing patient prognosis after radiotherapy. Compounds with antioxidant properties, such as vitamin E and molecular hydrogen (H2), have been described as effective agents for improving a range of health issues linked with oxidative stress, thereby potentially offering options for improved outcomes in patients undergoing radiotherapy. In the presented study, we used one-year-old Wistar rats (males) as an experimental model. The animals were irradiated with a single dose of 10 Gy in the chest area (4-5 Gy/min.). Subsequently, a six-week treatment with H2 (inhaled 4% H2 with air, 3 x daily for 30 min.) and vitamin E (1 x daily 100 mg/kg) was given. We detected a protective effect of H2 and vitamin E administration on irradiated hearts, where infarct size was significantly decreased after both treatments. Irradiation increased the level of aspartate aminotransferase by 70%, the activity of lactate dehydrogenase more than twice, and the level of uric acid in blood plasma by 20% compared to non-irradiated animals. Administration of H2 and vitamin E significantly reduced these values close to control group. When measuring the amount of malondialdehyde and superoxide dismutase activity, we observed the same trend of changes, but not significant. In the heart, we also observed a downregulation of 41 miRNAs caused by cardiac irradiation, with the most significant changes in miR-126-3p, miR-195-5p, miR-30e-5p, and miR-451a. Our findings indicate that administration of H2 or vitamin E could serve as a promising therapeutic approach to mitigate oxidative stress post-irradiation. Furthermore, our results suggest that H2 administration demonstrates a comparable efficacy to vitamin E in this regard, suggesting its potential as an equally effective radioprotective agent. Moving forward, we plan to continue detailed monitoring of radiation-altered miRNAs and search for its protein targets to reveal the mechanism of radiation-induced heart damage.

**Keywords:** radiation, oxidative stress, molecular hydrogen
**Funding:** This project was financed by grants: APVV-19-0317, VEGA 2/0092/22.
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**S3-**10
**Title: Effects of artificial light at night and high-fat diet on cardiovascular system in hypertriglyceridemic rats**

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**Abstract:**
Artificial light at night (ALAN) disrupts the 24-h rhythm of cardiovascular and metabolic variables, and high-fat diet (HFD) consumption is known to induce metabolic disorders such as dyslipidaemia, which may exacerbate ALAN-induced perturbations in cardiometabolic health. In our study, we aim to analyse the effects of ALAN, HFD and their combination on cardiovascular variables monitored by telemetry in male hereditary hypertriglyceridemic (HTG) rats. HTG rats were exposed to ALAN (2 lx) for five weeks. The control group was kept in standard conditions, light phase (L) 12 h 150 lx/ dark phase (D) 12 h 0 lx. After 5 weeks, the ALAN and control groups were fed HFD for three weeks. We focused on systolic blood pressure (SBP), pulse pressure (PP) and heart rate (HR) to evaluate the effects of ALAN, HFD and their combination on the cardiovascular system. ALAN increased SBP in the D ($p = 0.013$) and L ($p = 0.017$) phases and dampened the circadian rhythm ($p = 0.005$) after five weeks. ALAN had no direct effect on PP values but reduced the power of the PP circadian rhythm ($p = 0.014$). HR spontaneously decreased over time in both groups during the D phase ($p < 0.001$), however the decrease was more pronounced in the ALAN group ($p = 0.030$). The opposite effect was observed during L, ALAN attenuated the spontaneous decrease in HR observed in the control group ($p < 0.05$). ALAN also dampened the circadian rhythm of HR ($p = 0.006$) and reduced its amplitude ($p = 0.007$). HFD induced a significant increase in SBP ($p < 0.05$). HFD also increased HR differently in the D and L phases, but HR tended to normalise after three weeks. HFD decreased PP after one week in both L and D, after two and three weeks the effects were phase dependent ($p = 0.014$), while PP increased in L phase, opposite PP persistently decreased in D phase. HFD did not affect the circadian rhythm and amplitude of SBP, HR and PP. ALAN and HFD had no additive effects on SBP, PP and their circadian rhythm. ALAN in combination with HFD attenuated the HFD-induced increase in HR ($p < 0.05$). In conclusion, our study shows that ALAN disrupts the circadian rhythm of SBP, PP and HR, while the combined effects of ALAN and HFD have no additive effects on SBP and PP. However, ALAN in combination with HFD blunted the HFD-induced increase in HR, suggesting that opposite mechanisms may be activated at their simultaneous action.

**Keywords:** ALAN, diet, circadian, telemetry, rat
**Funding:** Study was supported by APVV-21-0223 and ACCORD (the Operation Program of Integrated Infrastructure for the project, Advancing University Capacity and Competence in Research, Development and Innovation, ITMS2014+: 313021X329, co-financed by the European Regional Development Fund) projects.
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**S3-**41

**Title: Angiopoietin-like 4 improves left ventricular function in a pig model of heart failure with preserved ejection fraction**

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**Abstract:**
Heart failure with preserved ejection fraction (HFpEF) represents more than half of heart failure cases worldwide and is associated with high morbidity and mortality. The pathophysiology of HFpEF is characterized by diastolic dysfunction but involves cardiac fibrosis, hypertrophy, inflammation, and microvascular alterations. We previously showed that angiopoietin-like 4 (ANGPTL4) is induced by hypoxia and maintains vascular integrity at reperfusion of acute myocardial infarction and stroke. This study evaluates whether ANGPTL4, by preserving vascular integrity, improves left ventricular and vascular function in a hypertensive-induced HFpEF pig model. Sixteen chronically instrumented pigs received a continuous infusion of the potent vasoconstrictor, angiotensin II (IV, 30 ng/kg/min), for 38 days. After 35 days, the pigs were randomly treated with an intracoronary injection of placebo (n=9) or ANGPTL4 (n=7, 75 µg/kg). Left ventricular and vascular function were investigated through hemodynamic measurements, echocardiography, and vascular function tests before the start of angiotensin II infusion (Day 0), before treatment (Day 30), and one (Day 36) and three days (Day 38) after treatment. Samples were then collected for biological and histological analyses of cardiomyocytes, fibrosis, hypertrophy, oxidative stress, and vascular density. Mean arterial pressure significantly and similarly increased in both groups, accompanied by left ventricular hypertrophy and fibrosis. Left ventricular systolic function was preserved while diastolic function diminished (increased left ventricular end diastolic pressure, E/A ratio, E/e' septal ratio, and E/e' lateral ratio) similarly in the two groups after several weeks of angiotensin II infusion. Concomitantly, increases in blood flow due to reactive hyperemia (30 s), acetylcholine (IV, 3 µg/kg), and nitroglycerin (IV, 30 µg/kg) significantly decreased. Administration of ANGPTL4 significantly improved diastolic function parameters and endothelium-dependent responses to acetylcholine but had no effect on mean arterial pressure, hypertrophy, fibrosis, and endothelium-independent responses to nitroglycerin. In conclusion, intracoronary administration of ANGPTL4 improves left ventricular diastolic function and coronary endothelial function in a hypertensive, animal model of HFpEF.

**Keywords:**
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**S3**-44
**Title: Characterisation of a new model of doxorubicin-induced HFrEF in Ren-2 transgenic rats**

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**Abstract:**
Introduction: Anthracyclines, including doxorubicin (DOXO), are still one of the most effective anticancer drugs, however they exhibit cardiotoxicity with inherent risk of irreversible cardiomyopathy leading to heart failure with reduced ejection fraction (HFrEF). Present pharmacological strategies are less effective for this type of HFrEF, hence there is an urgent need for novel therapeutic strategies. The prerequisite for success is a thorough understanding of the pathophysiology of this HFrEF form, which obviously requires application of an appropriate animal model of the disease. Purpose and Methods: The aim of this study was to comprehensively characterise a novel model of cardiorenal syndrome, i.e. DOXO-induced HFrEF with nephrotic syndrome, in which DOXO was administered to Ren-2 transgenic rats (TGR) via five intravenous injections in a cumulative dose of 10 mg/kg of body weight. Results and conclusion: We have shown that the new model adequately mimics the cardiac remodelling described as “eccentric chamber atrophy” and myocardial damage typical for DOXO-related late cardiotoxicity and obvious sings of nephrotic syndrome, without major impairment of the peritoneum, lungs and liver. This pattern corresponds well to a clinical situation of cancer patients receiving anthracyclines, where HF develops with some delay after the anticancer therapy. Hence, the new model is optimal for studying the pathophysiology of chemotherapy-induced HFrEF and evaluation of long-term effectiveness of new therapeutic strategies.

**Keywords:** doxorubicin, TGR, HFrEF, nephrotic syndrome
**Funding:** Supported by MH CZ - DRO (“Institute for Clinical and Experimental Medicine - IKEM, IN 00023001”)
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**S3**-48
**Title: Metabolic therapy in heart failure.**

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**Abstract:**
Heart failure (HF) is associated with a decrease in mitochondrial function, depriving cardiomyocytes of the energy necessary to support calcium cycling and contraction. We performed a metabolic therapy of transverse aortic constriction (TAC) -induced HF by treating mice with a combination of B vitamins and studied its impact on calcium handling and ultrastructural changes in myocytes isolated from respective hearts. All procedures were approved by the Federal Ministry of the Republic of Austria. Symptomatic HF was induced in male C57BL/6N mice by TAC operation, while SHAM-operated mice served as healthy controls. After 4 weeks, mice with developed HF were randomly divided into nontreated (TAC) and VitB-treated groups. The progression of HF was monitored by echocardiography. After 8 weeks of treatment animals were sacrificed. Myocytes were enzymatically isolated, and either fixed in a modified Karnovsky solution and processed for electron microscopy or loaded with fluo-4 to perform confocal Ca-imaging during electrical field-stimulation. HF induced qualitative and quantitative ultrastructural changes in the mitochondrial population, namely, degradation and vascularization of mitochondrial cristae, and an increase in the number, clustering, and size of mitochondria. Myofibrils were disorganized and intracellular membrane systems were downregulated. Areas rich in cytoplasm were present in the cells, but only rarely were they occupied with ribosomes. Metabolic therapy induced positive changes in a subpopulation of cardiomyocytes, with increased regularity in mitochondria that contained densely packed organized mitochondrial cristae. In addition, an increase in the formation of t-tubules, dyads, sarcoplasmic and rough endoplasmic reticulum (ER), and Golgi apparatus was observed. Growth regions full of cytosolic ribosomes and rough ER, with the expansion of the sarcolemma, were numerous. In line with the ultrastructural changes, the amplitude of calcium transients and sarcoplasmic reticulum Ca2+ load in myocytes isolated from vitamin B cocktail-treated animals was partially rescued. In conclusion, metabolic treatment of HF showed positive changes at the ultrastructural level that translated to changes in cardiomyocyte function. Treatment of mitochondrial function is therefore a promising target for future clinical studies.

**Keywords:** heart failure
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**S3-**50
**Title: Omecamtiv Mecarbil: Balancing Benefits and Risks in Enhancing Cardiac Contractility**

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**Abstract:**
Omecamtiv mecarbil (OM) is a promising novel drug for improving cardiac contractility. We tested the therapeutic range of OM and identified previously unrecognized side effects. The Ca2+ sensitivity of isometric force production (pCa50) and force at low Ca2+ levels increased with OM concentration in human permeabilized cardiomyocytes. OM (1 μM) slowed the kinetics of contractions and relaxations and evoked an oscillation between normal and reduced intracellular Ca2+ transients, action potential lengths and contractions in isolated canine cardiomyocytes. Echocardiographic studies and left ventricular pressure–volume analyses demonstrated concentration-dependent improvements in cardiac systolic function at OM concentrations of 600–1200 μg/kg in rats. Administration of OM at a concentration of 1200 μg/kg was associated with hypotension, while doses of 600–1200 μg/kg were associated with the following aspects of diastolic dysfunction: decreases in E/A ratio and the maximal rate of diastolic pressure decrement (dP/dtmin) and increases in isovolumic relaxation time, left atrial diameter, the isovolumic relaxation constant Tau, left ventricular end-diastolic pressure and the slope of the end-diastolic pressure–volume relationship. Moreover, OM 1200 μg/kg frequently evoked transient electromechanical alternans in the rat in vivo in which normal systoles were followed by smaller contractions (and T-wave amplitudes) without major differences on the QRS complexes. Besides improving systolic function, OM evoked diastolic dysfunction and pulsus alternans. The narrow therapeutic window for OM may necessitate the monitoring of additional clinical safety parameters in clinical application.

**Keywords:** Omecamtiv mecarbil, cardiac contractility
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**S3-**52

**Title: Three-dimensional Genome Architecture in Cardiac Muscle Cells: Pathophysiological Implications in DMD- and LMNA-Dilated Cardiomyopathy**

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**Abstract:**
Mutations in the nuclear A-type lamins- (LMNA gene) and dystrophin (DMD gene)-encoding genes cause dilated cardiomyopathy, characterized by compromised cardiac contractility resulting in poor left ventricular function and associated with a high incidence of conduction defects and arrhythmias. A physical continuum links the extracellular environment to the nuclear interior, crucial for mechanotransduction and structural support. Key components include the LINC complex, connecting the nuclear envelope to the cytoskeleton; dystrophin (DMD gene), linking actin to the basal membrane; and nuclear lamins A/C (LMNA gene), linking the LINC complex to chromatin. Disruptions in these components in cardiomyocytes, as seen in DMD or LMNA gene mutations, can impair mechanotransduction, leading to genome conformation changes and dysregulated cardiac gene expression, ultimately contributing to dilated cardiomyopathy pathogenesis. To address this question, we have examined relevant in vitro and in vivo models corresponding to each mutation associated with the disease. Abnormal diastolic calcium levels and contractions have been observed in human induced pluripotent stem cell-derived cardiomyocytes (hiPSCs-CMs), along with impaired cardiac function in mice. Calcium modulation plays a crucial role in gene expression, particularly in cardiac remodeling. Additionally, global changes in genome organization may contribute to conduction defects by deregulating the expression of ion channel genes. To establish correlations between functional and structural abnormalities observed in hiPSCs-CMs and mice, we are investigating three-dimensional genome organization at various scales (chromosome painting, lamina-associated domains FISH). This analysis is closely linked to gene expression studies (RNA-seq, ATAC-seq). Integrating functional and genomic studies aims to enhance our understanding of the pathogenesis of dilated cardiomyopathy associated with LMNA and DMD gene mutations, shedding light on common molecular signatures and/or specific molecular characteristics.

**Keywords:** DCM, Genome, hiPSCs-CMs, Mouse Models
**Funding:** Région Occitanie - Association Institut de Myologie
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**Session 4 "NOVEL STRATEGIES FOR CARDIOPROTECTION ASSOCIATED WITH MYOCARDIAL ISCHEMIA REPERFUSION"**

**S4**-45

**Title: Fluorescence lifetime imaging microscopy (FLIM) on isolated cardiomyocytes from cold acclimated rats**

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**Abstract:**
Objective: Despite the recent advances in research and therapy, ischemic heart disease remains the most common cause of death and comorbidity worldwide. Many promising targets that have been demonstrated in animal models have failed in clinical trials. Recently, we developed a unique cardioprotective model of moderate cold acclimation (8±1ºC, 5 weeks) that reduces the extent of myocardial infarction and improves mitochondrial function without any negative side effect. Cold environment increases organismal energy demands and triggers a complex adaptive process which may shift energy and cell redox balance. To get more understanding to the mechanism of cold-elicited cardioprotection, we applied fluorescence lifetime imaging microscopy (FLIM) to assess a redox and metabolic status on isolated cardiomyocytes by lifetimes of free and protein-bound NAD(P)H pools. Methods: Male Wistar rats were exposed to a new cardioprotective regimen of acclimation 9±1ºC for 10 days and controls were kept at 24±1ºC. Cardiomyocytes (CDMs) were freshly isolated using Langendorff apparatus and subjected to 30-min stabilisation (21%O2), 60-min hypoxia (1%O2) and 60-min reoxygenation (21% O2) protocol using the ÖKOLab chamber, Zeiss LSM880 NLO confocal microscope. NAD(P)H-lifetime has been detected using Time-Correlated Single Photon Counting (TCSPC) system with two-photon excitation at 740 nm, 80MHz Chameleon Ti:sapphire pulsed laser (Coherent, Inc.). The analysis was performed in SPCImage software (Becker&Hickel) and TTTR software through the fitting-free phasor approach. Results: FLIM of NAD(P)H did not show any difference between control and cold acclimated CDMs during stabilisation. Hypoxia and reoxygenation insult caused a significant shift towards shorter mean lifetime in control cardiomyocytes, which indicates a reduction in oxidative metabolism and an increased reliance on glycolysis. Interestingly, 10 days of moderate cold acclimation preserved an original metabolic phenotype of isolated CDMs during hypoxia and reoxygenation. Increased amount hexokinase 2 protein in the mitochondrial fraction of the acclimated heart tissue may contribute to the cold-elicited preservation of mitochondrial metabolism during hypoxia/reoxygenation insult by preferential ADP supply to ATPsynthase. Conclusion: Our data show that cardioprotective regimen of cold acclimation prevents the metabolic shift in acclimated cardiomyocytes.

**Keywords:** Cold acclimation, cardioprotection, FLIM
**Funding:** Czech-BioImaging large RI project LM2023050 and e-INFRA LM2018140 Projects of Large Research, Development and Innovations Infrastructures
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**S4**-47

**Title: Impact of electrical field stimulation of neonate rat cardiomyocytes on AMPK signaling and NAD metabolism**

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**Abstract:**
Introduction Patients whith HFpEF are characterized by a severely altered exercises capacity. Combination of therapies and exercise training protocol could improve their cardiac function. The cardioprotective AMP-activated kinase (AMPK) is a sensor for energy stress that has been proposed as a key mediator for the beneficial effects of exercise training on cardiac and muscular function. SIRT1 of the sirtuin deacetylase family is a NAD-dependent signaling pathway that is also activated by energy stress and can increase mitochondrial biogenesis. Synergy between AMPK and SIRT1 signaling has been described in skeletal muscles cells but rarely studied in contracting cardiomyocytes. Objective We aim to decipher the link between AMPK and NAD-dependant signaling in relationship with contractile activity in cardiomyocytes. Method Neonatal rat ventricular cardiomyocytes (NRVC) were paced via electrical field stimulation at different time using IONOptix MyoPacer for electric field stimulation. AMPK activation was determined by western blot for phospho-AMPK and phospho ACCA (Acetyl-CoA carrboxylase), a target of AMPK. NAD was quantified by the alcohol dehydrogenase cycling assay. NRVC were treated with different doses of nicotinamide riboside (NR), a vitamin B3 precursor of NAD. Results Ongoing analyses show that pacing of cardiomyocytes at 3 or 4 Hz resulted in an increase in AMPK and ACCA phosphorylation level between 1h and 3h depending on the pacing rate and maturation of NRVC (3 days or 4 days after plating) when compred to unpaced NRVC. NAD levels declined after 1h of pacing but were restored at 3h when compared to unpaced NRVC. NR administration to the cell medium at the time of pacing or match time point in unpaced cells increased the cellular levels of NAD compare to non treated cells . Future experiments will assess the impact of pacing with and without NR treatment on glucose uptake in NRVC. Conclusion Therapeutic strategies based on combination of exercise training protocols and nutrient-based stimulation of energy metabolism such as vitamin B3 stimulation could help patients suffering HFpEF to recover cardiovascular and exercise capacities. Our future studies will asses theses strategies in vivo in rodent model of HFpEF.

**Keywords:** Neonatal cardiomyocytes; Nicotinamide-riboside; AMPK; Myopacing
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**S4-**22
**Title: Anti-inflammatory effect of molecular hydrogen inhalation on isoproterenol-induced heart failure**

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**Abstract:**
Introduction: Heart failure (HF) remains a significant global health issue causing high mortality, morbidity, and diminished quality of life. Oxidative stress and inflammation are among the main mechanisms involved in the formation and development of HF. These subsequently lead to myocardial defects, blood congestion, and hypoperfusion. One of the potential strategies to alleviate HF symptoms is the suppression of inflammatory process. Molecular hydrogen (H2) represents a new therapeutic promise due to its antioxidative and anti-inflammatory properties. Administered via inhalation or as H2-rich water, H2 modulates signaling pathways, suppresses cytokines, and activates antioxidant factors. Materials and Methods: Intraperitoneal administration of isoproterenol is considered a reliable and proven model of HF induction. In this study, six-month-old male Wistar rats were divided into four groups: control (C), isoproterenol-induced HF (ISO), HF with H2 treatment (ISO+H2), and HF with vitamin C treatment (ISO+C). The ISO groups received isoproterenol (50 mg/kg) for 5 consecutive days to induce HF. Five days post-ISO injection, the ISO+H2 group was exposed to H2-enriched air for 30 minutes daily for 6 weeks (4% of H2 in air), while the ISO+C group received vitamin C (75 mg/kg) in drinking water ad libitum. After the treatment, biochemical parameters (lipids, glucose, uric acid, etc.) were measured in blood plasma and the expression of pro-inflammatory proteins (IL-6, TNF-a, NF-kB) was assessed in heart tissue by Western blot method. Results: Administration of ISO caused an unsignificant increase of uric acid, aspartate aminotransferase, alanine transaminase, and significant increase of glucose in blood plasma. On the other hand, H2 administration non-significantly reduced these parameters to C group values. Expression of inflammatory proteins was significantly increased in ISO group. Both treatment groups showed downregulated protein levels, however, more significantly in the ISO + H2 group. Conclusion: Based on the results, this study shows that mostly H2 treatment attenuated the expression of pro-inflammatory proteins and ameliorated biochemical parameters in isoproterenol-induced HF, suggesting its therapeutic potential in cardiovascular diseases. We can therefore conclude that H2 inhalation can represent a new treatment strategy in mitigating HF pathology.

**Keywords:** heart failure, isoproterenol, molecular hydrogen
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**S4-**28
**Title: New prevention and treatment options for radiation-induced heart disease**

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**Abstract:**
Radiation-induced heart disease (RIHD) is a known complication of mediastinal radiotherapy applied in oncological diseases of the lungs, breasts, or Hodgkin's lymphoma. Clinical data show that up to 37% of these patients suffer from some kind of cardiovascular disease, and in the case of Hodgkin's lymphoma, this number is even higher, up to 55%. RIHD involves structural and functional abnormalities of the pericardium, coronary vessels, myocardium, valves, and conduction system. The underlying pathological mechanisms are complex and mainly related to endothelial cell damage, oxidative stress, and inflammation. Radiation can cause cardiomyocyte death, tissue fibrosis, and ultimately heart failure. To overcome these complications, it is necessary to look for specific therapeutic interventions, which are still lacking. One of the substances with a radioprotective effect could be molecular hydrogen (H2), which is characterized by antioxidant, anti-inflammatory, and anti-apoptotic effects in various diseases. In vitro as well as in vivo studies have shown that H2 has preventive or therapeutic effects on radiation damage, including RIHD. H2 could be effective in alleviating RIHD through various mechanisms, e.g. selective neutralization of hydroxyl radicals, protection against inflammatory and apoptotic damage, antifibrotic and antihypertrophic effects, etc. The work of our research team shows that the administration of H2 to Wistar rats with mediastinal irradiation with a single dose of 10 Gy (4-5 Gy/min.) effectively reduces heart damage (lactate dehydrogenase), improves blood biochemical (lipid profile, albumin, creatinine, alanine transaminase, aspartate aminotransferase, glucose) and oxidative stress (malondialdehyde, 8-hydroxy-2'-deoxyguanosine, catalase, superoxide dismutase, uric acid) parameters, and inflammatory damage (tumor necrosis factor alpha, nuclear factor kappa B, interleukin 6). This study also reveals that H2 administration on RIHD rat model also normalizes misexpressions of selected miRNAs (miRNA-1, -15b, -21). The results of this study confirm that the administration of H2 represents a promising treatment strategy for patients undergoing mediastinal irradiation with radiation-induced cardiac damage. However, in order to clarify other mechanisms of H2 action and to verify the effectiveness of H2 therapy in clinical practice, further studies are needed, especially focused on the still unclear mechanism of its biological effect.

**Keywords:** irradiation, heart disease, molecular hydrogen
**Funding:** This work was funded by grants VEGA (2/0092/22) and APVV (19-0317)
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**S4-**58

**Title: Evaluation of N-acetylcysteine's protective effects on biochemical parameters following experimentally induced myocardial infarction.**

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**Abstract:**
Myocardial infarction (MI) remains a leading global cause of morbidity and mortality among all cardiovascular diseases. Prognosis after MI is influenced by a several risk factors, including hypertension, which worsens the consequences of MI by inducing the release of oxidative and inflammatory mediators in the heart. The aim of the study was to determine the effects of hypertension and the antioxidant N-acetylcysteine (NAC) on biochemical and molecular parameters in an experimental model of myocardial infarction. In two groups of 12-week-old spontaneously hypertensive rats (SHR), MI was induced by ligation of the left descending coronary artery. One group received NAC (25mg/kg/day) for 4 weeks prior to ligation. Sham operations were performed as controls. Seven days after surgery, proinflammatory cytokines were measured in plasma using Bioplex kit. Levels of nitric oxide synthase (NOS) activity, endothelial NOS (eNOS), inducible NOS (iNOS), and nuclear factor kappa B (NF-κB) protein expression were evaluated in the ischemic, border, and non-ischemic zones of the heart. Concentration of conjugated dienes, a marker of oxidative stress, was measured spectrophotometrically in the heart tissue. Our results show that the most significant changes after myocardial infarction occurred in the infarcted and border zones. We observed an increase in reactive oxygen species, a decrease in the total activity of NOS, and concurrent increase in expression of the inductive form of NOS. The antioxidant properties of NAC were confirmed by a reduction in conjugated diene concentration, which positively correlated with decreased NF-κB expression. Preventive administration of N-acetylcysteine with an antioxidant effect partially reverse the pathological consequences of MI by increasing total NOS activity, decreasing iNOS expression, reducing oxidative stress, and lowering inflammatory markers.

**Keywords:** myocardial infarction, hypertension, N-acetylcysteine, NO
**Funding:** APVV-22-0271, VEGA 2/0131/24
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**S4-**63
**Physical exercise as a form of "remote preconditioning ": molecular mechanisms of cardioprotection**

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**Abstract:**
Although ischemic preconditioning (PC) is the most robust phenomenon protecting the heart of all animal species against ischemia/reperfusion (I/R) injury, its application in humans is limited by technical requirements, short-term duration and unpredictable occurrence of AMI. However, some other forms of "conditioning" do not require invasive approach. We explored non-invasive intervention in the adult male Wistar rats *in vivo*: voluntary exercise-induced PC (EPC) aimed to increase cardiac resistance to I/R *ex vivo*. For EPC, rats were placed in the cages equipped with wheels for 2 weeks free running, while control sedentary animals stayed in the standard cages. At the beginning and the end of the protocol, heart structure and function were evaluated by ECHO cardiography, and except reduced body weight and heart rate, no structural or functional changes were revealed. The efficacy of EPC was tested in the Langendorff-perfused normo- and hyperglycemic hearts exposed to 30 min global ischemia/2 hrs reperfusion, focused on the postischemic recovery of function, arrhythmogenesis and extent of lethal injury (infarct size, IS, TTC staining). In parallel groups, heart tissue samples were processed (WB) to investigate the levels and activity of several proteins involved in "pro-survival" RISK cascade. EPC significantly reduced contractile dysfunction, IS and the incidence and severity of reperfusion arrhythmias, the effects were less expressed in the hyperglycemic hearts. These effects were comparable with those we have observed in the rat hearts subjected to a remote PC on a. femoralis *in vivo*. Cardioprotection was associated with a significant up-regulation of selected pro-survival RISK proteins, such as PKB (reduced by hyperglycemia), PKCε, eNOS, anti-apoptotic and anti-oxidative effects, as well as with an increased levels of β-3 AR mRNA.
Beneficial effects of sub-chronic running suggest its potential that could be used in the management of ischemic heart disease.

**Key words:** ischemic heart injury, physical exercise, molecular mechanisms of protection
**Funding:** Slovak Grant Agency VEGA SR 2/0104/22, APVV-19-0540, APVV-20-0242.

**S4-**25

**Title: The effect of dimethyl fumarate on gene expression of NRF2 and its target genes in the heart and liver of borderline hypertensive rats**

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**Abstract:**
Objective The nuclear factor NRF2, encoded by the Nfe2l2 gene is a transcription factor regulating cellular homeostasis and several cytoprotective pathways. NRF2 regulates more than 250 target genes, mainly genes encoding antioxidant enzymes, and proteins involved in cell repair, but also proteins controlling iron metabolism. The study aimed to determine the effect of the activator of NRF2, dimethyl fumarate (DMF), on gene expression of Nfe2l2 and selected target genes of NRF2 in left heart ventricle (LHV) and the liver of borderline hypertensive rats (BHR). Methods In our study, 10-week-old BHR male rats were used. The rats were divided into control group to which 0.5% DMSO was administered (Cont, n =7) and a DMF-treated group (DMF, n =7) to which DMF diluted in 0.5% DMSO at the dose 30 mg/kg/day was administered for 6 weeks. Systolic blood pressure and heart rate were measured using tail-cuff plethysmography. The activity of nitric oxide synthase was determined in LHV and liver using the conversion of [3H]-L-arginine. The concentration of conjugated dienes was determined spectrophotometrically at 233 nm. The gene expression was determined using a two-step reverse transcription quantitative polymerase chain reaction (RT-qPCR). Various biochemical parameters were determined in plasma using Mnchip Celercare V5. Results DMF did not affect blood pressure but reduced the weight gain of the animals. Plasma markers of liver function (aspartate transaminase, alanine transaminase, alkaline phosphatase) and glycemia were not affected by DMF, plasma cholesterol, lactate dehydrogenase and α- hydroxybutyrate dehydrogenase concentration were significantly increased. The concentration of conjugated dienes (CD, a marker of oxidative damage) in the liver was not changed, while it was significantly reduced in the heart. In the liver, DMF decreased Nfe2l2 gene expression, while the expression of NRF2 target genes (Hmox1, Sod1, Gpx4, Fpn1 and Fth1) was not affected. On the other hand, in the heart, DMF significantly increased the gene expression of Nfe2l2, and the expressions of Hmox1, Sod1, Gpx4, Fpn1 and Irp1. Conclusion Our results show that the effect of DMF on gene expression of Nfe2l2, its target genes and oxidation status is tissue-specific, with significant differences between heart and liver. The results also suggest a possible negative effect of long-term activation of NRF2 function on the heart due to possible induction of a hyperreductive state.

**Keywords:** NRF2, dimethyl fumarate, heart, liver
**Funding:** This study was supported by the grant APVV-22-0296.
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**S4-**12

**Title: The effect of remote ischemic preconditioning on the resistance of the heart against ischemia-reperfusion injury in aging rats. Study of molecular mechanisms**

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**Abstract:**
The effect of age on reduced tolerance to ischemia-reperfusion (I/R) injury and adaptative mechanisms has been demonstrated in several studies in human and animal hearts. One of the most studied forms of cardioprotection is remote-ischemic preconditioning (RIPC), mainly for its possible clinical use. Positive effect of RIPC has already been found in elderly patients. However, little is known about its effect and molecular basis in elderly animals. Our work focuses on clarifying the effect of RIPC on the resistance of heart against I/R injury and identifying proteins involved in protective pathways in aging 13 months old rats. In Langendorff-perfused hearts exposed to 30-min I/120-min R without or with prior RIPC. RIPC (3 cycles, 5-min I/5-min R) was applied on the hind limb of anesthetized rats. We measured infarct size (IS), susceptibility to ventricular arrhythmias and recovery of contractile function (LVDP). In parallel groups, LV tissue was sampled for the detection of protein levels of RISK and pro/anti-apoptotic pathways. Application of RIPC caused decrease in myocardial IS and LVDP recovery as well as recovery of coronary flow after I/R. However, RIPC did not significantly affect the arrhythmia score. Positive cardioprotective effect of RIPC was associated with increased phosphorylation of GSK3β and decreased apoptotic activity of myocardial cells (Bax/Bcl-2). On the other hand, most of proteins of the RISK pathway (including MAPK kinases) were not activated by RIPC (Akt, eNOS, PKCε, ERK1/2, JNK2). Therefore, our next step will investigate whether RIPC exerts its effect through the SAFE pathway which could activate GSK3β in 13 months old rats. As a result, RIPC provided protection of the heart against I/R injury in 13 months old rats. Therefore, even at this age, RIPC appears to be still an effective and clinically easy-to-use form of cardioprotection. Although, we still need to better investigate its molecular pathways.

**Keywords:** remote preconditioning, aging, molecular mechanisms
**Funding:** APVV-19-0540, APVV-21-0194; VEGA 2/0141/18, VEGA 2/0104/20, VEGA 2/0159/24
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**S4-**7
**Title: Role of sGC stimulator in the treatment of heart failure**

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**Abstract:**
Objective: Heart failure (HF) is characterized by progressive reduction in cardiac output and occurrence of malignant arrhythmias. It is well known for its high prevalence and mortality. Since current therapies are not effective enough, attention has been lately directed to the nitric oxide (NO)/ soluble guanylyl cyclase (sGC)/ cyclic guanosine monophosphate (cGMP) pathway, which may be impaired in the patients with HF. The aim of the present study was to evaluate the efficacy of sGC stimulator in a model of hypertension and HF. Methods: To evaluate potential cardioprotective effect of sGC stimulator BAY41-8543 (3mg/kg/day for 30 weeks), either alone or in combination with an angiotensin converting enzyme inhibitor (ACEi) Trandolapril (0.25mg/kg/day for 30 weeks), we used male normotensive HanSD rats (n=17), male hypertensive heterozygous Ren-2 transgenic rats (TGR) (n=16) and male TGR rats with volume overload induced by aortocaval fistula (ACF) (n=27). Age of animals at the end of the experiment was 40 weeks. Left ventricle tissue and blood plasma were used for further analyses. Results: The sGC stimulator caused a significant decrease in rats' mortality due to volume overload. Besides that, in the left ventricle of TGR rats, the sGC stimulator significantly elevated levels of the antioxidant enzyme superoxide dismutase 1, and levels of matrix metalloproteinase 2, total and phosphorylated connexin 43, as well as protein kinase C epsilon, implicated in remodeling of extracellular matrix and intercellular communication. This effect of the sGC stimulator was not observed in the TGR ACF group. Conclusion: These results support the concept that sGC stimulators might represent a class of drugs suitable for combating heart failure, and may possess anti-arrhythmic properties. However, additional research is required to validate these potential benefits.

**Keywords:** sGC stimulator, heart failure, hypertension
**Funding:** This work was supported by APVV-21-0410, VEGA 2/0006/23 and EXCELES LX22NPO5104.
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**S4-**2

**Title: HIF-1α limits myocardial infarction by promoting mitophagy in mouse hearts adapted to chronic hypoxia**

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**Abstract:**
Transcriptional factor HIF-1α is known to contribute to cardioprotection against ischemia/reperfusion injury. Adaptation to chronic hypoxia (CH) is a cardioprotective phenomenon associated with HIF-1α stabilization. Nevertheless, its precise role in protective changes induced by CH remains incompletely understood. This study aimed to elucidate whether partial Hif1a deficiency would nullify the cardioprotective benefits of CH, while also investigating its impact on mitochondrial function and dynamics. Adult male wild type and heterozygous Hif1a knockout mice were adapted to CH or maintained under normoxic conditions. Physiological responses to CH were evaluated, and myocardial infarction was induced in isolated perfused hearts. Expression analyses, mitochondrial respiration measurements, and electron microscopy were conducted to assess mitochondrial characteristics. We revealed a reduction in infarct size in chronically hypoxic wild-type mice in comparison to their normoxic counterparts. In contrast, this protective effect of CH was absent in mice displaying partial Hif1a deficiency. Additionally, diminished mitochondrial respiration and altered mitochondrial ultrastructure were observed exclusively in chronically hypoxic wild-type mice compared to their normoxic counterparts. To explain the presence of a higher number of enlarged mitochondria accompanied by reduced mitochondrial mass, we assumed the occurrence of mitophagy. We performed microtubule-associated light chain protein 3 assay to monitor autophagy in the presence and absence of lysosomal protease inhibitor leupeptin. Remarkably, augmented autophagosome formation appeared solely in chronically hypoxic wild type mice. Finally, we pretreated the mice with mitochondrial division inhibitor (mdivi-1) and demonstrated that cardioprotective effect of CH in wild-type mice was abolished by mitophagy inhibition. These collective findings indicate the pivotal role of HIF-1α-regulated mitochondrial processes within cardiac myocytes during adaptation to CH, and importantly, they highlight its significance in chronic hypoxia-induced myocardial protection against ischemia/reperfusion injury through promotion of mitophagy.

**Keywords:** cardioprotection, chronic hypoxia, HIF-1alpha, mitochondria
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**S4-**31

**Title: Effect of flavonoid quercetin on myocardial ischemia-reperfusion injury, molecular signaling, and antioxidant status in aging rats**

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**Abstract:**
Objective: Quercetin (QCT) is a natural flavonoid known for its positive effects on the cardiovascular system. Previous studies have demonstrated its cardioprotective effects on myocardial ischemia-reperfusion (I/R) injury in young animal models. Considering the prevalence of myocardial I/R injury among the elderly, our study aims to explore the impact of QCT on myocardial I/R injury in aging 24-month-old Wistar rats. Methods: QCT was administered orally to rats at a dose of 20 mg/kg/day for 6 weeks. After 6 weeks, the animals were euthanized; their hearts were removed, and perfused according to the Langendorff method (30 min ischemia/120 min reperfusion). Assessment of infarct size was performed by TTC staining. The effect of QCT on selected molecular signaling pathways in the heart was analyzed by Western blotting, focusing on the RISK signaling pathway (Akt, PKC-ε, eNOS, and GSK-3β), endogenous antioxidant enzymes (SOD1 and SOD2), and markers of apoptosis (Bax/Bcl-2). We also investigated the effect of QCT on oxidative stress in blood plasma, focusing on the lipid peroxidation marker TBARS, the protein oxidation marker AOPP, the antioxidant capacity marker FRAP, the protein glycosylation level marker fructosamine, and the carbonyl stress marker AGEs. Results: Our results demonstrated that chronic application of QCT did not produce a beneficial cardioprotective effect on the recovery of hearts after ischemia. On the contrary, we observed a trend of worsening the recovery of cardiac functional parameters due to the influence of QCT. Significantly increased levels of FRAP and AOPP parameters suggest a pro-oxidant action of QCT in the aging animal model. Administration of QCT also failed to activate the RISK signaling pathway and caused a decrease in the Bax/Bcl-2 ratio, suggesting its anti-apoptotic effect. Conclusion: Our findings suggest that QCT does not have cardioprotective potential in elderly individuals that may be, at least partially, caused due to insufficient activation of the RISK pathway as well as inhibited antioxidant potential of QCT in aging individuals. On the other hand, QCT showed antiapoptotic potential in aging individuals. Thus, administration of QCT for the purpose of cardioprotection in the elderly appears to be not promising. The research was supported by grants: APVV-21-0194 and VEGA 2/0159/24.

**Keywords:** quercetin, ischemia, reperfusion, cardioprotective effect
**Funding:** The research was supported by grants: APVV-21-0194 and VEGA 2/0159/24.
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**S4-**19

**Title: Ischemia-reperfusion injury during heart preservation: static cold ischemia versus normothermic perfusion machine, with colchicine or placebo preconditioning**

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**Abstract:**
Introduction: In the present era, heart transplantation is constrained by limited availability of donor organs. Heart preservation common practice involves static cold storage (SCS), but it contributes to the development of ischemia-reperfusion injury (IRI), leading to a higher risk of primary graft failure. To mitigate the incidence of primary graft failure, both drug-based and infusion-related cardioprotective strategies seem pertinent. Colchicine has been promising in myocardial infarction settings in mice and humans. Additionally, devices for ex vivo normothermic perfusion (EVNP) have been developed to limit ischemia.
Objective: This study aims to compare SCS vs EVNP on IRI development and effect of colchicine preconditioning by using a porcine model.
Materials and Methods: Hearts were explanted and preserved during 4 hours with 2 different strategies; 12 hearts at 4°C in Custodiol (SCS group) and 12 hearts at 34°C on ex vivo heart perfusion system OCS® (EVNP group). 6 pigs in each group were preconditioned with colchicine. Then, all hearts undergo one hour of warm reperfusion with whole blood on ex vivo heart perfusion system. Perfusate samples are collected at various times during warm reperfusion, and biopsies are performed at the end of the experiment.
Results: Body weight, warm ischemia time and lactatemia at baseline were comparable between groups. Veino-arterial lactate difference during warm reperfusion were not significantly different. After 60min of warm reperfusion, hearts in OCS group showed significantly higher cardiac troponin I (cTnI) level in perfusate during warm reperfusion (OCS: 214.7 ng/ml vs IF: 74.0 ng/ml, U=16, $p<0.01$). But H-FABP level in perfusate were not significantly different. In perfusate at end of warm reperfusion, IL-6 (OCS: 7.4 pg/ml vs IF: 0.6 pg/ml, U=0, $p<0.0001$) and TNFa (OCS: 0.6 pg/ml vs IF: 0.2 pg/ml, U=20.5, $p<0.01$) were significantly higher in OCS group. According to colchicine preconditioning, no significant differences were observed on cTnI, H-FABP, pro and anti-inflammatory cytokines levels. Single-channel recordings of RyR2 in lipid bilayers, histological exploration and oxidative stress measurement are in process.
Conclusion: Regarding perfusate markers, EVNP seems to induce more myocardial damage. However histological exploration, oxidative stress measurement and ryanodine receptor activity analysis are needed to conclude on organ preservation strategies and colchicine preconditioning effect.

**Keywords:** organ preservation, ex-vivo perfusion, myocardial ischemic reperfusion injury, oxidative stress, ryanodine receptor
**Funding:** Bourse Alain Castaigne, Bourse ARCOTHOVA (SFAR), Bourse EACTAIC (European association Cardiac-Thoracic Anaesthesiology and Intensive Care)
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**Session 5 "NUTRITIONAL APPROACHES IN CARDIOMETABOLIC PROTECTION"**

**S5-**3

**Title: Lipid profile and nitric oxide production in experimental metabolic syndrome: Effects of natural polyphenolic substances**

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**Abstract:**
Objective: Nitric oxide (NO) plays a crucial role in the pathogeneses of metabolic syndrome. We aimed to determine NOS activity under conditions of different lipid profiles. Methods: In our studies, normotensive Wistar Kyoto (WKY), spontaneously hypertensive (SHR), obese SHR (SHR/cp), lean and obese Zucker rats have been used. In 12-week-old male rats, the lipid profile in plasma and NOS activity in the left ventricle (LV) and aorta have been determined. Simultaneously, the effect of different polyphenolic substances on NOS activity, including their mechanisms of action (expression of NOS isoforms, redox enzymes, and pro-inflammatory factors), have been studied. Results: We demonstrated that WKY and SHR had the same level of total cholesterol, triglycerides, HDL, and LDL. In SHR/cp, however, the level of total cholesterol, triglycerides, LDL, but also HDL increased significantly. Lean Zucker rats had a similar lipid profile to WKY. However, obese Zucker rats had all lipid parameters significantly higher than SHR/cp. NOS activity was significantly higher in the LV and aorta of SHR compared to WKY. In SHR/cp, however, NOS activity was comparable to that in WKY. This indicated that obesity reduced NOS activity in spontaneous hypertension. Lean and obese Zucker rats had comparable NOS activity in the heart, but it was significantly reduced in the aorta of obese Zucker rats. In accordance with these findings, polyphenol rich natural compounds like Lonicera caerulea L. and cornelian cherry varieties increased NOS activity in the aorta, while not affecting the activity in the LV. The reduced expression of pro-oxidative and pro-inflammatory factors were responsible for this effect rather than changes in the expression of individual NOS isoforms. Likewise, the polyphenol rich wine extract had a different effect on NOS depending on the basal level of NOS activity and pro-inflammatory factors in individual tissues. In conclusion, deteriorated lipid profile may reduce NOS activity, and natural polyphenolic substances have the ability to regulate it by different mechanisms, especially by reducing pro-oxidative and pro-inflammatory factors.
**Keywords:** lipids, nitric oxide, metabolic syndrome
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**S5-**36

**Title: Quercetin reduces fructose drinking in a model of fructose-induced insulin resistance**

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**Abstract:**
A general increase in calorie consumption, specifically refined carbohydrates and fructose, is clear and positively correlated with an alarming increase in metabolic syndrome. This study aimed to improve insulin sensitivity in fructose-treated animals by ingesting the flavonoid quercetin. After drinking a 10% fructose solution for 9 weeks, the rats developed several signs of insulin resistance. The effect of quercetin administered by gavage for 6 weeks (20 mg/kg/day in 1% methylcellulose solution) was monitored. Rats from the control groups also received a methylcellulose vehicle. The most striking result of quercetin treatment was the normalization of fructose solution drinking to the level of drinking water intake. In addition, quercetin supplementation significantly reduced plasma glucose and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) in fructose-consuming rats. Surprisingly, fructose ingestion did not elevate plasma uric acid, thiobarbituric acid reactive substances, nitrotyrosine, or advanced glycation end products fluorescence. Instead, a reduction in the above parameters was observed. Taken together, these results indicate that quercetin supplementation reduces fructose drinking and decreases plasma glucose and the HOMA-IR index. Furthermore, methyl cellulose, in combination with fructose, causes uric acid – lowering, antioxidant and anti-glycation effects. Thus, methyl cellulose possibly shifts fructose metabolism in favor of the utilization of antioxidant features of fructose. Our results call for using methyl cellulose in sweetened beverages and other sweetened food.

**Keywords:** Quercetin, Fructose-rich diet, Methyl cellulose
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**S5-**37

**Title: High-fat diet and its effect on nitric oxide bioavailability after experimentally induced myocardial infarction**

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**Abstract:**
Myocardial infarction (MI) is one of the leading causes of death worldwide. One of the biggest risk factors for cardiovascular diseases including MI is obesity. It promotes the development of atherosclerosis by systemic inflammation, abnormal lipid profile or adipokine secretion. Reduced bioavailability of nitric oxide, important vasodilator, influences the development of endothelial dysfunction, the first step towards atherosclerosis. In this study, we investigated the effect of high-fat diet on nitric oxide bioavailability after experimentally induced MI. Nine weeks old Wistar Kyoto rats were divided into two groups, one of which were fed with high-fat diet while the other continued with standard lab chow for four weeks. In week 13 the induction of MI was performed by the reversible ligation of the left descending coronary artery. The occlusion lasted 20 minutes. Seven days after surgery animals were sacrificed. Proinflammatory cytokines were determined by Bioplex kit in plasma. Total nitric oxide synthase (NOS) activity was detected by the conversion of [3H]-L-arginine to [3H]-L-citrulline in the infarcted zone, injured zone, and non-ischemic zone of the heart. Protein expressions of endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase and nuclear factor kappa B (NFκB) were measured by Western blot analysis also in the infarcted zone, injured zone, and non-ischemic zone of the heart. Rats fed a high-fat diet for 4 weeks in combination with MI showed increased level of tumor necrosis factor alpha and interleukin-6. Moreover, high-fat diet reduced total NOS activity and eNOS expression. NFκB was significantly increased after MI with an influence of high-fat diet only in the injured zone but surprisingly, the combination of a high-fat diet and MI caused a significant decrease in its expression in the infarct and the non-ischemic zone. In conclusion increased concentrations of proinflammatory cytokines as well as increased expression of inducible nitric oxide synthase and NFκB confirmed ongoing inflammation which may contribute to uncoupling of eNOS. Decrease in total NOS activity and eNOS expression may reduce nitric oxide bioavailability after MI and lead to further cardiomyocyte damage.

**Keywords:** infarction; high-fat diet; nitric oxide
**Funding:** This study was supported by APVV-22-0271 and VEGA 2/0131/24
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**S5-**24

**Title: Role of Matrix Metalloproteinases in the Mechanisms Underlying the Effects of Quercetin on Cardiac Function in Aged Zucker Diabetic Fatty Rats**

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**Abstract:**
Objectives: Diabetes-related cardiovascular pathology may be caused by multiple mechanisms, including dysregulation of matrix metalloproteinases (MMPs). These enzymes are involved in extracellular matrix degradation as well as in the regulation of several cellular processes. Quercetin (QCT) is a substance with preventive effects in the treatment of cardiovascular disease and diabetes. The aim of this study was to investigate the effects of chronic administration of QCT on changes in cardiac function in aged lean and obese Zucker diabetic fatty (ZDF) rats, and this was in the context of MMPs. METHODS: We used 1-year-old lean and obese ZDF rats with or without 6 weeks of quercetin (QCT) treatment. We investigated the effect of QCT on changes in cardiac function and on MMP-related changes and intracellular signaling. The effects of diabetes and QCT on cardiac function were investigated by assessing hemodynamic parameters of isolated perfused rat hearts. Myocardial MMPs activities were analyzed by gelatin zymography, specific protein levels were determined by Western blot analysis, and superoxide dismutase (SOD) activities were assayed using a colorimetric assay kit for superoxide dismutase activity. RESULTS: The results showed that obesity has a negative effect on hemodynamic parameters in the hearts of diabetic ZDF rats. This impairment of cardiac function was associated with alterations in MMP-2 and MMP-28 and attenuation of SOD antioxidant defense. QCT treatment had no positive effect on cardiac function or MMPs modulation. However, the application of QCT prevented the negative effect of diabetes on antioxidant defense via SOD and also led to activation of Akt kinase. CONCLUSION: In conclusion, alterations of MMP-2 and MMP-28 and attenuation of antioxidant defense via SOD are involved in the impairment of cardiac function due to obesity. QCT had no positive effect on the improvement of cardiac function or MMPs modulation. Nevertheless, its application mediated the activation of adaptive responses against oxidative stress via Akt kinase and the prevention of the negative effects of diabetes on antioxidant defense via SOD. These results provide new insights into the cellular mechanisms involved in the realization of the effects of diabetes and/or QCT treatment in the hearts of ZDF rats.

**Keywords:** Matrix metalloproteinases; Quercetin; Heart
**Funding:** This study was supported by grants VEGA 2/0169/24, and grant of Agency for Research and Development APVV-21-0194.
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**S5-**15
**Title: Quercetin as a potential cardioprotective agent in ischemia-reperfusion injury**

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**Abstract:**
Ischemia-reperfusion (I/R) injury represents a major cause of invalidity and death from cardiovascular disease. Since there are still no cardioprotective drugs on the market for prevention/treatment of I/R injury, searching for novel cardioprotective compounds is very much needed. Quercetin (QCT), a natural polyphenol enriched in human food, is a promising substance that exerts several beneficial effects in cardiovascular system including preventing cardiac I/R injury. Cardioprotective potential of QCT was largely documented in healthy young animals but only limited data are available regarding cardiac effects of QCT in presence of comorbidities, co-medications and in ageing subjects. The aim of the current study is to summarize data obtained in our experimental group documenting potential of QCT for preventing myocardial I/R injury in different experimental settings including presence of selected comorbidities, co-medications, and in aged subjects. QCT in the dose 20mg/kg/day was administered orally for 4/6 weeks to rats of different age and rats with selected comorbidities/co-medications. After the end of treatment hearts were isolated and ex vivo exposed to I/R (30-min global ischemia/2-hour reperfusion). Recovery of cardiac function and infarct size were assessed as the physiological outputs of the experiments. Molecular mechanisms of QCT action were evaluated as well. The results showed that QCT exerts cardioprotective effects in I/R injury in healthy young and doxorubicin-treated rats but it was inefficient in preventing I/R injury in aged rats and in rats with comorbidities (hypertensive/type 2 diabetic). In conclusion, QCT might be potentially cardioprotective in preventing myocardial I/R injury; however, ageing and/or presence of comorbidities may decrease or even abolish anti-ischemic effects of QCT.

**Keywords:** Ischemia-Reperfusion, Quercetin, Cardioprotection, Ageing, Comorbidities
**Funding:** The study was supported by grants: APVV-21-0194 and VEGA 2/0159/24
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**S5-**5

**Title: Cardiac ischaemia-reperfusion injury and plasma oxidative markers in hypertensive SHR rats after chronic administration of the flavonoid quercetin**

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**Abstract:**
Introduction: Quercetin (QCT) is a naturally occurring polyphenol that has been shown to have antioxidant and cardioprotective properties. In the absence of comorbidities, studies have shown that QCT protects the heart against ischemia-reperfusion (I/R) injury in rats. Given the incidence of cardiac I/R injury, e.g. myocardial infarction, in patients with various comorbidities, the aim of this study was to determine whether QCT can protect the heart against I/R even in the presence of comorbidities, namely hypertension, for which we used a recombinant strain of spontaneously hypertensive rats (SHR). In addition, QCT was tested on blood pressure and plasma oxidation levels. Methods: QCT (20 mg/kg/day) was administered orally to adult 3-month-old SHR rats for 6 weeks. Blood pressure was measured both at the beginning and at the end of the administration of QCT. I/R (30/120 min) was induced in isolated hearts by the Langendorff perfusion method after the animals were sacrificed. The recovery of cardiac functional parameters after ischemia was monitored. Infarct size was assessed at the end of I/R. Appropriate kits were used to analyse plasma markers of oxidative stress (AOPP, AGEs, FRAP, TBARS). Results: As a result of using QCT, we observed a positive trend in the recovery of cardiac function following I/R and also a trend towards a reduced increase in left ventricular end-diastolic pressure. All hearts with QCT recovered function after ischemia, whereas 22% of hearts without QCT did not recover function and went into permanent fibrillation. QCT did not affect infarct size or plasma oxidative stress markers. Finally, QCT had no effect on blood pressure in the rats. Conclusion: QCT seems promising in the prevention of I/R injury in hypertensive patients, mainly in terms of improving the electrical and mechanical function of the heart following I/R, but not in reducing the extent of the infarct size. It is unlikely that the systemic antioxidant effect of QCT is responsible for this cardioprotective effect in hypertensive subjects.
**Keywords:** quercetin, hypertension, oxidative stress
**Funding:** Supported by: APVV-21-0194 and 2/0104/20
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**S5-**1

**Carrot Genotypes Displayed Differential Pharmacological Profiles in Vascular and Metabolic Cells and Improve Blood Pressure and Reduces Aortic Root Lesions in an Atherosclerosis-Prone Genetic Mouse Model**

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**Abstract:**
Epidemiological studies have shown that carrot consumption may be associated with a lower risk of developing several metabolic dysfunctions. Carrots' genotype and growing conditions influence their potential properties to fight against cardiovascular and metabolic diseases. The present study evaluated the influence of carrot genotypes contrasted by root color (Bolero, Presto, Karotan, Deep Purple, Kintoki and Blanche des Vosges) growing under standard, water-restricted, biotic stress (*Alternaria dauci* inoculation), and combined stress conditions (water restriction and *A.dauci* inoculation).
Independently of varieties or growing conditions, all carrot extracts affected vascular cells' oxidative stress and apoptosis, and metabolic cells' oxidative stress and lipid accumulation. Three clusters were revealed and displayed beneficial properties mostly for adipocytes function, smooth muscle cells and hepatocytes, and endothelial cells and hepatocytes, respectively. Karotan and Presto varieties exhibited endothelial tropism while Blanche des Vosges targeted adipocytes. Carrots under biotic stress are more efficient in inducing beneficial effects, with the Bolero (Bo) variety being the most effective. However, extracts from carrots which grew under combined stress conditions had limited beneficial effects. Altogether, he Bo carrot variety exhibited vascular and hepatic tropism using cellular models of cardiometabolic diseases. (Soleti et al. Nutrients; 2020, 12(2):337)
Next, we evaluated the potential metabolic and cardiovascular protective effect of Bo, grown under two conditions (standard and biotic stress conditions (BoBS)), in apolipoprotein E-knockout ($ApoE^{-/-}$) mice fed with high fat diet (HFD). Both Bo and BoBS decreased plasma triglyceride and expression levels of genes implicated in hepatic de novo lipogenesis and lipid oxidation. BoBS supplementation decreased body weight gain, secretion of very-low-density lipoprotein, and increased cecal propionate content. Interestingly, Bo and BoBS supplementation improved hemodynamic parameters by decreasing systolic, diastolic, and mean blood pressure. Moreover, Bo improved cardiac output. Finally, Bo and BoBS substantially reduced the aortic root lesion area. These results showed that Bo and BoBS enriched diets corrected most of the metabolic and cardiovascular disorders in an atherosclerosis-prone genetic mouse model and may therefore represent an interesting nutritional approach for the prevention of cardiovascular diseases. (Soleti et al. Nutrients 2021, 13(4): 1181).

**Keywords:** carrot extracts, metabolic cells, vasculzar cells, carrot supplementation; hemodynamic parameter; atherosclerosis; high fat diet; $ApoE^{-/-}$
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**Session 6 "ADVANCES IN ATRIAL FIBRILLATION"**

**S6-**33

**Title: Pharmacokinetics of novel selective TRPV2 inhibitor SET2 and its acute cardiac effects**

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**Abstract:**
Inhibition of a transient receptor potential vanilloid 2 channels (TRPV2) has been depicted as a promising tool to affect the course and outcome of several diseases. From potential biomarker role in various types of cancer to modulation of immunological, neurological and heart diseases. However, all the proposed outcomes simply relied on the non-selective modulation of TRPV2. To conquer this limitation, new chemical probes are slowly emerging as selective modulators of TRPV2. In this study we describe the pharmacokinetics of the first-in-line selective inhibitor of TRPV2 - SET2. Firstly, we employed healthy Wistar rats that underwent cannulation of left carotid artery in order to gain access to the arterial blood. The pharmacokinetics of SET2 was then assessed through the intraperitoneal (i.p.) and oral route (p.o.) of administration after a single dose of SET2 (25 mg/kg). Urine excretion was monitored over 24 hours via metabolic cages. Plasma and urine concentration was determined using UHPLC-MS/MS platform which was previously developed. Next, we monitored acute cardiovascular effects – electrocardiogram (ECG) and blood pressure (during cmax and 2 hours after the SET2 application) as well as plasma markers of cardiac and renal damage. Peroral administration showed fast absorption into the plasma peaking between 15 min (cmax) to 90 min with gradual decline for 6 hours. SET2 maintained its inhibitory concentration for about 5 hours. Intraperitoneal application maintained its inhibitory concentrations for almost 3 hours without any acute effects on ECG nor blood pressure, therefore, rendering the compound safe and stable at the levels of acute cardiovascular effects. To our best knowledge, this is the first study characterizing pharmacokinetic parameters and acute cardiovascular effects of selective TRPV2 channels inhibitor, compound SET2, after i.p. and p.o. administration in healthy rats.

**Keywords:** TRPV2, SET2, cardiovascular effects, pharmacokinetic
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**S6-**53
**Title: Heart and Mind at Risk: Decoding Channel Disruptions in the Food Poisoning Enigma**

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**Abstract:**
Introduction: Ciguatera (CG) is a syndrome caused by Ciguatoxins (CTXs), natural phycotoxins produced by benthic dinoflagellates, among the most harmful known. CG results from food poisoning due to the consumption of contaminated fish meat along the food chain, from herbivorous to higher carnivorous fish. It is the most prevalent non-bacterial seafood poisoning worldwide, with an annual increase of 50,000 to 100,000 new cases, although only 2 to 10% are reported. CG presents with multiple undefined symptoms (gastrointestinal, cardiovascular, neurosensory, skin, and inflammatory) in the form of a polymorphic syndrome. CTXs are known to modify cellular membrane permeability, acting on ion channels (Na+, K+, Ca2+) essential for excitable cells like cardiomyocytes (CMs) and neurons (NRs). Objective: Our primary aim is to understand and characterize the impact of CTXs on human cardiac and neuronal physiology. We hypothesize that CTXs can influence intracellular calcium handling, affecting cardiac excitation-contraction coupling (ECC) and neuronal functional and molecular properties.

Methods: We differentiated human induced pluripotent stem cell-derived ventricular-like cardiomyocytes (hiPSC-CMs) and neurons of the central nervous system (hiPSC-NRs) from healthy and pathological patients and employed two types of purified CTXs (CTX1B and CTX3C). We investigated CTXs effects on cytosolic calcium, cellular morphology, CMs contractile properties, and RyR2 channel activity. Additionally, we used zebrafish embryos as a live model to monitor embryonic development, behavior and cardiac function.

Results: CTXs exposure resulted in intracellular calcium leaks, increased cardiac and neuronal basal calcium levels, and altered CMs and NRs single-cell morphology. It heightened RyR2 channel activity, reflected by a higher opening frequency and reduced closing time. CTX exposure also led to reduced contractions of hiPSC-derived cardiac sheets. In vivo studies with CTXs demonstrated high zebrafish embryos mortality, skeletal and physical malformations, and alterations in cardiac function and behavior.

Conclusion: We discovered that CTXs caused RyR2 aberrant kinetic properties, abnormal intracellular calcium handling, reduced beat rate, and altered hiPSC-CMs/NRs morphology. In vivo studies confirmed cardiac and neuronal damage, with embryonic malformations and high mortality.

**Keywords:** Ciguatera, hiPSC-CMs, hiPSC-NRs, RyR2, calcium.
**Funding:** This work was supported by grants of MUSE CIBSEEA, ANR MUSAGE and the “Institut National pour la Santé et la Recherche Médicale”.
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**S6-**61

**Title: Integrated Strategies for Enhancing the Maturity of hiPSC-Derived Cardiomyocytes: A Molecular, Metabolic, and Morphological Approach**

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**Abstract:**
In recent years, cardiomyocytes derived from human-induced pluripotent stem cells (hiPSC-CMs) have proven invaluable for modeling inherited cardiac diseases and advancing patient-specific, non-invasive therapies. However, a significant challenge lies in the cultivation of mature 2D cardiac tissues that accurately reflect human heart physiology. This is due to hiPSC-CMs often displaying immature functional and morphological traits, such as a rounded shape and inconsistent beating rates. Given the limited in-depth studies on enhancing maturity, our objective is to integrate various strategies—molecular, metabolic, and morphological—to emulate some of the key physiological characteristics of adult ventricular cardiomyocytes. Our approach includes molecular maturation, achieved by adding specific small molecules (cyclic AMP and 3,3',5-Triiodo-L-thyronine) to the culture medium. Metabolically, we aim to transition the cells from a purely glycolytic metabolism, characteristic of immature cells, towards a more physiological metabolism where fatty acids contribute to 60-70% of cardiac energy production. Additionally, we use micropatterned coverslips to restrict cell growth and promote fiber-like alignment in hiPSC-CMs, thereby enhancing cell-cell contact and promoting maturation. By assessing the organization of sarcomeric proteins (cardiac troponin I, α-actinin, and myosin heavy chain) through immunofluorescence and multiphoton second harmonic generation imaging—and evaluating contractility parameters via video-edge detection—our preliminary results show promising improvements, particularly in the alignment of sarcomeres in hiPSC-CMs. However, the treatment with fatty acids to enhance β-oxidation has shown mixed results, with no significant improvement in the surface area or organization of the hiPSC-CMs sarcomeres.

**Keywords:** hiPSC-CMs, maturation, fatty-acid, small-molecules, micropattern
**Funding:** ANR
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**Session 7 "NOVEL TARGETS FOR VASCULAR DISEASES"**

**S7-**17

**Title: Can H2S be a new mechanism for the vascular effects of sildenafil in healthy and oxidative stress conditions?**

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**Abstract:**
Objective. Hydrogen sulfide (H2S) is a gasotransmitter that regulates several functions in the vascular system, including vascular tone, modulation of endothelial cells, and smooth muscle cell proliferation. In addition to its concentration-dependent relaxant effects, H2S also inhibits phosphodiesterase enzyme activity. Sildenafil is a drug with similar pharmacological mechanisms to H2S, inhibits the PDE5 enzyme that causes vasodilation and is widely used in the treatment of erectile dysfunction. Previously, we showed that H2S contributes to the relaxing effects of sildenafil in penile tissue, whose nitric oxide (NO)-mediated relaxant effects are well known. Although sildenafil has been reported to cause relaxation in the aorta, the role of H2S in the endothelium-independent mechanism has not been fully elucidated. In this study, we investigated the H2S-induced effects of sildenafil on vascular tone in mice aorta. Methods. The effect of sildenafil (1nM) on endogenous H2S formation in the aorta under healthy and pyrogallol-induced (0.1mM) oxidative stress conditions was investigated with methylene blue assay. The H2S-dependent effects of sildenafil on vascular tone in mice aorta were investigated by isolated organ bath experiments on myograph. Statistical analysis was conducted using ANOVA followed by the Bonferroni post-hoc test. Results. Sildenafil significantly increased L-cysteine-induced H2S production and aminooxyacetic acid (AOAA) inhibited sildenafil-induced increase in H2S production under both healthy and oxidative stress conditions ($p<0.001$, one-way ANOVA, n=5), indicating that sildenafil increases H2S levels through inducing endogenous H2S synthesis in mice aorta. Sildenafil significantly increased L-cysteine relaxation ($p<0.05$, $p<0.001$, two-way ANOVA, n=5), it also reversed the decreased relaxation responses under conditions of oxidative stress ($p<0.05$, $p<0.001$, two-way ANOVA, n=5). AOAA inhibited Sildenafil-induced L-cysteine-mediated relaxation responses under both healthy and oxidative stress conditions ($p<0.01$, $p<0.001$, two-way ANOVA, n=5). Conclusion. Further, our results suggest a new concept that the combination of L-cysteine with sildenafil may be a new therapeutic approach in cardiovascular diseases. Since H2S has antioxidant, anti-inflammatory, and vasodilatory effects, we suggest that our findings may pave the way for further investigation into the role of sildenafil in vascular pathologies where oxidative stress is involved.

**Keywords:** Hydrogen sulfide; Sildenafil, PDE-5
**Funding:** The study was supported by the grant No. VEGA-2/0157/21 and BAP-21428
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**S7**-60

**Title: Effect of deuterium depleted water on cardiac nitric oxide synthase activity in normotensive and hypertensive rats.**

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**Abstract:**
Objective: Deuterium-depleted water (DDW), also known as light water, has a lower concentration of deuterium than occurs naturally. There are currently several studies showing a beneficial effect of DDW on cancer and metabolic syndrome as well. The aim of our study was to determine the effect of DDW on blood pressure, relative heart weight and cardiac nitric oxide synthase (NOS) activity in normotensive Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) treated with 15% fructose for three weeks. Methods: In this study, 48 male adult 12-week-old normotensive WKY and age-matched SHR were used. The experiment consisted of 8 groups (n=6 in each group): control group, group treated with DDW or fructose and the group treated with DDW and fructose concomitantly. Blood pressure was measured by tail-cuff plethysmography. Total NOS activity was determined by measuring the formation of L-[3H] citrulline from L-[3H] arginine in the heart. Results: Blood pressure and relative heart weight were significantly higher in SHR compared to WKY. Neither DDW nor fructose had any significant effect on blood pressure and relative heart weight in WKY or SHR. DDW increased cardiac NOS activity in both WKY and SHR. Although there was a decreasing trend in NOS activity after fructose treatment in WKY, finally no significant changes were determine in both strain studied. Concomitant treatment of DDW with fructose increased cardiac NOS activity even above the control level. This increase was significant in spontaneously hypertensive rats. Conclusion: In conclusion, deuterium-depleted water was able to increase cardiac NOS activity in both normotensive and hypertensive rats which may have serious cardioprotective effects in different cardiac disorders.

**Keywords:** deuterium depleted water, nitic oxide
**Funding:** Supported by grants APVV-22-0271 and VEGA 2/0122/24.
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**S7**-59

**Title: Effect of low dose L-NAME pretreatment on NO/ROS balance and vasoactivity in L-NAME/salt-induced hypertensive rats**

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**Abstract:**
Objective: NADPH oxidase–dependent reactive oxygen species (ROS) overproduction and decreased nitric oxide (NO) bioavailability lead to vascular dysfunction and development of hypertension. Methods: The goal of our study was to analyze an effect of salt diet and NO synthase inhibition with NG-nitro-L-arginine methyl ester (L-NAME) on blood pressure (BP), arterial reactivity, NO production, as well as ROS level in adult rats pretreated with low dose of L-NAME (2 mg/kg/day) for three weeks. Higher dose of L-NAME (40 mg/kg/day), or salt diet (8% NaCl), or combination of both were applied for the following four weeks. Results: Administration of L-NAME in low dose had no effect on BP but enhanced the expression of NO synthase. Both higher dose of L-NAME and salt diet elevated BP, decreased NOS activity, and impaired the endothelium-dependent arterial relaxation. However, salt diet did not increase ROS production and sympathoadrenergic arterial contractions in low dose L-NAME-pretreated rats. Combination of salt diet with higher dose of L-NAME did not evoke additive decrease of NOS activity, but it caused elevation of CD concentration and NOX2 protein expression. Conclusion: These findings indicate that chronic low dose of L-NAME treatment has a potential to trigger adapting mechanisms deserving further investigation.

**Keywords:** nitric oxide, salt diet, L-NAME
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**S7**-57

**Title: GWAS meta-analysis in SCAD, a women predominant ischemic heart disease, reveals common variants and genes related to artery integrity and tissue-mediated coagul**

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**Abstract:**
Cardiovascular disease is the leading cause of death in women but sex-specific forms of the risk of heart disease remain under-studied. Spontaneous coronary artery dissection (SCAD) affects a young, predominantly female population, and arises from hematoma formation leading to coronary artery dissection, rather than atherosclerotic plaque rupture as in coronary artery disease (CAD). Through a meta-analysis of 8 GWAS including 1917 cases and 9292 controls of European ancestry, we identified 16 risk loci, including 11 new. Functional annotations pointed at enrichment in enhancer marks of arteries, specifically smooth muscle cells and strong candidate genes, such as tissue factor gene (F3) on Chr1 near rs1146473 (OR=1.32, P=5.8e-9). F3 is novel for SCAD or any cardiovascular disease with high posterior probability (PP=94%) of colocalization as an eQTL for F3 in arteries, supporting the genetic risk is likely through lower F3 expression, a biological mechanism consistent with hematoma formation in coronary arteries. Bayesian gene regulatory networks constructed from expression and genetics data indicated the extracellular matrix organisation in arteries as the biological function where most prioritized genes clustered. Overall, we report evidence for substantial polygenicity for SCAD ($h2 = 0.71 \pm 0.11$) and shared genetic basis with several neurovascular diseases (e.g intracranial aneurysm: LDSC correlation coef. RG=0.22; P=2.0e-4). Intriguingly, for 6 loci, colocalization analyses showed that SCAD and CAD are likely to share the same causal variants with high PP (>80%) but involving opposite risk alleles. In addition, a negative genetic correlation was found between SCAD and CAD (RG=-0.12; P=3.7e-3), including after conditioning on blood pressure (mt-COJO: RG_CAD/SBP=-0.19, P=4.6e-6). Mendelian randomization analyses using genetic determinants of main cardiovascular risk factors for associations with either SCAD or CAD were significant for higher BP and increased risk for SCAD (SBP: BETA=0.05, P=7.6e-6, DBP: BETA=0.10, P=1.9e-6) and CAD (SBP: BETA=0.04, P=8.6e-49; DBP: BETAI=0.06, P=1.6e-44) but not for BMI, lipids, or type 2 diabetes, while we confirmed these traits as genetic risk factors for CAD. Our results set the stage for future investigation of novel biological pathways relevant to both SCAD and CAD and potential therapeutic and preventive strategies specifically targeting this ischem

**Keywords:** cardiovascular diseases, complex diseases, GWAS,
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**S7**-42

**Title: Angiogenic and lymphangiogenic effects of mesenchymal stem cell extracellular vesicles through transfer of microRNAs and proteins, including ITGα5 and NRP1**

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**Abstract:**
Objectives: Mesenchymal stem/stromal cell (MSC)-derived extracellular vesicles (EVs) mediate pro-regenerative effects in damaged ischemic tissues by regulating angiogenesis. MSC-EVs modulate the functions of cells, including endogenous mature cells, progenitor cells, and stem cells, resulting in the restoration of blood flow. However, the mechanisms underlying such MSC-EV activity remain poorly understood. The present study analyzes the biological effects of bone marrow (BM)-derived MSC-EVs on endothelial cells (ECs) in ischemic tissues under both in vitro and in vivo conditions and elucidates the molecular mechanisms underlying tissue repair. Methods: MSC-EVs were isolated from murine BM-derived MSCs and their morphological, antigenic, and molecular composition in terms of protein and microRNA levels were evaluated to investigate their properties. Results: Global proteomic analysis revealed the presence of proteins in MSC-EVs that regulate pro-regenerative pathways, including integrin α5 (Itgα5) and neuropilin-1 (NRP1), which are involved in lymphangiogenesis. MSC-EVs were also enriched in microRNAs that regulate angiogenesis, TGF-β signaling, and processes that control cellular adhesion and interactions with the extracellular matrix. The functional effects of MSC-EVs on capillary ECs in vitro included an increase in capillary-like tube formation and cytoprotection under normal and inflammatory conditions by inhibiting apoptosis. Notably, MSC-EVs also enhanced capillary-like tube formation of lymphatic ECs, which may be regulated by Itgα5 and NRP1. Moreover, in a mouse model of critical limb ischemia, MSC-EVs enhanced the recovery of blood flow in ischemic muscle tissue, which was accompanied by increased vascular density in vivo. This pro-angiogenic effect was associated with an increase in nitric oxide (NO) production via activation of endothelial NO synthase in ischemic muscle. Interestingly, MSC-EVs enhanced lymphangiogenesis, which has never been reported before. Conclusion: This study provides evidence for pro-angiogenic and novel pro-lymphangiogenic roles of MSC-EVs on ECs in ischemic tissues. These roles are mediated by their protein and miRNA molecular cargoes. The results identify Itgα5 and NRP1 on MSC-EVs as potential therapeutic targets for promoting lymphangiogenesis.
**Anna ŁABĘDŹ-MASŁOWSKA , J Nanobiotechnology. 2024; 22: 60.**

**Keywords:** Pro-angiogenic, pro-lymphangiogenic, extracellular vesicles, ischemia
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**S7-**34

**Title: Blood pressure changes in patients with spinal cord injury during orthostatic challenge and their attenuation by compression aids**

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**Abstract:**
Objective: Patients after complete cervical spinal cord injury (SCI) suffer often from autonomic dysregulation. Impaired sympathetic control over cardiovascular system leads to the blood redistribution and in consequence to the hypotension during orthostasis. Since rehabilitation by verticalization is often used in these patients, orthostatic hypotension (eventually leading to a syncope) represents a challenge both for patient and for caretakers. In such cases, compression aids (CA) are used as a non-pharmacological therapy since they are supporting venous return and thus prevent the orthostatic hypotension. Methods: The aim of the study was to evaluate the effect of CA on the baroreflex control over the cardiovascular system during verticalization in SCI patients. Blood pressure (BP) was continuously recorded by photoplethysmography in 10 subjects with cervical SCI in three consecutive positions: sitting, verticalization with CA and verticalization without CA. From the BP record, sequences of beat-to-beat systolic, diastolic and pulse pressures (SBP, DBP, PP) and inter-beat intervals (IBI) were detected. Causal coherence from SBP to IBI (Coh) and baroreflex sensitivity (BRS) were calculated by autoregressive bivariate model. Results: All variables - SBP, DBP, PP, and BRS - significantly decreased during verticalization and this decrease was attenuated in case when CA were used. Coh did not change during verticalization without CA, but increased with CA. Use of CA supported venous return and consequently stroke volume (expressed as PP increase), which probably prevented the impairment of the baroreflex BP control during verticalization. Conclusion: Compression aids may be successful in improvement of BP regulation during verticalization in cervical SCI patients.

**Keywords:** orthostasis; compression aids; causal analysis
**Funding:** Supported by grant no. MUNI/A/1547/2023.
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**S7-4**

**Title: Extracellular vesicles derived from primary cilia induce oxidative stress in human endothelial cells**

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**Abstract:**
Objective: Primary cilia play a key role in maintaining homeostasis in the cardiovascular system. A decrease in the number and length of primary cilia present in endothelial cells is associated with ciliopathies. However, molecular mechanisms of cilia-related diseases are not elucidated. Disruption of primary cilia can be achieved by pharmacological interventions as well as by the release of extracellular vesicles (EVs), which cause decapitation of these cilia. Here, we investigate the role of EVs released from primary cilia in modulating endothelial function. Methods: EVs were isolated and characterized from human vein endothelial cells with intact primary cilia and those where primary cilia were pharmacologically removed using chloral hydrate. Subsequently, we assessed the effects of these EVs on cultured endothelial cells and on ex vivo mesenteric arteries from ApoE knock-out mice. Results: We demonstrate that pharmacologically-induced loss of primary cilia leads to increased release of large and small EVs, which show an enrichment of primary cilia markers. These EVs impair endothelial function by promoting cytosolic reactive oxygen species (ROS) production, affecting endothelial cell migration, and increasing permeability. Furthermore, large but not small EVs from deciliated cells did not affect nitric oxide release but increased cytosolic ROS production in cells and mesenteric arteries. These effects were not prevented by inhibiting ROS sources with allopurinol and apocynin in cells, but interestingly, they were prevented in mesenteric arteries. Mitochondrial ROS production were not affected by EV treatment. Additionally, EVs from ciliated cells had a protective effect on HUVEC, promoting endothelial cell migration and preventing the increase in endothelial cell permeability induced by TNF-α. Finally, large EVs from patients with obstructive sleep apnea syndrome, who display endothelial dysfunction, overexpressed primary cilia markers. Conclusion: Taken together, these findings highlight the role of primary ciliary EVs in endothelial dysfunction through increased oxidative stress and suggest that EVs may serve as a potential biomarker for cardiovascular disease.

**Keywords:** Extracellular vesicles; endothelial function; cilia
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**S7**-16
**Title: Isolated Rat Aorta Model in Doxorubicin Toxicity Study – pilot data**

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**Abstract:**
The isolated aortal ring is widely used model in preclinical testing of drugs with effects on the vessels. Changes of its tension after drug administration predict the drug´s vasoactive effects, but may also reveal potential vascular toxicity. Anthracycline cytostatic doxorubicin, used in the treatment of numerous cancers, often causes systemic vasculitis. However, the mechanisms of doxorubicin vascular toxicity are not fully understood yet. This pilot study focused on the potential effects of doxorubicin on vascular reactivity. The thin (2 mm) aortal rings from Wistar rats were randomly immersed in Krebs-Henseleit (K-H) solution (PHYS group, negative control; CaCl2 2.49 mM, 37 °C, constant O2 saturation) or in K-H solution with doxorubicin (DOX group; 1 μM). After 3-hrs incubation, the aortal ring was fixed on the myograph (DMT751-Mini-Tobs, Denmark), immersed in K-H solution and tension was continuously recorded. After setting the initial tension (10 mN, prestretch), the aortal ring was stabilized (10 min) and prestretched again (10 mN). The physiological functions of the ring were verified by cumulative dose-response of phenylephrine (PHE) and then acetylcholine (ACE) in gradually increasing doses (3 nM, 10 nM, 30 nM, 0.1 μM, 0.3 μM, 1 μM, 3 μM, 10 μM). Doses were administered in 5 min intervals. Then, the ring was washed out 3x by the K-H solution. If necessary, the standard tension (10 mN) of the ring was set again. Next, a single dose of PHE (2 μM) was administered. After 10 min, L-NAME (L-N-Nitro arginine methyl ester; 300 μM) was added to determine the change in tension upon NOS inactivation (20 min). Dose response curves were constructed using Prism 10 (GraphPad) software. No significant difference between DOX and PHYS groups was detected in response to PHE and ACE. The change in tension upon NOS inactivation was expressed as percentage of previously induced tension by PHE. In the DOX group, NOS inactivation lead to increase in tension to 106.7±2.9%. In PHYS group, tension upon NOS inactivation increases to 106.2±4.1%. There is no significant difference in the effect of NOS inactivation between the groups. The results reveal no significant effect of DOX (1 μM) on adrenergic and cholinergic stimulation and NOS inactivation in aortal rings. A follow-up study will focus on the potential impact of chronic or acute cardiovascular drugs administration on the effects of doxorubicin on vascular reactivity.

**Keywords:** doxorubicin, aorta, rat, L-NAME
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**S7-**19

**Title: Sex differences in cardiac function and remodeling induced by early abdominal aortic constriction in Wistar rats.**

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**Abstract:**
Pressure overload-induced cardiac remodeling can result in life-threatening arrhythmias or heart failure. Despite progression in diagnostics and treatment, the role of sex in cardiac remodeling is not fully elucidated. Early postnatal abdominal aortic constriction (AAC) is a unique experimental model with the potential to mimic congenital heart diseases with elevated workload. Using this model, the study aimed to determine the impact of increased pressure load applied in the early postnatal period on the left ventricular (LV) geometry and function impairment in adult Wistar rats, emphasizing sex differences. AAC was induced by ligation of the aorta (internal diameter of the ligature 0.25 mm) in the subdiaphragmatic suprarenal region on postnatal day 2. In control groups, the aorta was exposed but not constricted. Cardiac function and geometry were assessed by echocardiography. Cardiac electrophysiology was evaluated by optical mapping, and myocardial fibrosis was assessed by immunohistochemistry. At postnatal day 90, AAC resulted in LV dilatation (LVDd: 9.26±0.23 mm vs. 8.10±0.12 mm and 8.15±0.27 mm vs. 7.15±0.09 mm, respectively) and LV wall thickening in both males and females with AAC compared to controls. Enlargement of LV expressed as LVW/BW was similar in males and females compared to sham-operated animals (LVW/BW ratio increased by 103 ± 9 % and 99 ± 10 %, respectively). AAC also led to a decrease in systolic function in both males and females (FS: 30.3±1.4 % vs. 41.6±1.0 % and 33.4±1.8 % vs. 43.2±0.9 %, respectively) and a decrease in heart rate. However, cardiac output was not altered in both sexes. RVW/BW and lung/BW ratios were increased in AAC animals compared to controls and were higher in males than in females with AAC, indicating progressive cardiac dysfunction in AAC males. AAC-induced cardiac remodeling was also associated with higher levels of myocardial fibrosis in males than in females. Moreover, conduction velocity (CV) in males with AAC decreased compared to controls (CVL 80±3 vs. 92±3 cm/s and CVT 35±2 vs. 54±3 cm/s) but was preserved in females compared to controls (CVL 104±8 vs. 109±8 cm/s and CVT 66±7 vs. 59±6 cm/s). Our data show that early postnatal exposure to pressure overload leads to similar alterations in cardiac geometry in males and females. However, AAC-induced cardiac remodeling exhibits sex-related differences resulting in pro-arrhythmogenic alterations in males but not females.

**Keywords:** Cardiac remodeling, sex differences
**Funding:** Supported by the Czech Health Research Council: NU21J-02-00039.
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**S7**-21

**Title: Extracellular vesicles carrying the NLRP3 protein are actively involved in the vascular inflammation associated with early atherosclerosis in metabolic syndrome**

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**Abstract:**
Objective: Atherosclerosis is a chronic low-grade inflammatory disease related to obesity and diabetes. Extracellular vesicles (EV) from metabolic syndrome (MetS) have been reported as important players in the earlier stages of atherosclerosis. NLRP3-Inflammasome is a multimeric cytosolic protein complex activated in response to cellular metabolic stress, and its implication in atherosclerosis development is not fully understood. We sought to uncover actionable targets that could help to refine the diagnostic values of MetS patients by taking advantage of EVs bearing NLRP3-Inflammasome to support the inflammatory hypothesis of atherosclerosis. Methods: Large-EVs (lEVs) were isolated from the plasma of non-MetS subjects and MetS patients and from human atherosclerotic lesions by using differential high-speed centrifugations. Characterization of plasmatic and atheroma lEVs was performed by Western blot and transmission electron microscopy. In addition, the number and expression of specific cell origin markers were determined by flow cytometry. The contribution of NLRP3 in the effects of EVs on human aortic endothelial cells (EC) permeability, human aortic smooth muscle cells (SMC) proliferation and migration, and macrophage secretome was analyzed by its pharmacological inhibition using the specific inhibitor MCC950. Also, the pathological relevance of NLRP3 carried by EV in human atherosclerotic lesions was investigated. Results: Our results indicate that circulating lEVs are enriched in NLRP3 inflammasome components, mainly in MetS lEVs. Furthermore, increased levels of NLRP3+ lEVs correlated with metabolic risk factors associated with obesity and insulin resistance. lEVs from MetS patients, but not from non-MetS subjects, displayed an atheroprone role by promoting EC permeability and proinflammatory cytokine secretion by SMC and macrophages. In addition, MetS-LEVs increased SMC proliferation and migration. Pharmacological inhibition of NLRP3 by MCC950 prevented the effects induced by MetS-lEVs. Furthermore, human advanced plaques demonstrated an accumulation of NLRP3+-lEVs and their implication in complicating atherosclerotic lesions by altering endothelial permeability. Conclusion: Our findings highlight the role of NLRP3-bearing EVs as a potential biomarker and target for potential therapeutic strategies in atherosclerosis-related diseases leading to major adverse cardiovascular events.

**Keywords:** Extracellular vesicles, Atherosclerosis, Inflammation
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**S7**-65
**Title: Extracellular vesicles as novel targets against vascular diseases associated to metabolic syndrome**

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**Abstract:**
Abdominal obesity is frequently associated with insulin resistance, elevated blood pressure, and dyslipidemia consisting in low levels of HDL-cholesterol and high levels of triglycerides; this cluster of events is denominated metabolic syndrome (MetS). MetS patients have increased risks to develop cardiovascular diseases and type 2 diabetes. Extracellular vesicles (EVs), small structures released from cells are now considered veritable entities for diagnosis, prognosis, and therapeutics. In particular, circulating EVs are increased in MetS patients and could participate in the development of vascular diseases.

We have shown that large EVs (lEVs) from MetS patients induced an increase endoplasmic reticulum stress reflected by an increase in phosphorylation of eIF2a and nuclear translocation of ATF6, which were prevented in the presence of TUDCA (Safiedeen et al. Antoxid Redox Signal 2017). Similarly, lEVs from MetS patients caused a reduction in NO release from endothelial cells, which was abolished when ER stress was inhibited by TUDCA. Fas/FasL interaction participated in the response induced by lEVs. In addition, neutral SMase activation induced directly ER stress which, in turn, increased both cytosolic and mitochondrial ROS. All of these events lead to reduction of NO release and the subsequent impairment of endothelium-dependent vasorelaxation.

Circulating levels of small EVs (sEVs) positively correlated with anthropometric and biochemical parameters including visceral obesity, glycaemia, insulinemia, and dyslipidemia (Ali et al. Metabolism 2021). Treatment of HAoECs with sEVs from MetS patients decreased NO production through the inhibition of the endothelial NO-synthase activity. Injection of MetS-sEVs into mice impaired endothelium-dependent relaxation induced by acetylcholine. Furthermore, MetS-sEVs increased DHE and MitoSox-associated fluorescence in HAoECs, reflecting enhanced cytosolic and mitochondrial ROS production. Patients with MetS had elevated circulating levels of lipopolysaccharide (LPS) in plasma. LPS was at least partly carried by circulating sEVs. Pharmacological inhibition and down-regulation of TLR4, as well as sEV-carried LPS neutralization, results in a substantial decrease of ROS production induced by MetS-sEVs.

Take together these results evidence lEVs and sEVs from MetS patients as potential new biomarkers for this syndrome, and suggest that FasL and LPS carried by EVs are promising therapeutic targets against the development of vascular dysfunction during MetS.

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